ABSTRACTS

June 26-27 Geno-MIC Workshop & Symposium

INVITED KEYNOTE

REIMAGINING ENERGY: HOW IMAGERY AND EMOTION HAVE SHIFTED ENERGY CONVERSATIONS

Lianne M. Lefsrud

Past energy discussions were framed around facts, science, and logical arguments – cognitive legitimacy. Over the past decade, all that has changed. Discussions have become increasingly centred on values and emotions. Moral legitimacy now dominates. To understand this changing dynamic, I examine the words and images that people choose to 'talk' about different resource industries including pipelines, how negative or positive these are, and the effect this has on subsequent conversations about resource development.

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INVITED KEYNOTE

DESIGN FEATURES OF OFFSHORE OIL PRODUCTION PLATFORMS INFLUENCE THEIR SUSCEPTIBILITY TO BIOCORROSION

Kathleen E. Duncan[1], Irene A. Davidova[1], Heather S. Nunn[1], Blake W. Stamps[1,2], Bradley S. Stevenson[1], Pierre J. Souquet[3], and Joseph M. Suflita[1].

Offshore oil producing platforms are designed for efficient and cost-effective separation of oil from water. However, design features and operating practices may create conditions that promote the proliferation and spread of biocorrosive microorganisms. The microbial communities and their potential for metal corrosion were characterized for three oil production platforms that varied in their oil-water separation processes, fluid recycling practices and history of microbially influenced corrosion (MIC). Microbial diversity was evaluated by 16S rRNA gene sequencing, and numbers of total bacteria, archaea, and sulfate-reducing bacteria (SRB) were estimated by qPCR. The rates of ^{35}S sulfate-reduction (SRA) were measured as a proxy for metal biocorrosion potential. A variety of microorganisms common to oil production facilities were found, but distinct communities were associated with the design of the platform and varied with different locations in the processing stream. Stagnant, lower temperature (< 37 °C) sites in all platforms had more SRB and higher SRA compared to samples from sites with higher temperatures and flow rates. However, high (5 mmol/L) levels of hydrogen sulfide and high numbers (10^7/mL) of SRB were found in only one platform. This platform alone contained large separation tanks with long retention times and recycled fluids from stagnant sites to the beginning of the oil separation train, thus promoting distribution of biocorrosive microorganisms. These findings tell us that tracking microbial sulfate-reducing activity and community composition on off-shore oil production platforms can be used to identify operational practices that inadvertently promote the proliferation, distribution and activity of biocorrosive microorganisms.

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MICROBIOLOGICAL, CHEMICAL AND CORROSION ANALYSES OF SOLIDS SAMPLES COLLECTED FROM A LEAKAGE SITE OF A HEAVY OIL TRANSPORTING PIPELINE

Mohita Sharma and Lisa M. Gieg

Microbial and chemical analyses were conducted on samples collected from a pinhole leakage site in a heavy oil transporting pipeline to assess the potential for microbiologically influenced corrosion (MIC). A total of 24 samples were collected from 4 locations representing a leakage area (Location 1), an area adjacent to leakage area (Location 2), a non-corroded area (Location 3) and sediments collected from the pipeline (Location 4). Water chemistry, XRD (X-ray diffraction) analyses, microbial community analysis, and corrosion assays were performed on these samples. Notably, all samples were comprised of microorganisms that were indicative of an anoxic environment, including fermenters, methanogens, sulfur-/thiosulfate-reducers, and known biofilm-forming organisms - all metabolisms known to play a role in MIC. XRD results showed an enrichment of $FeCO_3$ at/near the leak site, and elevated levels of acetate at/near the leak site (17-20 mM) compared to the non-leak locations (2-10 mM). Collectively the data suggested that fermentative organisms producing organic acids, biofilm-forming organisms, and organisms able to withdraw electrons directly from the steel contributed to the corrosion at this terminal. Corrosion assays performed on these samples after incubation for one month showed the formation of localized pits of up to 20 µm in samples collected from Location 1, up to 4 µm in samples collected from Locations 2 and 3, and up to 10 µm in samples collected from Location 4. This suggests that microbial community present in the sediments may be responsible for formation of pinholes in the pipeline. However, investigations related to microbial community composition of planktonic and sessile samples collected from the corrosion assay incubations are currently in progress to confirm this hypothesis.

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SAMPLING AND CHEMICAL ANALYSIS OF PETROLEUM PRODUCTS

Ali Modir-Rousta[1], Christina Bottaro[1], and Kelly Hawboldt[2]

Timely and accurate sampling, handling, and transport of samples taken from field installations such as offshore platforms are critical in ensuring the samples are representative of the source. However, a protocol that requires costly and/or hazardous preservatives, is labour intensive or overly complex, or does not account for possible compound reactivity as pH, temperature, and pressure changes can be difficult to implement, this is especially in remote locations where there can be significant delays in transporting samples to the lab and the samples are complex in chemistry. As the sample matrix (chemistry) becomes more complex (such as produced water), costly and time-consuming sample treatment is required. We have developed a sampling technique called molecularly imprinted polymer (MIP) thin film to simplify the sample preparation steps. In this technique a thin film polymer which consists of monomer, crosslinker, solvent system and template is designed, tuned and fabricated to target a group of compounds that share comparable properties. Finally, we developed several analytical methods using MIP technique, we can selectively pick up target analytes from complex matrices. In this work, we present a high throughput method for extraction and analysis of 16 PAHs in produced water using our newly developed MIP-PAH combined with headspace-GC-FID technique. We have achieved up to 80% recovery and sub ppb LOD for this group of compounds. Since this technology is transferable to other class of compounds, we managed to include phenols, thiophenes, nitrosamines and compounds of interest in produced water for MIC project.

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GENETIC PREVALENCE OF MULTIDRUG RESISTANCE EFFLUX PUMPS IN A MODEL MICROBIOLOGICALLY INFLUENCED CORROSION COMMUNITY

Damon Brown and Raymond J. Turner

Increased resistance of microbes in a microbiologically influenced corrosion (MIC) community towards industrial biocides (i.e. antiseptics) such as glutaraldehyde, tetrakis-hydroxymethyl phosphonium sulfate (THPS), 2,2-dibromo-3-nitrilopropionamide (DBNPA), etc., has been hypothesized to originate from horizontal gene transfer (HGT) events of multidrug resistance efflux pumps (MDREPs). MDREPs have previously been studied in antibiotic resistant microbial strains. MDREPs are frequently contained on transmissible plasmids or transposons, allowing the rapid spread of these genes in a biofilm. A mixed model community is being created to study the transfer of MDREPs. This model community will be comprised of six species, each previously identified in, or associated with, MIC and each representing a different metabolic process. The chosen species (and metabolic roles) are: *Acetobacterium woodii* (acetogen), *Bacillus subtilis* (fermentation), *Desulfovibrio vulgaris* (sulfate reducing), *Geoalkalibacter subterraneus* (iron reducing), *Thauera aromatica* (denitrification/hydrocarbon degradation) and *Pseudomonas putida* (biofilm pioneering).

This work focuses on the design of unique primers to target MDREPs known to efflux biocides for each member of the model community. Additionally, the successful construction of a model MIC community will be done to standardize MIC testing procedures. Here we show the primers, which have been designed for select MDREP genes. The results of our initial PCR amplification experiments will also be presented.

By monitoring and quantifying the copies of these genes with respect to 16S rRNA gene copy numbers using quantitative PCR (qPCR) in the model community, we should be able to determine which MDREPs are being transferred within the community in response to treatments of different biocides. This will provide any easy, DNA-based screen to assess a field sample's potential for resistance towards different biocides.

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MEASUREMENT OF MICROBIAL ACTIVITY AS A DIAGNOSTIC TOOL FOR THE DETECTION AND MITIGATION OF BIOCORROSION IN THE ENERGY SECTOR

Irene Davidova and Joseph M. Suflita

The rapid development of molecular tools focused attention on new and different ways to assess microbial communities. Metagenomics and qPCR–based analysis continue to offer tremendous insight on microbial community structure and cell quantification, but are generally unable to measure actual microbial activity associated with a process of interest. Quite often, the most abundant organisms are not necessarily the most metabolically active. Metabolic activity and specifically, microbial respiration depends on the prevailing physico-chemical conditions, oxygen status, nutrients, availability of electron donors/acceptors, sediments structure, water flow rate and other ecological variables. As a result, microbial activities tend to be patchy in distribution and form pockets where ambient conditions are most favorable. In the case of sulfate reduction, an important process closely associated with metal biocorrosion in the oil and gas industry, a sensitive but underutilized radiotracer method is available for directly assessing microbial respiratory activity. A measure of the rate of bioconversion of $^{35}SO_4^{2-}$ to $H_2{}^{35}S$ in reservoir samples or along surface processing facilities allows operators to pinpoint "hot spots" of sulfate reduction. Investigative attention can then be focused on why problem areas are particularly acute and mitigation efforts can be more directly targeted. The commercially available radioisotope technique is also useful for the rapid evaluation of biocide efficacy. By directly measuring the rate of sulfate reduction, it is possible to customize the

choice and the amount of biocide(s) for control of model organisms or complex microbial communities under near *in situ* conditions. While all tools have their interpretational limits, the direct assay of sulfate reduction activity is a powerful augment to molecular tools in the larger biocorrosion diagnostic and mitigation arsenal.

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ISOTHERMAL PCR FOR RAPID MIC DIAGNOSIS

Natalie M. Rachel and Lisa M. Gieg

The current process to analyze microbial genetic profiles within pipelines involves many steps and manipulations, including: sample collection, preservation, and transport to a laboratory for processing. While this process is effective, it is not ideal for rapid diagnosis and furthermore, multi-step protocols introduce additional variables that may skew results. We seek to develop a means to genetically diagnose microbially influenced corrosion (MIC) on-site, rapidly, with as few manipulations as possible and that can be performed on-site.

Recombinase Polymerase Amplification (RPA) is an isothermal, commercially available genetic amplification biotechnology that was selected to investigate applicability to MIC detection. Benchmarking experiments were established by selecting a genetic target known for playing a role in MIC: *dsrAB*, which encodes the diagnostic sulfite reductase in sulfate reducing bacteria (SRB). Specifically, primers were designed for the extensively-studied SRB, *Desulfovibrio vulgaris* Hildenborough (DVH). Reactions proceed within 25-40 minutes at 37°C and produced high yields of the gene fragment. In an attempt to keep primer numbers low and achieve broad, diverse genetic coverage, a series of primers was prepared targeting DVH containing a varying number of mutations to investigate the tolerance required for reproducible amplification. We have determined that 15-18% of the primer sequence can vary from the template sequence and still bind reliably to the target. Knowing this, RPA-compatible primers based on a large-scale collection and bioinformatic analysis of hundreds of *dsrAB* sequences were also designed, to capture a broad array of SRBs from a variety of different samples. This methodology is being expanded to other genes diagnostic of MIC activities.

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A CARBON NANOTUBE BASED ELECTROCHEMICAL SENSOR FOR SULFIDE DETECTION

Xin Pang and Chao Shi

Microbiologically influenced corrosion (MIC) is considered one of the major causes of oil and gas pipeline failures, costing billions of dollars annually. Despite the increasing research attention towards this area, in-situ detection and monitoring of MIC in real time has presented difficulties, due to the complexity of the corrosion processes resulted from the activities of microorganisms such as sulfate-reducing bacteria (SRB). CanmetMATERIALS has been engaged in the development of electrochemical biosensor for the detection of MIC for over a decade. Recently, we incorporated advanced nanomaterials into the probe design to improve sensor performances, for fast and sensitive detection of H_2S. As SRB can act as a catalyst in the reduction of sulfate producing H_2S, the activity of SRB and the extent of MIC can be detected and monitored by measuring the concentration of sulfide in presence. In this work,

single-walled carbon nanotubes (SWCNTs) were functionalized using a conductive polymer for the fabrication of a nanocomposite sensing layer. An effective cross-linking agent was identified to enhance the water stability of the sensor. Fast and sensitive detection of sulfide was achieved, attributing to the large active surface area of the carbon nanotubes and excellent conductivity of the nanocomposite sensing layer. The sensor developed paved the way for further development of online sensors for monitoring MIC.

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INVITED KEYNOTE

AN ONLINE BIO-PROBE FOR MONITORING MICROBIAL GROWTH FOR APPLICATION IN OIL AND GAS SYSTEMS

Tesfa Haile[1], Yongxu Chen[2], and John Wolodko[2]

Currently, there are no online microbial growth monitoring tools regularly used by conventional and non-conventional oil and gas operators in Canada. Existing commercially available probes used for biofilm monitoring are biological sample collection devices for subsequent off-line microbiological measurements. These sample devices include the Bio-probe HCA200, Teledyne Corman Biofilm Probe, Caproco Biofilm Probe and CorrOcean Bio-probe. Flush mounted coupons are also routinely used to measure bacterial density offline. The most common methods used by the industry are serial dilution, followed by Biological Activity Reaction Test (BART™ Testers). Genomic tools; such as qPCR are also being considered by several pipeline operators in an effort to accurately monitor microbes associated with microbiologically influenced corrosion (MIC). At present, BIoGeorge and ALVIM, electrochemical biofilm activity monitoring system, were found to be the only online probes recognized by the petroleum industry but are not regularly utilized by Canadian operators.

Microbial cells within a biofilm can be considered as a suspension of spherical capacitors, each containing conducting cytoplasm. When an electric field is applied to a suspension of cells in an aqueous ionic solution, the ions in the cytoplasm are forced to move causing the cells to polarize. The magnitude of the cells field induced separations is measured by its capacitance (C) in pico-Farads (pF) at various frequencies. In a biofilm system the number of microbial cells will increase over time resulting in increase in capacitance proportionally. The goal of the present work is to develop a bio-capacitor based microbial growth monitoring device that can be coupled with existing corrosion monitoring tools to predict biofouling and microbiologically influenced corrosion (MIC) real-time.

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INVITED KEYNOTE

MICROBIOLOGICALLY INFLUENCED CORROSION ISSUES – AN INDUSTRY END-USER POINT OF VIEW

Tony Semerad

A 2016 NACE IMPACT study indicated that the Worldwide wastage of metallic materials caused by corrosion reaches about $2.5 trillions UDS annually. About 20% of this wastage is caused by microbiologically influenced corrosion (MIC); i.e., about $100 billions USD. In the Oil & Gas Operations MIC is one of the leading causes of equipment failures.

MIC is an electrochemical process where metal wastage (corrosion) is initiated, propagated and accelerated by the activities of microorganisms in the presence of water. In most cases, MIC is driven by the synergic action of several types of microbial communities that create complex self-supporting colonies. Usually, MIC is complemented by other corrosion processes that are active on the metallic surfaces. The environmental and operating conditions must be conducive to the proliferation and activity of microbial consortia participating in such destructive processes.

MIC is usually a localized attack, which is characterized as isolated pitting. The unpredictability of occurrence and corrosion rate makes detection, forecast, prevention and management of MIC very challenging. In addition, H_2S generation (souring), flow restriction (biofouling, plugging and deposition of reaction products), and fluid degradation usually accompany the MIC process.

The aim of this presentation is to give an overview of learnings, recent developments, and proposed remedies for this challenging MIC problem.

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INVITED KEYNOTE

RELIABILITY AND INTEGRITY MANAGEMENT OF OFFSHORE STRUCTURES

Rouzbeh Abbassi

Significant attention should be given to predict the occurrence of pitting corrosion in offshore structures. This presentation provides a brief review of the current understanding of the pitting corrosion mechanism and investigates the factors affecting pitting corrosion. A development of a novel methodology for an optimum maintenance programme by integrating a Risk-based Maintenance (RBM) approach with risk assessment strategy will be presented. The developed methodology is applied to a case study in an offshore oil and gas production facility. A sensitivity analysis using developed methodology proved that pitting corrosion is a critical factor for structural deterioration. A novel probabilistic methodology will be presented to precisely predict the depth of pitting corrosion for structural steel in marine and offshore environments. The propose model combines an understanding of corrosion phenomenological model and empirical model calibrated using real-world data. The results of an accelerated laboratory experiment for pitting corrosion in order to understand the practical significance of pitting mechanism, and to realistically predict the long-term service life of the steel structures will be provided and discussed. This includes a series of pitting corrosion tests on stainless steel specimen with different thickness. This experimental investigation will help to modify ASTM G48 procedure. Finally, the results of numerical modelling of the stainless-steel specimen with varying thickness subjected to different level of pitting corrosion deterioration will be shared. This numerical model using Finite Element Analysis (FEA) on corroded and un-corroded steel specimen to predict the ultimate tensile strength and validation with experimental data will be presented.

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VULNERABILITY ANALYSIS OF MIC IN OIL AND GAS OPERATIONS

Mohammed Taleb-Berrouane, Faisal Khan, and Kelly Hawboldt

Microbiologically Influenced Corrosion (MIC) is proposed to be a factor in approximately 30-40% of total corrosion-related failures. Assessing MIC is complex due to factors such as microbial growth rates, and attachment to the metal surfaces. In the literature, there is little published work attempting to predict the process equipment or pipeline segment, in which, the MIC could predominately manifest. On this basis, the present paper introduces a novel risk-based ranking tool to identify and rank process equipment based on the vulnerability with regard to MIC. The proposed tool ranks the vulnerability using a set of indicators such as MIC detectability, accessibility for in-line inspection, and distance from points of sampling. The tool provides an early recognition tool to map MIC most sensitive locations in the process circuit. To test and verify it, an offshore oil rig is taken as a case study. The obtained results have proved its effectiveness and advanced attributes. This tool, once implemented, would help in MIC sampling, diagnosis, and prediction processes.

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MECHANISTIC MODELLING OF MICROBIAL INFLUENCED CORROSION IN OIL AND GAS PRODUCTION OPERATIONS

Abdul-Waris Dauda, Ibraheem Adeoti, and Faisal Khan

Microbiologically Influenced Corrosion (MIC) is known to be regular mechanism in oil and gas operations and is becoming an increasingly important branch of research in corrosion. MIC is a complex phenomenon involving range of parameters, complex interactions, and diverse in discipline. The mechanistic pathways leading to quantifying corrosion and microbial growth rates are sparsely investigated or discussed in the open literature. Sulfate reducing bacteria, SRB, has been identified and studied as one of major microbes in MIC. Diffusion of nutrients required for bacterial growth is an important factor in the subsequent MIC. In this study, the effect of biofilm thickness on microbial nutrient diffusion; on the overall corrosion mechanism; and on the corrosion rate are investigated. This work uses the Biocatalytic Sulfate Reduction (BCSR) model in studying/predicting the corrosion mechanism. The combination of the nutrient diffusion; pitting rate model (BCSR) can give better insight into the mechanism, and prediction of corrosion rates. The rate of nutrient diffusion to/through biofilms of varying thickness can indicate microbial growth rate (e.g. limited diffusion could indicate limited growth), and subsequent corrosion rates.

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IMPACT OF FES IN THE MECHANISTIC MODELLING OF MICROBIOLOGICALLY INFLUENCED CORROSION RATE

Nonso Ezenwa[1], Faisal Khan[1], Kelly Hawboldt[1], Torben Lund Skovhus[2], Richard Eckert[3]

Microbiologically influenced corrosion (MIC) is a form of corrosion impacting pipelines and equipment used in oil and gas operations. In production and processing systems where sulfate is present, MIC is predominantly caused by the sulfate reducing bacteria (SRB) and sulfate reducing archaea (SRA), generally referred together as sulfate reducing prokaryotes (SRP). To better manage assets operating in various environments, a reliable determination of the more MIC pitting rate is needed. Some models for MIC pitting rate have been proposed based on sulfate diffusion in the biofilm. Though these models are useful, none incorporates the effect of iron sulfide (FeS), an important product of MIC, into the estimation of the pitting rate. Recent studies have provided a better understanding of the formation of FeS as an MIC product as well as its structure and properties. This knowledge is applied here to mathematically develop a model from the Gu-Zhao-Nesic model, considering the conductivity of FeS as an influencing parameter of the pitting rate. The proposed model predicts that the MIC pit depth over time follows an exponential curve which was validated using data from the literature. It is tested and verified using earlier published laboratory and field results.

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THE MICROBIOLOGICALLY INFLUENCED CORROSION ONTOLOGY (MICON)

Elvira Mitraka and Robert Beiko

Robust data curation practices and conceptual model standards have become a necessity. Ontologies are used as backbones for databases and data management. They represent knowledge of a specific domain in a manner that is understandable by machines and humans alike.

The Microbiologically Influenced Corrosion Ontology (MICON) will become the connecting point among standard operational protocols in on- and off-shore oil pipelines, pipeline maintenance, sample extractions, metagenomics analysis, and various other related wet lab and computational procedures. It is already interoperable with other well established ontologies in different domains and will expand even more to cover all the aspects of microbiologically influenced corrosion. It includes both unique terms and imported terms from other established and curated ontologies. It also includes logical definitions and axioms, which will enable for smarter searches in the database, as well as automatic categorization of samples and analysis outputs. The Standard Operational Protocols (SOPs) have been translated into ontological processes, so that every step has a machine readable representation in the ontology. The metadata fields are in place, according to the minimum information requirements for the samples. We have also established textual definitions for a variety of corrosion specific terms, standardizing the exact meaning and facilitating communication between different experts.

The database that is being built using MICON will allow for smarter searches, interoperability between data sources and precisely annotated and curated data. Other interested parties, outside of this project, that also use the aforementioned ontologies, will be able to easily compare their analyses with MICON data.

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ASSESSING INTERACTION CHANNELS FOR KNOWLEDGE TRANSFER BETWEEN ACADEMIA AND INDUSTRY – PRELIMINARY RESULTS FOR THE MIC COMMUNITY

Nicholas Bak[1], John Wolodko[1], Lianne Lefsrud[2]

The linkages and benefits of university-industry (U-I) interactions and collaborations have been studied for a number of years. Documented benefits from both perspectives are often both physical and intangible, and significant resources are often expended to help facilitate meaningful and productive partnerships. Among the varying fields of U-I research, our team has focused on an area known as "Interaction Channels" which represent the various ways industry and academia can interact and pass on knowledge. Our research plan integrates a bibliometric approach analyzing co-authorships of peer-reviewed and conference papers in conjunction with a traditional survey approach. By analyzing the frequent co-authorship groupings from academic journals, NACE Corrosion Conferences, and the various SPE conferences, we profile these three venues based on the frequency of U-I co-authorship. Combining these methods, we will highlight the most influential interaction channels for knowledge transfer from academic researchers to industry in a growing research field such as the use of genomics in Microbiologically Influenced Corrosion (MIC). Our targeted participants are from across the MIC value chain, with academia being represented by university and government researchers and industry being represented by suppliers/service companies and end users. Using survey methodologies, we plan to have participants rank the channels by importance, as well as suggest and rank various barriers that impede the transfer of knowledge. By analyzing these barriers, we hope to help bridge the gap and provide academia and industry with the most effective channels to transfer research into practise. This presentation provides a summary of the existing literature on best practises and barriers on U-I interaction, and a recent bibliometric analysis on the role of conferences in transferring knowledge from between the academic community and industry. Conclusions from this work will then be used to develop our own hypotheses specifically for the MIC field which will then be tested using future surveys.

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POSTER ABSTRACTS

THE GENO-MIC DATABASE

Sayna Hajiloo and Robert Beiko

The amount and variety of data being generated by the geno-MIC project requires customized solutions to facilitate storage, analysis/reanalysis, retrieval, and visualization. The geno-MIC Database aims to integrate multiple project data sources, and utilize the Microbiologically Influenced Corrosion Ontology (MICON) to support smarter search and easier accessibility to the specific data for researchers, industry partners and the public.

The geno-MIC database is implemented using the PostgreSQL management system and encompasses relational and non-relational (NoSQL) components to support both structured (i.e. project data compliant with MICON) and unstructured data such as sequence reads. Key features under development include automated import of project data, execution of standard analysis pipelines (e.g., QIIME2 for marker-gene analysis) and retention of intermediate results, connections to visualization tools, export of data, and data privacy and security.

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WHAT'S IN YOUR BUG BOTTLE?

16S RRNA GENE SEQUENCING OF ENRICHED AND UNENRICHED OILFIELD SAMPLES

Lisa M. Gieg, Courtney Toth, and Oscar Montoya

Microbial communities in oilfield systems can be identified and monitored using a variety of approaches. Growth-dependent 'bug bottles' that enumerate specific groups of microorganisms, and growth-independent molecular microbiological methods (MMM), such as quantitative PCR and 16S rRNA gene sequencing, are the most commonly used approaches. While bug bottles are simple to use and relatively low cost, it is well known that growth-based tests are only able to capture (grow) a very low percentage (e.g., 1-5%) of all the microorganisms that may be present in a given sample. For this reason, potential problematic microorganisms in an oilfield system may be missed if only bug bottles are used. Because MMM do not rely on growth, they can be far better at identifying all the microorganisms in a sample thus this approach can help detect potential microbiological problems earlier on in an oilfield system. In this work, we compared bug bottle assays (focussing on sulfate-reducing bacteria (SRB) and acid-producing bacteria (APB)) and 16S rRNA gene sequencing (16S) to assess the microbial community composition of a produced water and pigging sample. Each sample was subject to 16S analysis directly, or first incubated using the most-probable number test or BART^{TM} growth-based tests followed by 16S analysis of the grown (enriched) community. Direct 16S analysis on both samples did not reveal the presence of SRB (<0.5% of the total microbial community). In contrast, the growth based tests revealed the presence of 10^3 to 10^6 SRB, but 16S sequencing either revealed only low percentages of known SRB (e.g., 5%) and in one case, no recognizable SRB despite yielding counts of 10^6 SRB. Using 16S analysis, the microbial community composition present in the growth based tests did not mimic that of the original sample. This study confirms that growth-based bug bottles do not accurately represent the microbial community composition of a given sample, and cautions that what is 'growing' in bug-bottles may not be what is intended. Bug bottles do have value in monitoring trends in oilfield systems, but we recommend that these are complimented with non-growth based MMM to give a more accurate view of microbial community compositions.

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ISOLATION AND PURIFICATION OF A SULFIDE OXIDIZING ENZYME FOR ADHERENCE ONTO AN AMPEROMETRIC BIOSENSOR

Danika Nicoletti, Mohita Sharma, and Lisa Gieg

Detection of sulfide levels can give insight into the risk of Microbiologically Influenced Corrosion (MIC) due to the metabolic activity of sulfate-reducing microorganisms (SRM) in pipelines and other infrastructure associated with oil recovery. This makes a portable device like an amperometric biosensor for online detection of sulfide production in pipeline samples an interesting prospect for the oil and gas industry.

One promising approach for creating a biosensor is to adhere an enzyme to a sensor that carries out a desired metabolic activity; here, a sulfide oxidase serves as the key enzyme for sulfide detection. This project thus involves isolating a sulfide oxidizing enzyme from either pure cultures or enrichment cultures of sulfide-oxidizing microorganisms (SOM). The two pure strains chosen, *Thiobacillus denitrificans* and *Thiomonas intermedia*, were selected based on their known sulfide oxidizing activity. Environmental samples (wastewater sludge and produced water) were also enriched in selective media that included thiosulfate as the electron donor.

To determine SOM activity in enrichment cultures, Ion Chromatography (IC) measurements over 14 days of incubation showed a depletion of thiosulfate paired with an increase in sulfate within 4-7 days, varying between treatments and environmental samples. 16S rRNA gene sequencing in consecutive transfers showed an increase in *Thiobacillus* spp., *Betaproteobacteria* spp., and *Gammaproteobacteria* spp., and others, all of which are known to contain SOM taxa. Protein measurements confirmed growth of enrichment cultures in the presence of thiosulfate in comparison to controls, and enzyme assays with the same cultures showed depletion of sulfide. The establishment of these pure and enrichment cultures of SOM will be utilized in the next step of this research project; isolating and purifying an enzyme specific for sulfide, that can be used as a component of a sulfide biosensor for future field use in corrosion detection.

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EFFECTS OF DNA AMPLIFICATION PRIMERS, POLYMERASES, AND BIOINFORMATICS PIPELINES IN THE IDENTIFICATION OF MICROORGANISMS IN A MOCK COMMUNITY

Oscar Montoya, Natalie Rachel, and Lisa M. Gieg

With the advent of high throughput sequencing and the continuous drop in nucleic acid sequencing costs, amplicon sequencing of universally conserved phylogenetic marker genes (e.g., 16S and 18S rRNA genes) is now a commonly-used practice in industry and academia for monitoring microbial communities. The main advantages of this approach are that it is culture-independent, low cost, and gives relatively fast and accurate results. However, the molecular sequencing approach is subject to biases introduced at different stages of sample processing such as through DNA extraction protocols, choice of amplification primers, and polymerases selected for the polymerase chain reaction (PCR). Additionally, a plethora of computational biology tools have been made available to users. Modifications in the listed factors can drastically affect the results obtained from conserved phylogenetic marker genes. In this work, a mock community consisting of 11 microorganisms was created and a portion of their 16S rRNA genes were sequenced using Illumina MiSeq. Three sets of commonly used universal paired-end primers were used during the first round of PCR (initial DNA amplification step) to determine the effects of primer selection on amplifying the different taxa in the mock community. We also contrasted the effect of using high fidelity versus non-high fidelity polymerases during the second round of PCR (barcode attachment step). Finally, three bioinformatics pipelines for conserved phylogenetic marker genes were used to process the results. Mock

communities should be included in all sequencing runs to help ensure the quality of sequencing of all environmental samples.

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PRELIMINARY ASSESSMENT OF ABIOTIC ELECTROCHEMICAL RESPONSE FOR A PROPOSED BIOFILM DETECTION SYSTEM

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Microbiologically Influenced Corrosion (MIC) is a complex form of localized corrosion caused by the activity of microorganisms within a biofilm attached to a metallic substrate. Developing a system which can characterize the formation and evolution of these biofilms offers a potential way to detect the onset and location of potential MIC threats. This study examines the potential for using a two-electrode system to characterize biofilm initiation and growth using the Electrochemical Impedance Spectroscopy (EIS) technique. In the first part, a series of preliminary experiments were conducted on a variety of electrode materials to determine the response to abiotic conditions (DI water and ASTM G106 standard solution). EIS data was fit to a number of standard electrical circuit models to determine impedance and capacitance values for each electrode combination. The second part of the presentation outlines the next steps that will be conducted in assessing the biotic response of this novel system. Strains of sulphate-reducing bacteria (SRB) and iron reducing bacteria (IRB) will be introduced to the parallel plate system under controlled conditions, and the growth kinetics (cell counts over time) will be quantified using both Biological Activity Reaction Tests (BARTS) and spread plate method. The biotic growth kinetics will then be correlated to the EIS response to determine a calibration curve for various microbial species and detection system parameters (materials and fitting models). Future extensions to this work will also be discussed including the use of micromachined Inter-Digitated Electrodes (IDE) to achieve higher sensitivity readings, and the benefits of coupling the biofilm monitoring system with real-time corrosion measurements to identify and quantify the impact of localized MIC.

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REVIEW AND ANALYSIS OF MICROBIOLOGICALLY INFLUENCED CORROSION: CHEMICAL ENVIRONMENT IN OFFSHORE FACILITIES

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The chemical environment (e.g. produced water, soured oil, limited oxygen environments) plays an important role on microbial activities and corrosion product which impact MIC. The interaction of the chemistry (produced water, crude oil etc.) and microbial behaviour complicates the understanding of chemical species transformation and partitioning behavior in gas, water, and oil and impact on corrosion. This is further complicated by the corrosion by-products which have a feedback loop on both microbial and chemical reactions and phase behaviour. This work presents a review of the complex chemical transformations which include a wide range of chemical species resulting from biotic and abiotic processes. These chemical species can have growth, inhibitory, or synergistic effects on microbial activities causing MIC. The groups of microbes associated with MIC are analysed based on nutrients, physico-chemical conditions, and metabolic products as well as the chemical environment in the offshore oil and gas operations such as produced water systems, oil and gas separators, and flowlines. Microbial activities are found

to overlap with chemical/electrochemical processes leading to corrosion. This work further describes the links between chemical environment, environmental factors, electrochemical, and microbial processes. This study will inform further investigation on the chemical environment impacting MIC. This includes the on-going kinetic-equilibrium model of MIC chemistry/chemical environment (e.g. produced water, crude oil etc.). The model is proposed to capture four categories/environments; sulfur environments (e.g. soured waters, sour crude), nitrogen environments (e.g. fluids from reservoirs being treated for souring), oxygen rich environments, and environments where carbon dioxide is present. The partitioning behaviour of these environments will be modeled separately and then integrated.

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DEVELOPMENT ANALYSIS METHODS OF INDICATOR SPECIES FOR MICROBIOLOGICALLY-INFLUENCED CORROSION

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Inorganic species (e.g. thiosalts, sulfate, polythionates, nitrogen compounds etc.) impact environmental quality and have negative economic consequence on offshore operations e.g., anaerobic reduction can produce corrosive hydrogen sulfide and oxidation can lead to acidification of the environment and mobilization of toxic metals. Understanding the chemistry of the reservoir and process fluids throughout the oil extraction, processing or transport systems is key to devising strategies to limit the reductive or oxidative reactions and their associated effects. The complexity of these fluids requires a range of analytical techniques suited to analysis organic and inorganic species. We have used capillary zone electrophoresis with ultraviolet-visible (CZE-UV-Vis) in two strategies, one with direct detection and the other with indirect detection, for the determination of these ionic constituents of produced water. Both CZE methods were developed with reverse-polarity, employing hexamethonium hydroxide to modify the capillary surface chemistry and reduce electroosmotic flow and analysis time. Background electrolyte (BGE) components (pH and flow modifiers, chromophoric probes) were considered for each method. For indirect detection, selection of pyromellitic acid (PMA) as the chromophoric probe was based on its high molar absorptivity (high sensitivity) and good mobility match for the ions of interest, as well as its low potential for oxidizing analytes. Other factors that were taken into consideration include: the capillary length and its internal diameter. Optimization efforts and results will be presented.

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ELECTROCHEMICAL BEHAVIOUR OF ZN-NI ALLOY FILMS AS A REPLACEMENT OF ZN ANTI-CORROSIVE COATINGS DEPOSITED FROM CITRATE BATHS

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The anticorrosive coating is an effective approach for protecting the steel structures/machinery against corrosion in process industries. Electrodepositions of zinc and zinc-nickel alloy films on steel substrate under the various level of deposition conditions from baths containing potassium citrate were studied. The effects of plating variables such as bath composition and current density on the coating composition, morphology, corrosion and mechanical property were systematically investigated. The electrochemical and mechanical behaviour of Zn-Ni deposit obtained at 60mA/cm^2 from citrate bath exhibited lower corrosion current (I_{corr}), and less negative corrosion potential (E_{corr}) compared to pure Zn and Zn-Ni alloy coatings from the non-citrate bath. Crystallite size of Zn-Ni coating deposited from citrate bath is 19.64 nm, and Ni content of the coating is 8.3 wt%. The morphological properties and crystalline phase structure of the alloy coating were examined by scanning electron microscopy (SEM) and X-ray diffraction (XRD). The topographical structure of the coatings was analyzed by atomic force microscopy (AFM). The dominant γ-$NiZn_3$ (815) and γ-Ni_2Zn_{11} (330) (631) plane orientation in zinc-nickel alloy films improved corrosion resistance. Zn-Ni films with smaller grain size with uniform coating had increased impedance modulus and improved corrosion resistance.

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