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Toward improved monitoring and control of microbiologically influenced corrosion (MIC)
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EPA Project Officer: Bala Krishnan
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Project Amount: $98,139
Research Category: Pipeline Corrosion Detection and Monitoring

Objective(s) of the Research Project: Biofilm bacterial communities from a bench-scale flow loop ("bioloop") designed to provide a model system for the examination of pitting corrosion in pipelines are being assayed using PLFA and DNA-based molecular methods to determine which bacteria may be key members in corrosion-producing biofilms. An emphasis is placed on characterization of sulfate-reducing bacteria (SRB) due to their known potential for corrosion, however, the more general screening also planned for this project will allow detection of other types of bacteria that may promote corrosion.

Progress Summary/ Accomplishments:

Results of the project to date were presented at the 16th Annual International Petroleum & Biofuels Environmental Conference by Dr. Jennifer Busch-Harris. The 7th quarter goals of progress on molecular analysis were met. DNA was extracted, amplified, and cloned from another sample type (BioStrips). Results were negative for the detection of archaea from all BioStrips using primers that had detected archaea in BioTraps #3 and 6. A bioloop #6 coupon clone library was constructed and a 96-well plate of clones sent for sequencing.

MIC is a major factor in leaks of oil-field pipelines as well as damaging a variety of above-ground and below-ground structures. Fluids emitted from such structures can be harmful to the environment and to human health, necessitating immediate and expensive clean-up procedures. Better means of identifying and monitoring the microbes responsible for MIC, the goals of this project, will aid in the prevention of MIC.

Molecular analysis:
DNA extraction from BioStrips. Tris-EDTA SDS lysis buffer (pH 8.0) was added to tubes containing the BioStrips, vortexed, then sonicated as per the protocol for extraction of DNA from the BioTraps. The addition of lysis buffer containing EDTA was performed in order to chelate the iron filings present in half the BioStrip samples, as iron is known to be very inhibitory to PCR.
PCR amplification from pooled coupon DNA and BioStrip DNA. DOE Joint Genome Institute recommended primers for eubacterial 16S rRNA gene sequence amplification (http://my.jgi.doe.gov/general/protocols/SOP_16S18S_rRNA_PCR_Library_Creation.pd

f), DGGE primers (Muyzer et al., 1998), and other primers in use by the Duncan lab for amplification of 16S rRNA gene sequences were used to optimize the conditions required for amplification from the coupon DNA. DNA extracted from BioStrips was successfully amplified using DGGE primers and the JGI primers.

DGGE of BioStrips. DGGE (Muyzer et al., 1998) was performed to compare the communities from the BioStrips---differences in the banding pattern were seen for samples from bioloop #1 vs. #2 vs. #3, but samples from #4, 5, and 6 did not produce strong enough bands to make reliable comparisons.

Cloning and sequencing: PCR products were amplified from DNA extracted from the Bioloop 1, 2, 3, 4, 5, and 6 BioStrips using 16S rRNA eubacterial primers 27F and 1391R and cloned into the TOPO-TA vector (Invitrogen, Calsbad, CA). The manufacturer's instructions were followed for maximizing the clone diversity from a pool of different sequences. Colonies were picked into single wells of a 96-well plate containing tryptone-yeast extract glycerol broth with ampicillin (Elshahed et al., 2003) grown overnight at 37°C, and stored at -85°C for DNA isolation and sequencing was performed. Cloning and /or picking more plates from coupon samples was repeated in order to obtain approximately equal sample sizes for the coupon libraries. Plates containing clones from coupons were sent to Microgen (Oklahoma City, OK) for sequencing. Plates containing clones from the BioStrips were archived at -80^{o}C.

PCR amplification to detect archaea. Repeated tests showed no PCR amplification was obtained for any of the BioStrip samples using the archaeal small subunit rRNA primers ARC333 and 958R (Gieg et al., 2008).

Dr. Beatriz Monica Perez-Ibarra. Dr. Perez-Ibarra has been acquiring greater proficiency in the molecular techniques used for this project under the direct supervision of Dr. Duncan. She was responsible for performing the research on the BioStrip samples and assisted on performing the research on the coupons.

Publications/ Presentations: "Microorganisms Associated with Pitting Corrosion in a Model Flow Cell System": Jennifer Busch-Harris*, Robert H. Webb & Gary Jenneman, ConocoPhillips, Bartlesville, OK; Kathleen Duncan, University of Oklahoma, Norman, OK; Kerry Sublette, University of Tulsa, Tulsa, OK. 2009 16th Annual International Petroleum & Biofuels Environmental Conference. Houston, TX - November 3-5, 2009
* presenter

Future activities:
Major objectives for the next period are to continue analyses of 16S rRNA and *dsrA* gene sequences from coupon samples and begin analysis of the BioTrap samples. Statistical analyses will be made rates of pitting corrosion, PLFA and DNA test results in order to evaluate the relationship of the microbial community to localized corrosion.

Supplemental Keywords: pitting corrosion, sulfate-reducing bacteria, molecular probes, protection of groundwater and land, oil-field pipelines, pollution prevention, microbiology, petroleum industry, pipeline transportation.

Relevant Web Sites: No Web site has been established as part of the project.

Elshahed, M. S., J. M. Senko, F. Z. Najar, S. M. Kenton, B. A. Roe, T. A. Dewers, J. R. Spear, and L. R. Krumholz. 2003. Bacterial diversity and sulfur cycling in a mesophilic sulfide-rich spring. Appl. Environ. Microbiol. 69: 5609–5621.

Gieg, L. M.; Duncan, K. E.; Suflita, J. M. 2008. Bioenergy production via microbial conversion of residual oil to natural gas. Appl.Environ. Microbiol. 74:3022–3029.

Muyzer, G., T. Brinkhoff, U. Nubel, C. Santegoeds, H. Schafer, and C. Wawer. 1998. Denaturing gradient gel electrophoresis (DGGE) in microbial ecology, p. 1–27. In A. D. L. Akkermans, J. D. van Elsas, and F. J. de Bruijn (ed.), Molecular microbial ecology manual, vol. 3.4.4. Kluwer Academic Publishers, Dordrecht, The Netherlands.