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**Toward improved monitoring and control of microbiologically influenced corrosion (MIC)**
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**EPA Project Officer: Bala Krishnan**
**Project Period: 4/01/08-3/31/09**
**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:**

The first quarter goals of successful operation of the bioloops and distribution of bioloop samples to project participants was accomplished. Upon visual inspection, sample Biotraps and mild steel strip coupons from the bioloops showed the presence of bacterial biofilms and iron sulfide deposits, a potential indicator of microbially influenced corrosion (MIC). Preliminary analysis of effluents from the bioloops indicates success in altering the microbial community in response to treatments with biological control chemicals. Replicate bioloops showed a very high degree of similarity in microbial community profiles, indicating treatment effects could be identified. 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, which are the goals in this project, will aid in the prevention of MIC.

We wish to acknowledge the significant role of Dr. Jennifer Busch Harris, ConocoPhillips Bartlesville Technology Center, in generating the data for this project and contributing to this report.

Source of microorganisms.

Inoculum for the experiment was obtained from scraping (e.g. "pigging") a three-phase pipeline conducting oil, oil-field brine, and gases from production wells on the North Slope of Alaska to a central processing facility. The 1 L sample was immediately placed in a sterile glass container and purged with nitrogen gas to maintain the ambient anaerobic conditions. The sample container was shipped to the Bartlesville Technology Center (BTC) Corrosion Laboratory and enrichment for sulfate-reducing bacteria (SRB) was performed using a synthetic brine medium containing sulfate.

Test run to establish time period for corrosive activity.

Prior to the start of this experiment, a test run was conducted using the same culture at the same conditions as the planned experiment. The coupon analysis of this test indicated that after 30 days of continuous flow of the working reservoir culture through the loops corrosive pitting and biofilm formation by viable microorganisms was present. These initial findings were used as a guideline for establishing the timeline to use for this test.

Operation of bench-scale flow loops.

Six biofilm flow loops were constructed using 1.3 cm and 1.9 cm (ID) schedule 40 PVC pipe. A sterile, 8-L glass nutrient reservoir contained degassed synthetic brine. The nutrient reservoir brine was continuously purged of $O_2$ using $N_2$:$CO_2$ (90%:10%). The synthetic brine was pumped into a working reservoir inoculated with an enrichment from a pipeline sample (see above) and grown as a batch culture until soluble sulfide concentrations reached as high as 50-65 mg/L, indicating success in enriching for SRB.

Once the working reservoir sulfide concentrations attained these sulfide concentrations the culture was pumped through the flow loops for one month. Biological control chemicals were used to test effects on biofilm formation and microbial community profile. Two different chemicals were used, A and B, and each chemical was diluted into deionized water and pumped into the test loops via a syringe pump. Two flow loops were control loops that were devoid of any chemical treatment, two flow loops were injected with chemical A, and two flow loops were injected with chemical B.

Sampling.

The flow loops contained four coupon sites, each with a pair of mild steel strip coupons, and an additional spool of pipe at the end of the loop for installation of Biotraps and Biostrips, composed of Bio-Sep material. The Bio-Sep materials (beads and wafers) were heat treated and the traps rinsed in methanol to remove residual biomarkers before placing in the bioloop. After 30 days of continuous flow, the effluent fluid was collected from each loop along with the Bio-Sep sampling devices and coupons. The coupons and Bio-Sep sampling devices were distributed for analysis to the BTC, Microbial Insights, and OU, as detailed in the QAPP.

Biofilm viability.

The coupons were sampled for biofilm viability analysis by MPN at the ConocoPhillips facility. MPN bacterial enumerations were performed from the bacterial suspension (prepared by sonication in sterile buffer) in commercially available Postgate's modified media (sulfate reducing bacteria), thioglycollate, and media to detect general heterotrophic acid producing bacteria. The MPN bottles were incubated at $40^oC$, and will be monitored for positive results after 28 days incubation.

Sample preparation for molecular analysis.

As per the QAPP, samples were immediately processed upon receipt (e.g. fluids were filtered) and the processed samples were preserved at -70$^{o}$C. During processing, 3 mL of each of the 6 effluent samples, a sample from the working reservoir, and wash buffer sterile control sample were removed and processed in parallel separately (e.g. centrifugation, PowerSoil DNA protocol as detailed in the QAPP) for preliminary screening purposes.

Molecular analysis: preliminary screening.
PCR performed using eubacterial 16S rRNA gene sequence primers (GMSF, 907R, for DGGE, Muyzer et al. 1998) demonstrated that amplifiable DNA was successfully extracted from the effluent and working reservoirs. No amplification was observed from the wash buffer control sample, indicating lack of contamination of the wash buffer and during processing. The PCR products were run on a denaturing gradient gel (DGGE) and showed differences among microbial communities from the working reservoir, no treatment, treatment A, and treatment B. In contrast to the differences among treatments, duplicate samples for the same treatments showed very similar profiles.

**Publications/ Presentations:** No publications or presentations have been made during this time period, except for in-house progress reports made at the University of Oklahoma and Bartlesville Technology Center.

**Future activities:**
As per the project schedule, major objectives for the next quarter are that analyses of pitting corrosion, 16S rRNA and *dsrA* gene sequences from coupon and Bio-Sep samples, and PLFA will be initiated, but are not expected to be completed during this time period. An abstract will be submitted during the next quarter to the 15$^{th}$ Annual International Petroleum & BioFuels Environmental Conference, reporting on preliminary findings.

**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.

References cited
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.