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**Toward improved monitoring and control of microbiologically influenced corrosion (MIC)**

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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:**

The 2nd quarter goals of measuring pitting corrosion, initial analysis of PLFA data, and progress on molecular analysis were met. The pitting corrosion measurements used the planar surface profilometer which provides a 3D map of the coupon surface. A significant finding was that inhibitor chemical treatments increased pitting rates on the coupons as compared to the untreated loop coupons. Viable biomass (PLFA) was higher on treated coupons and the pitting rate showed a linear trend with biomass. PLFA profiles differed with treatment, demonstrating a shift in the microbial community composition. Preliminary 16S rRNA gene sequence data obtained from SRB MPN series inoculated from coupons demonstrated that two *Desulfovibrio* species (as well as other bacteria) were a component of the coupon biofilm. *Desulfovibrio* species are a type of SRB and hence of particular importance for this study. 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.

### Analysis of pitting corrosion on metal coupons from the BioLoops

Our primary goal was to measure localized corrosion and pitting severity. COP has a planar surface profilometer and software package useful in determining pitting corrosion by scanning the coupon surface. A linear surface profilometer allows for a 3D scan of the full coupon surface and for a more precise and detailed measurement of localized corrosion. Some of the features include:

- 3D Non-Contact Profiling
- Chromatic aberration interferometer sensor
- Y (lateral) axis range of 10 cm
- Lateral resolution of 1.3 $\mu$ m
- Z (height) axis range of 300 $\mu$ m or 3 mm
- Z axis resolution of 0.06 $\mu$ m

The linear surface profilometer enables more accurate pit determination based on pit description criteria, such as pit width and pit depth. The entire coupon is scanned, then profiled. With the upgraded computer software package (MountainsMap), the surface profile data is analyzed and a pit “definition” applied in order to filter the pits. The computer quantifies the number of pits based on the required filter information for pit identification, such as a pit that is 50 microns or greater in width with a pitting rate of 20 mpy or higher. MountainsMap will only count those pits that meet the requirements. A mean and maximum pit size, mean and maximum pit rate, and the size and depth distribution is provided. Human error is removed from the equation and chemical evaluation more fairly measured.

A secondary goal was to obtain weight loss measurements on the coupons so as to calculate general corrosion rates due to actual metal loss. The coupons were weighed prior to being installed into the flow loops and exposed to experimental conditions. Upon removing the coupons from the loops, the coupons were cleaned using inhibited acid. The weight loss in the time period of the experiment is converted to mils and the corrosion rate is calculated as mils per year.

The primary conclusion from the corrosion assessment of the coupons was that the inhibitor chemical treatments increased pitting rates on the coupons in the treated loops as compared to the control (untreated) loop coupons, in contrast to the effect the inhibitor chemical treatment had on the general corrosion rate determined by coupon weight loss. Furthermore, pitting rate was proportional to the viable biomass obtained from the sample matrix.

### PLFA analysis:

Total picomoles of PLFA and detailed PLFA profiles for coupons, BioSep beads, and BioStrips from the bioloops were provided by Microbial Insights, Inc. (Rockville, TN). This information allowed us to compare viable biomass levels and shifts in microbial community composition among bioloops subjected to different treatments. It was noteworthy that viable biomass levels for each of the three sample types was higher for the treated bioloops than the control, untreated bioloops. Therefore, it appeared that the corrosion inhibitors stimulated microbial cell numbers. A second noteworthy finding was that the PLFA profiles showed that corrosion inhibitors shifted the PLFA profiles of the microbial communities on the coupons--coupons from control untreated bioloop had a high proportion of "Nsat"s and PLFA associated with Firmicutes. Coupons from the

bioloops treated with corrosion inhibitor A had a lower proportion of Nsats and Firmicutes and a much higher proportion of PLFAs associated with proteobacteria. Coupons from bioloops treated with corrosion inhibitor B appeared more like the coupons from untreated bioloops at this level of analysis.

#### Molecular analysis:

APB, GAnH, and SRB MPN series were inoculated from the coupons at BTC and incubated per standard protocols. After scoring, the MPN vials were shipped to OU for DNA extraction. Fluid was removed aseptically from the vials, the cells pelleted by centrifugation, and washed 2x in isotonic sterile saline before storing at -20°C. DNA was extracted from the subsets of the SRB and APB samples using the protocol detailed in the QAPP. PCR performed using eubacterial 16S rRNA gene sequence primers (GMSF, 907R, for DGGE, Muyzer et al. 1998). The PCR products were run on a denaturing gradient gel (DGGE) and showed differences among microbial communities from the SRB coupons, paired effluent samples, and the working reservoir.

As a preliminary examination of the differences among the samples, 31 DGGE gel bands were cut out and soaked in PCR-grade water in order to elute DNA. The eluted DNA was reamplified using the DGGE primers and a portion run on a DGGE gel in order to detect whether a single band at the correct location had been isolated. PCR reactions meeting the quality criteria were cleaned and submitted for sequencing, other bands were recut, reamplified, and rerun on DGGE. 22 sequences were obtained, including sequences from the coupons SRB series similar to those of two *Desulfovibrio* (SRB) species.

However, sequence quality was not as high for all cut-out bands as desired, so it was decided to focus on cloning PCR products.

**Publications/ Presentations:** No publications or presentations have been made during this time period.

#### **Future activities:**

As per the project schedule, major objectives for the next quarter are that analyses of 16S rRNA and *dsrA* gene sequences from coupon and Bio-Sep samples will be continued but are not expected to be completed during this time period. Initially, a comparison of methods of DNA extraction from the coupons will be performed to choose the optimal method. More detailed analyses will be made rates of pitting corrosion, PLFA and DNA test results so as 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.

#### 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.