

**Period Covered by the Report: 10/01/08-12/31/08**

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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, NCE requested**

**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:**

A meeting was held October 23, 2008 at the University of Tulsa among the investigators in order to report on progress towards goals. The 3rd quarter goals of progress on molecular analysis were met. A protocol was developed that permitted extraction of DNA from single coupons. Initial tests of PCR amplification of the coupon DNA were performed. Preliminary 16S rRNA gene sequence data obtained from the working reservoir indicated that the dominant 16S rRNA sequences was very similar (98-99%) to that *Desulfovibrio indonensis*. The type strain of *D. indonensis* (Feio et al., 1998) was isolated from a severely corroded metal surface. Sequences similar to those of two other species of SRB were found. 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 contributing to this project.

#### Molecular analysis:

Extraction of DNA from single coupons: The protocol of the MOBIO PowerMax Soil DNA Isolation Kit was followed with a few modifications; namely the inclusion of additional lysing beads for more effective lysis (Lysing Matrix A beads, MP BioMedical), prefacing the bead-beating step by a 5 minute sonication step performed at 40°C and including a polyacryl carrier (Molecular Research Center, Inc) to enhance the

precipitation of small quantities of DNA. Controls included unused coupons taken directly from their paper envelopes and sent through the entire extraction process and PCR amplification.

PCR amplification from single coupons. 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.pdf](http://my.jgi.doe.gov/general/protocols/SOP_16S18S_rRNA_PCR_Library_Creation.pdf)), 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. Most samples were able to be amplified directly, however, due to low DNA yields from some coupons, some of samples required a nested PCR amplification in order to obtain sufficient product.

Cloning and sequencing: PCR products were amplified from DNA extracted from the working reservoir 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 until DNA isolation and sequencing was performed.

Analysis of sequence data: The sequences were aligned using the greengenes NAST-aligner and examined for chimeric sequences using the Bellerophon program available through the greengenes website (version 3, [http://greengenes.lbl.gov/cgi-bin/nph-bel3\\_interface.cgi](http://greengenes.lbl.gov/cgi-bin/nph-bel3_interface.cgi)). The taxonomic affiliation of the sequences was determined by the RDP Classifier (Wang et al., 2007) and by the closest match to sequences in the GenBank database by BLASTN (Altschul et al., 1997)

**Publications/ Presentations:** None during this quarter.

**Future activities:**

As per the project schedule, major objectives for the next quarter are to continue analyses of 16S rRNA and *dsrA* gene sequences from coupon samples but are not expected to be fully completed during this time period. 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

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