Period Covered by the Report: 4/01/09-3/31/10
Date of Report: 6/4/10
EPA Grant Number: X83242801
Toward improved monitoring and control of microbiologically influenced corrosion (MIC)
Investigators: Kathleen E. Duncan (PI, kathleen.e.duncan-1@ou.edu), Bruce A. Roe, Kerry L. Sublette, Gary Jenneman.
Institutions: University of Oklahoma (KED, BAR), University of Tulsa (KLS), Conoco-Phillips Bartlesville Technology Center (GJ).
EPA Project Officer: Bala Krishnan
Project Period: 4/01/08-9/30/10 (with NCE)
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:
1. Analysis of the pattern of pitting corrosion on metal coupon samples from the BioLoops.
As well as standard weight loss measurements, a planar surface profilometer was used to provide a 3D map of the coupon surface. The significance of measuring pitting corrosion using the profilometer is that a more precise and detailed measurement of localized corrosion, indicative of biocorrosion, can be obtained. A significant finding was that coupon pit morphology and distribution appeared to vary with treatment. Practical applications are that particular corrosion inhibitors may be contraindicated if they prove to increase the rate or number of deep pits.
2. PLFA analysis. Viable biomass levels and shifts in microbial community composition were assayed by analysis of PLFA from coupons, BioTraps, and BioStrips. A significant finding was that biomass levels were higher for each sample type for the treated bioloops than the untreated bioloops. Biomass levels from BioTraps and BioStrips that contained carbon steel shavings were lower than those sampling devices from the same bioloop but did not contain metal shavings. This has a practical application in that particular corrosion inhibitors may be contraindicated if they increase microbial biomass levels, however, previous research suggests that the association between microbial biomass and degree of corrosion is complex.
3. DNA extraction and PCR amplification from other sample types. DNA was successfully extracted from BioTraps, BioStrips, and filtered effluent samples and used in PCR amplification reactions. Initial preparation of the samples prior to processing by the addition of Tris-EDTA buffer and sonication were key elements. A practical application

is that BioTraps, BioStrips, and effluents are more easily collected and processed for molecular analysis than are coupons, which also are in demand for corrosion analysis.
4. DNA sequence analysis of microbial communities on coupons. Results from the coupon bacterial 16S rRNA clone libraries confirmed that different microbial communities are growing on samples obtained from BioLoops subjected to different treatments. The communities contain a high proportion of deltaproteobacterial SRB, including *Desulfovibrio* species that have previously been implicated in biocorrosion. Sequences similar to that of *D. indonesiensis* were by far the most abundant in the working reservoir while sequences similar to that of *D. alkalitolerans* were more abundant on the coupons. Both of the type strains are strongly associated with biocorrosion. Other dominant groups of sequences were similar to those of Firmicute, Synergistes, Beta- and Gammaproteobacteria obtained from previous studies of oil facilities. A practical application is that these laboratory studies were performed with diverse microbial communities similar to those found in oil facilities, therefore providing a more realistic test situation for biocides and corrosion inhibitors. Further sequence data and analysis will determine the association between specific microbial communities and higher levels of pitting corrosion. Practical application of this information will allow targeting of microbes strongly associated with corrosion. Comparisons of the microbial composition from different sample types obtained from the same BioLoops will allow design of better sampling protocols, as some types of samples more quickly provide higher levels of microbial biomass. A decrease in the period required for sample collection and increased test sensitivity due to greater biomass, would have practical application to monitoring and controlling biocorrosion.
5. Methanogen sequences from two BioTraps. Repeated tests showed a positive signal for archaea was obtained by PCR amplification using archaeal small subunit rRNA primers for bioloop #3 and #6 BioTraps, but not from any of the coupons or BioStrips using the same primers and conditions. Sequences were found to be most similar (>99%) to uncultured *Methanolobus* from a Niigata oil well (AB243813). Significance: H_2-utilizing methanogens have been implicated in producing pitting corrosion, but appear not to be a major component in this set of experiments.
6. In addition to the investigators listed above, two scientists contributed substantially to the success of this project.
Dr. Jennifer Busch Harris (ConocoPhillips Bartlesville Technology Center) was the lead scientist in setting up the microbial inoculum for the BioLoops and operating the BioLoops, as well as coordinating the distribution of the BioLoop sample devices to the various participants. She also performed the corrosion measurements, MPN studies, and was integral to the data analysis and generation of reports.
Dr. Beatriz Monica Perez-Ibarra joined the Duncan laboratory in January 2009 as a postdoctoral fellow funded by Consejo Nacional de Ciencia y Tecnología (CONACyT, Mexico) and has been assisting in the DNA-based portion of the research. Her efforts allowed additional types of samples to be analyzed.

Publications/ Presentations: As reported in the quarterly reports, two presentations have been made during this time period: one (Dr. Jennifer Busch-Harris, presenter) at 2009 16th Annual International Petroleum & Biofuels Environmental Conference. Houston, TX, the other (Dr. Kathleen E. Duncan, presenter) an in-house seminar on Feb.

12, 2010 to the Department of Botany and Microbiology, University of Oklahoma, Norman, OK.

Future activities: Research is in progress to use pyrosequencing to create sequence libraries from the BioTrap and effluent samples, and if time and funding permit, the BioStrip as well. Comparison of the microbial community profiles from these three sample types with the clone libraries from the coupon samples will allow us to determine whether the more quickly and easily obtained and processed sample types (e.g. effluent, BioTrap, BioStrip) can be used as a surrogate for the coupon samples. QPCR techniques will be developed to assess the number of *D. indonesiensis* and *D. alkalitolerans* as they were found to be the primary SRB on the coupons and their proportions varied with treatment. A manuscript is in progress which describes the corrosion patterns, PLFA characteristics and 16S rRNA gene sequence affiliations of the microbial communities on coupons with respect to the treatments experienced by the bioloops. At least one more manuscript will be generated after pyrosequencing is completed comparing the microbial community profiles and PLFA characteristics from the various sample types.

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.