

# 5<sup>th</sup> Quarterly Report

## Paraffin Control in Oil Wells Using Anaerobic Microorganisms

**Period Covered by the Report:** October 15, 2006 to Jan 15, 2007

**Date of Report:** April 20, 2007

**EPA Grant Number:** X83-2428-01

**Title:** Paraffin Control in Oil Wells Using Anaerobic Microorganisms

**Investigators:** J. M. Suflita and L. M. Gieg

**Institution:** The University of Oklahoma

**EPA Project Officer:** Bala Krishnan

**Project Period:** 10-15-06 to 10-14-07 (Year 2)

**Project Amount:** \$149, 298

**Research Category:** Petroleum Environmental Technology, Wellbore Cleanout

### Objective(s) of the Research Project:

Paraffins that form waxy deposits upon removal from reservoirs have been implicated in numerous oil field problems leading to reductions in oil recovery. In oil reservoirs, anaerobic conditions usually predominate. Thus the addition of anaerobic microbial populations that can definitively biodegrade paraffins under such conditions may be of great use to treat wax accumulations. Our aim is to evaluate the feasibility of using anaerobic microbial consortia to biodegrade waxy hydrocarbons in order to ameliorate paraffin accumulations in oil reservoirs.

### Progress Summary/ Accomplishments:

For this project, we have been cultivating microbial populations from a variety of sources for the potential to degrade and treat waxy paraffins under anaerobic conditions. Enrichment cultures derived from hydrocarbon-contaminated marine sediments in San Diego Bay have shown enhanced levels of sulfate reduction when C<sub>28</sub>, C<sub>40</sub>, or C<sub>50</sub> is provided as the paraffinic substrate relative to substrate-free controls. These cultures continue to be transferred and cultivated for potential paraffin-treating activity. The paraffin-utilizing enrichments are also able to utilize alkanes as low as C<sub>6</sub> (hexane) based on sulfate reduction measurements. Molecular biology analyses were used to help identify the organisms in these enrichments responsible for anaerobic paraffin decay, which included affiliation with several known hydrocarbon-degrading sulfate-reducing members of the delta proteobacteria. Enrichments set up from other marine sediments under methanogenic conditions (e.g., no added electron acceptor) have also shown enhanced levels of methane production relative to controls. This effect was most dramatically observed when C<sub>28</sub> was supplied as the sole paraffin source, but some enhanced methane levels were also measured when C<sub>40</sub>, C<sub>50</sub>, or a commercially-available high molecular weight waxy mixture, Polywax (~ C<sub>30</sub> to C<sub>100</sub>, Polywax 655, Supelco) was supplied as the paraffin substrate. These enrichments also continue to be cultivated as potential paraffin-treatment cultures.

During this reporting period, we endeavoured to detect and identify metabolites that may be formed during the anaerobic decay of paraffins. Thus, all of the sediment-free, highly enriched populations described above were assayed for potential metabolites using organic extraction and GC-MS analysis (e.g. *Gieg & Suflita, 2002*); however, we were not able to detect or identify any known putative anaerobic metabolite (fumarate addition products or related alkanolic acids, e.g. *Callaghan et al., 2006*). Therefore, we have been exploring the use of different strategies to help in the detection and identification of some possibly novel metabolites. For example, the methanogenic enrichments were amended with fully deuterated C<sub>28</sub> in order to help with the identification of any labelled metabolites that may be produced during anaerobic paraffin degradation. Further, during the incubation period, these cultures will be amended with BESA (bromoethanesulfonic acid), a known inhibitor of methanogenesis in an attempt to allow putative metabolites to accumulate in the cultures and thus allow for easier detection.

Previously, we described other freshwater sediment-containing incubations that showed enhanced levels of sulfate reduction in the presence of crude oil or Polywax relative to substrate-unamended controls. During this reporting period, these cultures were transferred into fresh medium and incubated in the presence of a “soft” paraffin sample pulled from a paraffinic reservoir in Kingfisher County, OK. Analyses are ongoing to determine whether the anaerobic populations are able to utilize “real” paraffin as a growth substrate. Finally, another set of anaerobic oil-degrading enrichments is showing promise in paraffin reduction. Bunker C crude oil-degrading sulfate-reducing cultures derived from the sunken USS Arizona were incubated in the presence of the Bunker C crude oil or with Polywax. Several of the cultures reduced the same amount of sulfate in the Polywax-amended cultures as with the crude oil-amended cultures. These cultures will continue to be monitored for enhanced levels of sulfate reduction in the presence of paraffins.

### **Publications/ Presentations:**

Gieg, L. M., Duncan, K. E., Suflita, J.M. 2006. Anaerobic Paraffin Biodegradation. *In:* Abstracts of the 11<sup>th</sup> International Symposium on Microbial Ecology, Vienna, Austria, August 20 - 25 (poster presentation).

### **Future activities:**

Enrichment and monitoring of the above-described cultures for the ability to degrade waxy paraffins under anaerobic conditions will continue. Experiments will be conducted to determine the nutritional requirements of some of the enrichment cultures to improve growth and deduce salinity tolerance. In the next phase of the project, more mature enrichments will be challenged with field paraffins and paraffinic oils. Some cultures will also be established under more thermophilic conditions using field samples such as oil production waters. The concentration of paraffins will be assessed in addition to measures of electron-accepting processes.

**Supplemental Keywords:** paraffin treatment, anaerobe, biodegradation, oil field reservoir

**Relevant Web Sites:** Not applicable at this time.

**References:**

- Callaghan, A.V.; L. M. Gieg; K. G. Kropp; J. M. Suflita; L. Y. Young. 2006. Comparison of mechanisms of alkane metabolism under sulfate-reducing conditions among two bacterial isolates and a bacterial consortium. *Appl. Environ. Microbiol.* 72: 4274-4282.
- Gieg, L.M. and J.M. Suflita. 2002. Detection of anaerobic metabolites of saturated and aromatic hydrocarbons in petroleum-contaminated aquifers. *Environ. Sci. Technol.* 36: 3775-3742.

Figure 1

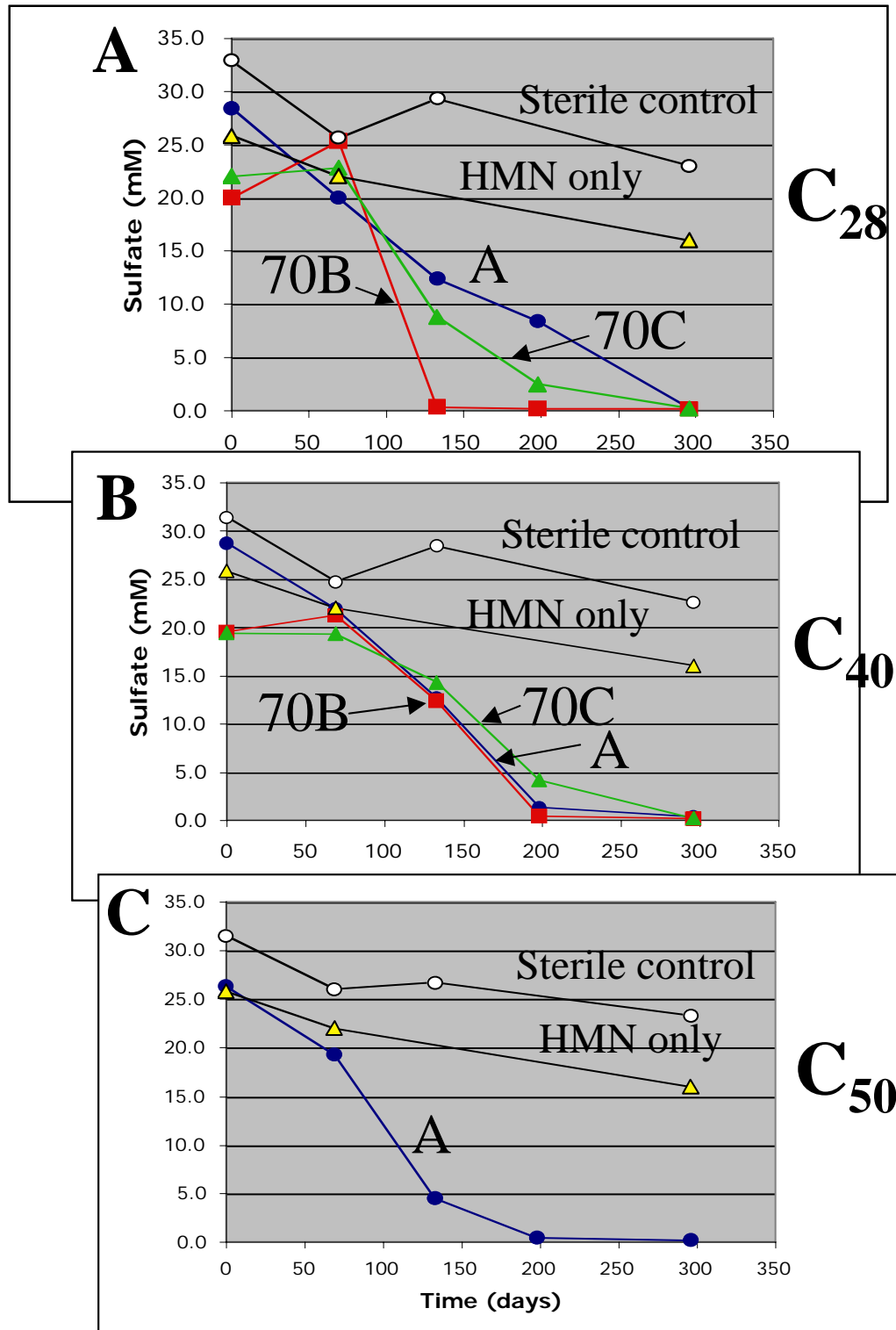


Figure 2

NJ, 16S rRNA 1320 bp,  
#s >800 out of 1000 bootstrap  
replicates. 3/30/06.

0.02

