

6th Quarterly Report

Paraffin Control in Oil Wells Using Anaerobic Microorganisms

Period Covered by the Report: January 15, 2007 to April 15, 2007

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Title: Paraffin Control in Oil Wells Using Anaerobic Microorganisms

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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 as well as from offshore oil platform operations in Brazil have shown enhanced levels of sulfate reduction when C₂₈, C₄₀, or C₅₀ is provided as the paraffinic substrate relative to substrate-free controls. These cultures continue to be transferred and cultivated for potential paraffin-treating activity. Some of the paraffin-utilizing enrichments are also able to utilize alkanes as low as C₆ (hexane) based on sulfate reduction measurements. Molecular biology analyses of some of the sulfate-reducing cultures 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 San Diego Bay 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 C₂₈ was supplied as the sole paraffin source, but some enhanced methane levels were also measured when C₄₀, C₅₀, or a commercially-available high molecular weight waxy mixture, Polywax (~ C₃₀ to C₁₀₀, Polywax 655, Supelco) was supplied as the paraffin substrate. Cloning exercises

performed with this active methanogenic culture showed that it contained cells closely related to *Syntrophus aciditrophicus* strain SB and other *Syntrophus* species as well as to the hydrogenotrophic methanogenic genus *Methanoculleus*. These marine-derived enrichments continue to be cultivated as potential paraffin-treatment cultures.

Freshwater sediment-containing incubations have also shown enhanced levels of sulfate reduction in the presence of crude oil or Polywax relative to substrate-unamended controls. In the previous 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. Several of the cultures have now shown enhanced levels of sulfate reduction in the presence of the field paraffin relative to paraffin-free controls and continue to be amended with sulfate as it becomes depleted. In addition, Bunker C crude oil-degrading sulfate-reducing cultures derived from the sunken USS Arizona incubated in the presence of Polywax showed enhanced levels of sulfate reduction relative to substrate-free controls. These cultures have also been transferred to fresh medium containing field paraffin (as above) and continue to be monitored for paraffin-utilizing activity.

In the past reporting period, we endeavoured to detect and identify metabolites that may be formed during the anaerobic decay of paraffins. 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 (i.e. fumarate addition products or related alkanolic acids, *Callaghan et al., 2006*). Thus, we have been exploring other methods to help identify paraffin metabolites, including using inhibitors of electron accepting processes, using labelled substrates, and using different organic solvent mixtures as extractants. Results from these strategies will be described in a subsequent report.

Publications/ Presentations:

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

Gieg, L.M., Davidova, I.A., Duncan, K.E., Suflita, J.M. 2007. Paraffin Control in Oil Wells Using Anaerobic Microorganisms. Abstract submitted for oral presentation at the upcoming IPEC meeting to be held in Houston, TX, November.

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. Oil field production water samples will be obtained and tested for paraffin-degrading activity under thermophilic conditions. The

concentration of paraffins will be assessed in addition to measures of electron-accepting processes. Strategies to help aid in metabolite identification will continue to be explored.

Supplemental Keywords: paraffin treatment, anaerobic, 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

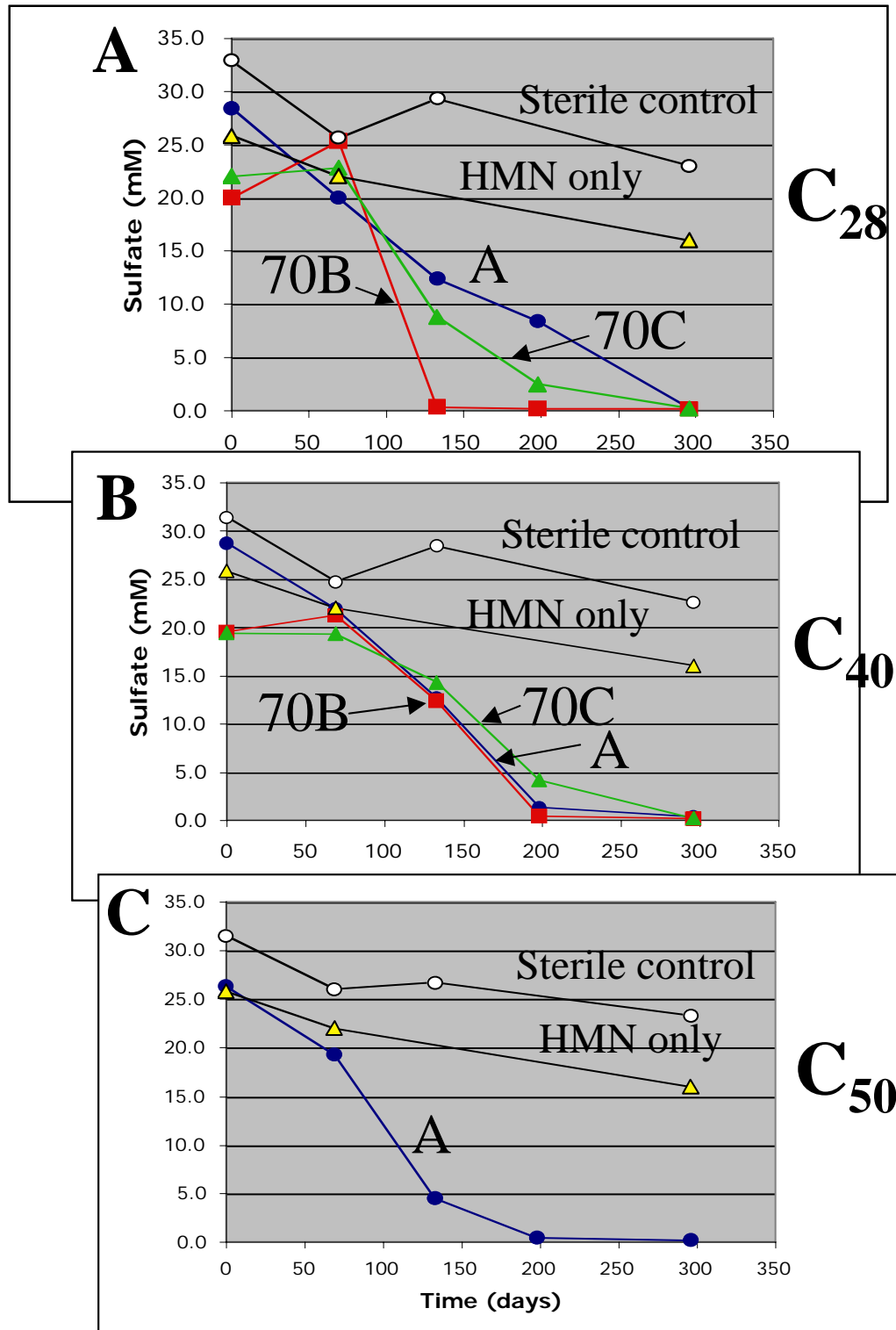


Figure 2

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

0.02

