

Annual Summary (Year 2)

Paraffin Control in Oil Wells Using Anaerobic Microorganisms

Period Covered by the Report: October 15, 2006 to October 14, 2007

Date of Report: November 15, 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. For this project, our aim was 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. To date, we enriched for cultures from five different freshwater or marine sources that have in some way been impacted with petroleum. From these sources, we were able to establish enrichments under either sulfate-reducing and/or methanogenic conditions that are capable of utilizing long-chain alkanes as carbon sources based on enhanced levels of sulfate consumption or methane production relative to substrate-unamended controls. Our strategy was to initially enrich the environmental samples on pure paraffins (such as C₂₈, C₄₀, C₅₀), a commercial wax mixture (Polywax 655, C₃₀ to C₁₀₀, Supelco), or a crude oil (ANS from Alaska's North Slope). Promising cultures were repeatedly transferred into fresh medium containing these paraffins, paraffinic oil, or field paraffins sampled from an oil well in Oklahoma (C₂₄-C₇₀). We were successful in enriching for anaerobic paraffin-degrading activity in all of the samples assessed. Table 1 shows an overall summary of the sources of potential paraffin-utilizing anaerobes, and the substrates on which we have obtained promising enrichments thus far. These cultures continue to be transferred and cultivated.

Table 1. Sources of paraffin-utilizing enrichments, incubation conditions, and the substrates utilized under sulfate-reducing and/or methanogenic conditions.

Source	Salinity	Incubation Temperature	Substrates used under sulfate-reducing conditions	Substrates used under methanogenic conditions
Gas condensate-contaminated aquifer	Freshwater	22°C	ANS crude oil, Polywax, Field paraffins	ANS crude oil, Field paraffins
Oil-contaminated marine harbor sediments	Marine	33°C	C ₂₈ , C ₄₀ , C ₅₀	C ₂₈ , (C ₄₀ , C ₅₀) ²
Production water from Alaska North Slope oilfield operations ¹	Brackish	55°C	(Paraffinic oil, Field paraffins) ²	Paraffinic oil, Field paraffins
Biofilm culture from Bunker C fuel leak from USS Arizona Monument	Marine	33°C	Polywax, Paraffinic oil, Field paraffins ³	N/A
Oil platform tank	Marine	33°C	C ₂₈ , C ₄₀	N/A

¹ See text below

² Weak activity

³ Sulfate-unamended controls also showed similar amounts of sulfate reduction, so further transfers are necessary for more definitive results

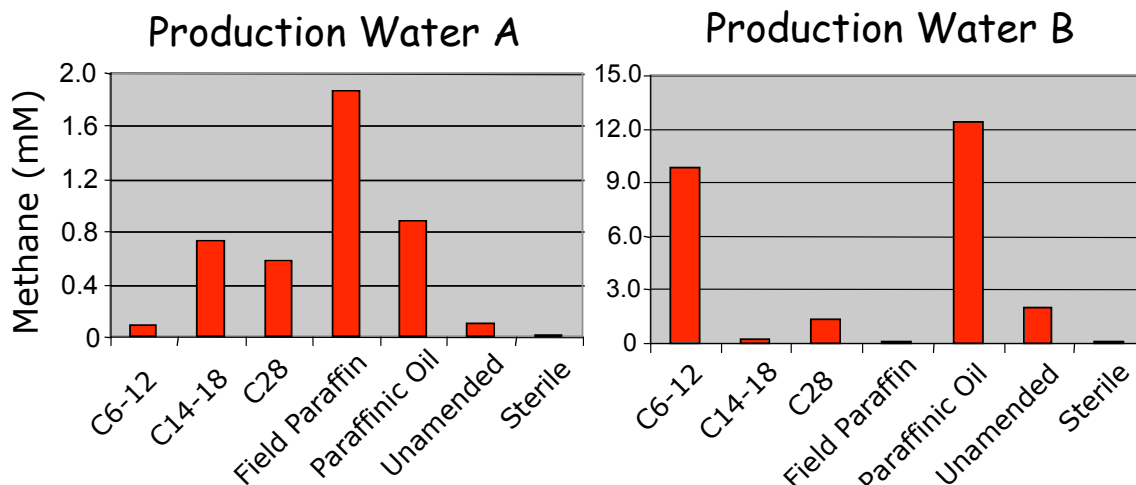
N/A = Not applicable to this environmental sample

Throughout this project, molecular analyses were used to help identify the organisms in some of the enrichments responsible for anaerobic paraffin decay. Denaturing gradient gel electrophoresis (DGGE) analysis, cloning, and sequencing of the 16S rDNA (~1500 bp) amplified from the enrichments were used to assess the diversity and identity of the requisite bacteria. A comparison of the 16S rDNA clone library from the C₂₈, C₄₀, and C₅₀-degrading enrichment cultures revealed close affinity to several known hydrocarbon-degrading sulfate-reducing members of the delta proteobacteria. Cloning exercises performed with the active methanogenic culture revealed that cells closely related to *Syntrophus aciditrophicus* strain SB and other *Syntrophus* species as well as to the hydrogenotrophic methanogenic genus *Methanoculleus* were present in the enrichments.

In our last quarterly report, we described the establishment of sulfate-reducing and methanogenic enrichment cultures from two Alaska North Slope oilfield production water samples on a variety of different paraffinic substrates at 55°C. A small amount of sulfate reduction was observed in cultures enriched from one of the production water samples, but no sulfate reduction occurred in the cultures established with the other water sample. Instead, methanogenesis appeared to be the preferred electron-accepting process in the enrichments. Figure 1 shows the results of these enrichments under methanogenic conditions after a 4-month incubation period. We found enhanced levels of methane production relative to substrate-free controls when paraffinic oil or field paraffins were

added as the carbon source. All cultures producing enhanced levels of methane will be transferred to confirm this activity and monitoring for methane will continue.

Figure 1. Methane production in two thermophilic oilfield production water samples amended with various paraffinic carbon sources after a 4-month incubation.



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. Oral presentation at the 14th IPEC Meeting, Houston, TX, November 6 – 9.

Future activities:

This project will continue as a no-cost extension. Maintenance and monitoring of the above-described cultures will be ongoing. During the enrichment process, we evaluated the ability to degrade waxy paraffins by monitoring changes in electron accepting processes. As this project progresses, we will start to also examine changes in the paraffin profiles by high temperature gas chromatography as well as measure changes in the physical properties of the paraffinic mixtures (i.e. oils or field paraffins) as a result of anaerobic biodegradation under the varying conditions and couple these changes with

sulfate consumption and methane production. Further, identifying metabolites of anaerobic paraffin decay has remained elusive thus we will continue explore strategies to detect and elucidate the structures of biodegradation products.

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

Relevant Web Sites: Not applicable at this time.