# 9th Quarterly Report (No-Cost Extension)

## Paraffin Control in Oil Wells Using Anaerobic Microorganisms

**Period Covered by the Report:** October 16, 2007 to January 14, 2008
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**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:** 10-16-07 to 10-15-08 (Year 3)
**Project Amount:** No-cost extension
**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. We have assessed cultures from five different freshwater or marine sources that have been impacted with petroleum. From these sources, we established enrichments under either sulfate-reducing and/or methanogenic conditions that are capable of utilizing a variety of paraffinic substrates based on enhanced levels of sulfate consumption or methane production relative to substrate-unamended controls. All of the enrichment cultures and the specific paraffinic substrates used by each were described in the previous report (2-Year Annual Report). One of the more promising paraffin-utilizing enrichments was derived from a gas condensate-contaminated aquifer wherein the anaerobic microbial populations were historically exposed only to relatively short chain hydrocarbons (i.e. up to $C_{15}$). However, electron acceptor-based monitoring results of these enrichments have suggested that the extant aquifer flora have long-chain paraffin-utilizing ability. In initial enrichments, we challenged the microbes from the aquifer with either Alaska North Slope (ANS) crude oil, Polywax (commercial wax mixture, $C_{30}$ to $C_{100}$), or a mixure of the two and found enhanced levels of sulfate reduction in the hydrocarbon-amended versus substrate-unamended incubations. Incubations demonstrating this activity were transferred into fresh medium and the cultures were

supplied with a mixture of "field" paraffins acquired from an Oklahoma oilfield. In the paraffin-amended incubations, enhanced levels of both sulfate consumption and methane production were observed relative to the unamended incubations. The results from these enrichments (after about 1 year incubation) are shown in Figure 1A (sulfate consumption) and 1B (methane production). These enrichments will be transferred again with field paraffins as the substrate and the remaining paraffin fraction will be quanitified using high-temperature GC.

In our last 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. We have now transferred these cultures and continue to monitor for methane production.

**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 is continuing as a no-cost extension. Maintenance and monitoring of all enrichments cultures are ongoing. During the enrichment process, we have been evaluating the ability of the cultures to degrade waxy paraffins by monitoring changes in electron accepting processes. We are now starting to examine changes in the paraffin profiles by high temperature GC 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 are continuing to explore strategies to detect and elucidate the structures of biodegradation products.

**Supplemental Keywords:** paraffin treatment, anaerobe, biodegradation, oilfield reservoir

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

**Figure 1.** Sulfate consumption (A) and methane production (B) in freshwater aquifer-derived enrichments incubated in the absence (unamended) or presence of field paraffins after approximately 1 year of incubation. Enrichment substrates 1, 2, and 3 indicate the results from incubations that were originally enriched on ANS crude oil, Polywax, or ANS + Polywax, respectively. The values above each bar in the graphs indicate the average sulfate or methane concentrations in triplicate incubations.