

7th Quarterly Report

Paraffin Control in Oil Wells Using Anaerobic Microorganisms

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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. We have established numerous enrichments from hydrocarbon-contaminated marine sediments (i.e. from San Diego Bay and offshore oil platform operations in Brazil) that utilize long-chain alkanes under sulfate-reducing and/or methanogenic conditions. Under sulfate-reducing conditions, we observed enhanced levels of sulfate consumption when C₂₈, C₄₀, or C₅₀ were added as carbon sources to the enrichments relative to substrate-unamended controls. 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. Under methanogenic conditions, enhanced levels of methane were produced in San Diego Bay-derived enrichments 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 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. These marine-derived enrichments continue to be cultivated as potential paraffin treatment cultures.

We had previously shown that freshwater sediment-containing incubations also showed enhanced levels of sulfate reduction in the presence of crude oil or Polywax relative to substrate-unamended controls. During the previous reporting period we transferred these enrichments into fresh medium 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 and methane production in the presence of the field paraffin relative to substrate-free controls. Figure 1 shows enhanced levels of sulfate consumption relative to substrate-free controls (A) as well as enhanced levels of methane production (B) from these enrichments that were transferred from either ANS oil, Polywax, or ANS/Polywax-enriched cultures. The field paraffin-amended incubations continue to be maintained with sulfate as needed and monitored further for sulfate and methane concentrations. In future work, we will examine the paraffin components of these enrichments to determine which paraffins are being consumed by the microbial consortia.

During the past 3 months, we received production water samples from two different oil fields on Alaska’s North Slope and established enrichments with these samples on numerous paraffinic carbon sources under both sulfate-reducing and methanogenic conditions. These carbon sources included mixtures of alkanes (C_6 to C_{12} and C_{14} to C_{18}), C_{28} , field paraffins (from Kingfisher Co., OK), and paraffinic oil (Alpine). These enrichments, which are brackish, are being incubated at 55°C, a thermophilic temperature similar to the formation from which they were derived. Sulfate reduction and methane production is ongoing in these enrichments.

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:

All enrichments continue to be monitored for enhanced levels of sulfate reduction and/or methane production. For enrichments currently incubating with field paraffins, we will examine the changes in paraffin profiles as a result of microbial activity using GC analysis and will also have the paraffins examined for changes in physical properties such as the wax appearance temperature. Strategies to help aid in identifying metabolites produced during anaerobic paraffin decay will continue to be explored.

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

Relevant Web Sites: Not applicable at this time.

See Figure 1 below.

Figure 1. Net sulfate consumption (A) and methane production (B) from freshwater sediment-derived enrichments incubated with field paraffins after a 9-month incubation.

