

1st Quarterly Report:

Microbial Enhanced Energy Recovery Via The Production Of Methane From Residual Hydrocarbons In Oklahoma Reservoirs

Period Covered by the Report: December 1, 2006 to March 1, 2007

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EPA Grant Number: X83242801

Title: Paraffin Control in Oil Wells Using Anaerobic Microorganisms

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Project Period: 12-01-06 to 11-30-07

Project Amount: \$80,000

Research Category: Pollution Prevention

Objective(s) of the Research Project: New technology is needed to recover the rather sizable amount of energy that is inherent in Oklahoma domestic oil reservoirs. A novel approach to this problem is the bioconversion of hydrocarbons entrained in marginally producing fields to methane gas as a cleaner-burning energy source. Thus, this project is designed to evaluate the utility of using an anaerobic bacterial consortium capable of converting oil in petroliferous reservoirs to methane and carbon dioxide. We will evaluate the efficiency and rate of oil bioconversion to methane by a methanogenic oil-degrading inoculum and delineate the tolerance of the organisms to select ecological variables.

Progress Summary/ Accomplishments: Past work in our laboratory showed that microorganisms enriched from a gas condensate-contaminated aquifer were able to biodegrade crude oil under methanogenic conditions (*Townsend et al., 2003*). We used this consortium in a preliminary study to examine whether hydrocarbons trapped in residual oil-bearing core material sampled from a marginal well (sampled from Nowata County, OK) could be also be converted to methane. Indeed, we found that in the presence of the residual oil, the inoculum produced from 1500 to 2200 μmol methane within a year, at a rate approximating 0.25 μmol methane/d/g core relative to uninoculated controls (*Suflita et al., 2004*). We found that the inoculum was readily transferred (10% vol/vol) to incubations containing freshly crushed core material, with enhanced methane production continuing at comparable rates (ranging from 0.15 - 0.42 μmol methane/d/g core) relative to core-free incubations. In the initial experiments, some incubations contained core material that was crushed finely, whereas others contained core pellets. The latter incubations were characterized by a longer lag period before the onset of methanogenesis so we questioned whether the inoculum was mass transfer limited. To assess this, we established incubations containing core material crushed to varying grain sizes ranging from < 149 μm to > 1.18 mm. Despite this core grain size variability, we found no significant differences in the rates or extents of methane production, suggesting that the culture was not mass transfer limited. However,

when the inoculum was supplied with formation oil only (i.e. not trapped in core material), the rate and extent of methane production was much lower, suggesting that the core material itself might be providing a nutritional requirement for the methanogenic consortium or that a solid surface is necessary for consortial activity. In a separate experiment, we assessed whether the organisms in the inoculum could grow in response to the residual oil substrate. To do this, we set up incubations containing the same amounts of crushed core material but added different amounts of inoculum (5, 10, or 20% by volume). Within the first part of the experiment (up to 100 days), we found that methane was produced at rates roughly proportional to the amount of inoculum (20%, 0.36 $\mu\text{mol CH}_4/\text{d/g}$ core; 10%, 0.18 $\mu\text{mol CH}_4/\text{d/g}$ core; 5%, 0.07 $\mu\text{mol CH}_4/\text{d/g}$ core). However, after that time, the rates of methane production were essentially equivalent at approximately 0.5 $\mu\text{mol CH}_4/\text{d/g}$ core, indirectly suggesting that the inoculum grew in response to the hydrocarbon substrate. In a parallel experiment, we examined methane production in incubations containing the same inoculum size but different amounts of core as substrate, ranging from no core to 20 g of core. As might be expected, the presence of more oil-bearing core material produced a greater amount of methane, in roughly proportional amounts: from 20 g core, about 750 $\mu\text{mol CH}_4$ were produced, from 10 g core, about 380 $\mu\text{mol CH}_4$ were produced, and from 5 g core, about 200 $\mu\text{mol CH}_4$ were measured. This observation suggested that oil trapped within the core is serving as the substrate for methane production. A time course experiment to measure changes in the hydrocarbon profiles during residual oil methanogenesis is ongoing.

Publications/ Presentations:

None during this reporting period.

Future activities: We will continue to transfer the residual oil-degrading inoculum under different conditions (i.e. salt concentrations, temperature) and monitor methane production. Hydrocarbon depletion will be assessed over time. Tools of molecular biology will be used to help identify the microorganisms present in the methanogenic inoculum.

Supplemental Keywords: Anaerobic biodegradation; Methanogenesis; Marginal Wells, Enhanced Energy Recovery

Relevant Web Sites: Not applicable at this time.

References:

Suflita, J. M., Davidova, I.A., Gieg, L.M., Nanny, M. & Prince, R.C. Anaerobic hydrocarbon biodegradation and the prospects for microbial enhanced energy production. *In* R. Vazquez-Duhalt and R. Quintero-Ramirez (ed.), *Petroleum Biotechnology. Developments and Perspectives*, Vol. 151, Elsevier Science, Amsterdam (2004).

Townsend, G.T., Prince, R.C. & Suflita, J.M. Anaerobic oxidation of crude oil hydrocarbons by the resident microorganisms of a contaminated anoxic aquifer. *Environ. Sci. Technol.* 37, 5213-5218 (2003).

