

2nd Quarterly Report:

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

Period Covered by the Report: March 2, 2007 to May 31, 2007

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EPA Grant Number: X83242801

Title: Paraffin Control in Oil Wells Using Anaerobic Microorganisms

Investigators: J. M. Suflita

Institution: The University of Oklahoma

EPA Project Officer: Bala Krishnan

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: In the previous report, we described initial experiments performed with a methanogenic consortium that was able to produce high levels of methane when incubated with sandstone core material containing residual oil relative to core-free controls. The inoculum appeared to grow in response to the trapped oil, was not mass transfer limited when incubated with core grains of various sizes, and incubations containing more core material produced more methane in proportional amounts. These results suggested that it was the biodegradation of hydrocarbons by the consortium that was driving the observed methane production. To test this hypothesis, we performed a time course experiment to monitor both the loss of hydrocarbons and concomitant methane production. Hydrocarbons in the residual oil were quantified following the organic extraction of duplicate incubations. Prior to extraction, a known quantity of a standard, C₂₄D₅₀, was added. Extracts were analyzed by gas chromatography-mass spectrometry and the ratios of the integrated peak areas of *n*-alkanes (ranging from C₁₂ to C₃₀) relative to that of the extraction standards were calculated. On this basis, we found that approximately 2/3 of the *n*-alkane fraction of the residual oil was depleted over the course of four months when the inoculum was added to the core material relative to sterile or uninoculated controls (Table 1). The alkane loss indeed correlated with methane production (Figure 1).

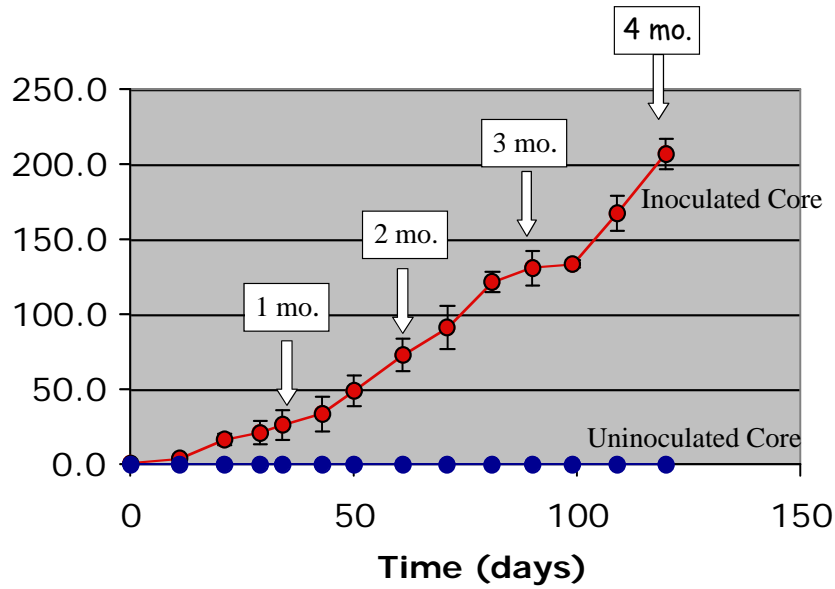
Currently, incubations for salt and temperature tolerance of the consortium have

been established and nucleic acid-based analyses to determine the members of the consortium are ongoing and will be the focus of a future report.

Table 1. Loss of *n*-alkanes from methanogenic residual oil incubations over time.

Time (months)	<i>n</i> -Alkanes/C ₂₄ D ₅₀ ratio	
	Inoculated Core	Controls (Sterile & Uninoculated Cores)
0	10.1	7.9 (+/- 0.4)
1	10.7 (+/- 1.4)	-
2	8.1 (+/- 0.9)	7.2 (+/- 0.1)
3	6.5 (+/- 0.6)	-
4	3.4 (+/- 0.7)	8.0 (+/- 0.8)

Figure 1. Methane produced during residual oil biodegradation by a methanogenic consortium.



Publications/ Presentations:

Gieg, L.M., Duncan, K.E., Suflita, J.M. 2007. Bioenergy Production: The Conversion of Residual and Unconventional Oil to Natural Gas. Abstract submitted for an oral presentation at the IPEC meeting to be held in Houston, TX, November.

Future activities: We will continue to evaluate the residual oil-degrading inoculum under different conditions (i.e. salt concentrations, temperature) and monitor methane production. Tools of molecular biology will be used to help identify the microorganisms present in the methanogenic inoculum. Nutritional requirements for optimum methane productions will be determined.

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

Relevant Web Sites: Not applicable at this time.