

7th Quarterly Report (no-cost extension)

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

Period Covered by the Report: June 1, 2008 to August 31, 2008

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EPA Grant Number: X83242801

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

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Project Period: 12-01-06 to 11-30-07 + no-cost extension till 11/30/08

Project Amount: \$80,000 + no-cost extension

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. Our goal is to evaluate the efficiency and rate of oil bioconversion to methane by a methanogenic oil-degrading inoculum, identify the consortial members, and delineate the tolerance of the organisms to select ecological variables.

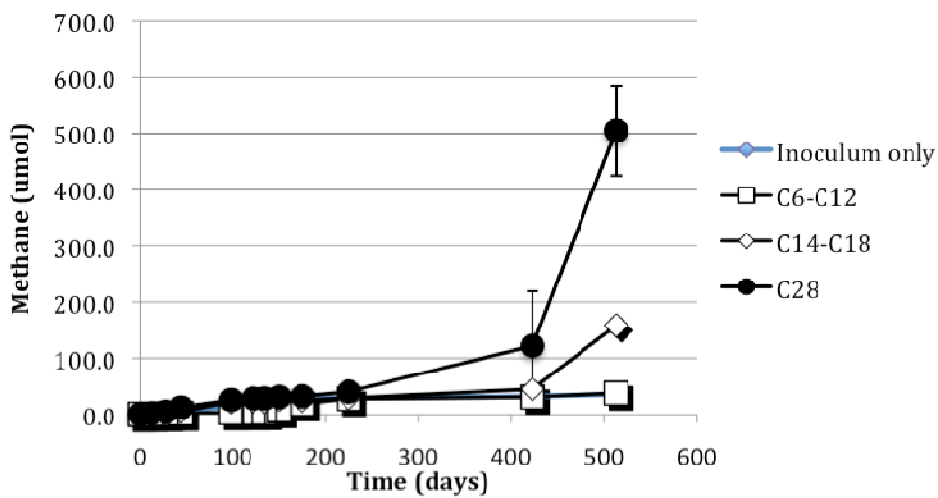
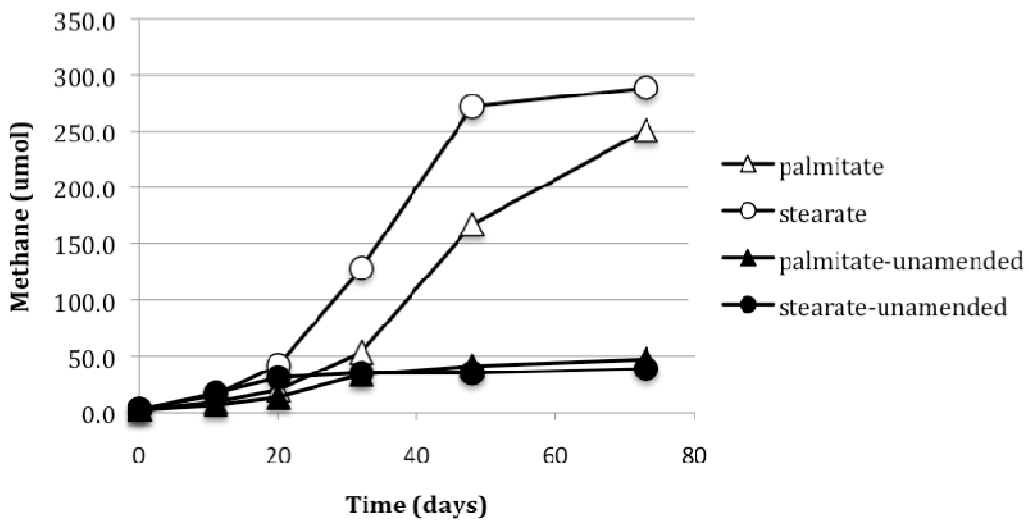
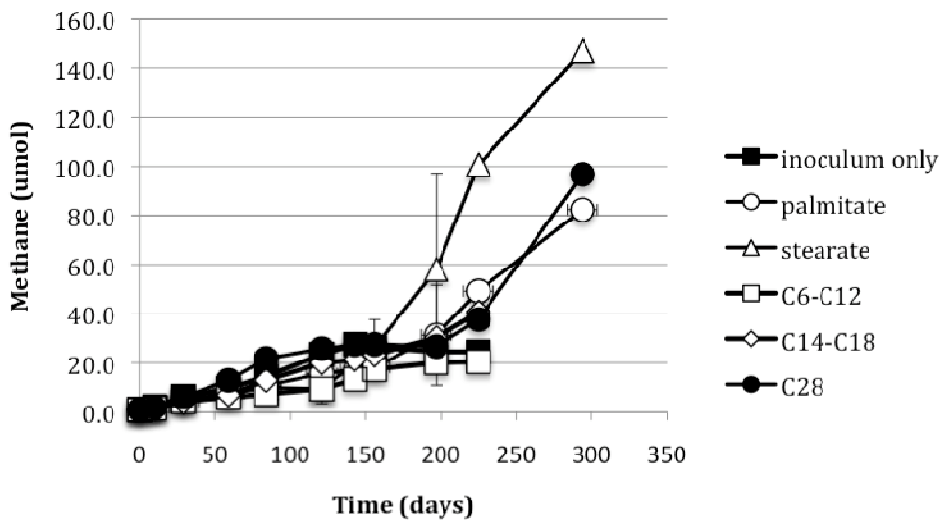
Progress Summary/ Accomplishments:

In the previous report, we noted that work done partially as a result of this funding was published in a reputable microbiology journal (Gieg *et al.*, 2008, Appl. Environ. Microbiol. 74: 3022-3029), wherein we showed that oil entrained in cores sampled from marginal fields could be biotransformed to methane by an anaerobic consortium. In addition, we showed that the anaerobic consortium was able to biotransform shale components to methane gas, offering proof of concept that the microbial inoculum may also find application in bioenergy recovery from shale deposits.

The anaerobic, methanogenic culture capable of degrading oil continues to be maintained on crushed residual oil core material since it is known that the organisms can utilize alkane constituents within the oil. We have begun carrying out studies with specific substrates (alkanes and other potential substrates) in order to determine which organisms in the consortium are actually carrying out various biotransformation reactions. For example, in the bioconversion of a hydrocarbon to methane, at least two but probably more, different species are required to interact in order to make the metabolic reactions energetically favorable (syntrophy). Thus, we aim to identify which organisms are enzymatically activating the hydrocarbon, which organism(s) are involved in metabolizing some of the resulting metabolites (such as fatty acids), and which

methanogens are ultimately using any generated substrates such as acetate and/or H₂. We identified a variety of species present in the consortium using tools of molecular biology, but do not know the metabolic activity of each species (except for the methanogens, Gieg *et al.*, 2008). Thus, we established enrichment cultures from the anaerobic consortium on substrates such as alkanes (C₆ - C₁₂ mix; C₁₄-C₁₈ mix, C₂₈) and the fatty acids palmitic (hexadecanoic) acid and stearic (octadecanoic) acid. Figure 1 (top graph) shows the results of the initial incubations with these various substrates, wherein the cultures incubated with palmitic acid, stearic acid, or C₂₈ produced enhanced levels of methane relative to control incubations containing the inoculum only with no added substrate. Incubations with the alkane mixtures (C₆-C₁₂ or C₁₄-C₁₈) did not produce significant amounts of methane above that measured in the inoculum-only controls during the incubation period (approximately 1 year). However, all of the incubations were further transferred following the year of incubation, and the results of the transfers are also shown in Figure 1. The middle graph shows the continued production of methane produced during the decomposition of palmitate and stearate relative to the substrate-free controls. Near stoichiometric amounts of methane were produced within a relatively short period of time (70 days). In contrast, transferred cultures incubating with C₆-C₁₂, C₁₄-C₁₈, or C₂₈ required a much longer incubation time (over 400 days) before any significant methanogenesis was observed relative to inoculum only controls (Figure 1, bottom graph). Incubations with the C₆ to C₁₂ mixture did not show significant methane production relative to the controls, even after approximately 500 days of incubation. These cultures will continue to be cultivated and monitored under methanogenic conditions, but characterizing the community members will require time and effort beyond of the scope of the no-cost extension grant period.

Figure 1 (below). Methane production from a variety of substrates by the residual oil-utilizing inoculum. Top graph: Initial transfers from the residual oil-amended incubations to more specific substrates; Middle graph: Continued transfers (second transfers) of the inoculum with palmitate or stearate; Bottom graph: Continued transfers (second transfers) of the inoculum with C₆ - C₁₂ mixture, C₁₄ - C₁₈ mixutre, or C₂₈ as the substrate.



Publications/ Presentations:

Gieg, L.M., Duncan, K.E., Suflita, J.M. 2008. Bioenergy production via microbial conversion of residual oil to natural gas. *Applied and Environmental Microbiology* 74: 3022-3029.

Gieg, L.M., Duncan, K.E., Suflita, J.M. 2007. Bioenergy production via the conversion of residual hydrocarbons to methane. Oral presentation given at the IPEC meeting in Houston, TX, November 6-9.

Future activities: Continued monitoring and cultivation of the oil-utilizing cultures and final report preparation.

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

Relevant Web Sites: Not applicable at this time.

