

Annual Report 2007

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

Period Covered by the Report: September 1, 2007 to November 31, 2007

Date of Report: December 30, 2007

EPA Grant Number: X83242801

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

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, identify the consortial members, and delineate the tolerance of the organisms to select ecological variables.

Progress Summary/ Accomplishments: In previous reports, we presented data from numerous experiments showing that oil trapped in a sandstone core could be biotransformed to methane by an anaerobic consortium. We found the the consortium could biodegrade oil components to methane equally well in the presence or absence of sulfate as an alternate electron acceptor and in conditions of up to 2% salt, observations which may have important implications for application of the inoculum into reservoirs. During this reporting period, we performed a series of cloning and sequencing exercises (based mainly on 16S rRNA gene sequences) to identify the members of the methanogenic consortium. Universal eubacterial or archaeal primers for amplification of the 16S rRNA gene were used to determine bacterial or archaeal members (e.g., methanogens), respectively. Some methanogens were also identified using primers specific for the amplification of the methyl coenzyme A reductase (*mcrA*) gene, a critical component in the methanogenesis pathway. Amplification of DNA extracted from the residual oil-degrading consortium using the universal archaeal primers revealed only sequences affiliating closely with *Methanosaeata* sp., methanogens that utilize acetate (but not H₂/CO₂) as the methanogenic substrate. Amplification exercises using the *mcrA*-specific primers further revealed the presence of H₂/CO₂-utilizing methanogens most closely affiliating with *Methanoculleus* and *Methanobacterium* sp. Cloning and

sequencing of the 16S rRNA gene using the eubacterial primers revealed the presence of several organisms affiliating with a variety of fermentative, syntrophic, and sulfate-reducing bacteria. Many of the cloned eubacterial sequences have no known cultured representatives, so the organisms in the consortium may represent new species. Figure 1 (below) is a phylogenetic tree showing the eubacterial sequences amplified from the oil-degrading consortium.

Publications/ Presentations:

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: We will continue to determine the potential broad utility of the residual oil-degrading inoculum by examining methane production from a variety of other petroliferous materials. A manuscript based on the data collected from this project is currently being drafted.

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

Relevant Web Sites: Not applicable at this time.

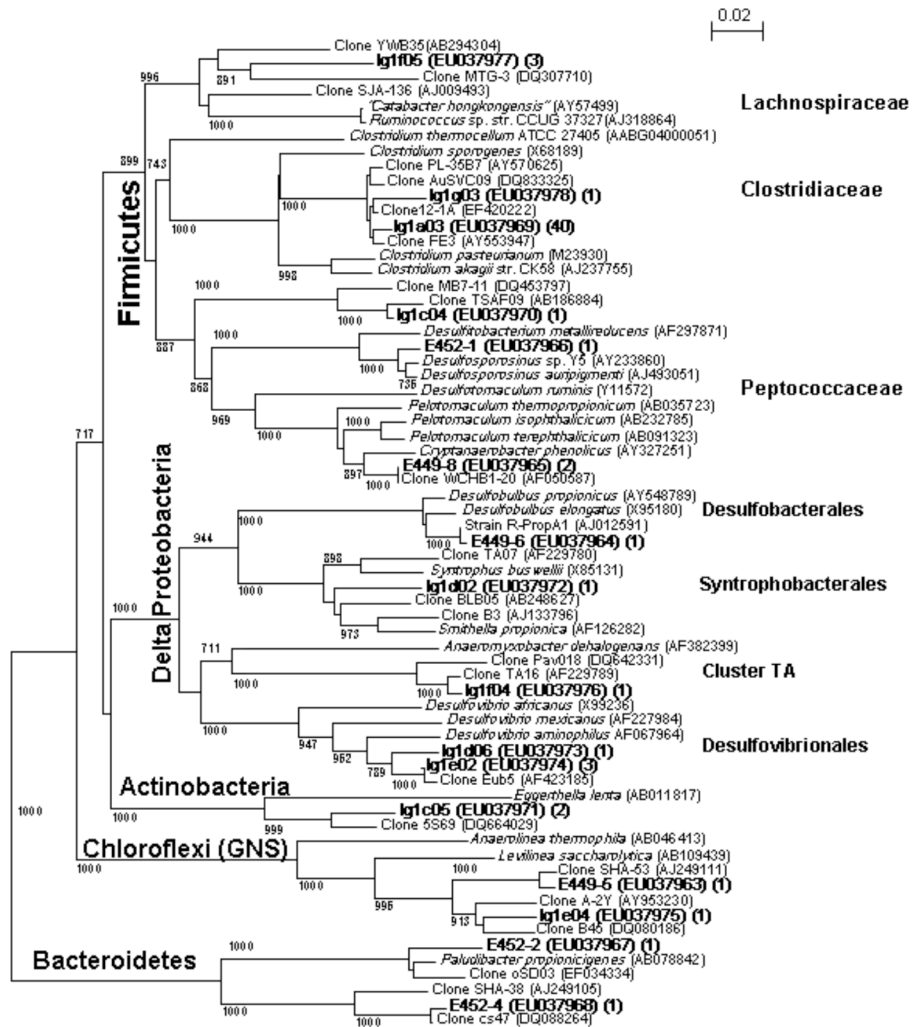


Figure 1. Phylogenetic relationships of eubacterial clones from the oil-utilizing methanogenic consortium (in bolded text) with respect to related sequences. The tree is constructed from approximately 800 bp 16S rRNA gene sequence using the neighbor-joining algorithm. One thousand bootstrap replications were performed; only values greater than 750 are shown. The number in parentheses following the

accession number indicates the total number of clones represented by the OTU.