

Continuation of an Investigation into the Anaerobic Intrinsic Bioremediation of Whole Gasoline – Annual Report

Period Covered by the Report: 3-1-01 to 2-28-02

Date of Report: 3-20-02

EPA Grant Number: R827015-01-0

Title: Continuation of an Investigation into the Anaerobic Intrinsic Bioremediation of Whole Gasoline – Annual Report

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Project Period: March 1, 2001 to February 28, 2002 (N/C Ext. to August 28, 2002)

Project Amount: \$111,344

Research Category: Intrinsic bioremediation/natural attenuation

Objectives of the Research Project: The primary objective of this project is to investigate the ability of microorganisms from contaminated aquifers to degrade a variety of hydrocarbon molecules under different electron accepting conditions. Specifically, the degradation of several alicyclic compounds with either sulfate or carbon dioxide as electron acceptor is examined. In addition, the ability of hydrocarbons in several crude oils to stimulate sulfate reduction and methanogenesis is examined. Compounds contained in the oils are noted for their relative susceptibility to anaerobic microbial decay and the preferred electron acceptor similarly examined.

Progress Summary/Accomplishments:

The biodegradation of petroleum hydrocarbons by anoxic aquifer sediments previously contaminated by natural gas condensate was examined under methanogenic and sulfate-reducing conditions. To explore the substrate range of the resident microbiota, two crude oils, rich in high molecular weight constituents, were chosen as substrates: artificially weathered Alaska North Slope crude, an oil that is depleted in low molecular weight alkanes and monoaromatic compounds but retains its normal complement of mid-to-heavy range hydrocarbons (C₁₅-C₃₄), and Alba crude oil, an oil that is naturally depleted in n-alkanes of all sizes but retains most of its polycyclic aromatic hydrocarbons. We examined in detail the change in crude oil composition over a 400+ day incubation period to determine the resident microbiota's substrate preference when presented with a petroleum hydrocarbon mixture and the role of sulfate in the biodegradation of individual hydrocarbon components.

Weathered Alaska North Slope oil amendments resulted in a drastic increase in both methanogenesis and sulfate reduction. Alba crude oil amended incubations displayed a relatively modest stimulation of methanogenesis and sulfate reduction (Figure 1).

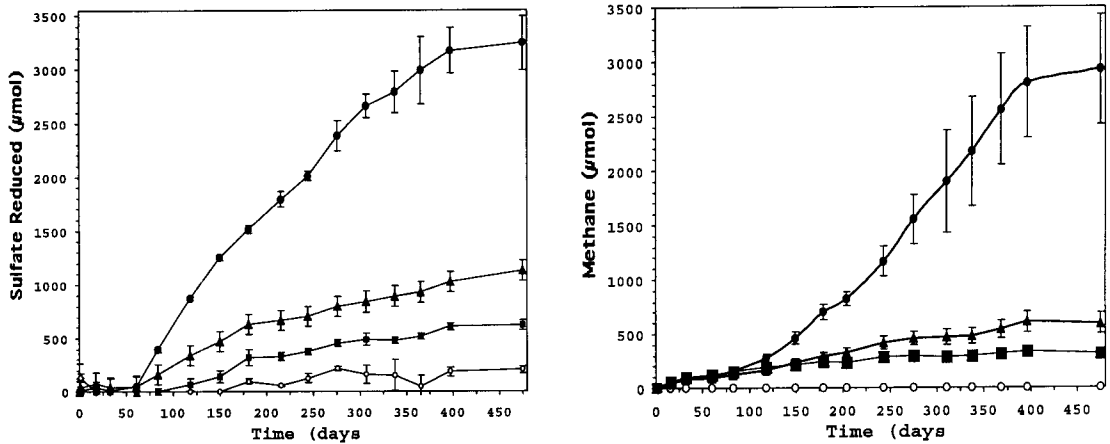


Figure 1. Cumulative methanogenesis and sulfate reduction in incubations amended with weathered Alaska North Slope Crude (◼) and Alba (◻) compared to autoclaved (○) and unamended (◻) control incubations.

Hydrocarbon analysis revealed that the n-alkane fraction of the weathered Alaska North Slope crude, consisting predominately of hydrocarbons fourteen to thirty-four carbons in length, was completely biodegraded under both sulfate-reducing and methanogenic conditions after thirteen months of incubation (Figure 2). Unlike the unbranched n-alkanes, the branched isoprenoid alkanes pristane and phytane were not biodegraded under either condition. The time course for degradation of two representative alkanes, heptadecane (C₁₇H₃₆) and triacontane (C₃₀H₆₂), is also given in Figure 2. As illustrated by these examples, the entire range of n-alkanes was consumed simultaneously with only slightly reduced rates for the longer chain congeners. The n-alkanes were biodegraded approximately twice as fast in the presence of sulfate as they were in its absence. Nevertheless, by the end of the incubation period, all unsubstituted n-alkanes originating from the weathered Alaska North Slope crude were completely consumed under both conditions.

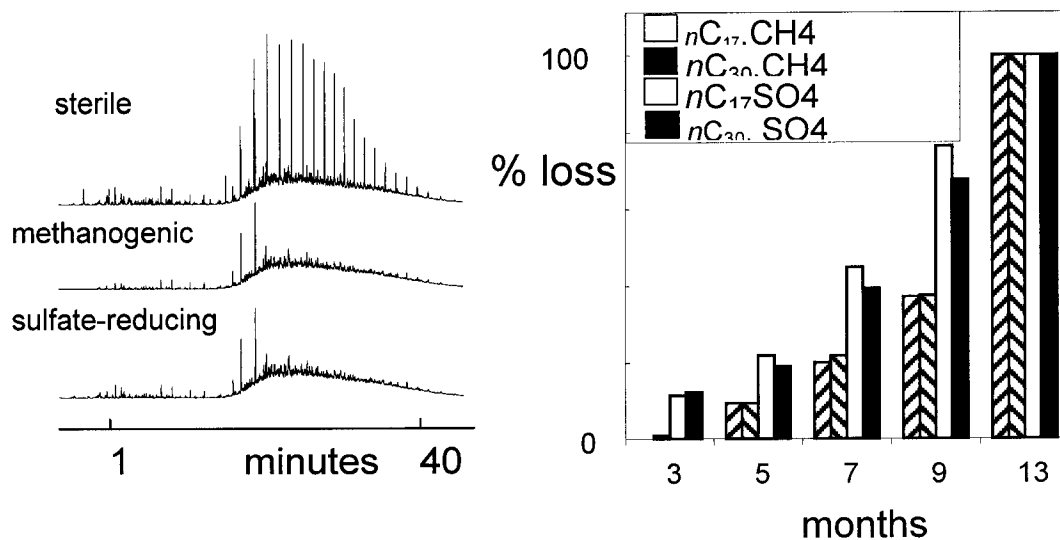


Figure 2. n-Alkane biodegradation in weathered Alaska North Slope crude oil. (A) Chromatograms of residual oil after 13 months of incubation. (B) Time course of biodegradation of two n-alkanes.

Polycyclic aromatic hydrocarbons on the USEPA priority pollutant list were also examined. In sulfate reducing incubations amended with either oil, naphthalene, 2-methylnaphthalene, and 2-ethylnaphthalene were biodegraded in that order of preference (Figure 2). In incubations amended with Alba oil, the coeluting 2,6- and 2,7-dimethylnaphthalenes were also biodegraded. The 1-methyl- and 1-ethyl-naphthalene congeners as well as the dimethyl-substituted naphthalenes within these incubations were recalcitrant. No biodegradation of naphthalene or its alkylated congeners was seen in incubations held under methanogenic conditions. We believe this is the first report of 2-ethylnaphthalene and of 2,6- and 2,7-dimethylnaphthalene biodegradation under anaerobic conditions

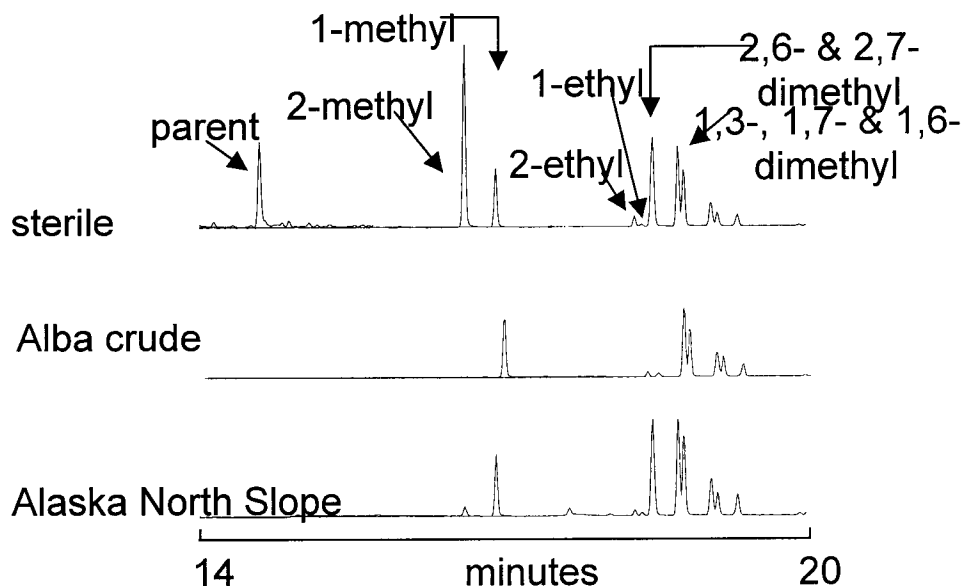


Figure 3. Selective biodegradation of naphthalene, 2-methylnaphthalene, and 2-ethylnaphthalene in crude oil amended incubations held under sulfate reducing conditions after 14 months.

These results indicate that n-alkanes, regardless of their size, are relatively labile in anaerobic compartments and their biodegradation in terrestrial environments is not limited by electron acceptor availability. Polycyclic aromatic hydrocarbons, on the other hand, are much more recalcitrant, and their biodegradation involves biodegradative activities that are both sulfate-dependent and congener-specific. This information should be useful in judging the extent of *in situ* biodegradation of crude oil in terrestrial environments and in making decisions regarding risk-based corrective actions.

We report that these aquifer sediments harbor microorganisms are able to biodegrade a wide range of high molecular weight alkanes, even though they are not significant components of the native contamination, under both sulfate-reducing and methanogenic conditions. Selected naphthalenes were also biodegraded exclusively under sulfate-reducing conditions. These results demonstrate the presence of diverse petroleum biodegradative activities in anaerobic terrestrial environments that transcends previous hydrocarbon exposure. This study, which took advantage of complex hydrocarbon mixtures as substrate and unenriched microbial communities as inoculum, shed light on the extent to which *in situ* biodegradative processes are acting upon residual contamination and hopefully will influence decisions regarding the need for remediation.

Publications/Presentations:

Rios-Hernandez, L. A., Gieg, L. M., and Suflita, J. M. Biodegradation of an Alicyclic Hydrocarbon by a Sulfate-Reducing Enrichment from a Gas Condensate-Contaminated Aquifer. In preparation.

Townsend, G. T., Prince, R. C., and Suflita, J. M. The Anaerobic Oxidation of Crude Oil Hydrocarbons by the Resident Microorganisms of a Contaminated Anoxic Aquifer. In preparation.

Townsend, G. T., Prince, R. C., and Suflita, J. M. Anaerobic Biodegradation of Alicyclic Constituents of Gasoline and Natural Gas Condensate by Bacteria from an Anoxic Aquifer. In preparation.

Supplemental Keywords:

groundwater, hydrocarbons, biodegradation, alicyclic, alkanes, sulfate reduction, methanogenesis, ethylcyclopentane, anaerobic, community analysis, denaturing gradient gel electrophoresis, gas chromatography/mass spectrometry (GCMS), PAH's, bacteria, bioremediation, pathway, intermediates, microbial ecology, aquifer, oil, gas condensate, contamination, gasoline, fumarate addition