Continuation of an Investigation into the Anaerobic Intrinsic Bioremediation of Whole Gasoline – Fifth Quarterly Progress Report

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Sediment from a gas condensate contaminated aquifer was used as the inoculum in laboratory incubations in which whole gasoline or gas condensate was used as the substrate. The gasoline mixture contained five alicyclic or methylsubstituted alicyclic hydrocarbons that were monitored over time as well as several C_2-substituted alicyclic molecules compounds. In the presence of sulfate, the alicyclic fraction was degraded with a shorter lag time and to a greater extent than replicate incubations conducted under methanogenic conditions. When natural gas condensate was present, similar biodegradation profiles were observed with the naturally occurring alicyclic compounds being more amenable to decay in the presence of sulfate. We believe this is the first laboratory report demonstrating the anaerobic biodegradation of alicyclic hydrocarbons. These findings suggest that similar to aerobic systems, complex anaerobic microbial communities exist and are active in hydrocarbon contaminated environments.

Sediment from the gas condensate contaminate aquifer was used as an inoculum to assess the ability of microorganisms to degrade two crude oils under methanogenic and sulfate reducing conditions in the laboratory. Alaska North Slope (ANS) crude oil served as the substrate while Alba oil, which is naturally depleted in alkanes was used in separate incubations. Methanogenesis and sulfate reduction were stimulated in the presence of both oils but the ANS oil had a greater stimulatory effect apparently resulting from the presence of relatively labile alkanes. Moreover, the presence of sulfate allowed a greater degree of biodegradation of the polycyclic aromatic hydrocarbon fraction in both oils. These findings indicate that n-alkanes are relatively labile under either sulfate reducing or methanogenic conditions but polycyclic aromatic hydrocarbons are relatively more recalcitrant and their degradation appears to be dependent on the presence of sulfate. This information should be useful for assessing the limits of crude oil biodegradation in terrestrial environments and in making predictions regarding the capacity of the resident microflora to mitigate the contamination levels of particular pollutants.

Sediment and groundwater from the gas condensate contaminated aquifer was used to develop an enrichment capable of degrading ethylcyclopentane (ECP) with sulfate as electron acceptor. Several putative intermediates of ECP metabolism were identified and

used to propose a pathway, which appears to be initiated by an anaerobic fumarate addition mechanism, similar to that identified for several hydrocarbon molecules. Analysis of the microbial community in the ECP degrading enrichment was analyzed by denaturing gradient gel electrophoresis (DGGE) identifying at least three different organisms. The sequences belong to the *Bacteroides, Syntrophobacter,* and *Desulfotomaculum* genera. Organisms in the genus *Desulfotomaculum* are not known for their ability to degrade hydrocarbons but they do oxidize aliphatic monocarboxylic and dicarboxylic acids coupled to sulfate reduction. These results support the suggestion that the *Desulfotomaculum* sp. is responsible for the degradation of ECP in the enrichment culture.