Characterization of Microbial Communities Mediating Anaerobic Biodegradation of Petroleum Hydrocarbons along a Depth Transect in NAPL Zones

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Abstract. Persistent contaminant source zones are formed in soils by petroleum liquids released into the subsurface. Microbially mediated depletion of light non- aqueous phase liquids (LNAPL) is gaining regulatory acceptance as a method for managing impacted sites; however, the fundamental microbiology of anaerobic hydrocarbon degradation at field sites remains poorly understood. Thus, we performed a depth-resolved characterization of the mixed microbial communities present at a former refinery in Wyoming. Seventeen soil cores were collected from the impacted site, frozen on dry ice and subsampled at 6-inch intervals for analysis of biogeochemical parameters. Multi-level sampling systems were installed at the core sites to monitor contaminants in the aqueous and gas phases as well as oxygen, methane and carbon dioxide levels. Diesel and gasoline range organics present in the cores and in water samples, were analyzed. Inorganic dissolved ions, pH, and ORP were also measured. DNA was extracted in triplicate from each subsample corresponding to the study's center core (21 samples). Total Archaea and Eubacteria were quantified via 16S rRNA gene-targeted qPCR. Microorganisms present at selected depth intervals were identified via 454 pyrosequencing of both eubacterial and archeal 16S rRNA genes. Results indicate that at the study site, the majority of the hydrocarbon contamination is found between 5 and 12 feet below ground surface (bgs). The presence of methane in the vadose zone and relatively high sulfate concentrations in water samples suggest that both methanogenesis and sulfate reduction are likely driving LNAPL depletion processes. Interestingly, the quantity of eubacterial 16S rRNA genes dominate the quantity of archaeal 16S rRNA genes at most sampled depths, with the exception of a zone found at the center of the contaminant smear (7 - 11.5 ft bgs), where these quantities are roughly equal. The latter can be interpreted as evidence of syntrophism, which has been reported in other hydrocarbon biodegradation studies. Pyrosequencing results will contribute to further elucidating the spatial correlation between microbial communities and geochemical parameters. Understanding the effects these relationships have on LNAPL depletion rates will inform development of advanced remediation technologies.

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