

Mineralization of PAHs in coal–tar impacted aquifer sediments and associated microbial community structure investigated with FISH

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Abstract

The microbial community structure and mineralization of polycyclic aromatic hydrocarbons (PAHs) in a coal–tar contaminated aquifer were investigated spatially using fluorescence in situ hybridization (FISH) and in laboratory-scale incubations of the aquifer sediments. DAPI-detected microbial populations in the contaminated sediments were three orders of magnitude greater than nearby uncontaminated sediments, suggesting growth on coal–tar constituents in situ. *Actinobacteria*, β - and γ -*Proteobacteria*, and *Flavobacteria* dominated the in situ aerobic ($>1 \text{ mg l}^{-1}$ dissolved oxygen) microbial community, whereas sulfate-reducing bacteria comprised 37% of the microbial community in the sulfidogenic region of the aquifer. Rapid mineralization of naphthalene and phenanthrene were observed in aerobic laboratory microcosms and resulted in significant enrichment of β - and γ -*Proteobacteria* potentially explaining their elevated presence in situ. *Firmicutes*, *Flavobacteria*, α -*Proteobacteria*, and *Actinobacteria* were also enriched in the mineralization assays, but to a lesser degree. Nitrate- and sulfate-limited mineralization of naphthalene in laboratory microcosms occurred to a small degree in aquifer sediments from locations where groundwater chemistry indicated nitrate- and sulfate-reduction, respectively. Some iron-limited mineralization of naphthalene and phenanthrene was also observed in sediments originating near groundwater measurements of elevated ferrous iron. The results of this study suggest that FISH may be a useful tool for providing a much needed link between laboratory microcosms and groundwater measurements made in situ necessary to better demonstrate the potential for natural attenuation at complex PAH contaminated sites.

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1. Introduction

Polycyclic aromatic hydrocarbon (PAH) contamination is a common environmental challenge found at creosote works, coal gasification sites, coking industries, and petroleum refineries. PAHs tend to associate with dense nonaqueous phase liquids (DNAPLs) that coat soils surfaces and

pool on impermeable layers deep in aquifer systems resulting in a persistent source of groundwater contamination. US EPA has identified 16 PAHs as priority pollutants. All 16 PAH compounds are susceptible to aerobic biodegradation. Low-ring PAHs have also been shown to degrade in anaerobic incubations of contaminated harbor, estuarine, and aquifer sediments (e.g. Rockne et al., 2000; Hayes and Lovley, 2002). Because of the difficulties associated with removing DNAPLs from the subsurface, natural attenuation of PAH-contaminated sites as a remediation strategy is of interest (Landemeyer et al., 1998; Ong et al., 2001).

To implement natural attenuation at PAH-contaminated sites, better methods for documenting the capacity

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of the indigenous microbial consortia to transform PAHs into innocuous byproducts are needed. Others have noted that PAH mineralization in soils and sediments globally is dominated by a limited number of bacterial taxonomic groups including nocardioforms, sphingomonads, *Burkholderia*, pseudomonads, and *Mycobacterium* (Johnsen et al., 2002). This suggests that the use of specific molecular 16S rRNA probes may be one approach for tracking the presence of PAH-degrading bacteria in complex systems in real time. Studies that employ molecular approaches such as FISH to link laboratory microcosm data to the field-scale may also be more appropriate for assessing natural attenuation than traditional monitoring approaches (Richardson et al., 2002; Eriksson et al., 2003).

This research explores the use of fluorescence in situ hybridization (FISH) as a tool to support the occurrence of natural attenuation of PAHs in a coal-tar impacted aquifer by linking the intrinsic condition to mineralization of PAH compounds exhibited in laboratory microcosms. The objectives were (1) to determine whether changes in the aqueous geochemical environments exhibited in situ may be related to oxygen-, nitrate-, iron-, or sulfate-limited degradation of PAHs as evidenced by laboratory microcosms under defined oxidation–reduction conditions, and (2) identify whether shifts in the structure of the intrinsic microbial community relative to nearby pristine conditions (a) reflected the aqueous geochemistry observed in groundwater measurements and (b) were related to growth of microbial taxa in laboratory microcosms with site sediments under the dominant redox processes related to PAH mineralization. The relationship between groundwater geochemical trends, the presence of PAHs, and the response of the intrinsic microbial community provided evidence for mixed aerobic and anaerobic metabolic processes in situ. The applications and limitations of these methods to intrinsic remediation at PAH-contaminated sites are discussed.

2. Materials and methods

2.1. Former manufactured gas plant (FMGP) study site

Coal-tar PAH and BTEX contamination resulting from gas manufacturing operations and possible gasoline spillage pervades the aquifer underlying an FMGP site located in Northwestern Iowa (Fig. 1). The aquifer hydrogeology, contaminant plume extents, and aqueous geochemistry, have been presented in detail in a previous work (Rogers et al., 2007a). The shallow semi-confined aquifer system is comprised of four primary geologic units that underlay a top layer of mixed fill including (1) loess, (2) fine-grained silty alluvium, (3) highly transmissive coarse alluvium, and (4) glacial till (Fig. 1). Because of the thinning of the alluvium layer and thickening of the overlying loess, there is a region of sharp hydraulic gradient between the site and nearby river. Coal-tar DNAPLs and the resultant plume are confined primarily to the alluvial sediments.

Changes in groundwater biogeochemistry within the contaminated region of the aquifer relative to groundwater sampled immediately up-gradient suggested microbial activity on coal-tar constituents. Aerobic oxidations were evidenced by rapid depletion of dissolved oxygen as groundwater moved through the region of coal-tar DNAPLs (Table 1 and Fig. 2). Anaerobic transformations of the coal-tar constituents in the contaminated aquifer and further down the hydraulic gradient may have included denitrification, dissimilatory nitrate reduction, metals (iron and manganese) reduction, and sulfidogenesis, respectively. Nitrate reduction was evidenced by the depletion of nitrate from the groundwater corresponding to increasing concentrations of nitrite and ammonia that depleted further down the hydraulic gradient. Metal reductions were evidenced by increased aqueous concentrations of ferrous iron and manganese(II). Further down-gradient where measured groundwater oxidation–reduction potentials (ORPs) were -247 mV, hydrogen sulfide concentrations increased in response to declining sulfate concentrations. Methane production was not detected within the contaminated region of the aquifer.

2.2. Sediment core sampling

Eight continuous sediment cores were extracted from ground surface to glacial till using direct push technology. The sediments were selected based on contaminant exposure history and aqueous geochemical measurements as described in Table 1 and shown in Figs. 1 and 2. Sediment cores were obtained in 1.2 m sections that were sheathed in sterile plastic tubing. Alternate sections of each core were either retained for microbial analysis or used for characterization of the porous media (Rogers et al., 2007a). Core sections retained for microbial analysis were selected within the alluvial layer, and generally of the same elevation; however, differences in ground surface elevation resulted in slight variations of the sample elevations.

Each 1.2 m sediment core section for microbial analysis was cut in half upon extraction from the ground using a hack saw that was cleaned with soapy water and sterilized with 70% ethanol between cuts. Using a sterile spoon, the top 2.5 cm from one of the core section halves was carefully removed and discarded. Approximately 5–10 g sediments from the center of the sediment core, at least 1 cm from the plastic tubing, were aseptically removed, preserved in 4% paraformaldehyde–PBS (pH 7.2), and stored on ice (maximum holding time was 2 days). The remaining 0.6 m core section halves were capped, sealed with electrical tape, and maintained at 4 °C until used in batch microcosms as described below.

2.3. Mineralization of ^{14}C -radiolabeled substrates

Aquifer sediments were assayed for mineralization of ^{14}C -labeled PAH compounds. Assays were designed to determine whether the addition of electron acceptors

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