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Succession of microbial functional communities in response to a pilot-scale ethanol-blended fuel release throughout the plume life cycle

Jie Ma [a, b], Ye Deng [c, d], Tong Yuan [d], Jizhong Zhou [d, e, f], Pedro J.J. Alvarez [b, *]

[a] State Key Laboratory of Heavy Oil Processing, Beijing Key Lab of Oil & Gas Pollution Control, China University of Petroleum-Beijing, Beijing 102249, China
[b] Department of Civil and Environmental Engineering, Rice University, Houston, TX 77005, USA
[c] Research Center for Eco-Environmental Science, Chinese Academy of Sciences, Beijing 100085, China
[d] Institute for Environmental Genomics, and Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019, USA
[e] State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China
[f] Earth Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94270, USA

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ABSTRACT

GeoChip, a comprehensive gene microarray, was used to examine changes in microbial functional gene structure throughout the 4-year life cycle of a pilot-scale ethanol blend plume, including 2-year continuous released followed by plume disappearance after source removal. Canonical correlation analysis (CCA) and Mantel tests showed that dissolved O_2 (which was depleted within 5 days of initiating the release and rebounded 194 days after source removal) was the most influential environmental factor on community structure. Initially, the abundance of anaerobic BTEX degradation genes increased significantly while that of aerobic BTEX degradation genes decreased. Gene abundance for N fixation, nitrification, P utilization, sulfate reduction and S oxidation also increased, potentially changing associated biogeochemical cycle dynamics. After plume disappearance, most genes returned to pre-release abundance levels, but the final functional structure significantly differed from pre-release conditions. Overall, observed successions of functional structure reflected adaptive responses that were conducive to biodegradation of ethanol-blend releases.

1. Introduction

Microorganisms play vital roles in key biogeochemical cycles in virtually all of our planet's environments, thus comprising the backbone of most ecological systems (Zhou et al., 2014). Therefore, unraveling microbial responses to environmental perturbations is a central goal for environmental microbiologists (Allison and Martiny, 2008; Shade et al., 2012). As a typical environmental perturbation, fossil fuel releases pose a big threat to groundwater biosphere, which despite constituting the largest terrestrial freshwater biome, it remains amongst the least explored habitats on earth (Griebler et al., 2014). The growing use of ethanol as transportation biofuel is increasing the likelihood of encountering it in fuel releases, where it may hinder the natural attenuation of co-occurring contaminants such as benzene, toluene, ethylbenzene and xylenes (BTEX) (Corseuil et al., 1998; Ma et al., 2013b; Powers et al., 2001a, 2001b). Therefore, it is important to understand how ethanol-blended fuel releases influence the succession and functioning of indigenous microbial communities in impacted aquifers, and the associated microbial functional structure and bioremediation processes.

The impacts of ethanol-blended fuel releases on microbial phylogenetic structure have been investigated via 16S rRNA pyrosequencing (Ma et al., 2013a), denaturing gradient gel electrophoresis (DGGE) (Capiro et al., 2008; Elazhari-Ali et al., 2013) and automated ribosomal intergenic space analysis (ARISA) (Nelson et al., 2010). Although these 16S rRNA-based studies provided useful taxonomic and phylogenetic information regarding resulting microbial population shifts, little is known about the associated changes in functional structure and metabolic potential.

Several individual functional genes such as *mcrA* (methanogenesis), *fhs* (acetogenesis), *aps* (sulfate reducing), *nirK* and *nirS* (nitrate reducing), PHE and TOD (BTEX aerobic degradation), and *bssA* (BTEX anaerobic degradation) have been previously investigated in aquifers impacted by ethanol blends (Beller et al., 2008; Capiro et al., 2008; da Silva and Corseuil, 2012; Feris et al., 2008; Ma et al., 2013a). However, these studies provided only partial

* Corresponding author.
E-mail address: alvarez@rice.edu (P.J.J. Alvarez).

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information regarding a limited number of functional genes. A more comprehensive characterization of microbial functional structure is needed. As a high-throughput functional gene microarray, GeoChip is well suited for this purpose and it has been successfully applied to characterize microbial functional diversity in a variety of environments (Chan et al., 2013; Hazen et al., 2010; Wang et al., 2009; Zhou et al., 2014, 2012).

Another knowledge gap relates to how the microbial community responds after the contaminant source is removed and the plume is attenuated or remediated, since previous research has mainly focused on microbial responses before (baseline) and after the contamination occurs. Improved understanding of the microbial response after source removal may help optimize site management strategies for biofuel releases.

In this study, GeoChip 4.6 was used to characterize the dynamics of microbial functional structure in response to 1) a pilot-scale, continuous (two-year) ethanol blend release, and 2) its subsequent shut-off and natural attenuation over two additional years. Therefore, the succession of the microbial community was considered throughout the life cycle of the plume. The pilot-scale experiments were unique in that they are of sufficient scale such that more realistic three-dimensional contaminant plumes can be established, but at a small enough scale to provide sufficiently controlled experimental conditions. Chemical concentrations (e.g., ethanol, benzene, toluene, methane, acetate, butyrate, and butanol) and environmental variables (e.g., temperature, pH, redox potential, and dissolved oxygen) were monitored to enhance the interpretation of GeoChip data.

2. Materials and methods

2.1. Pilot-scale model aquifer system

An 8-m^3 (3.7 m × 1.8 m × 1.2 m) pilot-scale continuous-flow tank packed with fine grain southeast Texas sand (Circle Sand; Houston, Texas) was used in this study (Fig. S1 in the supporting information). Tap water was added from the "inlet" (Fig. S1) at 170 L/day (average seepage velocity of 2.5 ft/day) to obtain a water table elevation of about 70 cm from the bottom of the tank. The groundwater retention time in this model tank was around 4 days. The total aquifer thickness was 115 cm and the depth of the water table was 45 cm below ground surface. The ethanol blend solution was a water solution containing 10% (v/v) ethanol, 50 mg/L benzene, 50 mg/L toluene and 24 000 mg/L of sodium bromide (NaBr). The blend solution was continuously injected into the tank from the ethanol blend injection port (22.5 cm below the water table) at a rate of 0.4 L/day for 10 months. NaBr was added as a conservative tracer, and to maintain a solution density to reach neutral buoyancy with the flowing groundwater. The added NaBr was diluted by the tank flow to less than 2000 mg/L (measured at groundwater sampling well, see Fig. 1), which was within the typical tolerance range of soil bacteria (Atlas and Bartha, 1997). The groundwater sampling well was at the same depth as the ethanol blend injection port (22.5 cm below the water table). Details on the tank construction and packing methods can be found in (Ma et al., 2011) and (Ma et al., 2012).

2.2. Release stages and plume life cycle

This pilot-scale release experiment lasts for 4 years, which could be divided into 4 experimental stages (Fig. 1). General information for each stage can be found in Table 1. Stage 1 was the pre-release baseline. Stage 2 began with the continuous ethanol blend release (10% ethanol + 50 mg/L benzene + 50 mg/L toluene) and lasts for 2 years. Stage 3 followed the removal of ethanol from the continuous release, resulting in continuous exposure to 50 mg/L benzene and 50 mg/L toluene continues for 8 months. This mimicked the earlier removal of ethanol than BTEX at contaminated sites (Corseuil et al., 2011; Freitas and Barker, 2013; Freitas et al., 2011a, 2011b; Mackay et al., 2006; Spalding et al., 2011). Stage 4 was the return to initial conditions (benzene and toluene removed from the tank influent), when clean water flowed through the aquifer material for 4 months. At the end of each experimental stage, sand samples were collected for GeoChip and soil property analysis, and groundwater samples were collected for chemical and geochemical analysis. The sampling date can be found in Table 1.

2.3. Analysis of groundwater pollutants and geochemical parameters

For chemical analysis, four replicate groundwater samples were collected from the groundwater sampling well using 50 mL syringes at the end of each experimental stage. Ethanol, methane, acetate, propionate, butyrate and butanol were measured by GC-FID (Hewlett Packard, Palo Alto, CA, USA). Ethanol, acetate, propionate, butyrate, and butanol were measured by liquid injections while methane was measured by headspace injections. The detection limits (aqueous concentration) were 1 mg/L for ethanol, acetate, and propionate, 2 mg/L for butyrate and butanol, and 0.1 mg/L for methane. Benzene and toluene were pre-concentrated by Purge and Trap System (Tekmar, Vernon, BC, Canada) and measured by GC–MS (Agilent, Santa Clara, CA, USA) with a detection limit of 10 μg/L (aqueous concentration). Details on chemical measurement methods can be found in Ma et al., 2011.

Groundwater geochemical parameters at Stage 1 and 2 (pH, dissolved O_2, temperature, and redox potential) were monitored by a YSI 600XLM groundwater quality probe (YSI, Yellow Springs, Ohio, USA) which was installed 15 cm upstream from the groundwater sampling well (Fig. 1). After Stage 2, this probe broke, thus no redox data was available since then. For Stage 3 and 4, the temperature was measured by a Pen-Style Thermometer (Taylor Precision Products, Oak Brook, IL, USA); dissolved O_2 (DO) was measured by a Dissolved Oxygen AccuVac® Ampules kit (Hach, Loveland, CO, USA); pH was measured by a Pocket pH Tester (Davis Instruments, Vernon Hills, IL, USA). Groundwater geochemical data can be found in Table 1.

2.4. Sand sampling and analysis

Sand samples were collected in 5 replicates from a depth of 5–30 cm below water table (50–75 cm below the sand surface, Fig. S1) on the same day when groundwater samples were collected. Details on sand sampling method can be found in (Ma et al., 2013a). Dry sand samples were sent to the Soil, Water and Forage Testing Laboratory at Texas A&M University for the measurement of soil pH, total organic carbon content, conductivity, nitrate-nitrogen, P, K, Ca, Mg, S, and Na (Table S1). Details on soil analytical methods can be found in the supporting information.

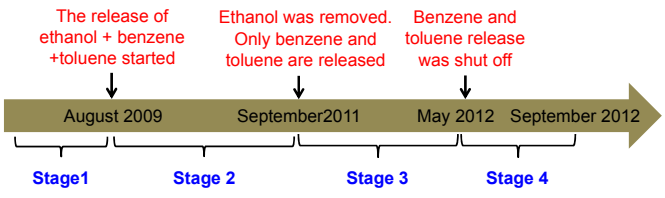

Fig. 1. Timeline of the pilot-scale release experiment.