WATER RESEARCH 68 (2015) 479–486

Available online at www.sciencedirect.com

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Increased fermentation activity and persistent methanogenesis in a model aquifer system following source removal of an ethanol blend release

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ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form
6 October 2014
Accepted 10 October 2014
Available online 17 October 2014

Keywords:
Groundwater
Ethanol
Source removal
Toxicity
Fermentation
Methanogenesis

ABSTRACT

The increased probability of groundwater contamination by ethanol-blended fuel calls for improved understanding of how remediation efforts affect the fate and transport of constituents of concern, including the generation and fate of fermentation byproducts. A pilot-scale (8 m^3) model aquifer was used to investigate changes in the concentrations of ethanol and its metabolites (methane and volatile fatty acids) after removal of the contamination source. Following the shut-off of a continuous release of a dissolved ethanol blend (10% v:v ethanol, 50 mg/L benzene, and 50 mg/L toluene), fermentation activity was surprisingly stimulated and the concentrations of ethanol metabolites increased. A microcosm experiment showed that this result was due to a decrease in the dissolved ethanol concentration below its toxicity threshold (~2000 mg/L for this system). Methane generation (>1.5 mg/L of dissolved methane) persisted for more than 100 days after the disappearance of ethanol, despite clean air-saturated water flowing continuously through the tank at a relative high seepage velocity (0.76 m/day). Quantitative real-time PCR showed that functional genes associated with methane metabolism (*mcrA* for methanogenesis and *pmoA* for methanotrophy) also persisted in the aquifer material. Persistent methanogenesis was apparently due to the anaerobic degradation of soil-bound organic carbon (e.g., biomass grown on ethanol and other substrates). Overall, this study reflects the complex plume dynamics following source removal, and suggests that monitoring for increases in the concentration of ethanol metabolites that impact groundwater quality should be considered.

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http://dx.doi.org/10.1016/j.watres.2014.10.023

1. Introduction

Fuel ethanol is increasingly being used as a blending agent for gasoline, which increases the likelihood of ethanol blend releases during fuel transportation and storage. A primary concern associated with ethanol-blend releases has been that the presence of ethanol may increase the persistence and region of influence (and thus the exposure risk) of co-occurring or pre-existing toxic aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylenes (BTEX) (Corseuil et al., 1998; Gomez et al., 2008; Mackay et al., 2006; Rasa et al., 2013; Zhang et al., 2006). However, as higher ethanol blends (e.g. E85) are introduced, there is growing interest in discerning potential impacts from ethanol degradation byproducts (Ma et al., 2013b).

Ethanol biodegradation rapidly depletes the available dissolved oxygen and other electron acceptors, creating an anaerobic/fermentative environment. Under these conditions, ethanol can be fermented to volatile fatty acids (e.g., acetic, propionic and butyric acids), butanol, methane, and carbon dioxide (Ma et al., 2013b; Powers et al., 2001). Some of these metabolites could impact public safety, groundwater quality or natural attenuation processes. For example, high generation of methane may pose an explosion hazard or enhance BTEX vapor intrusion (Freitas et al., 2010; Jewell and Wilson, 2011; Jourabchi et al., 2013; Ma et al., 2014, 2012; Sihota et al., 2013; Wilson et al., 2013). Butanol is a regulated compound in drinking water in several states in the U.S. (Nelson et al., 2010). The accumulation of acetate could hinder the thermodynamic feasibility of anaerobic benzene degradation, increasing the length of benzene plumes (Corseuil et al., 2011). Volatile fatty acids (particularly butyric acid) generate odor that could compromise groundwater aesthetic quality (Ma et al., 2011). The accumulation of volatile fatty acids may also decrease groundwater pH, possibly facilitating heavy metal dissolution into groundwater (Brown et al., 2010). Therefore, improved understanding of the generation and fate of ethanol metabolites is critical to enhance risk assessment of releases of current and future biofuel blends.

Whereas several studies have quantified the formation of ethanol metabolites following discrete or continuous release of ethanol blends (Capiro et al., 2008, 2007; Corseuil et al., 2011; Feris et al., 2008; Ma et al., 2011; Mackay et al., 2006; Nelson et al., 2010; Spalding et al., 2011), previous studies have overlooked how the system responds following source removal, which is usually the first step to remediate a contaminated site. This motivated us to investigate changes in the concentrations of ethanol and its anaerobic metabolites (i.e., acetate, propionate, butyrate, butanol, and methane) following the shut-off of a continuous release of an ethanol blend solution in a model aquifer system. To support data interpretation, quantitative real-time PCR (qPCR) and functional gene microarray (GeoChip) were used to assess changes in the abundance of selected functional genes associated with methanogenesis, methanotrophy, and extracellular polymeric substance (EPS) production.

2. Materials and methods

2.1. Pilot-scale aquifer system and release experiment

An 8 m^3 (3.7 m × 1.8 m × 1.2 m) pilot-scale continuous-flow tank packed with fine grain sand was used in this study (Fig. 1). Tap water was added 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 total aquifer thickness was 115 cm and the depth of the water table was 45 cm below the sand surface. Tap water amended with 10% (v/v) ethanol, 50 mg/L benzene, 50 mg/L toluene and 24,000 mg/L of sodium bromide (NaBr) was continuously injected into the channel at 22.5 cm below the water table at a rate of 0.4 L/day. NaBr was added as a conservative tracer, and to maintain a solution density to reach neutral buoyancy with the flowing groundwater (Ma et al., 2011). The added NaBr was diluted by the tank flow to less than 2000 mg/L (measured at groundwater sampling well B, Fig. 1), which is within the typical tolerance range of soil bacteria (Atlas and Bartha, 1997). NaBr tracer test showed that the groundwater travel time between the injection point and sampling port B was less than 1 day. Details on the tank construction and packing methods can be found in Ma et al., 2012 and Ma et al., 2011.

Figure S1 (in the supporting information) shows the timeline of the release experiment. The continuous release (flow rate 0.4 L/day) of the ethanol blend solution began on August 17th 2009 and lasted for 2 years. On September 5th 2011, ethanol was removed from the blend solution and the solution containing 50 mg/L benzene and 50 mg/L toluene continued to be released at the same flow rate (0.4 L/day) for 8 months. This stage, which mimics the earlier removal of ethanol than hydrocarbons (Corseuil et al., 2011), investigated how the disappearance of ethanol affects the fate of benzene and toluene. Note that the added benzene and toluene was diluted by the tank inflow to less than 0.1 mg/L (measured at well B). Thus, the presence of benzene and toluene in groundwater

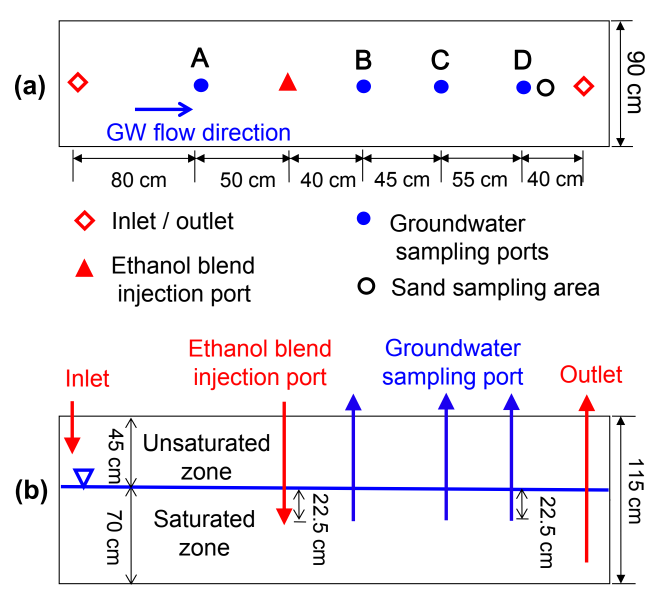

Fig. 1 – Plan view (a) and profile view (b) of the aquifer tank.