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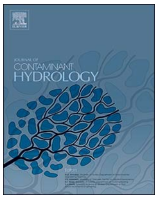

Anaerobic biodegradation of dissolved ethanol in a pilot-scale sand aquifer: Variability in plume (redox) biogeochemistry

Heather C. McLeod[a], James W. Roy[a,b,*], Gregory F. Slater[a], James E. Smith[a]

[a] *School of Geography and Earth Sciences, McMaster University, Hamilton, Ontario, Canada*
[b] *Water Science and Technology Directorate, Environment and Climate Change Canada, Burlington, Ontario, Canada*

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ABSTRACT

The use of ethanol in alternative fuels has led to contamination of groundwater with high concentrations of this easily biodegradable organic compound. Previous laboratory and field studies have shown vigorous biodegradation of ethanol plumes, with prevalence of reducing conditions and methanogenesis. The objective of this study was to further our understanding of the dynamic biogeochemistry processes, especially dissolved gas production, that may occur in developing and aging plume cores at sites with ethanol or other organic contamination of groundwater. The experiment performed involved highly-detailed spatial and temporal monitoring of ethanol biodegradation in a 2-dimensional (175 cm high × 525 cm long) sand aquifer tank for 330 days, with a vertical shift in plume position and increased nutrient inputs occurring at ~Day 100. Rapid onset of fermentation, denitrification, sulphate-reduction and iron(III)-reduction occurred following dissolved ethanol addition, with the eventual widespread development of methanogenesis. The detailed observations also demonstrate a redox zonation that supports the plume fringe concept, secondary reactions resulting from a changing/moving plume, and time lags for the various biodegradation processes. Additional highlights include: i) the highest dissolved H_2 concentrations yet reported for groundwater, possibly linked to vigorous fermentation in the absence of common terminal electron-acceptors (i.e., dissolved oxygen, nitrate, and sulphate, and iron(III)-minerals) and methanogenesis; ii) evidence of phosphorus nutrient limitation, which stalled ethanol biodegradation and perhaps delayed the onset of methanogenesis; and iii) the occurrence of dissimilatory nitrate reduction to ammonium, which has not been reported for ethanol biodegradation to date.

1. Introduction

Petroleum fuel spills represent a long-term supply of toxic organic contaminants to groundwater resources (Wiedemeier et al., 1999), raising public concerns over drinking water supplies and the aquatic ecosystem health of receiving surface waters. The addition of ethanol to gasoline to serve as a fuel oxygenate (typical blends > 10% ethanol to gasoline by volume) has become common practice in North and South America (Powers et al., 2001), in part because ethanol is a non-toxic, renewable biomass-based resource. Unfortunately, with the widespread adoption of these alternative fuels, numerous inadvertent releases of both denatured ethanol (95% ethanol by volume; e.g., Spalding et al., 2011) and blended fuels (as noted by Corseuil et al., 1998) have occurred during their production, handling and storage. The concern for ethanol contamination of groundwater from such releases stems primarily from secondary impacts related to its biodegradation in the subsurface. For instance, preferential biodegradation of ethanol may limit the degradation of more toxic petroleum compounds, such as benzene, while also boosting their solubility in groundwater via cosolvency (e.g., Corseuil et al., 1998; Mackay et al., 2006). Groundwater geochemistry changes associated with ethanol biodegradation may also cause the release of sediment-associated compounds, such as arsenic and various metals (e.g., Ziegler et al., 2015), that may then impair groundwater quality. Finally, explosion hazards from methane produced by the intensive biodegradation of ethanol are also a public safety concern (e.g., Spalding et al., 2011; Ma et al., 2012).

Natural attenuation (including biodegradation) of organic contaminant plumes in groundwater has been abundantly studied over the past several decades, from landfill leachate plumes (e.g., Baedecker and Back, 1979; Cherry et al., 1979; Lyngkilde and Christensen, 1992; van Breukelen and Griffioen, 2004) to petroleum hydrocarbons (e.g., Baedecker et al., 1993; Cozzarelli et al., 1999; Anneser et al., 2008) and chlorinated solvents (e.g., Lendvay et al., 1998; Chapelle and Bradley, 1998). This has contributed to the development of the redox zonation concept (as described by Christensen et al., 2000) and more recently the plume fringe concept (as described by Meckenstock et al., 2015), and

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[*] Corresponding author at: Environment and Climate Change Canada, 867 Lakeshore Rd, L7S1A1 Burlington, ON, Canada.
E-mail address: jim.roy@canada.ca (J.W. Roy).

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brought about recognition of the complexity of mass transfer and microbial consortia relationships occurring at the sub-plume scale, especially in mixing zones (Meckenstock et al., 2015). However, there still remains a generally poor understanding of the internal functioning of anaerobic plume cores, as well as the dynamics in attenuation processes caused by changing flow and groundwater chemistry conditions (Meckenstock et al., 2015). Ethanol, as a readily biodegradable compound available at high dissolved concentrations in affected groundwater (Powers et al., 2001), may result in more vigorous natural attenuation. This may lead to substantially more biogenic gas production in the plume core and the possibility of differences in the spatial-temporal biogeochemistry (redox) patterns than occurs for plumes of petroleum hydrocarbons or other organic compounds. Indeed, numerous studies have shown the prevalence of reducing conditions and methanogenesis, with accumulations of acetate and other volatile fatty acids (VFA), driven by high ethanol content from groundwater contaminated by denatured ethanol or blended fuels in lab columns (e.g., Corseuil et al., 1998; Nelson et al., 2010) and pilot-scale laboratory tank experiments (Capiro et al., 2007; Ma et al., 2011), and at spill sites (Spalding et al., 2011) and experimental-release field sites (Mackay et al., 2006; Corseuil et al., 2011; Freitas and Barker, 2013). Methane transfer to the vadose zone above a plume has also been reported (e.g., Rasa et al., 2013; Ma et al., 2012; Sihota et al., 2013). However, the associated dissolved and trapped gas behavior within the plume has not received much attention thus far.

In this study, the biodegradation processes that may develop in an anaerobic unconfined sand aquifer following groundwater contamination with dissolved ethanol were simulated at a field representative-scale. The experiment was implemented in a highly-instrumented, two-dimensional (175 cm high × 525 cm long), pilot-scale laboratory tank filled with uniform sand. Dissolved ethanol, along with low concentrations of two petroleum compounds (methyl tert-butyl ether (MTBE) and toluene), was continuously injected into the synthetic aquifer for 330 days. Temporal and spatial monitoring within the tank comprised aqueous geochemistry (including dissolved gases) and trapped gas contents, and was more detailed than previously performed to date (and as called for by Meckenstock et al., 2015). The resulting conditions were representative of a developing and aging plume core with substantial bioavailable ethanol mass. In addition, several changes occurred in the midst of the experiment. First, the nutrient load to the groundwater, affecting inflowing nitrate and phosphate concentrations, was increased on Day 93. And second, an unintentional occurrence between Days 126 and 147 was a shift in the vertical positioning of the ethanol input. Thus, this study may also provide broader insights into the dynamics of natural attenuation mechanisms subject to changes in groundwater flow and background biogeochemistry, which will be relevant to plumes of other organic contaminants.

In this manuscript, the focus is on the spatial and temporal patterns in redox geochemistry that developed from the ethanol biodegradation processes; the fate of the two petroleum compounds will not be discussed in detail here. Continual monitoring of dissolved gas pressure changes during this experiment have been reported already (Roy et al., 2017), while the resultant biogenic gas production and trapped gas dynamics will be addressed in a subsequent manuscript.

2. Methods

The pilot-scale synthetic aquifer consisted of 36.5 metric tonnes of M49 medium silica sand (Opta Minerals) in a stainless steel tank (600 cm long × 240 cm wide × 180 cm deep; Fig. 1 and Fig. S1) located at the Canada Centre for Inland Waters in Burlington, ON, Canada. The sand was packed damp in 5-cm lifts, achieving an overall average porosity of ~38%. The tank was divided lengthwise into three separate lanes, each 80 cm wide with an open reservoir or head tank (*HT*, 80 cm wide × 75 cm long) located at one end and separated from the sand-packed aquifer by a steel perforated plate affixed with geotextile (Nilex). Only one lane was used in this study (Lane 3; Fig. S1A), which was packed to be homogeneous. Instrumentation and wells installed during packing are described below. The tank was wetted using de-chlorinated tap water inoculated with a microbial community from a field site with previous ethanol contamination. This entrapped a residual gas phase, which was partly dissolved in the experiment of McLeod et al. (2015). Final position of the water table was established ~5 cm below the sand surface, with the capillary fringe extending to the sand surface. Further details on sand properties and packing, and inoculation procedures are provided in Supporting information.

Simulated groundwater for this experiment was composed of de-chlorinated tap water amended with NH_4NO_3 and K_2HPO_4 (for nutrient supply) at 310 μM and 70 μM, respectively, up to Day 93, after which these concentrations were doubled. It was gravity fed into the *HT*, which was equipped with a brass float valve to maintain the up-gradient constant head boundary condition. Groundwater was removed at the end of the lane, 520 cm from the *HT* (Fig. 1 and Fig. S1), from one fully-screened, 4-inch PVC "withdrawal well" (*WW*). A constant withdrawal pump rate was applied to maintain an average linear groundwater velocity of ~21 cm/day, which corresponds to a pore volume (*PV*; sand-filled section = 2770 L) passed every 25.4 days, approximately.

An ethanol mixture stock solution was prepared in 23.0 L glass bottles every second day using the simulated groundwater and high purity (> 99%) chemical grades of ethanol (Commercial Alcohols), methyl tert-butyl ether (MTBE; Sigma Aldrich) and toluene (Fisher Chemical), at concentrations of 89,900, 2350 and 2250 μM, respectively. Ethanol was added as the primary organic substrate, whereas MTBE and toluene were added at significantly lower concentrations. The dissolved plume was created by adding the ethanol mixture to three fully-screened 4-inch diameter PVC "injection wells" (*IWs*) installed as a fence across the lane, 30 cm down-gradient of the *HT* (Fig. 1 and Fig. S1). To start the experiment, each *IW* was spiked with a single concentrated dose of the ethanol mixture and an inorganic tracer (KBr), to produce a step-wise, rather than gradual, increase in concentrations. Subsequently, the ethanol mixture was added to each *IW* at a rate of 2.3 ± 0.3 mL/min and was thoroughly mixed within the well via continuous and rapid internal circulation. Note that the ethanol concentration in the *IW* was always < 0.6% by volume (4730 mg/L), thus avoiding any drastic changes in the viscosity, density or interfacial tension of the aqueous phase (Antonella and Leij, 2012). The ethanol mixture was added to the tank from August 17, 2012 (Day 0, $PV = 0.0$) to July 12, 2013 (Day 330; $PV = 13.0$). There was a small accidental spill of the ethanol mixture into the *HT* on Day 103. An experiment timeline is shown in Fig. 2.

Thirty in situ groundwater samplers, each consisting of a 3-inch stainless steel, 40-μm porous cup (Chand Eisenmann Metallurgical), were embedded horizontally in the sand within the middle of the lane during packing. Each was connected to stainless steel tubing that extended to surface along the tank wall and had a dedicated 30-mL HDPE syringe for purging and sampling. These samplers were distributed at six vertical depths (15, 45, 75, 105, 135 and 165 cm) below the sand surface and along five sampling arrays located 70, 155, 205, 320 and 445 cm from the *HT* (0 cm; Fig. 1). Groundwater samples were collected on July 11, 2012 (Day −37, $PV = -1.5$) to characterize the initial background conditions prior to ethanol addition, and then monthly to bimonthly throughout the experiment. These were analyzed for dissolved ethanol, ammonium, anions (including nitrate and phosphate), major cations (including iron and manganese), and volatile fatty acids (*VFAs*, semi-quantitative results only) at the Canada Centre for Inland Waters. Details regarding sample preservation, storage and analytical methods (including detection limits), are provided in the Supporting information (Table S2). Spatial interpolations of geochemical data between sampling locations was performed by a linear triangulation grid method, which honors the data exactly, using Surfer Software (Golden Software Inc.).