WATER RESEARCH 68 (2015) 479–486

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

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

Jie Ma [a,1], *William G. Rixey* [b], *Pedro J.J. Alvarez* [a,*]

[a] *Department of Civil and Environmental Engineering, Rice University, 6100 Main St., Houston, TX 77005, USA*
[b] *Department of Civil and Environmental Engineering, University of Houston, 4800 Calhoun St., Houston, TX 77204-4003, USA*

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.

* *Corresponding author*. Tel.: +1713 348 5903; fax: +1713 348 5203.
E-mail address: alvarez@rice.edu (P.J.J. Alvarez).
[1] Current address: State Key Laboratory of Heavy Oil Processing, Beijing Key Lab Oil & Gas Pollution Control, China University of Petroleum-Beijing, 18 Fuxue Rd., Beijing 102249, China.
http://dx.doi.org/10.1016/j.watres.2014.10.023
0043-1354/

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

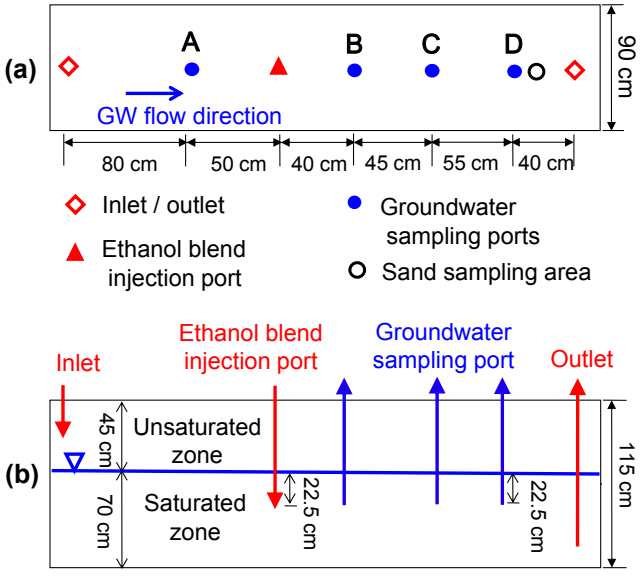

Fig. 1 – Plan view (a) and profile view (b) of the aquifer tank.

did not significantly affect microbial fermentation and methanogenic activities. On May 4th 2012, the release of benzene and toluene mixture was shut off. For this experiment, groundwater sampling began on September 5th 2011 and continued until neither ethanol nor its metabolites (volatile fatty acids, butanol, and methane) were detected.

2.2. Chemical analysis

Four replicate groundwater samples were collected from each sampling well A, B, C and D, which were at the same depth as the ethanol/benzene/toluene injection port (22.5 cm below the water table, Fig. 1b). Ethanol, methane, acetate, propionate, butyrate, and butanol were measured by GC-FID (Agilent Technologies Inc., Santa Clara, CA). The detection limits were 1 mg/L for ethanol, acetate and propionate, 2 mg/L for butyrate and butanol, and 0.1 mg/L for methane. Details on groundwater sampling and chemical measurement can be found in Ma et al. (2011).

2.3. Microcosm experiment

A series of microcosm experiments containing different concentrations of ethanol was conducted to assess potential inhibitory effects of high ethanol levels and to characterize the distribution of degradation products at different ethanol concentrations. To set up the microcosm, ~1.3 L tank water was collected from the sampling well D and ~800 g sand was collected from a depth of 50–75 cm below ground surface near well D in October of 2012 (Fig. 1). Microcosms were set up in an anaerobic chamber. The sand was homogenized before use. Fifteen grams of sand were mixed with 30 mL tank water and various concentrations of ethanol (0, 500, 1,000, 1,500, 2,000, 3,400, 10,000, 40,000, 80,000 mg/L). The mixture was placed in sterile 125-mL serum bottles and sealed with gas-tight butyl rubber stoppers and aluminum crimp caps. Four replicate microcosms were prepared for each ethanol concentration. The microcosms were incubated at room temperature (24 °C) for 133 days. Methane concentrations in the headspace were monitored using GC-FID. On the last day of the microcosm experiment (day 133), water samples in the microcosm were collected to measure dissolved concentrations of acetate, propionate, butyrate, and butanol.

2.4. Sand sample collection and qPCR analysis

For qPCR analysis, sand samples were collected in four replicates from a depth of 50–75 cm below ground surface near the groundwater sampling well D (Fig. 1) after ethanol was removed from the release solution (September 5th 2011, Fig. S1). Details on sand sampling can be found in Ma et al., 2013a. DNA was extracted from 0.25 g of sand using a PowerSoil DNA Isolation Kit (MOBIO, Carlsbad, CA, US). Absolute quantification was used to enumerate the gene copy number for two functional genes associated with methane metabolism: 1) methyl coenzyme-M reductase gene (*mcrA*) for methanogens and 2) methane monooxygenase gene (*pmoA*) for methanotrophs. The target genes, primer sequences, and DNA standards for calibration are given in Table S1 of the supporting information. The practical quantification limits for

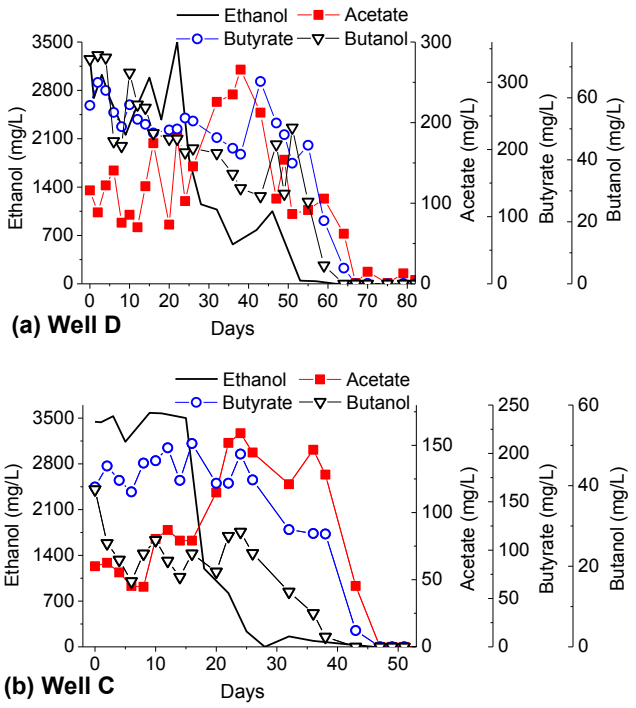

Fig. 2 – Changes in the concentrations of ethanol and its degradation byproducts at groundwater sampling well D (a) and C (b) after the ethanol release was shut off (t = 0 day).

mcrA gene and *pmoA* gene were 1000 copies/g dry sand. Melting curve analysis was conducted after the thermal cycle was completed to ensure that no nonspecific PCR products were generated. Details on qPCR method can be found in the supporting information.

2.5. Sand-associated organic carbon

Sand samples were collected in five replicates from a depth of 50–75 cm below ground surface near the groundwater sampling well D on three different dates: 1) August 7th 2009 (pre-contaminated baseline samples); 2) September 5th 2011 (samples exposed to ethanol blend release for 2 years) and 3) September 2nd 2012 (one year after ethanol was removed from the release solution). Total organic carbon content of sand samples was measured using an Elementar VarioMax CN analyzer (Elementar Analyses system GmbH, Hanau, Germany) with the primary furnace temperature set at 650 °C at Soil, Water and Forage Testing Laboratory of Texas A&M University. The detection limit for total organic carbon was 0.002%.

2.6. GeoChip analysis

GeoChip was used to characterize functional gene structure of sand collected on August 7th 2009, September 5th 2011 and September 2nd 2012 (same samples used for organic carbon measurement, Fig. S1). Triplicate samples at each time point were used for GeoChip analysis. DNA was extracted from 10 g sand using a modified PowerMax Soil DNA isolation kit (MOBIO, Carlsbad, CA, US) with several modifications (see

supporting information) to increase DNA yield. Details on the DNA extraction method, GeoChip experiment and GeoChip data processing can be found in the supporting information.

3. Results and discussion

3.1. *Faster ethanol degradation following source removal*

After the ethanol-blended fuel release was shut off in the model aquifer system, dissolved ethanol concentrations gradually decreased to zero within 60 days at sampling wells C and D (Fig. 2). Unlike ethanol, the concentration of acetate at well D first increased 2–2.5 fold and then decreased below detection (<1 mg/L) within 90 days (Fig. 2a). Similar to acetate, butyrate and butanol concentrations at well D also temporarily increased and their peak concentrations (301 and 45 mg/L, respectively) appeared following the acetate peak concentration (Fig. 2a). Under methanogenic conditions, ethanol is first metabolized to acetate and hydrogen, which can then be transformed to methane and carbon dioxide (ITRC, 2011; Ma et al., 2013b). Acetate can also be transformed to butyrate and butanol (ITRC, 2011). The increases in acetate, butyrate, and butanol concentrations corroborate that the fermentative biodegradation of ethanol was temporarily stimulated following the shut-off of the ethanol release. The transient accumulation of acetate was also observed at well C, but there was no obvious increase in butyrate and butanol concentrations following the increase in acetate concentration (Fig. 2b). Well C is closer to the source zone (ethanol/benzene/toluene injection port) (Fig. 1) and the travel time was apparently insufficient to observe this progression of microbial transformation processes.

3.2. *Decreasing the source-zone concentration of ethanol enhances fermentation activities*

A microcosm experiment confirmed that faster ethanol degradation following source removal was attributed to a decrease in the dissolved ethanol concentration below its toxicity threshold (~2000 mg/L for this system). A series of microcosms with different initial ethanol concentrations was prepared to assess changes in degradation byproducts distribution and potential inhibitory effects at high concentrations. Four parameters were inferred from the microcosm experiment: 1) methane accumulation after 133 days of incubation (Fig. 3a), 2) the acclimation time for methane generation (Fig. 3b),) methane generation rate (Fig. 4), and 4) total dissolved concentrations of ethanol metabolites after 133 days of incubation (Fig. 5). Microcosms with 1000–2000 mg/L of ethanol exhibited the highest ethanol biodegradation activity. Higher initial ethanol concentrations (>3400 mg/L) resulted in lower metabolite accumulation (Figs. 3a and 5) and slower methane generation rate (Fig. 4). When ethanol concentrations reached 40,000 mg/L, the generation of volatile fatty

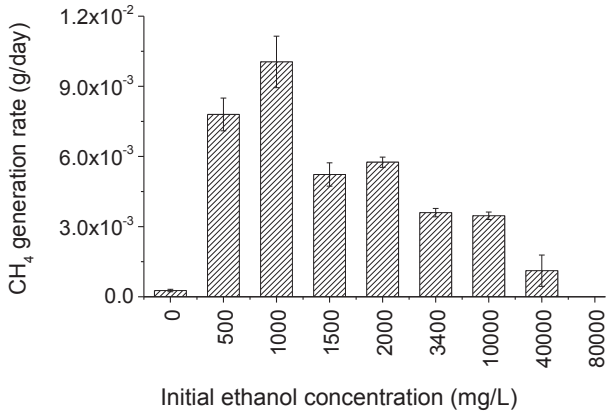

Fig. 4 – Methane generation rates in the microcosm experiments. The time ranges that were used to estimate methane generation rate were: day 5–20 for 0 mg/L, day 5–15 for 500 mg/L, day 5–20 for 1000 mg/L, day 8–50 for 1500 mg/L, day 8–60 for 2000 mg/L, day 8–60 for 3400 mg/L, day 8–40 for 10,000 mg/L, day 60–133 for 40,000 mg/L, and day 0–133 for 80,000 mg/L. The regression fitting plots can be found in the supporting information (Figure S2–S8).

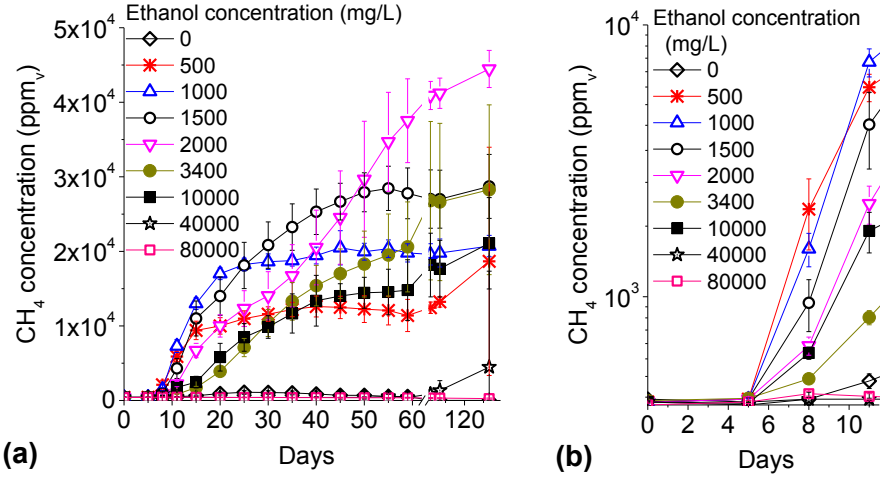

Fig. 3 – Methane accumulations in the headspace of microcosms. (a) is the complete incubation period (133 days) and (b) is the first 12 days (semi-log scale). Curves represent different microcosms with different initial ethanol concentrations.

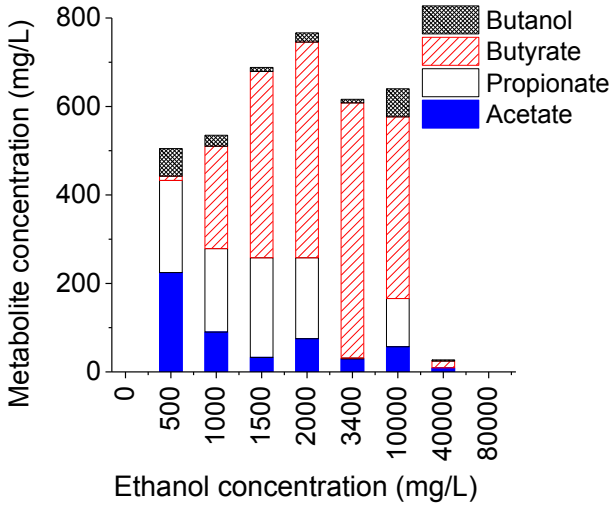

Fig. 5 – Dissolved metabolite concentrations in microcosms after 133 days of incubation. The microcosm containing 2000 mg/L ethanol has the highest total concentration of dissolved ethanol degradation byproducts.

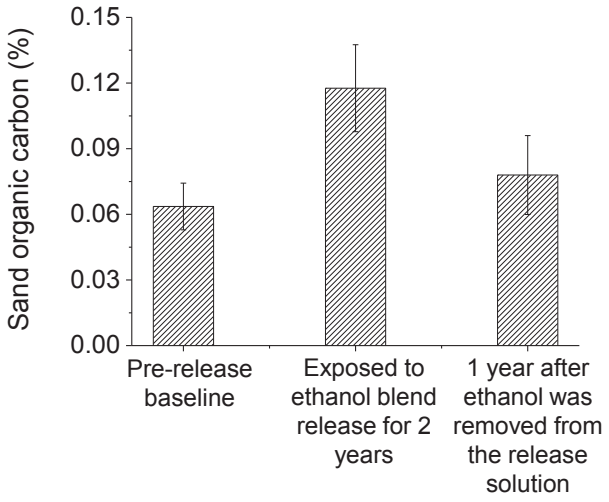

Fig. 6 – Sand-associated total organic carbon content from a depth of 50–75 cm below ground surface near the groundwater sampling well D. All three time points (August 7th 2009, September 5th 2011 and September 2nd 2012) were at the transition between summer and fall ($T = 27.9–29.1\ ^{\circ}C$).

acids, methane, and butanol were strongly inhibited. Fig. 2b also shows that the microcosm containing higher initial ethanol concentrations experienced longer acclimation time.

These results indicate that 40,000 mg/L (5.1% v:v) of ethanol could strongly inhibit microbial activity, which is consistent with the ethanol toxicity threshold reported by Igram and Vreeland (3.1% v:v) and Nelson et al. (6% v:v) (Ingram and Vreeland, 1980; Nelson et al., 2010). However, such a high concentration (>1% v:v) would likely occur for only high ethanol content fuels and would be confined to the capillary fringe near the source zone where a non-aqueous phase liquid (NAPL) phase exists (Freitas and Barker, 2011, 2013b; Stafford et al., 2009; Stafford and Rixey, 2011). Concentrations of dissolved ethanol in groundwater impacted by ethanol-blended fuels are usually lower than 10,000 mg/L (Corseuil et al., 2011; da Silva and Corseuil, 2012; Freitas and Barker, 2013a; Freitas et al., 2011; Spalding et al., 2011). Since the active zone for biodegradation is usually located within the dissolved plume (especially at the plume edge) rather than in the NAPL phase source zone, information on how ethanol concentrations lower than 10,000 mg/L affect microbial activities is of more practical significance to assess the environmental behavior of ethanol-blended fuel releases. Microcosm results showed that 3400 to 10,000 mg/L of ethanol exert partial inhibitory effects on ethanol biodegradation (for this system), corroborating previous studies with a wide variety of microbial cultures (Bringmann and Kühn, 1980; Eaton et al., 1982; Heipieper and Debont, 1994).

3.3. *Ethanol stimulated EPS production and increased sand-associated organic carbon content*

The release stimulated extracellular polymeric substance (EPS) production and increased the organic carbon content in the impacted aquifer materials. Fig. 6 shows the total organic carbon content of the sand samples collected on three different dates. The sand used in this study had relatively low level of organic carbon content (0.066 ± 0.011% on August 7th 2009), which increased significantly ($p < 0.05$) by 85% following exposure to the ethanol blend release for 2 years (September 5th 2011). A sand sample collected 1 year after the shut-off of ethanol release (September 2nd 2012) had 34% significantly ($p < 0.05$) lower organic carbon content than the sample collected on September 5th 2011, indicating a net consumption of sand organic carbon after the shut-off of ethanol release.

In addition to organic carbon content data, GeoChip analysis (a comprehensive functional gene microarray (He et al., 2007)) shows that, compared to the pre-contaminated sand sample (August 7th 2009), several functional genes involved in extracellular polymeric substance (EPS) production were significantly ($p < 0.05$) enriched in the sand sample exposed to ethanol blend release for 2 years (September 5th 2011, Fig. 7). The enriched EPS production genes included gene encoding for mannose-1-phosphate guanylyltransferase, LPS heptosyltransferase, NAD dependent epimerase dehydratase family protein, UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase, capsular polysaccharide biosynthesis protein, glycosyltransferase, tyrosine protein kinase. This corroborates that fuel ethanol releases could stimulate EPS production, thus increasing the sand organic carbon content.

3.4. *Persistent methane accumulation may be due to anaerobic degradation of remaining acetate and bound organic matter*

Methane persisted in the aquifer tank even several months after the disappearance of ethanol and its metabolites (i.e., acetate, butyrate and butanol). At well C, ethanol and its metabolites disappeared after day 47, while methane persisted in

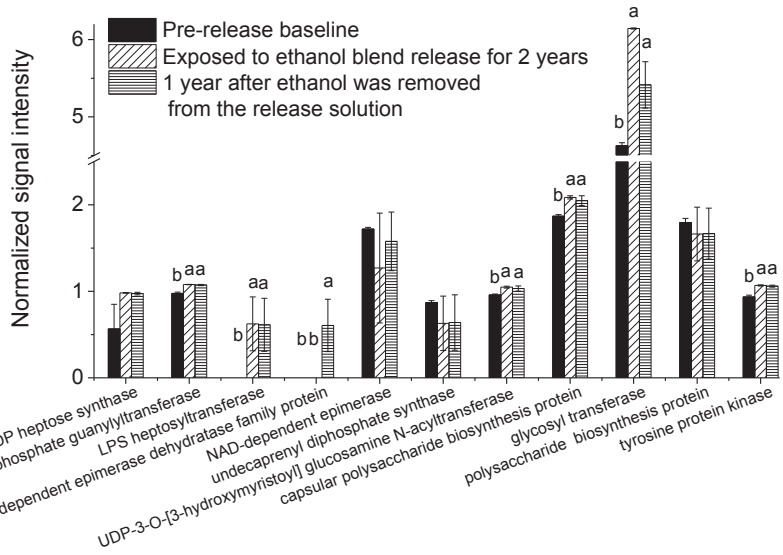

Fig. 7 – Normalized signal intensity of detected functional genes for extracellular polymeric substance (EPS) production. The data normalization method is described in the supporting information. Gene number is the protein ID number for each gene as listed in the GenBank database. Different letters indicate statistical differences at a *p* value of <0.05 among treatments by Fisher's least-significant-difference (LSD) test. No letter label was used for ADP heptose synthase, NAD-dependent epimerase, undecaprenyl diphosphate synthase, and polysaccharide biosysthesis proteins, because there was no statistical difference ($p > 0.05$) for these genes.

the groundwater until day 131 (Figure S9a). Similarly, at the well D, ethanol and its metabolites disappeared after day 89, while methane persisted until day 212 (Figure S9b). The persistence of dissolved methane was also reported in several field studies. At a site impacted by an accidental fuel ethanol spill, the ethanol concentration decreased from ~20,000 mg/L to below detection limit (0.1 mg/L) within the first 3 years, but 20 mg/L of dissolved methane was still detected even 6 years after the spill (Sihota et al., 2013; Spalding et al., 2011). At an experimental site impacted by a pulse injection of Brazilian gasoline which contains 24% (v:v) of ethanol, oversaturated dissolved methane (>24 mg/L) was continuously detected in the groundwater even 6.5 years after the spill, while ethanol disappeared 4 years after the spill (Corseuil et al., 2011). Based on these field studies and our pilot-scale experiment, it appears that methane persistence is likely to be common at sites impacted by ethanol blend fuel releases.

In addition to methane accumulation, functional genes associated with methane metabolism also persisted in the model aquifer materials. Two functional genes were analyzed: 1) methyl coenzyme-M reductase gene (*mcrA*) for methanogens and 2) methane monooxygenase gene (*pmoA*) for methanotrophs (Fig. 8). Ethanol releases stimulated strong methanogenic activity. Accordingly, the abundance of *mcrA* gene increases for four orders of magnitude from 1.0×10^3 copy/g dry sand (pre-contamination baseline, August 7th, 2009) to 6.4×10^6 copy/g dry sand (exposed to ethanol blended solution for 2 years, September 5th, 2011). However, even one year after the ethanol solution was shut off, a high abundance of *mcrA* gene (~10^6 gene copy/g dry sand) still existed in the aquifer, thus indicating the persistence of methanogens in this system. The *pmoA* gene showed a similar temporal trend as the *mcrA* gene.

Although this and several other studies corroborate that methane persistence may be a common phenomenon at fuel ethanol impacted sites, previous studies did not clarify why it happens. In this experiment, tap water was continuously injected into and flowed out of the tank, with an average seepage velocity of 0.76 m/day. Since methane is unlikely to be adsorbed and retained by aquifer materials, methane accumulating post shut off must have been generated from anaerobic degradation of other organic compounds. Figure S9 shows that ethanol metabolites (acetate, butyrate, and butanol) remained in the groundwater for a longer time than

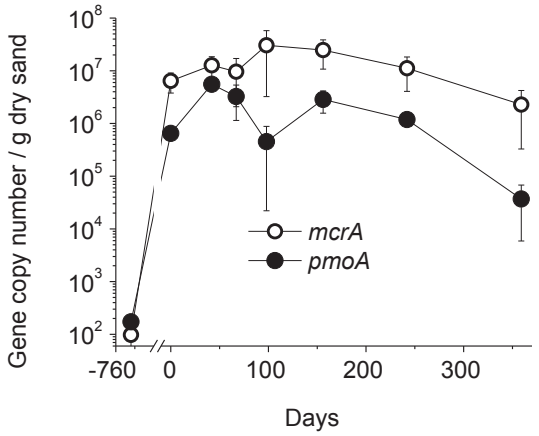

Fig. 8 – Changes in the copy numbers of methane cycling functional genes following the shut off of the ethanol release ($t = 0$ day). *mrcA* codes for methyl coenzyme-M reductase gene in methanogens and *pmoA* codes for methane monooxygenase in aerobic methanotrophs.

ethanol after the ethanol solution was shut off. Therefore, it is very likely that ethanol metabolites (especially acetate) contributed to the persistence of dissolved methane.

Interestingly, methane generation persisted for more than 80 days after the disappearance of dissolved acetate, butyrate, and butanol (Figure S9). Thus, there must have been an alternative carbon source to support the activity of methanogens. A potential alternative carbon source is organic carbon associated with the aquifer materials. Both GeoChip data and the sand organic carbon content data show that the ethanol blend release stimulated the growth of microorganisms that attach to aquifer material, excrete EPS and accumulate intracellular carbon reserves, which would increase the organic carbon content associated with the impacted aquifer materials (Figs. 6 and 7). Therefore, it can be inferred that the persistent generation of methane was supported by organic carbon bound to the aquifer material. In this study, the aquifer tank was packed with sand which has low sorption capacity for organic carbon such as EPS. Natural aquifers containing clay and silt lenses are likely to have much higher sorption capacity for organic carbon and thus may have even longer methane persistence time. Nevertheless, we cannot rule out the possibility that some ethanol trapped in the capillary fringe (and not detected in the groundwater) also contribute to methanogenesis.

4. Conclusions

Recently, there have been increasing concerns over the methane vapor intrusion risk associated with ethanol blend fuel releases (Freitas et al., 2010; Jewell and Wilson, 2011; Jourabchi et al., 2013; Ma et al., 2014, 2012; Sihota et al., 2013). This study shows that methane generation in groundwater impacted by ethanol blends would continue well beyond the disappearance of ethanol and its degradation products (e.g., acetate, butyrate, and butanol). Note that the groundwater velocity (0.76 m/day) in this sandy aquifer is relative fast compared to real aquifers. Therefore, at real contaminated sites with slower groundwater flow, methane persistence time could be much longer. These results suggest that, for site closure, long-term monitoring for methane should be considered to fully understand the vapor intrusion risk from methane, even when the ethanol source is removed and residual ethanol was attenuated.

Besides methane, other ethanol metabolites (e.g., acetate, butyrate, and butanol) may also impact groundwater quality (e.g., inhibit benzene biodegradation (Corseuil et al., 2011), cause aesthetic impacts (Ma et al., 2011), and facilitate heavy metal dissolution (Brown et al., 2010)). This study shows that after the ethanol source was removed, ethanol anaerobic degradation activities may be temporarily stimulated when the ethanol concentration decreases below its toxicity threshold (e.g., ~2000 mg/L for this system), thus resulting in transient accumulation of problematic ethanol degradation metabolites. Overall, this study reflects the complex plume dynamics following source removal, and suggests that long-term monitoring for the problematic ethanol metabolites that impact groundwater quality (beyond the disappearance of ethanol) should be considered.

Acknowledgments

This work was funded by the American Petroleum Institute (2012-106835). Jie Ma also received partial support from a scholarship from the China Scholarship Council.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.10.023.

REFERENCES

Atlas, R.M., Bartha, R., 1997. Microbial Ecology: Fundamentals and Applications. Benjamin Cummings, Redwood City, CA.

Bringmann, G., Kühn, R., 1980. Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Water Res. 14 (3), 231–241.

Brown, R.A., Zimmerman, M.D., Ririe, G.T., 2010. Attenuation of Naturally Occurring Arsenic at Petroleum Hydrocarbon–impacted Sites. Monterey, California.

Capiro, N.L., Da Silva, M.L.B., Stafford, B.P., Rixey, W.G., Alvarez, P.J.J., 2008. Microbial community response to a release of neat ethanol onto residual hydrocarbons in a pilot-scale aquifer tank. Environ. Microbiol. 10 (9), 2236–2244.

Capiro, N.L., Stafford, B.P., Rixey, W.G., Bedient, P.B., Alvarez, P.J.J., 2007. Fuel-grade ethanol transport and impacts to groundwater in a pilot-scale aquifer tank. Water Res. 41 (3), 656–664.

Corseuil, H.X., Hunt, C.S., Dos Santos, R.C.F., Alvarez, P.J.J., 1998. The influence of the gasoline oxygenate ethanol on aerobic and anaerobic BTX biodegradation. Water Res. 32 (7), 2065–2072.

Corseuil, H.X., Monier, A.L., Fernandes, M., Schneider, M.R., Nunes, C.C., do Rosario, M., Alvarez, P.J.J., 2011. BTEX plume dynamics following an ethanol blend release: geochemical footprint and thermodynamic constraints on natural attenuation. Environ. Sci. Technol. 45 (8), 3422–3429.

da Silva, M.L.B., Corseuil, H.X., 2012. Groundwater microbial analysis to assess enhanced BTEX biodegradation by nitrate injection at a gasohol-contaminated site. Int. Biodeterior. Biodegrad. 67, 21–27.

Eaton, L.C., Tedder, T.F., Ingram, L.O., 1982. Effects of fatty acid composition on the sensitivity of membrane functions to ethanol in Escherichia coli. Subst. Alcohol Actions/Misuse 3 (1–2), 77–87.

Feris, K., Mackay, D., de Sieyes, N., Chakraborty, I., Einarson, M., Hristova, K., Scow, K., 2008. Effect of ethanol on microbial community structure and function during natural attenuation of benzene, toluene, and o-xylene in a sulfate-reducing aquifer. Environ. Sci. Technol. 42 (7), 2289–2294.

Freitas, J.G., Barker, J.F., 2011. Oxygenated gasoline release in the unsaturated zone - Part 1: source zone behavior. J. Contam. Hydrol. 126 (3–4), 153–166.

Freitas, J.G., Barker, J.F., 2013a. Denatured ethanol release into gasoline residuals, Part 2: fate and transport. J. Contam. Hydrol. 148, 79–91.

Freitas, J.G., Barker, J.F., 2013b. Denatured ethanol, release into gasoline residuals, Part 1: source behaviour. J. Contam. Hydrol. 148, 67–78.

Freitas, J.G., Doulatyari, B., Molson, J.W., Barker, J.F., 2011. Oxygenated gasoline release in the unsaturated zone, Part 2:

Downgradient transport of ethanol and hydrocarbons. J. Contam. Hydrol. 125 (1–4), 70–85.
Freitas, J.G., Fletcher, B., Aravena, R., Barker, J.F., 2010. Methane production and isotopic fingerprinting in ethanol fuel contaminated sites. Ground Water 48 (6), 844–857.
Gomez, D.E., de Blanc, P.C., Rixey, W.G., Bedient, P.B., Alvarez, P.J.J., 2008. Modeling benzene plume elongation mechanisms exerted by ethanol using RT3D with a general substrate interaction module. Water Resour. Res. 44 (5), W05405.
He, Z.L., Gentry, T.J., Schadt, C.W., Wu, L.Y., Liebich, J., Chong, S.C., Huang, Z.J., Wu, W.M., Gu, B.H., Jardine, P., Criddle, C., Zhou, J., 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. Isme J. 1 (1), 67–77.
Heipieper, H.J., Debont, J.A.M., 1994. Adaptation of Pseudomonas putida S12 to ethanol and toluene at the level of fatty acid composition of membranes. Appl. Environ. Microbiol. 60 (12), 4440–4444.
Ingram, L.O., Vreeland, N.S., 1980. Differential effects of ethanol and hexanol on the Escherichia coli cell envelope. J. Bacteriol. 144 (2), 481–488.
ITRC, 2011. Biofuels: Release Prevention, Environmental Behavior, and Remediation. Interstate Technology & Regulatory Council, Biofuel Team, Washington, DC, USA.
Jewell, K.P., Wilson, J.T., 2011. A new screening method for methane in soil gas using existing groundwater monitoring wells. Ground Water Monit. Remediat. 31 (3), 82–94.
Jourabchi, P., Hers, I., Mayer, K.U., DeVaull, G.E., Kolhatkar, R.V., Bauman, B., 2013. Numerical modeling study of the influence of methane generation from ethanol-gasoline blends on vapor intrusion. In: The 2nd International Symposium on Bioremediation and Sustainable Environmental Technologies. Jacksonville, FL, US.
Ma, J., Luo, H., DeVaull, G.E., Rixey, W.G., Alvarez, P.J.J., 2014. Numerical model investigation for potential methane explosion and benzene vapor intrusion associated with high-ethanol blend releases. Environ. Sci. Technol. 48 (1), 474–481.
Ma, J., Nossa, C.W., Xiu, Z., Rixey, W.G., Alvarez, P.J.J., 2013a. Adaptive microbial population shifts in response to a continuous ethanol blend release increases biodegradation potential. Environ. Pollut. 178 (0), 419–425.
Ma, J., Rixey, W.G., Alvarez, P.J.J., 2013b. Microbial processes influencing the transport, fate and groundwater impacts of fuel ethanol releases. Curr. Opin. Biotechnol. 24 (3), 457–466.
Ma, J., Rixey, W.G., DeVaull, G.E., Stafford, B.P., Alvarez, P.J.J., 2012. Methane bioattenuation and implications for explosion risk reduction along the groundwater to soil surface pathway above a plume of dissolved ethanol. Environ. Sci. Technol. 46 (11), 6013–6019.
Ma, J., Xiu, Z., Monier, A., Mamonkina, I., Zhang, Y., He, Y., Stafford, B., Rixey, W., Alvarez, P., 2011. Aesthetic groundwater quality impacts from a continuous pilot-scale release of an ethanol blend. Ground Water Monit. Remediat. 31 (3), 47–54.
Mackay, D.M., De Sieyes, N.R., Einarson, M.D., Feris, K.P., Pappas, A.A., Wood, I.A., Jacobson, L., Justice, L.G., Noske, M.N., Scow, K.M., Wilson, J.T., 2006. Impact of ethanol on the natural attenuation of benzene, toluene, and o-xylene in a normally sulfate-reducing aquifer. Environ. Sci. Technol. 40 (19), 6123–6130.
Nelson, D.K., Lapara, T.M., Novak, P.J., 2010. Effects of ethanol-based fuel contamination: microbial community changes, production of regulated compounds, and methane generation. Environ. Sci. Technol. 44 (12), 4525–4530.
Powers, S.E., Hunt, C.S., Heermann, S.E., Corseuil, H.X., Rice, D., Alvarez, P.J.J., 2001. The transport and fate of ethanol and BTEX in groundwater contaminated by gasohol. Crit. Rev. Environ. Sci. Technol. 31 (1), 79–123.
Rasa, E., Bekins, B.A., Mackay, D.M., de Sieyes, N.R., Wilson, J.T., Feris, K.P., Wood, I.A., Scow, K.M., 2013. Impacts of an ethanol-blended fuel release on groundwater and fate of produced methane: simulation of field observations. Water Resour. Res. 49 (8), 4907–4926.
Sihota, N.J., Mayer, K.U., Toso, M.A., Atwater, J.F., 2013. Methane emissions and contaminant degradation rates at sites affected by accidental releases of denatured fuel-grade ethanol. J. Contam. Hydrol. 151 (0), 1–15.
Spalding, R.F., Toso, M.A., Exner, M.E., Hattan, G., Higgins, T.M., Sekely, A.C., Jensen, S.D., 2011. Long-term groundwater monitoring results at large, sudden denatured ethanol releases. Ground Water Monit. Remediat. 31 (3), 69–81.
Stafford, B.P., Capiro, N.L., Alvarez, P.J.J., Rixey, W.G., 2009. Pore water characteristics following a release of neat ethanol onto pre-existing NAPL. Ground Water Monit. Remediat. 29 (3), 93–104.
Stafford, B.P., Rixey, W.G., 2011. Distribution of fuel-grade ethanol near a dynamic water table. Ground Water Monit. Remediat. 31 (3), 55–60.
Wilson, J.T., Toso, M., Mackay, de Sieyes, N., DeVaull, G.E., 2013. What's the Deal with Methane at LUST Spill Sites? Part 2: Vapor Intrusion, LUSTline, Bulletin #72. New England Interstate Water Pollution Control Commission, Lowell, MA, US.
Zhang, Y., Khan, I.A., Chen, X.H., Spalding, R.F., 2006. Transport and degradation of ethanol in groundwater. J. Contam. Hydrol. 82 (3–4), 183–194.