Effects of Ethanol versus Methyl *tert*-Butyl Ether on Benzene, Toluene, Ethylbenzene, and Three Isomers of Xylene Natural Attenuation in Aquifer Columns

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Abstract: The increased use of ethanol as a replacement for the gasoline oxygenate, methyl *tert*-butyl ether (MTBE), may lead to indirect impacts related to natural attenuation of benzene, toluene, ethylbenzene, and the three isomers of xylene (BTEX) compounds. Ethanol could enhance dissolved BTEX mobility by exerting a cosolvent effect that decreases sorption-related retardation. This effect, however, is concentration dependent and was not observed when ethanol was added continuously (at 1%) with BTEX to sterile aquifer columns. Nevertheless, a significant decrease in BTEX retardation was observed with 50% ethanol, suggesting that neat ethanol spills in bulk terminals could facilitate the migration of pre-existing contamination. MTBE (25 mg/L influent) was not degraded in biologically active columns, and it did not affect BTEX degradation. Ethanol (2 g/L influent), on the other hand, was degraded rapidly and exerted a high demand for nutrients and electron acceptors that could otherwise have been used for BTEX degradation. Ethanol also increased the microbial concentration near the column inlet by one order of magnitude relative to columns fed BTEX alone or with MTBE. However, 16S-rRNA sequence analyses of dominant denaturing gradient gel electro-phoresis bands identified fewer species that are known to degraded BTEX when ethanol was present. Overall, the preferential degradation of ethanol and the accompanying depletion of oxygen and other electron acceptors hindered BTEX biodegradation, which suggests that ethanol could increase the length of BTEX plumes.

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Introduction

Monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene, and the three isomers of xylene (BTEX) are ubiquitous groundwater pollutants commonly associated with petroleum product releases. About 420,000 such releases have been confirmed in the U.S. from leaking underground tanks alone (U.S. EPA 2001). While significant advances have been made towards understanding the genetic and biochemical bases of BTEX biodegradation, little attention has been given to how differences in gasoline formulation affects BTEX fate and transport. In this regard, there is a recent initiative being considered to phase out methyl *tert*-butyl ether (MTBE) as a gasoline oxygenate (Federal Register 2000), due to its recalcitrance, ability to rapidly impact drinking water sources, and low taste and odor thresholds ($<5-40$ ppb). The most likely candidate to replace MTBE (which accounts for 80% of current oxygenate use) is ethanol (currently accounting for 15% of oxygenate use) (Powers et al. 2001a). Ethanol is also a renewable resource that can serve as a substitute fuel for imported oil. Therefore, an increase in the use of EtOH as a gasoline additive seems imminent, and a better understanding of its effects on BTEX migration and natural attenuation is warranted.

There are two abiotic (cosolvency related) effects of ethanol that could enhance BTEX migration. One well-studied effect relates to a decrease in the polarity of the aqueous phase, which facilitates the dissolution of BTEX from the fuel phase into groundwater (for review, see Powers et al. 2001b). The other effect, which is not as well understood, relates to how ethanol affects the equilibrium partitioning of BTEX between the sorbed and dissolved phases (Rao et al. 1990). This effect is more difficult to predict ab initio because of complex interactions between ethanol and the sorbent (e.g., soil organic matter with different domains). Thus, little is known about the conditions under which ethanol is likely to decrease sorption-related retardation and enhance BTEX transport during bulk flow.

The need to understand substrate interactions between BTEX and ethanol is very recent, and little research has been conducted on the potential effects of ethanol on BTEX biodegradation. Ethanol concentrations as low as 20 mg/L have been reported to inhibit aerobic benzene degradation in aquifer microcosms (Hunt et al. 1997). Similarly, ethanol was preferentially utilized and hindered toluene degradation in anaerobic aquifer microcosms under different electron accepting conditions (Corseuil et al. 1998). However, these batch experiments did not mimic the exposure conditions that can be encountered in aquifers undergoing natural attenuation, where continuous flow and geochemical transitions through different electron-accepting conditions are common. Thus, there is considerable uncertainty regarding the overall effect of ethanol on the natural attenuation of BTEX compounds.

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In this work, aquifer columns were used to evaluate the potential for ethanol to enhance BTEX migration by decreasing sorption-related retardation, and to study its effect on BTEX biodegradation and geochemical transitions in a flow-through system simulating natural attenuation. Phospholipid fatty acids (PLFA) analysis and denaturing gradient gel electrophoresis (DGGE) were also used to gain insight into how ethanol may affect the microbial community.

Materials and Methods

Retardation Factor Experiments

Small aquifer columns were used to conduct breakthrough studies and compare the effects of ethanol on BTEX retardation factors. Three glass columns (Kontes, 15 cm long, 1 cm i.d.) were packed with uncontaminated soil (f_{oc} = 0.024) as described elsewhere (Siegrist and McCarty 1987; Alvarez et al. 1998), to ensure that no air bubbles were trapped. All tubes and fittings in the flow train were made of Teflon or were Teflon lined to minimize sorption and volatilization losses. The feeds were dispensed from 100-mL gas tight syringes (SGE, Austin, Tex.) at a constant flow of 1 mL/h using a syringe pump (Harvard, Southnatick, Mass.). The tubing was adapted for sampling with a $\frac{1}{4}$ 28-adapter male lure lock fitting and a thin (30-gage) disposable syringe needle. The columns were fed continuously with benzene (5 mg/L), which is the most toxic of the BTEX compounds, toluene (5 mg/L), which is the one most commonly reported to biodegrade, and *o*-xylene (3 mg/L), which is frequently the most recalcitrant one (Gulensoy and Alvarez 1999). Two columns were fed also with ethanol (1 or 50% each) and the other column (without oxygenate amendment) was used as a baseline to evaluate changes in retardation factors. The selected ethanol concentrations are representative of expected levels near the source of gasohol (1%) or neat ethanol releases at bulk terminals (50%) (Powers et al. 2001a, b). All columns were poisoned with a biocide (Kanthon CG/IPC (Supelco©, Bellefonte, Pa.), diluted 1:100 to eliminate confounding effects due to biodegradation and isolate the effects of ethanol on sorption-related retardation. Samples were taken every hour by attaching the effluent end of the column train to collection vials (5 mL CG vial, Kimble) closed previously with Teflon-lined rubber septa and aluminum crimps. The samples were analyzed immediately after collection for BTX and ethanol using gas chromatography.

The hydraulic characteristics of the columns were determined from bromide tracer studies. A carbonate solution (1,000 mg/L) spiked with potassium bromide (1,500 mg/L) was fed continuously at 1 mL/h. The bromide breakthrough curve was used to calculate the effective porosity (η_e), dispersion coefficient (D), and retardation factors (R_f) by fitting the breakthrough data to the one-dimensional (1D) advection-dispersion equation (Domenico and Schwartz 1998)

$$C=\left(\frac{\mathrm{Co}}{2}\right)\mathrm{erfc}\left[\frac{\left(R_f x-\left(\frac{Q}{A\eta_e}\right)t\right)}{2\sqrt{\mathrm{DR}_f t}}\right]+\exp\left[\frac{\left(\frac{Q}{A\eta_e}\right)x}{D}\right]\mathrm{erfc}\left[\frac{\left\{R_f x+\left(\frac{Q}{A\eta_e}\right)t\right\}}{2\sqrt{\mathrm{DR}_f t}}\right] \quad (1)$$

where C=effluent concentration; Co=influent concentration; x=column length; t=elapsed time; Q=flow rate; A=cross sectional area; and erfc complementary error function. The effective porosity was estimated to be 0.45, yielding a pore velocity of 2.8 cm/h. One pore volume was exchanged every 5.7 h. The dispersion coefficient was estimated to be 0.7 cm^2/h. Retardation factors were 1.0 for ethanol, 1.6 for benzene, 2.8 for toluene, and 11.3 for *o*-xylene.

Table 1. Amendments to Influent of Large Columns

	Influent concentration (mg/L)[a]	Ethanol mineralization potential (mg/L)[b]
Electron acceptors		
Oxygen (O_2)	8	3.8
Nitrate (NO_3^-)	30	9.3
Sulfate (SO_4^{-2})	150	47.9
Carbonate (as $CaCO_3$)	1,000	306.7
Nutrients		
Ammonium (NH_4^+)	5.5	
Mg^{2+}	1.5	
PO_4^{-3}	0.06	
Ni(II),Cu(II), Zn(II),Co(II), Mo(IV)	0.002 each	
Electron Donors		
Total BTEX	~1	
Ethanol	~2,000 or none	
MTBE	~ 25 or none	

[a]Total iron [Fe(III)] concentration in the soil aquifer was 72 ppm.

[b]Based on the following stoichiometric requirements (in *g*-electron acceptor/*g*-EtOH): 2.09 for O_2, 3.23 for NO_3^-, 3.13 for SO_4^{2-}, and 3.26 for CO_2 (as $CaCO_3$).

Natural Attenuation Experiments

Five larger glass columns (120-cm long, 5-cm diameter) were used to investigate natural attenuation of benzene, toluene, ethylbenzene, *o*-*m*-*p*-xylenes (BTEX), ethanol and methyl *tert*-butyl ether (MTBE), and their potential interactive effects. The columns were equipped with eight sampling ports (at 2.5, 7.6, 14, 20, 40, 60, 80, and 100 cm from the inlet), and packed with a noncontaminated aquifer material (f_{oc}=0.01) collected from the Northwest Terminal in Tigard, Oregon. Uncontaminated material was used to avoid any potential bias of acclimated microbes towards ethanol or MTBE degradation. One column was fed BTEX at 1 mg/L total, to provide a baseline for the effect of MTBE or ethanol on BTEX attenuation. Three other columns were fed BTEX plus either ethanol (~2 g/L), MTBE (25 mg/L), or both. A sterile control column was also run to distinguish biodegradation from potential abiotic losses. This column was poisoned with Kathon biocide and fed BTEX plus ethanol and MTBE. Each column was fed continuously with carbonate-buffered synthetic groundwater (Table 1) purged with 95% air/5% CO_2. All columns were operated in the dark at 22°C. The columns were fed continuously in an upflow mode at ~7 mL/h using both a peristaltic pump (Masterflex Mod. 7519-15) to feed the mineral medium and a syringe pump (Harvard Apparatus Mod. 22) to feed the volatile organic compounds (i.e., BTEX, ethanol and/or MTBE). The ratio of the peristaltic to syringe pump rates was set at 20:1. Bromide tracer results indicated an effective porosity of about 0.37. The pore velocity in the packed columns was 2 cm/h. The dispersion coefficient was estimated to be 5 cm^2/h. Approximately six days were required to displace one pore volume.

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Analytical Procedures

Samples for BTEX, ethanol, MTBE, and methane analysis were injected in 5-mL vials previously capped with a 20-mm Teflon-coated septa and aluminum crimps. The vials were placed into a HP 19395A headspace sampler (Hewlett-Packard Co.) and equilibrated at 80°C for 30 min. The sampler was connected to an HP5890 gas chromatograph equipped with a 30-m HP5 column and a flame ionization detector (FID). The FID was connected to a HP 300 HPGC with Chem Station software to integrate the signal. Bromide, acetate, nitrate, and sulfate were analyzed in a Alcott 728 auto sampler ion chromatograph connected to an interface (HP35900E) and a conductivity detector (column Ionpac mod. AS4A). The hydrogen ion concentration (pH) of the samples was measured using a Fisher Scientific AB15 pH meter. Oxidation-reduction potential was measured at different points along the column with a microelectrode MI-16/800 connected to a 16-702 flow-thru pH reference and to the pH meter.

Microbial Characterization

Aquifer material samples from the inlet to the large columns and at 40-cm distance were characterized after five months of operation, when BTEX and ethanol concentration profiles along the length of the columns had stabilized. The total viable biomass concentration was analyzed by phospholipid fatty acid content (PLFA analysis) (White et al. 1997) and the dominant bacteria profiles were evaluated by a conserved region of the 16S rRNA gene analysis achieved by denaturing gradient gel electrophoresis (DGGE) (Muyzer et al. 1993). These analyses were performed by Microbial Insights, Inc. (Rockford, Tenn.). Sequence identifications were performed using the BLASTN facility of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and the similarity indices were obtained from the database of the Ribosomal Database Project (Maidak et al. 2000).

Results and Discussion

Effect of Ethanol on BTEX Retardation

The addition of ethanol to gasoline could affect the equilibrium partitioning for BTEX compounds between aqueous, fuel, and solid phases (i.e., the cosolvency effect). Specifically, ethanol could reduce the polarity of water allowing higher concentrations of moderately hydrophobic compounds (e.g., BTEX) in the aqueous phase (Heermann and Powers 1998). This cosolvent effect could also enhance the mobility of dissolved BTEX compounds by decreasing sorption-related retardation, as shown for methanol in M85 fuel (i.e., gasoline with 85% methanol) (Rixey 1994). Such effects, however, are concentration dependent, and were not observed when ethanol was fed continuously to sterile aquifer columns (as a cocontaminant with BTEX) at 10,000 mg/L (Fig.1). This is evident by the similarity of the breakthrough data from columns fed toluene alone or with 1% ethanol. Apparently, this oxygenate concentration is much lower than is required to create significant cosolvent effects (Heermann and Powers 1998; Powers et al. 2001b). Since this ethanol concentration is unlikely to be exceeded in gasohol-contaminated sites, adding ethanol to gasoline (e.g., <15% v/v) should not have a significant impact on BTEX retardation factors. Nevertheless, neat spills of ethanol (e.g., at a bulk terminal) could result in very high ethanol concentrations in a localized area, exerting a significant cosolvent effect

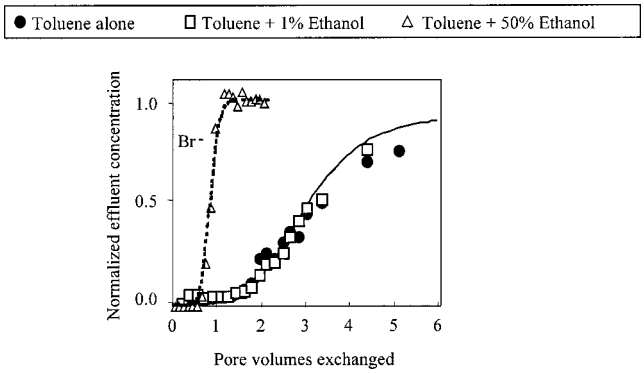

Fig. 1. Retardation of toluene in columns fed BTEX alone and with 1% or with 50% ethanol. Dashed and solid lines represent model fits [Eq. (1)] for the bromide tracer and toluene breakthrough data, respectively

that could exacerbate groundwater pollution by mobilizing preexisting petroleum product releases. At 50%, ethanol enhanced the migration of toluene, as well as of benzene and *o*-xylene (data not shown), which traveled unretarded at same velocity as the bromide tracer (Fig. 1).

Effect of MTBE and Ethanol on BTEX Biodegradation and Natural Attenuation

No significant decreases in BTEX, ethanol and MTBE concentrations (<8%) were observed in the sterile control column, showing that volatile losses were relatively minor. All tested compounds degraded under certain conditions, except for MTBE, which was recalcitrant in all experiments. MTBE has been recently reported to degrade under both aerobic and anaerobic conditions (Salanitro et al. 1994; Bradley et al. 1999; Hanson et al. 1999; Deeb et al. 2001; Finneran and Lovley 2001; Pruden et al. 2001). However, the ubiquity of such biodegradation capabilities has not been established, and MTBE is often found to be recalcitrant. The persistence of MTBE in aquifers is a major concern due to its potential toxicity and low taste and odor thresholds.

BTEX were completely degraded within the first 3 cm of the column inlet when fed alone or in the presence of MTBE [Fig. 2(a)]. The influent dissolved oxygen (~8 mg/L) was sufficient to support the aerobic degradation of the added BTEX compounds (~1 mg/L total). MTBE did not hinder the degradation of ethanol either (data not shown).

BTEX degradation was adversely affected by the presence of ethanol, which was preferentially utilized within 3 cm of the column inlet [Fig. 2(b)]. These results corroborate previous reports that ethanol is likely to be degraded faster than other gasoline constituents (Corseuil et al. 1998). Ethanol concentrations are expected to exceed 1,000 mg/L near the source of a gasohol release (Powers et al. 2001b). Thus, ethanol is prone to exert a significant biochemical demand for oxygen and other electron acceptors (e.g., nitrate and sulfate) compared to other soluble gasoline components. In this experiment, the degradation of ethanol depleted the system of oxygen, nitrate, and sulfate that would otherwise have been available for BTEX degradation (Fig. 3). Thus, even toluene, which is commonly reported to degrade under denitrifying and sulfate-reducing conditions (Alvarez and Vogel 1995; Corseuil et al. 1998; Heider et al. 1998) was recalcitrant in the ethanol-amended column. Note that the medium contained ammonium as nitrogen source (Table 1), which deters the use of nitrate as a nutrient.

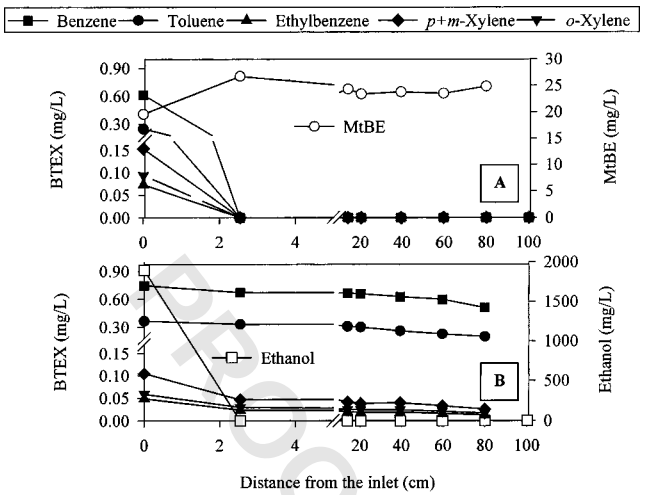

Fig. 2. BTEX concentration profiles in columns fed MTBE (panel A) or ethanol (panel B) after 110 days of operation

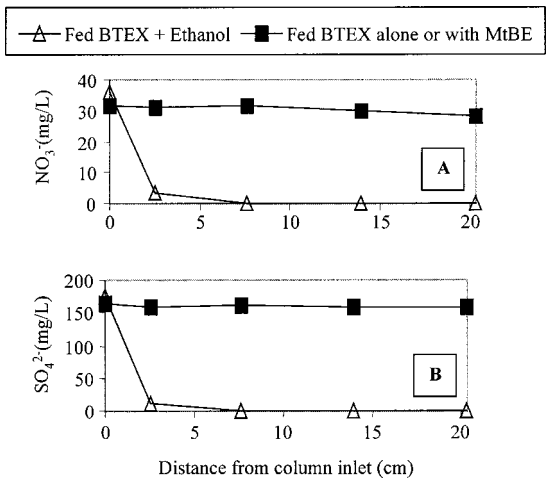

Fig. 3. Nitrate (panel A) and sulfate (panel B) concentration profiles in columns fed BTEX alone or with MTBE or ethanol after 110 days of operation

The high electron-acceptor demand exerted by ethanol created strongly reducing conditions (−300 mV) near the column inlet (data not shown). Such conditions decrease the thermodynamic feasibility of BTEX oxidation. The fast depletion of oxygen during ethanol degradation is particularly important for the fate of benzene, which is the most toxic of the BTEX compounds and degrades slowly or not at all under anaerobic conditions (Alvarez and Vogel 1995; Heider et al. 1998). Nevertheless, it should be kept in mind that ethanol itself is not a major water quality concern. Furthermore, unlike MTBE, ethanol is unlikely to spread over relatively large areas and persist for extended periods of time. Therefore, the adverse indirect impacts of ethanol on BTEX degradation are likely to be of relatively short duration and perhaps more manageable than the impacts associated with MTBE contamination.

Because of the high-oxygen demand exerted by gasohol spills, ethanol is likely to be degraded predominantly under anaerobic conditions. None of the products of anaerobic ethanol degradation is toxic, although some metabolites such as butyrate (Gottschalk 1986) could adversely affect the taste and odor of groundwater supplies. In addition, ethanol-derived acetate and other volatile fatty acids can cause a decrease in pH if they accumulate. This is shown in Fig. 4(a), where acetate was detected (up to 1,500 mg/L) only in the column fed BTEX plus ethanol. Acetate production caused a decrease in pH from 7.5 to as low as 6.2 [Fig. 4(b)], even though the medium was well buffered (1,000 mg/L as $CaCO_3$). Poorly buffered systems could experience a greater decrease in pH, which could inhibit microbial activity and the further degradation of ethanol and other compounds.

Another potential concern is the accumulation of ethanol-derived methane, which could represent an explosion hazard. Stoichiometric considerations suggest that (at 15°C) a 1,000-mg/L ethanol concentration could produce up to 0.77 L of methane within a one-liter pore volume (Powers et al. 2001a). In these experiments, methane was detected only in the column fed BTEX plus ethanol. Methane concentrations initially reached up to 1.8 mg/L when nitrate and sulfate were fed at 30 and 150 mg/L, respectively. Decreasing the influent nitrate and sulfate concentrations to 16 and 12 mg/L, respectively, stimulated methanogenesis, and the methane concentration increased up to 32 mg/L [Fig. 4(a)]. This value is higher than the solubility limit under 1 atm (~24 mg/L at 20°C), and suggests that some gasohol spills could pose an explosion risk if site-specific conditions favor extensive methanogenesis and methane accumulation (e.g., low concentration of alternative electron acceptors and adequate interspecies hydrogen transfer).

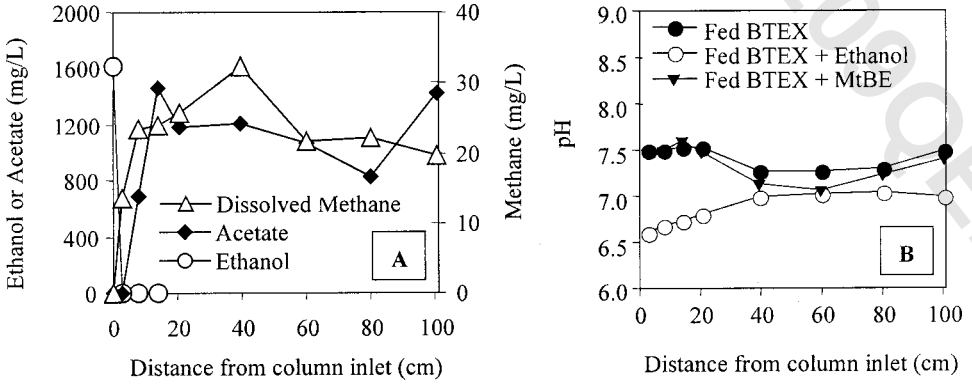

Fig. 4. Acetate and methane concentration profiles along the column fed BTEX plus ethanol (panel A), and pH profiles (panel B) after 542 days of operation

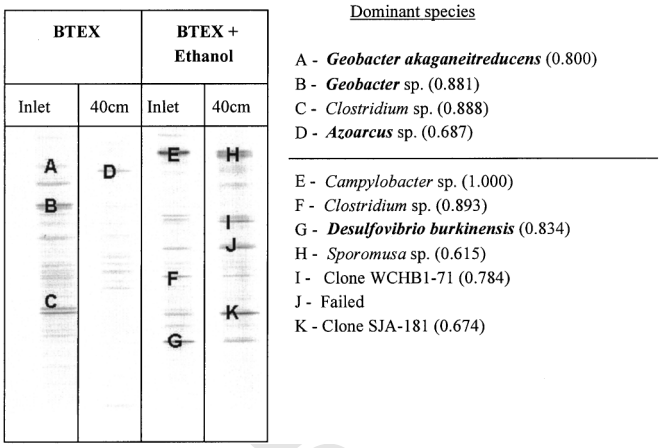

Fig. 5. DGGE gel image of bacterial 16S rRNA extracted from columns fed BTEX alone or with ethanol. Dominant species must constitute at least 1–2% of the total bacterial community to form a visible band. Genera that have been associated in the literature with BTEX degradation are written in bold. The similarity index (SI) is shown in parenthesis. Column samples were analyzed after 153 days of operation.

Microbial Analysis

Ethanol increased the total viable biomass concentrations by one order of magnitude near the column inlet, as determined by PLFA analysis. Specifically, the microbial concentration was about 2×10^7 cells/g-soil at the inlet of columns fed BTEX alone or with MTBE, compared to 3×10^8 cells/g-soil at the inlet of the column fed BTEX plus ethanol. This increase in biomass concentration did not significantly decrease the effective porosity, as indicated by bromide tracer studies (data not shown). In theory, microbial concentration (X) on the order of 3×10^8 cells/g-soil should decrease the effective porosity by less than 0.01%, assuming a dry cell weight (dcw) of 1.33×10^{-13} g (Bratbak 1985), a soil bulk density (ρ_{bulk}) of 1,600 g/L, and a biomass density (ρ_{cell}) of 1,100 g/L (Bratback and Dundas 1984) [i.e., the pore volume fraction occupied by the microorganisms $= X$ (dcw) (ρ_{bulk} /ρ_{cell})] (Clement et al. 1996).

The DGGE profiles showed diverse bacterial communities with several prominent bands appearing in the gel image (Fig. 5). Bacteria identification was based on the similarity index (SI) of 16S ribosomal RNA sequences. SI values above 0.8 are considered excellent matches, 0.6 to 0.7 are good, and below 0.5 are considered to be a unique sequence (Maidak et al. 2000). We recognize that such phylogenetic characterization does not necessarily correspond to expressed metabolic capabilities and activity, but this information can yield valuable (circumstantial) insight on catabolic potential.

Samples from the column fed BTEX alone showed dominant ribosomal sequences found in metal reducing bacteria that belong to the genus *Geobacter* (bands A and B, Fig. 5). This genus is commonly associated with anaerobic BTEX degradation under iron (III) reducing conditions (Anderson et al. 1998; Meckenstock 1999; Rooney-Varga et al. 1999). The other dominant band (C) was closely related to *Clostridium*. No species from this genus have been reported to degrade BTEX, although *Clostridium scalatogenes* is a fermentative anaerobe that can grow on ethanol and aromatic compounds such as naphthalene and phenanthrene (Zhang and Young 1997; Kusel et al. 2000). One band (D) was closely affiliated with the genus *Azoarcus*, which has been reported to degrade toluene and *m*-xylene under denitrifying conditions (Fries et al. 1994; Hurek and Reinholdhurek 1995; Elmen et al. 1997; Hess et al. 1997).

Samples from the column fed BTEX plus ethanol yielded several bands associated with a wide array of anaerobic microorganisms (Fig. 5). Acetogens that can feed on ethanol, such as *Clostridium* (Kusel et al. 2000) and *Sporomusa* (Kuhner et al. 1997) were dominant in these samples. Such strains could have been responsible for the observed production of acetate [Fig. 4(a)]. Interestingly, only one genus was detected that has been associated with BTEX degradation (i.e., band G, *Desulfovibrio*) (Rabus et al. 1996). Although DGGE cannot be used for quantitative evaluation of specific phenotypes, the lower frequency of genera that have been implicated with BTEX degradation suggests that ethanol could decrease the relative abundance of BTEX degraders. This may be related to the fact that more anaerobic bacteria can feed on ethanol, which is a common fermentation product, than on BTEX. Nevertheless, a more quantitative microbial ecology study would be needed to determine whether ethanol selects for bacteria that cannot degrade BTEX, or if it can also (fortuitously) stimulate the growth of bacteria that can degrade both ethanol and BTEX.

In conclusion, enhanced BTEX migration due to a decrease in sorption-related retardation is unlikely to occur at retail sites contaminated with gasohol (i.e., gasoline with 10% ethanol), but could be important when dealing with neat ethanol releases at bulk terminals. The preferential degradation of ethanol and the accompanying depletion of oxygen and other electron acceptors suggest that ethanol could hinder the natural attenuation of BTEX plumes. This is particularly important for the fate of benzene, which is the most toxic and the most recalcitrant of the BTEX compounds under anaerobic conditions. Nevertheless, it is unknown the extent to which ethanol is likely to increase the distance that benzene migrates before attenuating processes reduce benzene concentration to acceptable levels. Therefore, field studies should be conducted to quantify the effect of ethanol on plume length and improve our risk assessment and modeling capabilities.

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