Impact of Ethanol on Benzene Plume Lengths: Microbial and Modeling Studies

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Abstract: Recent legislation in several states has called for the removal of methyl *tert*-butyl ether (MTBE) from gasoline. In order to comply with Federal Clean Air Act requirements for carbon monoxide and ozone attainment, ethanol is being considered as a replacement for MTBE. The objective of this study is to evaluate the potential impact of ethanol on benzene plume lengths in subsurface environments following accidental spills of ethanol-blended gasoline. Two types of studies were conducted here. First, laboratory studies were performed using a pure culture indigenous to a gasoline-contaminated aquifer to evaluate the effect of ethanol on the rate of benzene biodegradation under aerobic conditions. Results from microbial studies showed that the biodegradation of 25 mg/L benzene was severely inhibited in the presence of 25 mg/L ethanol. While the enzymes responsible for benzene biodegradation by the culture were inducible, ethanol degradation appeared to be constitutive. Second, a two-dimensional model was developed to quantify the impact of ethanol on benzene plume lengths using weighted-average aerobic and anaerobic biodegradation rates for benzene in the presence and absence of ethanol. Model simulations indicated that benzene plume lengths are likely to increase by 16–34% in the presence of ethanol.

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Introduction

Fuel oxygenates have been used in the United States for over two decades. These compounds were initially added to gasoline to increase the octane rating, and more recently to fulfill gasoline oxygen content requirements of the Clean Air Act. Fuel oxygenates include methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), dissopropyl ether (DIPE), *tert*-butyl alcohol (TBA), methanol, and ethanol.

The addition of ethanol to gasoline in the United States was promoted in 1978 when a Nebraska group marketed gasohol, a gasoline containing 10% ethanol by volume. The purpose of this program was to increase gasoline availability during the oil embargo (Chevron 1996; USEPA 1998). In 1990, the Clean Air Act Amendments (CAAA) mandated the use of oxygenates in gasoline, either year round or seasonally, in certain air quality basins not meeting federal air quality standards for carbon monoxide and ozone. To meet the requirements of the CAAA, the EPA initiated the Oxyfuel Program in 1992 and the Reformulated Gasoline Program in 1995. The former required the use of gasoline with 2.7% oxygen by weight during winter months to control carbon monoxide emissions, and the latter required the use of gasoline with 2% oxygen throughout the year in ozone nonattainment areas. These oxygen contents correspond to 7.3 and 5.4% ethanol by volume, respectively. In 1992, 8% of all the gasoline sold in the United States contained ethanol. By 1998, ethanol-blended fuels accounted for approximately 15% of all oxygenated fuels in the United States, while the remainder was primarily MTBE-blended fuels (USEPA 1998). With recent problems involving the contamination of drinking water resources by MTBE, legislation in a few states has called for the removal of MTBE from gasoline. As a result, the use of ethanol-blended gasoline is expected to increase in the United States over the next several years in order to comply with CAAA oxygenate requirements.

One disadvantage related to the addition of ethanol to gasoline is the potentially negative impact of ethanol on the natural biodegradation of other gasoline constituents in contaminated soil and groundwater. Ethanol has been reported to biodegrade readily relative to other gasoline hydrocarbons and oxygenates under aerobic and anaerobic conditions. In addition, the preferential degradation of ethanol over other gasoline components could lead to the depletion of oxygen, other electron acceptors, and nutrients in contaminated subsurface environments, thereby inhibiting the biodegradation of other gasoline components. Of most importance is the potential impact of ethanol or benzene, toluene, ethylbenzene, and xylene (BTEX compounds) plume lengths. Prior to the widespread use of oxygenates in gasoline, bioattenuation

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was recognized as a critical mechanism for controlling the migration of dissolved gasoline hydrocarbon plumes (Rice et al. 1995; Salanitro 1993). Among the BTEX compounds, benzene is of most concern to regulators and water purveyors because of its documented toxicity and carcinogenicity (Dean 1985). Therefore, the overall goal of this study was to evaluate the impact of ethanol on the fate of benzene following accidental spills of ethanol-blended gasoline. Specifically, the objectives of this study were twofold:

1. To evaluate the impact of ethanol on the aerobic biodegradation rate of benzene using a culture indigenous to a gasoline-contaminated aquifer; and
2. To develop a two-dimensional model to quantify the impact of ethanol on benzene plume lengths in the field.

Materials and Methods

Microbial Degradation Studies

Culture

The pure culture used in the biodegradation experiments was isolated from a toluene-enriched culture from a gasoline-contaminated aquifer (Deeb and Alvarez-Cohen 1999, 2000). Identification of this culture using fatty acid methyl ester (FAME) analysis (Microbial ID, Inc., Newark, Del.) revealed that it was most closely related to *Rhodococcus rhodochrous*. 16S rRNA gene sequences of the first 500 base pairs followed by gene alignment comparisons in the MicroSeq Database by MIDI Labs suggested that the closest match for the strain was *R. coprophilus* (% of difference=4.56). The closest GenBank (%ID=99) and RDP (SR=0.96) matches were *Rhodococcus ruber*. This culture was therefore designated as *Rhodococcus* sp. RR1.

RR1 was grown aseptically in batch reactors consisting of either 250 mL clear glass bottles (Alltech Co., Deerfield, Ill.) or 385 mL side-armed Nephelo culture flasks (Bellco Glass, Inc., Vineland, N.J.) containing 50 and 100 mL, respectively, of a mineral salts medium with the following composition: 0.5 mg/L $CuSO_4 \cdot 5H_2O$; 1000 mg/L $NaNO_3$; 170 mg/L K_2SO_4; 37 mg/L $MgSO_4 \cdot 7H_2O$; 8.6 mg/L $CaSO_4 \cdot H_2O$; 530 mg/L KH_2PO_4; 1060 mg/L K_2HPO_4; 0.17 mg/L KI; 0.57 mg/L $ZnSO_4 \cdot 7H_2O$; 0.34 mg/L $MnSO_4 \cdot H_2O$; 0.124 mg/L H_3BO_3; 0.094 mg/L $CoMoO_4 \cdot H_2O$; 22.2 mg/L $FeSO_4 \cdot 7H_2O$; 9.8 mg/L H_2SO_4. The bottles were sealed with Teflon-lined Mininert valves (Alltech Co.) and spiked as needed with benzene at initial liquid concentrations of 40 mg/L. Pure oxygen was added to maintain approximately 15–21% oxygen by volume in the headspace. Following inoculation, growth reactors were maintained at 30°C in a controlled environment incubator/shaker at a mixing rate of 175 rpm. Stock solutions of RR1 were prepared by centrifuging liquid culture suspensions at 20,000 rpm for 20 min at 4°C using an Avanti J-30I centrifuge (Beckman Coulter) and by resuspending the cells in mineral salts media.

Experimental and Analytical Procedures

Benzene and ethanol substrate interaction studies were conducted in 385 mL Nephelo culture flasks. Bottles containing 100 mL of the mineral salts medium were sterilized by autoclaving for 20 min at 121 °C prior to use. Neat ethanol and benzene were added to the bottles using high precision 5–20 μL syringes (Hamilton Co., Reno, Nev.). Initial benzene concentrations were calculated using dimensionless Henry's constants with liquid and gas volumes as described elsewhere (Deeb and Alvarez-Cohen 1999). Following cell inoculation, the bottles were incubated at 30°C in the dark and shaken at 175 rpm (model No. 25, New Brunswick Scientific) for the duration of the experiment. The disappearance of benzene was monitored by headspace analysis using a Hewlett Packard 5880 gas chromatograph (GC) equipped with a flame ionization detector and a 0.75 mm×30 m glass capillary column (Supelco Co., Bellefonte, Pa.). The oven, injector, and detector temperatures of this gas chromatograph were fixed at 85, 250, and 300°C, respectively. Headspace samples (200 μL) were withdrawn from the bottles using Hamilton CR-700 series constant rate gas-tight syringes. Sampling proceeded at frequent intervals until the benzene concentrations in the bottles dropped below the detection limit of the gas chromatograph (1–50 μg/L for the different BTEX compounds). The disappearance of ethanol was monitored by removing a 0.5 mL sample of liquid culture from each bottle and by filtering the samples prior to analysis using 0.45 μm low protein binding hydrophilic LCR membranes (Millex-LH syringe driven filter units, Millipore, Burlington, Mass.). Five μL of the filtered sample were directly injected into a Varian 3400 gas chromatograph equipped with a flame ionization detector and a 6 ft by 1/8 in. by 0.085 in. column (1% AT-1000 on graphpac GB 80/100 mesh). The column, injector, and detector temperatures were kept constant at 110, 230, and 250°C, respectively. The detection limit for ethanol using this GC was approximately 1 mg/L.

Abiotic controls containing benzene and ethanol but no cells were used to monitor nonbiological losses of volatile compounds from the bottles. Killed controls were prepared by autoclaving the bottles for 45 min at 121°C. Experiments were performed in duplicate. Culture growth was monitored by measuring the increase in absorbance at 600 nm over time using a light spectrophotometer (Milton Roy Spectronic 20D, Spectronic Instruments, Inc., Rochester, N.Y.). A sample containing mineral salts media (but no cells) was used as a reference for the spectrophotometer prior to each absorbance measurement. In addition, cell counts were measured using CTC or DAPI stain methods described by Bhupathiraju et al. (1999). Samples of cells were obtained by sacrificing one bottle at the beginning and end of each experiment. Samples were preserved in a 20% glycerol solution and frozen at −80°C. Preserved cells were later stained and counted at a magnification of 100× using an Olympus BH-2 microscope.

Modeling Study

A model was developed to approximate the increase in benzene plume lengths in the presence of ethanol. The two-dimensional fate and transport equation derived by Domenico (1987) was used to calculate the predicted concentrations of ethanol and benzene in the subsurface. Several simplifying assumptions were made for purposes of this study. The model assumption and simulation results are presented and discussed in detail in Results and Discussion in this paper.

Results and Discussion

Microbial Degradation Studies

Microbial studies were performed under aerobic conditions to evaluate the impact of ethanol on benzene degradation rates by a pure culture designated RR1. RR1 was isolated from a BTEX-degrading consortium indigenous to a gasoline-contaminated aquifer (Deeb and Alvarez-Cohen 1999). This culture is a robust

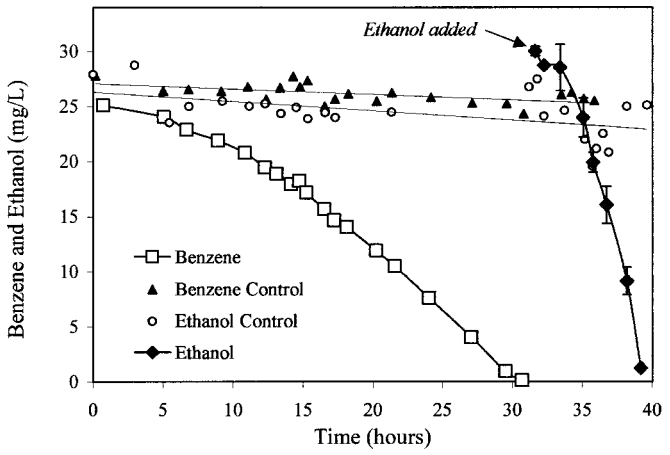

Fig. 1. Biodegradation of benzene followed by ethanol by culture RR1. Cells were grown on benzene to an initial density of 10^6 cells/mL. At completion of the experiment, the cell density increased to 10^7 cells/mL. The experiment was performed in duplicate with error bars depicting the range of duplicate samples.

BTEX degrader and has been shown to utilize benzene, toluene, and ethylbenzene effectively for growth (Deeb et al. 2000a,b). In this study, benzene degradation by RR1 was evaluated in the presence and absence of ethanol to understand the potential impacts of ethanol on benzene degradation rates.

Benzene-grown cells of RR1 (at low culture densities) readily degraded benzene and ethanol individually at initial substrate concentrations of 25–30 mg/L (Figs. 1 and 2). While the enzymes responsible for benzene degradation by RR1 are inducible (Deeb et al. 2000a,b), ethanol degradation by benzene-grown cultures was very rapid and proceeded without a significant lag period (Figs. 1–3), suggesting that the enzymes responsible for the degradation of ethanol by RR1 could be constitutive. This was supported by the observation that RR1 also degraded ethanol rapidly and without a lag period after the culture was grown on pyruvate

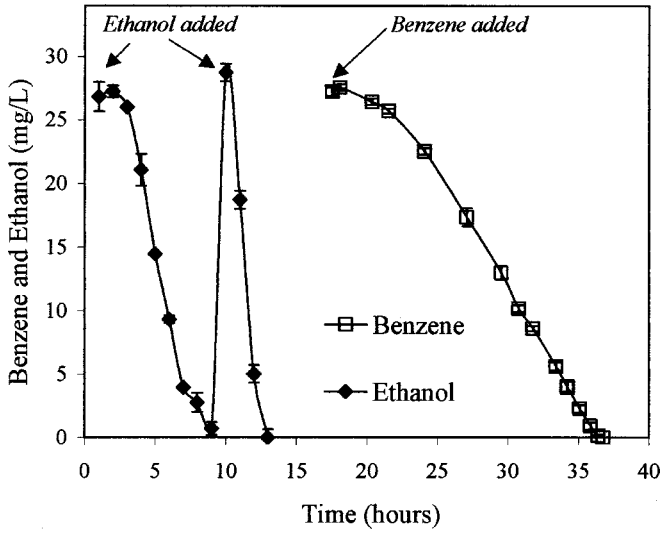

Fig. 2. Biodegradation of ethanol followed by benzene by culture RR1. Cells were grown on benzene to an initial density of 10^6 cells/mL. At completion of the experiment, the cell density increased to 10^7 cells/mL. The experiment was performed in duplicate with error bars depicting the range of duplicate samples.

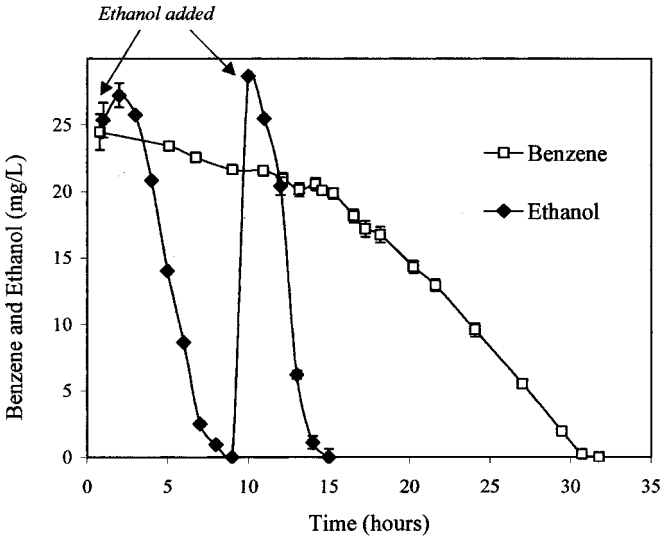

Fig. 3. Biodegradaiton of a mixture of ethanol and benzene by culture RR1. Cells were grown on benzene to an initial density of 10^6 cells/mL. At completion of the experiment, the cell density increased to 10^7 cells/mL. The experiment was performed in duplicate with error bars depicting the range of the duplicate samples.

(data not shown). Optical density measurements over the duration of each experiment increased significantly indicating that ethanol is used readily for growth by RR1.

Experiments that evaluated substrate interactions in mixtures of benzene and ethanol revealed that the presence of ethanol severely inhibited the biodegradation of benzene by benzene-grown cells of RR1 (Fig. 3). When a bottle containing 25 mg/L of benzene was spiked twice with ethanol at initial concentrations of 25 mg/L, benzene degraded very slowly until most of the ethanol was depleted from the bottles (Fig. 3). The observed lag in the biodegradation of benzene due to the presence of ethanol indicates that ethanol is preferentially degraded over benzene by RR1 although the culture was grown on benzene.

Similar experiments were performed to evaluate the impact of ethanol on the biodegradation of other BTEX components and similar results were obtained (data not shown). The degradation of toluene, ethylbenzene, and the xylene isomers were inhibited in the presence of ethanol.

Modeling Study

Model Assumptions

A model was developed to approximate the increase in benzene plume length in the presence of ethanol. Several simplifying assumptions were made including the following:

- The gasoline release was assumed to take place in porous media;
- The gasoline source was assumed to be continuous;
- The ethanol plume was assumed to reach some stable distance downgradient from the source;
- Gasoline aliphatic concentrations were assumed to be low in groundwater due to low alkane solubility; therefore, it was assumed that alkanes do not significantly impact the biodegradation of either ethanol or benzene;
- Ethanol concentrations near the source were assumed to be 4000 mg/L; this number is based on 5% ethanol content on a volume basis in gasoline with a 10-fold dilution factor. Actual

field source concentrations may be higher or lower than this value depending on the composition of the ethanol source;
- Benzene was assumed to enter the water table at a concentration of 8 mg/L which is representative of leaking underground storage tank source conditions (R. Weissenborn, personal communication, 1998); and
- In the absence of ethanol, benzene biodegradation was assumed to follow first order kinetics with a biodegradation rate coefficient λ. This parameter is a volume weighted-average biodegradation rate constant based upon assumptions of an anaerobic biodegradation rate within the heart of the plume and an aerobic biodegradation rate at the edge of the plume. Because of the low solubility of oxygen in water, most BTEX plumes are expected to become anaerobic over time in the absence of significant recharge or reaeration. For the purposes of this evaluation, it was assumed that in co-mingled BTEX/ethanol plumes, the biodegradation rate constant for benzene would be zero when the concentration of ethanol is over 3 mg/L. This assumption was based on aerobic batch studies that suggest that, when ethanol and BTEX are both available as substrates, benzene is not likely to degrade at a significant rate until the ethanol concentrations decrease below a threshold limit (Corseuil et al. 1998; this study) and on the expected insignificant anaerobic biodegradation rate of benzene. It was also assumed that, when the ethanol concentration falls below the threshold limit, or when the BTEX plume migrates past the ethanol plume, benzene biodegradation will commence at the given λ. It should be noted that the use of 3 mg/L as a threshold concentration, as well as the assumption that BTEX biodegradation is zero at ethanol concentrations above this limit, are both laboratory-based simplifying assumptions that have yet to be verified in the field.

Model Development

The two-dimensional (2-D) fate and transport equation derived by Domenico (1987) was used to calculate predicted concentrations of ethanol and benzene in the subsurface. Use of this equation to analyze plume behavior under a variety of hydrogeologic and geochemical conditions is widespread (McNabb and Dooher 1996, and references therein). The Domenico solution in two dimensions is given by the following equation:

$$C_{(x,y,t)}=\left(\frac{C_0}{4}\right)\exp\left\{\left(\frac{x}{2\alpha_x}\right)\left[1-\left(1+\frac{4R\lambda\alpha_x}{v}\right)^{1/2}\right]\right\}$$
$$\times\operatorname{erfc}\left[\frac{x-(v/R)t[1+(4R\lambda\alpha_x/v)]^{1/2}}{2[\alpha_x(v/R)t]^{1/2}}\right]$$
$$\times\left\{\operatorname{erf}\left[\frac{y+Y/2}{2(\alpha_y x)^{1/2}}\right]-\operatorname{erf}\left[\frac{y-Y/2}{2(\alpha_y x)^{1/2}}\right]\right\}$$

where C_0=source concentration [mg/L]; x=distance from the source in the longitudinal direction [m]; y=distance from the source in the lateral direction [m]; α_x=dispersivity in the x direction [m]; α_y=dispersivity in the y direction [m]; R=retardation factor [−] (this is a function of the partitioning coefficient of the constituent (K_{oc}), the organic content of the matrix (f_{oc}), the bulk density (ρ) of the matrix, and the effective porosity (n_e) of the aquifer material), λ=first order decay constant of the constituent [day^{-1}]; v=groundwater velocity [m/s] (this is a function of hydraulic conductivity (K), hydraulic gradient (i), and effective porosity (n_e) of the aquifer material); Y=the length of the source in the lateral direction [m].

Table 1. Biodegradation Rate Constants from Literature for Benzene and Ethanol under Range of Electronic Acceptor Conditions

	Anaerobic Pseudofirst-Order Rate Constant	
Compound	Electron acceptor	Degradation rate (day^{-1})
Ethanol	O_2	0.23–0.35[a]
	NO_3^-	0.53[a]
	Fe^{+3}	0.17[a]
	SO_4^{-2}	0.1[a]
Benzene	O_2	0.043–0.14[b]
	NO_3^-	0–0.045[c]
	Fe^{+3}	0–0.024[c]
	SO_4^{-2}	0–0.047[c]
	Methanogenic	0–0.052[c]
	Theoretical field	0.0062–0.00096[b]

[a]Estimated from Corseuil and Alvarez (1996); Corseuil et al. (1996) and Corseuil et al. (1998).

[b]Rathbun (1998).

[c]Aronson and Howard (1997).

The factors that typically govern the transport of a constituent are λ, v, R, and dispersivity (α_x and α_y). Among these factors, λ and v are the most influential parameters in determining the length of stable ethanol and benzene plumes. For the purposes of this modeling exercise, v was varied from 0.004 to 0.4 ft/day and f_{oc} was varied from 0.01 to 0.005. Dispersion is a scale dependent function of the matrix and the contaminant velocity. In this case, dispersion represents a much smaller contribution to plume stabilization than biodegradation although benzene has a higher retardation factor than ethanol ($R_{TBEX}>R_{ethanol}$) (Zogorski et al. 1997; Da Silva and Alvarez 2002). In this analysis, ethanol concentrations were first calculated for any given v and ethanol λ. The benzene λ was reduced to zero (i.e., no degradation of benzene) until the ethanol concentrations dropped below the threshold value (3 mg/L). Benzene and ethanol plume lengths were approximated based on iterative changes in the evenly spaced observation points along the x axis (down the center of the plume). This analysis was conducted for a range of groundwater velocities (0.004 ft/day–0.4 ft/day). Four velocity values were evaluated. For each velocity value, three f_{oc} values (0.2, 0.5, and 1.0%) were tested.

Biodegradation Rate Constants for Ethanol and Benzene

In the absence of ethanol, and at a distance at which the ethanol concentration drops below the threshold value, the maximum reported value for a theoretical benzene biodegradation rate in the field was used (0.0062 day^{-1}, Table 1). The benzene plume length was determined to be the distance from the source to the point where the benzene concentration falls below 0.001 mg/L (the primary MCL for benzene in California).

The ethanol λ reported in the literature is based upon laboratory results and is not necessarily reflective of field conditions. The average anaerobic laboratory derived λ for ethanol under a variety of reducing conditions is 0.28 day^{-1} which is equivalent to a half-life of 2.5 days (Table 1). At this rate, it is expected that the anaerobic biodegradation of ethanol reduces dissolved concentrations fairly rapidly, thereby attenuating the ethanol plume. Ethanol plume lengths calculated using this λ value were on the order of 10–30 ft which would not significantly elongate stable BTEX plume lengths. Laboratory-derived data should be adjusted in order to determine a λ which is more reflective of conditions in the field. This was accomplished here by addressing the three following issues.

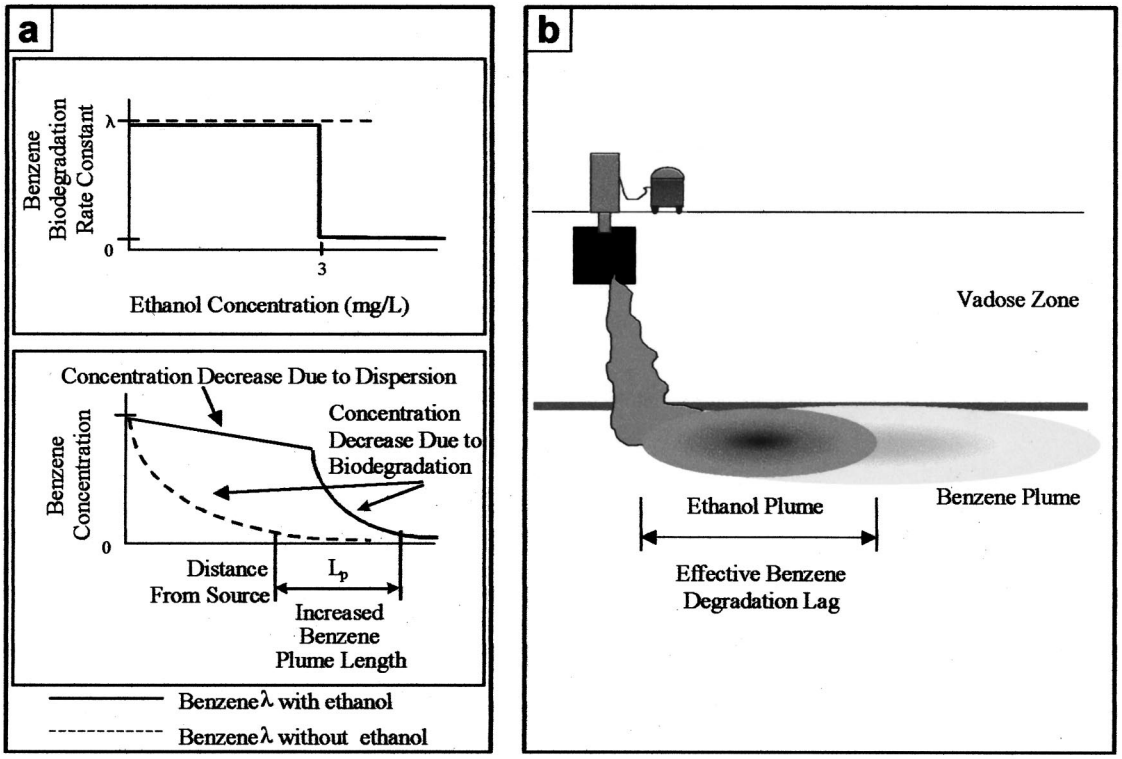

Fig. 4. (a) Schematic illustrating expected increase in benzene plume length due to benzene lag time resulting from presence of ethanol; (b) conceptual model of a co-mingled ethanol/benzene plume

1. The reported λ for ethanol was determined in a laboratory study at a temperature of 28°C. A temperature of 15°C is more representative of conditions in subsurface environments since groundwater temperatures are typically within 2°C of mean annual surface temperatures (Chapelle 1992). Realizing that, as a gross estimate, biodegradation rates can be reduced by one-half for every 10°C decrease in temperature (Gerhardt 1994), λ should then be adjusted for a change in temperature from 28 to 15°C.
2. The reported λ for ethanol was determined in a laboratory study based on liquid culture batch experiments performed using a relatively high concentration of active biomass. Biomass activities in field soils are generally much lower than those reported for laboratory cultures. In addition, only a small fraction of microorganisms within subsurface environments contacts dissolved contaminants, and only a fraction of those is expected to be capable of degrading ethanol. Based on these specifics, a magnitude of order decrease of a laboratory rate to account for the difference in biomass activity between the laboratory and the field appears to be a conservative assumption. The data in Table 1 support this assumption. Table 1 shows that the expected field biodegradation rate for benzene is at least an order of magnitude less than measured rates from laboratory experiments under a range of conditions.
3. Because of mass transfer limitations in subsurface material, degradation rate constants in the field are typically lower than those observed in the laboratory. Adjustments of laboratory-derived λ due to mass transfer limitations are difficult to quantify without extensive testing.

The literature-derived average ethanol biodegradation rate of 0.28 day^{-1} was therefore adjusted to 0.014 day^{-1}. This was based on an approximate 10°C difference in temperatures and an order of magnitude difference in the active microbial population between the laboratory and the field.

Model Simulation Results

The proposed model was applied to approximate the increase in benzene plume length in the presence of ethanol. Based on the assumptions listed above, a conceptual model of co-mingled benzene/ethanol plume behavior is presented in Fig. 4. Fig. 4 illustrates the probable increase in benzene plume length due to a lag time in benzene biodegradation that results from the presence of ethanol. It is expected that the resultant change in benzene plume length will be proportional to the lengths of ethanol plumes depicted by Fig. 4.

Model simulations showed that the ethanol biodegradation rate constant strongly influences the length of ethanol plumes in subsurface environments thereby impacting the length of benzene plumes. By using a literature-derived ethanol biodegradation rate constant, and by correcting for projected temperature and biomass activity in the field, stable benzene plumes were predicted by the model to travel approximately 27% further under typical California groundwater conditions when ethanol is present in gasoline (5% on a volume basis) (Table 2). Benzene plume lengths were generally observed to increase with increasing values of groundwater velocity and organic carbon content. For a range of tested parameters, benzene plume lengths were found to increase on average from 17 ($v=0.004$ ft/day and $f_{oc}=0.2\%$) to 34% ($v=0.4$ ft/day and $f_{oc}=1\%$) in the presence of ethanol (Table 2).

It is important to note that these plume lengths were approximated based on iterative changes in observation point spacing. The lowest observed value was used as the reported value. As a result, the predicted plume lengths may not be exact but they represent the best approximation based on the method used. In

Table 2. Increases in Benzene Plume Lengths Predicted by Model for Range of Groundwater Velocity and Organic Carbon Content Values

v in ft/day	PL2–PL1	Difference (%)	Plume length 1 (ft) (PL1)	Plume length 2 (ft) (PL2)
	f_{oc}	0.005		
0.004	5	6	1	22
0.04	26	33	7	27
0.4	220	280	60	27
	f_{oc}	0.002		
0.004	6	7	1	17
0.04	38	48	10	27
0.4	330	420	90	27
	f_{oc}	0.01		
0.004	3	4	1	19
0.04	17	22	5	27
0.4	145	195	50	34

Note: PL1=plume length 1: benzene in the absence of ethanol; PL2 =plume length 2: benzene in the presence of ethanol; v=groundwater velocity; f_{oc}=organic carbon content.

addition, the strong scale dependency (i.e., a large v will create a longer plume) results in an increasing margin of error with an increase in plume length.

Since this model was developed, several other models have been used to estimate possible increases in BTEX plume lengths following accidental spills of ethanol-blended fuels. A summary of key assumptions used for each of these models is presented in Table 3. In addition, predicted increases in benzene (or BTEX plume lengths) are presented in Table 3 and compared to values predicted by the model used in this study. In general, the presence of ethanol is predicted to cause increases in benzene plume lengths of between 7 and 150%. The values predicted by the model used here are within this range of values (McNabb et al. 1999; Molson et al. 1999; Schirmer et al. 1999).

The results of the laboratory studies presented here support the predictions of the model. Microbial degradation studies revealed that the presence of ethanol at concentrations as low as 25 mg/L severely inhibited the biodegradation of benzene by benzene-acclimated cultures, even under nonlimiting nutrient and oxygen conditions. Should the laboratory-observed preferential degradation of ethanol over benzene be realized in subsurface environments, the depletion of oxygen and nutrients could further limit the biodegradation of benzene and lead to even longer benzene plumes, especially in zones of low groundwater recharge.

The laboratory results presented here correlate well with a previous report that ethanol can be degraded faster than other gasoline constituents, and that the presence of ethanol inhibits BTEX biodegradation (Corseuil et al. 1998). However, these experiments were conducted in microcosms to which a high concentration of ethanol was added although the supply of oxygen and nutrients was limited. Results from column breakthrough experiments that simulate a natural attenuation flow-through system also support the conclusion that the presence of ethanol hinders the biodegradation of BTEX compounds, presumably by preferential degradation and consequent consumption of preferred electron acceptors and nutrients (Da Silva and Alvarez 2002). In these column studies, the degradation of ethanol was presumed to occur predominantly under anaerobic conditions because the oxygen demand exerted by the influent ethanol far exceeded the available dissolved oxygen. As expected, the lack of oxygen inhibited the

Table 3. Overview of Published Modeling Efforts to Assess Impact of Ethanol on Benzene Plume Lengths

Model Description	Increase in benzene plume length (%)	Reference
Steady state 2-D (X, Y) transport from a gasoline pool First-order decay of benzene when $C_{ethanol}<3$ mg/L First-order decay of ethanol	17–34	This study
2-D (X, Z) transport from a pool of gasoline Focus on co-solvency and interphase mass transfer Biodegradation not included	≤10 (for xylene, not benzene)	Heermann and Powers (1996)
Three-dimensional (3-D) aqueous transport Continuous slow release of gasoline (up to 3 gpd) to a growing NAPL pool at the water table First-order decay of ethanol and benzene Benzene degradation rate constant defined by inverse correlation to BOD concentration at the source	100	McNabb et al.
2-D transport (X–Z) from a gasoline source at the water table at residual saturation Decay rate used with O_2 as the sole electron acceptor (aerobic) Decay rate quantified using Monod kinetics Microbial growth incorporated	10–150	Molson et al. (1999)
3-D finite element model (BIONAPL) Aerobic and anaerobic (ethanol only) biodegradation assumed to be governed by Monod kinetics under electron acceptor-limiting conditions	7–114	Schirmer et al. (1999)

biodegradation of benzene in the columns resulting in an overall decrease in the attenuation of benzene and other BTEX components in the presence of ethanol.

Substrate interaction issues have been recognized to be of importance for evaluating the fate and transport of gasoline components in subsurface environments (Deeb and Alvarez-Cohen 1999, 2000; Deeb et al. 2001a,b; and others). Substrate interactions due to the use of ethanol in gasoline can vary with the source of ethanol in subsurface environments. Oxyfuel and RFG ethanol blends (7.3 and 5.4% ethanol by volume, respectively) are less likely to lead to large increases in benzene plume lengths as gasohol (10% ethanol by volume). The highest concentrations of ethanol encountered in groundwater will result following a spill of pure ethanol. This is a very likely scenario at fuel terminals where ethanol-oxygenated fuels are splash blended. For such a scenario, high concentrations of ethanol in source zones are likely to increase the mobility of other gasoline constituents in subsurface environments via co-solvency. In addition, the large amount of ethanol introduced into subsurface environments is likely to exert an exorbitant biochemical demand for oxygen and other electron acceptors that potentially inhibit the biodegradation of BTEX compounds and other gasoline components over large areas.

Conclusions

The overall objective of this study was to evaluate the potential impact of ethanol on benzene plume lengths in subsurface environments following accidental spills of ethanol-blended gasoline. Laboratory studies were performed using a pure culture indigenous to a gasoline-contaminated aquifer to evaluate the effect of ethanol on the rate of benzene biodegradation under nonlimiting oxygen and nutrient conditions. These studies showed that the biodegradation of benzene was severely inhibited in the presence of ethanol (25 mg/L) at concentrations much lower than those expected in co-mingled BTEX/ethanol plumes. The degradation of ethanol proceeded without a lag period by cultures grown on benzene or pyruvate, suggesting that ethanol biodegradation takes place constitutively. The observed preferential degradation of ethanol over benzene has implications regarding the impact of ethanol on benzene plume lengths in subsurface environments. A two-dimensional model was then developed in an attempt to quantify the impact of ethanol on benzene plume lengths using weighted-average aerobic and anaerobic biodegradation rates for benzene in the presence and absence of ethanol. Model simulations indicated that benzene plume lengths are likely to increase by 16–34% in the presence of ethanol.

The laboratory-observed and model-predicted negative impacts of ethanol on benzene plume lengths suggest that this area of research should receive significantly more attention before the use of ethanol in gasoline becomes a widely adopted practice. Field evaluations of the impacts of ethanol on benzene plume lengths are needed given the level of assumption required for this and for similar analyses.

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