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Enhanced anaerobic biodegradation of BTEX-ethanol mixtures in aquifer columns amended with sulfate, chelated ferric iron or nitrate

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Abstract

Flow-through aquifer columns were used to investigate the feasibility of adding sulfate, EDTA–Fe(III) or nitrate to enhance the biodegradation of BTEX and ethanol mixtures. The rapid biodegradation of ethanol near the inlet depleted the influent dissolved oxygen (8 mg l^{-1}), stimulated methanogenesis, and decreased BTEX biodegradation efficiencies from >99% in the absence of ethanol to an average of 32% for benzene, 49% for toluene, 77% for ethylbenzene, and about 30% for xylenes. The addition of sulfate, EDTA–Fe(III) or nitrate suppressed methanogenesis and significantly increased BTEX biodegradation efficiencies. Nevertheless, occasional clogging was experienced by the column augmented with EDTA–Fe(III) due to iron precipitation. Enhanced benzene biodegradation (>70% in all biostimulated columns) is noteworthy because benzene is often recalcitrant under anaerobic conditions. Influent dissolved oxygen apparently played a critical role because no significant benzene biotransformation was observed after oxygen was purged out of the influent media. The addition of anaerobic electron acceptors could enhance BTEX biodegradation not only by facilitating their anaerobic biodegradation but also by accelerating the mineralization of ethanol or other substrates that are labile under anaerobic conditions. This would alleviate the biochemical oxygen demand (BOD) and increase the likelihood that entraining oxygen would be used for the biotransformation of residual BTEX.

Introduction

The addition of ethanol to gasoline is likely to increase rapidly in the near future to diminish air pollution by automobile emissions and to meet renewable fuel requirements aimed at decreasing our dependence on fossil fuel (Powers et al. 2001a, b). Nevertheless, recent problems with surface and ground water contamination by methyl *tert*-butyl ether (MTBE) have made policy makers more cognizant of the need to consider the overall environmental impacts of gasoline additives. This is a timely issue because gasoline releases from leaking underground storage tanks are widespread, with over 443,568 releases confirmed in the USA as of 2003 (USEPA, 2004). Therefore, a better understanding of the potential impacts of ethanol on such groundwater pollution events and related remediation activities is warranted.

The biodegradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) can be hindered by the presence of ethanol, which is often degraded preferentially and contributes to the depletion of nutrients and electron acceptors (e.g., O_2) that would otherwise be available to support BTEX biodegradation (Da Silva and Alvarez 2002; Ruiz-Aguilar et al. 2002a). In addition, high ethanol concentrations (>10% v/v) expected initially near the source could exert a co-solvent effect that enhances BTEX solubility and migration (Da Silva & Alvarez 2002; Powers et al. 2001b; Rao et al. 1990). Therefore, ethanol may hinder BTEX natural

attenuation, which could result in longer BTEX plumes and a greater risk of exposure (Ruiz-Aguilar et al. 2002b). This could discourage the acceptability of natural attenuation at some sites, and stimulate a shift of cleanup decisions towards engineered remediation approaches.

BTEX bioremediation efforts often rely on the addition of oxygen and nutrients to stimulate aerobic biodegradation, with success often limited by the ability to distribute the stimulating materials throughout the contaminated zone (NRC 2000). However, aerobic bioremediation of ethanol-containing BTEX plumes could be technically difficult and prohibitively expensive. Specifically, ethanol would be present at much higher concentrations than BTEX, which would significantly exacerbate the biochemical oxygen demand (BOD) and nutrient requirements of the system, and possibly contribute to clogging due to excess microbial growth on ethanol if (high-yield) aerobic conditions are maintained. Therefore, anaerobic bioremediation strategies should be considered for the cleanup of gasohol releases, especially near the source zone, which is invariantly anaerobic.

Although slower than aerobic biodegradation, anaerobic microbial metabolism of toluene, ethylbenzene and xylenes is well documented (Ball & Reinhard 1996; Morgan et al. 1993). In addition, recent studies have shown that benzene, which is the most toxic of the BTEX compounds and the most recalcitrant in the absence of oxygen, can also be degraded anaerobically under nitrate-reducing (Burland & Edwards 1999; Coates et al. 2001), iron-reducing (Lovley et al. 1996), sulfate-reducing (Anderson & Lovley 2000; Coates et al. 1996), and methanogenic conditions (Ficker et al. 1999). Recent studies have suggested that anaerobic strategies for the *in situ* bioremediation of BTEX-contaminated aquifers may be as preferable as aerobic approaches because anaerobic electron acceptors are relatively inexpensive, can be easily added to the subsurface, and are chemically more stable than oxygen (Cunningham & Reinhard 2002; Finneran & Lovley 2001). Anaerobic ethanol biodegradation would also result in less biomass accumulation (and related clogging problems) because cell yield coefficients are significantly lower for anaerobic than for aerobic processes. However, the feasibility of stimulating the biodegradation of BTEX–ethanol mixtures through anaerobic electron acceptor amendments has not been evaluated.

This paper addresses the potential to enhance the anaerobic bioremediation of BTEX–ethanol mixtures by increasing the electron acceptor pool through the addition of sulfate, chelated Fe(III) or nitrate. Concentration profiles were compared along the length of flow-through aquifer columns to investigate geochemical transitions and spatial variation in biodegradation efficiency before and after biostimulation. The role of molecular oxygen as an adjunct electron acceptor under microaerophilic conditions was also addressed.

Materials and Methods

General Approach

Four aquifer columns were used to simulate the natural attenuation of BTEX–ethanol mixtures (phase 1, lasting 80 days) and to investigate the feasibility of enhancing biodegradation through the addition of anaerobic electron acceptors (phase 2, lasting 1 year). All columns were continuously fed with BTEX (i.e., benzene 0.5–10 mg l^{-1}, toluene 0.5–10 mg l^{-1}, ethylbenzene 0.2–2.5 mg l^{-1}, *m* + *p*-xylenes 0.2–2.5 mg l^{-1}, *o*-xylene 0.2–2.5 mg l^{-1}) and ethanol (30–110 mg l^{-1}) dissolved in synthetic groundwater. During phase 2, the influent to three of the columns was amended with either K_2SO_4 (218 mg l^{-1}), Fe[III]–EDTA (4.2 g l^{-1}) or $NaNO_3$ (171 mg l^{-1}) to stimulate anaerobic bioremediation of BTEX. Chelated-Fe(III) was used to facilitate its distribution and enhance its bioavailability, and it was added at similar concentrations used in other studies (Lovley et al. 1994, 1996). Influent electron acceptor concentrations were equivalent on an oxidation capacity basis (i.e., about 11 meq l^{-1}), and exceed the theoretical stoichiometric requirements for the mineralization of the added BTEX. The fourth column was poisoned with 15 mg l^{-1} of Kathon® CG/ICP biocide (5-Chloro-2-methyl-3(2H)-isothiazolone and 2-Methyl-3(2H)-isothiazolone solution; Sigma-Aldrich) and was used as a control to distinguish biodegradation from any potential abiotic losses (e.g., volatilization).

Glass columns (2.5-cm inner diameter, 30 cm long) were equipped with six sampling ports (located at 3, 6, 10, 15, and 25 cm from the inlet) to obtain concentration profiles, and were packed

with aquifer material from a BTEX-contaminated site in Travis Air Force Base, CA. The aquifer material sample was drained for 2 days inside a Coy anaerobic chamber ($N_2/CO_2/H_2$: 80/10/10 v/v), stored at 4 °C for three months, and homogenized prior to transferring it into the columns. Soil chemical and physical analyses were conducted by Minnesota Valley Testing Laboratories, Inc. (Table 1). The columns were packed as described elsewhere (Alvarez et al. 1998), to ensure that no air bubbles were trapped, and were continuously fed in an upflow mode at about 3 ml h^{-1} using a gas-tight syringe pump (Harvard Apparatus Mod. 22). Effective porosity (η_e) values ranged from 0.52 to 0.55. The flow velocities ($\sim 3 \times 10^{-6}$ m s^{-1}) were within the typical range of groundwater flow velocities (Domenico & Schwartz 1998). Approximately 1 day was required to displace one pore volume. Columns were operated in the dark and at room temperature (18–22°C).

BTEX and ethanol biodegradation efficiencies were determined for each column after 3 pore volumes had been exchanged following the replacement of a feed syringe. Efficiency was calculated as [(Co-C)/Co × 100%]. Whether differences in biodegradation efficiency were statistically significant was determined by a Kruskal–Wallis test (at 95% confidence; $p < 0.05$) using Minitab software version 13.1 (Minitab Inc., State College, PA). This non-parametric test, which ranks data from low to high and then analyzes the ranks (Lehmann 1975), is very robust to test differences in population medians (Johnson & Mizoguchi 1978). Two-sample Student's *t*-tests (Freedman et al. 1998) were also performed to determine if average BTEX biodegradation efficiencies were significantly different between the two phases.

Table 1. Physical–chemical characteristics of the aquifer material utilized

Component	Value
pH	6.9
Organic matter (%)	0.9
Salts electrical conductivity (mmhos cm^{-1})	0.2
Cation exchange capacity (meq [100 g]$^{-1}$)	4.2
P (mg l^{-1})	14
K (mg l^{-1})	70
Ca (mg l^{-1})	400
Mg (mg l^{-1})	240
Na (mg l^{-1})	70
S (mg l^{-1})	30
Zn (mg l^{-1})	0.8
Cu (mg l^{-1})	2.4
Mn (mg l^{-1})	70
Fe (mg l^{-1})	109
B (mg l^{-1})	0.6

Basal mineral medium

The mineral medium used to feed the columns was based on the synthetic groundwater recipe of Von Gunten & Zobrist (1993), except that sodium carbonate (3.9 mM) was added as a buffer and sodium nitrate was replaced by ammonium chloride (0.3 mM), a nitrogen source that cannot be used as electron acceptor. The medium composition was the following (in mg l^{-1}): KH_2PO_4 (531); K_2SO_4 (40); NH_4Cl (16); $MgCl_2 \cdot 6H_2O$ (12); $CaCl_2$ (6.7); $Ni(NO_3)_2 \cdot 6H_2O$ (0.002); $CuSO_4 \cdot 5H_2O$ (0.002); $ZnSO_4 \cdot 7H_2O$ (0.002); $CoSO_4 \cdot 7H_2O$ (0.002); $(NH_4)_6Mo_7O_{24}$ (0.001); and H_3BO_3 (0.0004). Appropriate electron acceptors were also added as described previously.

The medium was purged with air/CO_2 (95:5 v/v), and the influent dissolved oxygen concentration was about 8 mg l^{-1} during phase 1 and part of phase 2. However, oxygen was rapidly consumed near the inlet (as shown by potentiometric measurements) because the BOD exerted by ethanol greatly exceeded the available oxygen. Dissolved oxygen was not removed from the influent during the initial 133 days of phase 2 to simulate that the anaerobic core of BTEX plumes is often subject to some oxygen entrainment from surrounding, aerobic groundwater. Oxygen was removed from the influent (< 1 mg l^{-1}) for the remainder of phase 2 (which lasted over one year) by purging the medium with N_2:CO_2 gas (80:20 v/v) to evaluate its effect on BTEX biodegradation efficiency.

Analytical methods

Aqueous samples (1-mL) were collected directly from the columns sampling ports using a gas-tight syringe for further analysis of BTEX, ethanol, methane, acetate, sulfate, nitrate and dissolved iron (II). BTEX, ethanol and methane were analyzed by gas chromatography using a Hewlett Packard (Hewlett-Packard Co., Palo Alto,

CA) 5890 Series II gas chromatograph (GC) equipped with a HP 19395A headspace autosampler and flame-ionization detector (FID). Separation was achieved using a J&W Scientific DB-WAX column (Agilent Technologies, Palo Alto, CA) at 85–110 °C (1.66 °C/min).

Acetate, nitrate and sulfate were analyzed in a Dionex 4500i ion chromatograph with an AS4A separation column (Dionex Company, Sunnyvale, CA), followed by chemical suppression and conductivity detection. FE(ii) was analyzed by 1,10-Phenathroline method. The pH was measured at column sampling ports by inserting a microelectrode MI-16/800 (Microelectrodes (Inc., bedford, NH) connected to a 16–702 Flow-Through standard hydrogen electrode (SHE) reference (Beckman Instrument Inc., Fullerton, CA). Oxidation–reduction potential (ORP) was measured similarly using a microelectrode MI-16/800 connected to a 16–702 flow-thru she and to the pH meter.

Results and Discussion

Phase 1 – natural attenuation

BTEX and ethanol losses were small (< 20%) in the poisoned column (Figure 1), implying that high removal efficiencies in non-sterile columns were mainly due to biodegradation. Prior to ethanol addition, the influent dissolved oxygen (8 mg l^{-1}) exceeded the BOD exerted by the added hydrocarbons (total BTEX ca. 2 mg l^{-1}), and extensive BTEX biodegradation was observed (Figure 2a). This aquifer material was obtained from a BTEX contaminated site, and previous microcosm experiments had shown BTEX biodegradation activity under both aerobic and anaerobic conditions (Ruiz-Aguilar et al. 2002a).

BTEX biodegradation efficiencies decreased significantly following the addition of ethanol (Table 2). The preferential biodegradation of ethanol near the inlet (Figure 2b) rapidly consumed the dissolved oxygen that could have been otherwise available for BTEX biodegradation, and relatively high BTEX concentrations were detected in the effluent (> 1 mg l^{-1} total BTEX) even after 80 days of acclimation. The high electron acceptor demand exerted by ethanol created strongly reducing conditions, decreasing the ORP from

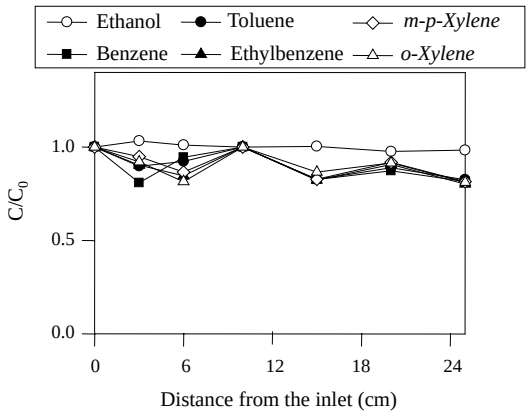

Figure 1. Normalized BTEX and ethanol concentrations in the poisoned column (control) (influent total BTEX = 30 mg l^{-1}).

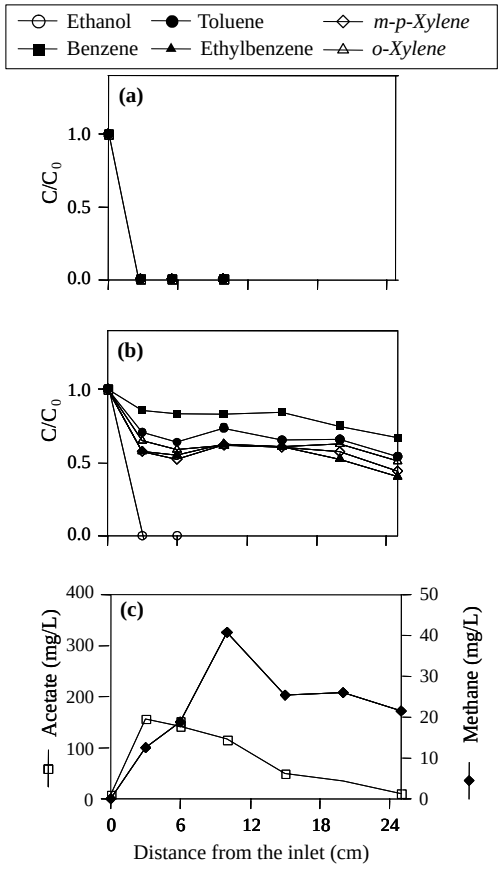

Figure 2. Normalized BTEX concentration profiles are shown in the absence of ethanol (influent total BTEX = 2 mg l^{-1}) (a), and after 80 days of ethanol addition (105 mg l^{-1}) (b) Panel (c) depicts acetate and methane concentration profiles in the column fed BTEX and ethanol.

Table 2. BTEX and ethanol biodegradation efficiencies in columns simulating natural attenuation (phase 1, before and after ethanol addition) and anaerobic biostimulation (phase 2). For these data, dissolved oxygen (8 mg l^{-1}) was present in the influent solution

Compound	Average Biodegradation (%) ± one standard deviation				
	Phase 1		*Phase 2* (electron acceptor added) + EtOH		
	None (no EtOH)	None (+ EtOH)	Sulfate (218 mg l^{-1} as K_2SO_4)	Fe(III)–EDTA (4.2 g l^{-1})	Nitrate (171 mg l^{-1}as $NaNO_3$)
Benzene	100	32 ± 5	72 ± 7	79 ± 7	75 ± 7
Toluene	100	49 ± 29	94 ± 20	90 ± 16	98 ± 2
Ethylbenzene	100	77 ± 26	74 ± 34	82 ± 26	91 ± 12
m + *p*-Xylenes	100	31 ± 28	77 ± 25	79 ± 24	74 ± 20
o-Xylene	100	26 ± 28	61 ± 35	82 ± 22	72 ± 20
Ethanol	NA	91 ± 20	99 ± 1	96 ± 17	99 ± 1

50 mV to about −100 mV near the inlet (data not shown). Lower ORP represents decreased thermodynamic feasibility of BTEX oxidation.

Acetate, which is a common byproduct of anaerobic ethanol biodegradation, transiently accumulated up to 160 mg l^{-1}, and its biodegradation coincided with methane production (Figure 2c). Apparently, acetate served as a substrate for methanogenesis, which lead to the accumulation of methane (42 mg l^{-1}) above its solubility limit (~24 mg l^{-1} at 1 atm and 20 °C). Whether extensive ethanol-induced methanogenesis could pose an explosion hazard was not investigated.

BTEX biodegradation is known to occur under methanogenic conditions (Ficker et al. 1999), but this process is relatively slow and the ubiquity of anaerobic BTEX degraders in methanogenic consortia has not been established. On the other hand, BTEX biodegradation appears to proceed more frequently and faster under more oxidizing conditions, which motivated us to investigate whether injecting selected electron acceptors could enhance anaerobic BTEX biodegradation in the presence of ethanol.

Phase 2 – anaerobic biostimulation

The addition of nitrate, sulfate or Fe(III)–EDTA to the ethanol-amended, methanogenic columns significantly enhanced BTEX biodegradation efficiencies (Table 2), even though influent BTEX concentrations were increased in phase 2 to about 30 mg l^{-1} total BTEX. The only exception was ethylbenzene, which had already exhibited high biodegradation efficiency during phase 1, leaving little room for improvement. The increased biodegradation of benzene from an average of 32% during phase 1 up to greater than 70% for all biostimulated columns is particularly noteworthy because benzene is commonly reported to be recalcitrant under anaerobic conditions (Cunningham et al. 2001; Da Silva & Alvarez 2002; Hutchins 1991; Ruiz-Aguilar et al. 2002a). Nevertheless, it should be noted that dissolved oxygen (8 mg l^{-1}) was present in the column influent during this stage to simulate oxygen entrainment into an anaerobic plume from surrounding aerobic groundwater.

The role that oxygen played in the biodegradation of benzene is unclear, although it is unlikely that sufficient oxygen was available to completely oxidize to CO_2 all the influent benzene (about 8 mg l^{-1}). This would have required about 24 mg l^{-1} dissolved oxygen, ignoring the BOD of ethanol and other BTEX compounds. Several studies have reported benzene biodegradation in anaerobic experiments where oxygen intrusion occurred on purpose (Alvarez & Vogel 1995; Durant et al. 1999) or inadvertently (Anid et al. 1993; Major et al. 1991). In such cases, molecular oxygen might have been used by the microbial consortia as a co-substrate during initial benzene biotransformations, which might have produced suitable substrates for anaerobic biodegradation (Yerushalmi et al. 1999). This untested hypothesis suggests that anaerobic electron acceptors such as sulfate, chelated Fe(III) or nitrate could enhance benzene biodegradation even in the absence of anaerobic benzene degraders by accelerating the mineralization of ethanol or other substrates that

are labile under anaerobic conditions. This would alleviate the BOD of the system and increase the likelihood that entrained oxygen would be used for the biodegradation of residual benzene as a subsequent aerobic (polishing) step.

Dissolved oxygen was subsequently purged from the medium (< 1 mg l^{-1}) to evaluate the role that it had played on BTEX biodegradation. Toluene, which is commonly reported to degrade under anaerobic conditions (Heider et al. 1998; Ruiz-Aguilar et al. 2002a) continued to be effectively removed in all columns; it was the only hydrocarbon to disappear ($> 99\%$) in the sulfate-amended (Figure 3a) and nitrate-amended columns (Figure 5a), and it also exhibited the highest biodegradation efficiency (85%) under Fe(III)-reducing conditions (Figure 4a). On the other hand, benzene biodegradation efficiencies decreased significantly ($< 30\%$) after purging the oxygen out of the media, even after 1 year of acclimation (Figures 3a, 4a, and 5a). In this case, benzene losses were partly due to increased volatilization and to some oxygen entrainment near the inlet (as indicated by ORP measurements, not shown), exacerbated by inten-

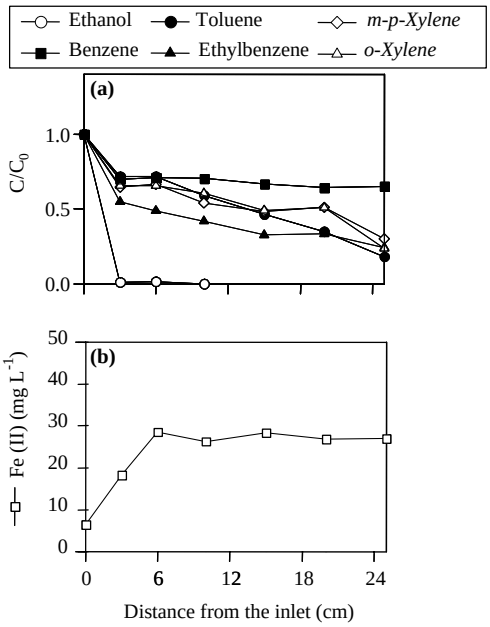

Figure 4. (a) Normalized BTEX and ethanol concentration profiles under iron-reducing conditions after 1 year of acclimation and, (b) Fe(II) concentration profile. Influent concentrations were 25 mg l^{-1} of total BTEX and 91 mg l^{-1} of ethanol. Influent DO was less than 1 mg/l.

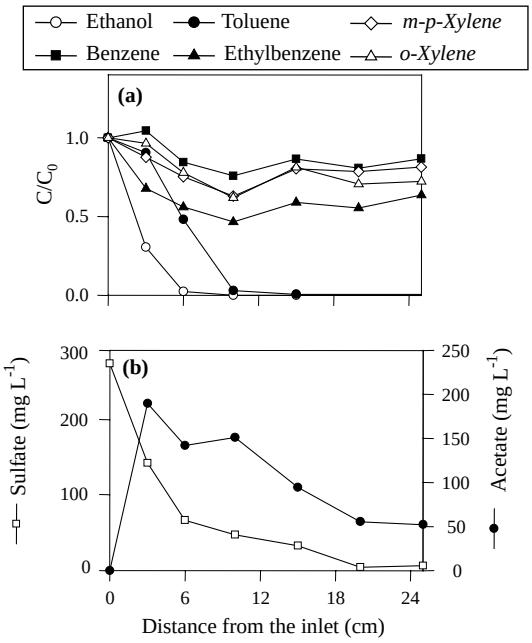

Figure 3. (a) Normalized BTEX and ethanol concentrations under sulfate-reducing conditions after one year of acclimation and, (b) Sulfate and acetate concentration profiles. Influent concentrations were 32 mg l^{-1} of total BTEX and 114 mg l^{-1} of ethanol. Influent DO was less than 1 mg/l.

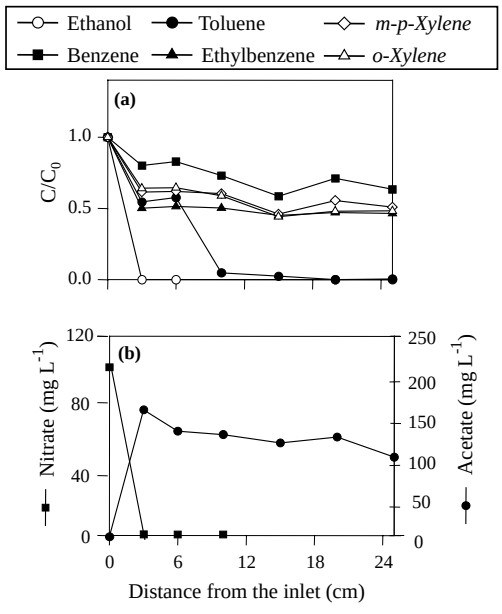

Figure 5. Normalized BTEX and ethanol concentrations under nitrate-reducing conditions after 1 year of acclimation (a) and, corresponding nitrate and acetate concentration profiles (b) Influent concentrations were 33 mg l^{-1} of total BTEX and 103 mg l^{-1} of ethanol. Influent DO was less than 1 mg/l.

sive sampling over 1 year. The fact that benzene concentrations remained constant downgradient of the first sampling port (e.g., Figure 3a) suggests that no significant benzene biodegradation should be attributed to strictly anaerobic biodegradation. Anaerobic benzene biodegradation is not always observed, and when it occurs it is often after acclimation periods greater than 1 year (Kazumi et al. 1997). The persistence of benzene in this experiment reflects that inducing the desired anaerobic respiration mode through electron acceptor amendment does not guarantee the presence and proliferation of anaerobic, benzene degrading microorganisms (Weiner & Lovley 1998).

These results corroborate previous studies where sulfate and nitrate injection was very effective in stimulating *in situ* toluene biodegradation, but not benzene (Cunningham et al. 2001; Reinhard et al. 1997). Unlike benzene, toluene has a methyl group that is electrophilic. This apparently facilitates nucleophilic attack by water (Altenschmidt & Fuchs 1991) or by anaerobic catabolic enzymes such as benzylsuccinate synthase (Heider et al. 1998), making it more labile under anaerobic conditions.

For all columns, electron acceptor utilization was relatively rapid near the inlet (Figures 3b, 4b, and 5b), and coincided with the highest ethanol biodegradation activity (Figures 3a, 4a, and 5a). In all cases, electron acceptor amendments fully inhibited methanogenesis, thus eliminating a potential risk of explosion. Under conditions typical of natural systems, sulfate [$E_h^0(W) = -0.22V$ for SO_4^{2-} to HS^-], ferric iron [$E_h^0(W) = +0.36V$ for $FeOOH_{(s)}$ to $FeCO_{3(s)}$], and nitrate [$E_h^0(W) = +0.42V$ for NO_3^- to N_2] are thermodynamically more favorable electron acceptors than CO_2 [$E_h^0(W) = -0.25V$ for CO_2 to CH_4] (Schwarzenbach et al. 1993), and sulfate-, iron-, or nitrate-reducing bacteria generally out-compete methanogens for hydrogen or other fermentation products that could be utilized as substrates to make methane (Anderson & Lovley 1997).

Sulfate, iron(III) and nitrate have different advantages and limitations for anaerobic biostimulation purposes (Table 3). Sulfate can accept more electrons than the other tested compounds (8 e^- equivalents per mole), and its injection has been shown to increase benzene biodegradation in groundwater contaminated with petroleum hydrocarbons (Anderson & Lovley 2000). However, it has a relatively low oxidation potential, and it is subject to a secondary drinking water standard (250 mg l^{-1}, based on taste, odor, and potential laxative effects) that could restrict its use. Fe(III) has a relatively high oxidation potential and its addition has also been shown to enhance benzene mineralization (Lovley et al. 1996). Chelated-Fe(III) could be used to overcome iron bioavailavility and solubility problems (Lovley et al. 1994). However, the relatively low electron assimilation capacity of Fe(III) (1 e^- equivalent per mole) could require the injection of large quantities, and the EDTA chelator could affect microbial activity (Haas & Dichristina 2002) or act as a competing carbon source that hinders the degradation of target pollutants.

Nitrate has the highest oxidation potential, and has a demonstrated high efficacy to stimulate alkylbenzene biodegradation *in situ* (Hutchins et al. 1991). Nonetheless, nitrate injection is subject to regulatory concerns due to its potential to cause methemoglobinemia, and it is often inefficient at stimulating benzene biodegradation under strictly anoxic denitrifying conditions (Hutchins et al. 1998). Note that multiple electron acceptors could be combined to increase the electron acceptor pool while meeting regulatory limits for drinking water (Cunningham et al. 2001; Durant et al. 1999). Whether this would result in synergistic interactions that improve the biodegradation efficiency of BTEX–ethanol mixtures remains to be determined.

A potential negative effect of biostimulation is the accumulation of biomass or precipitates that decrease aquifer porosity and permeability, hindering nutrient delivery and mass transport (Clement et al. 1996; Cunningham et al. 1991). In these experiments, occasional clogging was experienced in the column amended with EDTA–Fe(III), but not in the columns augmented with nitrate or sulfate. Apparently, iron precipitation caused a significant decrease in effective porosity that hindered permeability, which represents a serious practical limitation for the injection of chelated Fe(III) as a biostimulatory compound. It is unlikely that clogging was due to microbial growth because anaerobic microorganisms typically have low growth yields (Rittmann & McCarty 2001) and should not contribute to a significant decrease in porosity. For example, soil microbial concentrations rarely exceed

Table 3. Advantages and disadvantages of selected anaerobic electron acceptors utilized during biostimulation processes

Electron acceptor	Favorable attributes	Concerns
Sulfate	Highly soluble	H_2S can be bacteriostatic at concentrations > 200 mg l^{-1}
	High assimilative capacity (8 e^- equivalents accepted per mol of SO_4^{2-} reduced to H_2S)	Aesthetic (smell, taste) drinking water problems and potential laxative effect at 250 mg l^{-1}
	H_2S could complex and precipitate with inhibitory heavy metals	Low oxidation potentia*: $E_h^0(W) = -0.22V$ for SO_4^{2-} to HS^-
Iron (III)	High oxidation potential*: $E_h^0(W) = +0.36V$ for $FeOOH_{(s)}$ to $FeCO_3(s)$	
		Insoluble – must be added with a chelator (e.g., EDTA) which may be viewed as a co-contaminant or inhibitor of microbial activity
	No toxic byproducts	Clogging due to oxide precipitation
		Low assimilative capacity [only 1 e^- equivalent accepted per mol of Fe(III) reduced to Fe(II)]
Nitrate	Highly soluble	Nitrate is a regulated pollutant (MCL 10 ppm) and nitrite could be an undesirable byproduct (methamoglobinemia, bacteriostatic at ≥ 100 mg l^{-1})
	Can also be used as a nutrient	Permeability decrease due to $N_{2(g)}$ formation during denitrification
	High oxidation potential*: $E_h^0(W) = +0.42V$ for NO_3^- to N_2	
		Benzene is commonly recalcitrant under strictly anoxic denitrifying conditions
	High assimilative capacity (5 e^- equivalents accepted per mol of NO_3^- reduced to N_2)	

*Oxidation potential is for conditions typical of natural waters (pH = 7 and $[HCO_3^-]$ = 1 mM) (Schwartzenbach et al. 1993).

10^{11} cells g^{-1} (Atlas & Bartha 1998). This concentration would decrease porosity by less than 2%, assuming a dry cell weight (dcw) of 1.33×10^{-13} g (Bratbak 1985), a soil bulk density (ρ_{bulk}) of 1600 g l^{-1}, and a biomass density (ρ_{cell}) of 1100 g l^{-1} (Bratbak & Dundas 1984) (i.e., the pore volume fraction occupied by the microorganisms is calculated as $(X)(dcw)(\rho_{bulk}/\rho_{cell})$ (Clement et al. 1996).

Conclusions

The high BOD exerted by ethanol in gasoline could hinder the feasibility of aerobic bioremediation of aquifers contaminated by gasohol releases. Experiments with flow-through aquifer columns support the notion that anaerobic biostimulation through nitrate, sulfate, or chelated Fe(III) addition could be a practical approach to enhance the cleanup process, not only by facilitating anaerobic biodegradation of ethanol and some BTEX compounds (e.g., toluene) but also by alleviating the BOD of the system for more efficient biotransformation of any residual BTEX in a subsequent aerobic (polishing) stage.

The selection of appropriate electron acceptor(s) should consider whether the local groundwater chemistry is conducive to potential clogging due to mineral formation and precipitation (e.g., FeS). The potential accumulation of inhibitory byproducts (e.g., NO_2^- or H_2S) should also be considered. Nevertheless, the likely cost-effectiveness of anaerobic biostimulation is expected to outweigh these potentially preventable problems.

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