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Treatment of groundwater contaminated with PAHs, gasoline hydrocarbons, and methyl *tert*-butyl ether in a laboratory biomass-retaining bioreactor

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Abstract

In this study, we investigated the treatability of co-mingled groundwater contaminated with polycyclic aromatic hydrocarbons (PAHs), gasoline hydrocarbons, and methyl *tert*-butyl ether (MtBE) using an *ex-situ* aerobic biotreatment system. The PAHs of interest were naphthalene, methyl-naphthalene, acenaphthene, acenaphthylene, and carbazole. The gasoline hydrocarbons included benzene, toluene, ethyl benzene, and *p*-xylene (BTEX). Two porous pot reactors were operated for a period of 10 months under the same influent contaminant concentrations. The contaminated groundwater was introduced into the reactors at a flow rate of 4 and 9 l/day, resulting in a hydraulic retention time (HRT) of 32 and 15 h, respectively. In both reactors, high removal efficiencies were achieved for the PAHs (>99%), BTEX and MtBE (>99.7%). All the PAHs of interest and the four BTEX compounds were detected at concentrations less than 1 μg/l throughout the study duration. Effluent MtBE from both reactors was observed at higher levels; nevertheless, its concentration was lower than the 5 μg/l Drinking Water Advisory for MtBE implemented in California.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are major constituents of crude oil and fossil fuels and are present in creosote, a waste product of coal gasification (Freeman & Cattell 1990). These compounds are among the most frequently encountered environmental pollutants as a result of past mismanagement at town gas sites. Monocyclic aromatic hydrocarbons are also very frequently encountered environmentally as a consequence of leakage from underground gasoline storage tanks; inadvertent spills at production wells, refineries, and distribution terminals; improper disposal, and accidents during transport. Commonly encountered monocyclic aromatic hydrocarbons include benzene, toluene, ethyl benzene, and xylene (BTEX), while naphthalene, anthracene, and phenanthrene are among the many PAHs contaminating town gas sites. BTEX are relatively water-soluble as compared to PAHs, a property that enables them to migrate along the subsurface contaminating drinking water sources (Chen & Taylor 1997). However, PAHs are characterized by low water solubility, except for smaller molecules like naphthalene, and a high affinity for association with organic matter in soil and water (Kilbane II 1998). They are environmentally persistent due to their relative chemical stability and resistance to biodegradation.

Besides BTEX, gasoline may contain methyl *tert*-butyl ether (MtBE). MtBE is the most

common gasoline oxygenate used in reducing ozone (O_3) and carbon monoxide (CO) emissions in certain areas around the country that exceed the National Ambient Air Quality Standards (NAAQS). Due to its high water solubility, poor interaction with aquifer material, and relative recalcitrance in the environment, MtBE migrates faster and farther than BTEX in groundwater (Davidson & Creek 2000).

BTEX and PAHs exhibit toxic and carcinogenic properties; consequently, the US EPA has classified them as priority pollutants (Dean 1985; Fujikawa et al. 1993). Similarly, MtBE has been classified as a potential human carcinogen (USEPA 1997). Therefore, these contaminants must be reduced to a very low level in groundwater prior to their intended use. Among all remediation technologies for treating aquifers contaminated with BTEX, PAHs, and MtBE, bioremediation appears to be the most cost-effective, energy efficient, and environmentally sound approach. Extensive research has shown the capability of many microorganisms to degrade BTEX under aerobic (Deeb & Alvarez-Cohen 1999; Mason et al. 2000; Sedran et al. 2002; Van Agteren et al. 1998) anaerobic (Chen & Taylor 1997; Holliger & Zehnder 1996), and anoxic (Schmitt et al. 1996) conditions. Many PAHs were found to be amenable to biodegradation both aerobically (Bouchez et al. 1996; Harayama 1997; Van Agteren et al. 1998; Yuan et al. 2000) and anaerobically (Johnson & Ghosh 1998; Van Agteren et al. 1998). In addition, several species of white rot fungi have been found to attack and degrade a wide spectrum of PAHs (Gramss et al. 1999; Harayama 1997; Novotnỳ et al. 1999). Most of the research on the biodegradation of MtBE has focused on aerobic microbial transformation (Deeb et al. 2000; Fortin et al. 2001; Kharoune et al. 2001; Mo et al. 1997; Stocking et al. 2000; Wilson et al. 2001). However, a few studies investigated the anaerobic degradation of MtBE, mainly under sulfate-reducing (Somsamak et al. 2001) and methanogenic (Stocking et al. 2000) conditions.

In this study, we investigated the treatability of groundwater from a former manufactured gas plant (MGP) site located in Millville, NJ. The groundwater was contaminated with BTEX, MtBE, and *tert*-butyl alcohol (tBA) due to the presence of a gasoline spill in the same area as the MGP site and some PAHs from the abandoned MGP operations (naphthalene, 2-methylnaphthalene, acenaphthene, acenaphthylene, and carbazole). Two aerobic porous pot reactors were used to evaluate the biodegradation of these contaminants in the co-mingled plume. The objective was to demonstrate that real groundwater from the Millville MGP site contaminated with MtBE, tBA, BTEX, and PAHs can be treated to a concentration of 5 μg/l, the Drinking Water Advisory for MtBE implemented in California, or less on a consistent basis over a long period of time by aerobic treatment in *ex-situ* biomass-retaining reactors.

Materials and methods

Chemicals

tBA and the PAHs of interest (naphthalene, 2-methylnaphthalene, acenaphthene, acenaphthylene, and carbazole) were purchased from Aldrich, Milwaukee, WI. Benzene, toluene, ethyl benzene, *p*-xylene, MtBE, dichloromethane (DCM), and methanol were all purchased from Fisher Scientific, Pittsburgh, PA. All the above chemicals were greater than 99% pure except for 2-methylnaphthalene and carbazole, which were 97 and 96% pure, respectively.

Contaminated groundwater

Groundwater was collected in four 55-gallon stainless steel drums from the Superfund MGP site located at the Millville, NJ. Groundwater was collected every 2 months and stored under a blanket of nitrogen gas to prevent aerobic degradation prior to and during shipment to Cincinnati. Maximum concentrations of the contaminants are listed in Table 3 as reported to us by EPA Region 2. However, we measured higher concentrations of some of these compounds, especially the BTEX compounds, periodically prior to treatment. Maximum measured concentrations of benzene, toluene, ethyl benzene, and *p*-xylene in the contaminated groundwater drums were approximately 4,000, 26,000, 14,000, and 11,000 μg/l, respectively. A total of 20 drums were collected and shipped to Cincinnati at a frequency of four drums every 4 months. Relevant physical properties as well as Maximum Contaminant Levels (MCL) established by the US EPA of the contaminants of interest are listed in Table 1 below.

Table 1. Relevant physical properties and MCLs of targeted contaminants

Chemical	MCL (mg/l)[a]	logK_{ow}^{d}	Solubility (mg/l)[d]	Henry's Law Constant (atm-m^3/mole)[d]
MtBE	[b]	0.94	36,200	5.87×10^{-4}
tBA	[b]	0.35	Miscible	1.18×10^{-5}
Benzene	0.005	2.13	1,770	5.50×10^{-3}
Toluene	1	2.73	530	6.71×10^{-3}
Ethyl Benzene	0.7	3.15	169	8.75×10^{-3}
p-Xylene	10	3.15	180	5.70×10^{-3}
Naphthalene	[c]	3.34	31	4.24×10^{-4}
2-Methyl naphthalene	[c]	4.00	25	5.00×10^{-4}
Acenaphthene	[c]	3.96	3.8	1.20×10^{-4}
Acenaphylene	[c]	3.72	3.93	2.80×10^{-4}
Carbazole	[c]	3.72	1.2	8.60×10^{-7}

[a]USEPA 2003.
[b]MCL not established.
[c]Cumulative concentration not exceeding 0.4 μg/l.
[d]Lide 2005 (25 °C).

Media and culture conditions

The reactors were seeded with a mixture of a bacterial culture growing on MtBE, BTEX, and ethanol in continuous flow bioreactors and a second culture previously grown on crude oil. The crude oil culture had been preserved by freezing in glycerol and storing at −70 °C. The culture was thawed prior to inoculation. All the aforementioned microbial cultures were previously grown under aerobic conditions and at ambient temperature before being used to inoculate the reactors (Morrison et al. 2001; Pruden et al. 2001; Zein et al. 2004). An acclimation period of 2 weeks was required before efficient biodegradation of the target contaminants was observed in the two reactors. The only biomass that was wasted was the amount removed during sampling; thus, the sludge age was extremely long in both reactors.

Experimental design

Two porous pot reactors were used over the 10-month period of this study, each constructed of 304-stainless steel with an internal diameter of 21.6 cm and a height of 30.5 cm. Each reactor contained a 0.48 cm thick filter grade polyethylene porous pot (Atlas Minerals and Chemicals, Mertztown, PA) with a mean flow pore size of 18–28 μm for the retention and concentration of biomass. The porous pot was built in the form of a cylinder, 19.1 cm in internal diameter and 29.2 cm in overall height, welded to a 21.6 cm diameter base plate (Wilson et al. 2001). The total volume of each reactor was 8 l, while the volume of the porous pot within was 6 l. Biomass in the reactors was maintained completely mixed using magnetically coupled variable speed mixers (Autoclave Engineers, Erie, PA). The reactor setup is illustrated schematically in Figure 1 below.

Contaminated groundwater buffered with sodium carbonate (0.3 g/l) and an acidified nutrient solution containing salts and vitamins (Table 2) essential for biological growth were fed to each reactor using a 2 rpm Masterflex pump (Cole Palmer, Chicago, IL) controlled by electronic

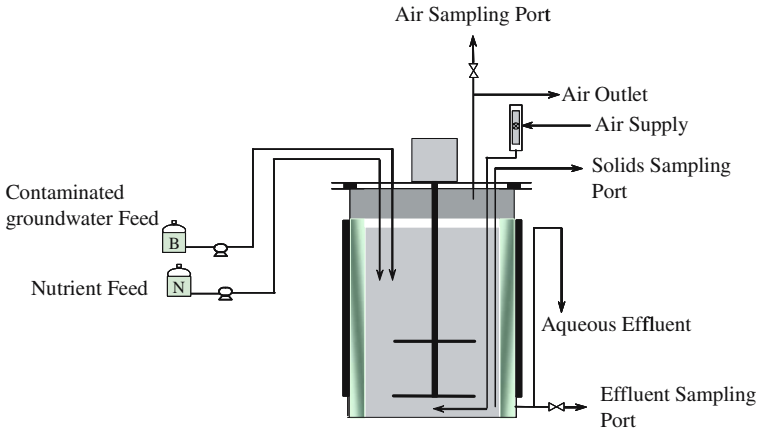

Figure 1. Schematic of the porous pot reactor.

Table 2. Target nutrient concentrations in the porous pot reactors

	Chemical	Concentration (mg/l)	Essential Nutrient	Concentration (mg/l)
Macronutrients	K_2HPO_4	6.90	P	4.00
	$(NH_4)_2SO_4$	93.00	N	19.73
	$FeCl_2{\cdot}4H_2O$	17.25	Fe^{++}	4.85
	$CaCl_2{\cdot}2H_2O$	22.50	Ca^{++}	6.13
	$MgSO_4{\cdot}7H_2O$	69.60	Mg^{++}	6.87
Micronutrients	$CuSO_4{\cdot}5H_2O$	0.11	Cu^{++}	0.03
	$Na_2MoO_4{\cdot}2H_2O$	0.15	Mo	0.06
	$MnSO_4{\cdot}H_2O$	0.13	Mn^{++}	0.04
	$ZnCl_2$	0.23	Zn^{++}	0.11
	$CoCl_2{\cdot}6H_2O$	0.42	Co^{++}	0.10
Vitamins	4-Aminobenzoic acid	0.0151		
	Biotin	0.0059		
	Cyanocobalamine	0.0003		
	Folic acid dihydrate	0.0059		
	Nicotinic acid	0.0151		
	Pantothenic acid	0.0151		
	Pyridoxine hydrochloride	0.0303		
	Riboflavin	0.0151		
	Thiamine hydrochloride	0.0151		
	Thioctic acid	0.0151		

timers (Lindberg Enterprises Inc., San Diego, CA) to obtain the desired flow rates. The C:N:P ratio for both reactors was 23:20:4. The total flow rate delivered to the reactors was 0.4 and 0.2 l/h, accounting for hydraulic retention times (HRT) of 15 and 32 h, respectively. The nutrient solution flow represented 6 and 13% of the total flow, respectively.

The groundwater feed was spiked starting from day 136 of the study with a solution of the PAHs, BTEX, MtBE, and tBA to make up for their loss from the original samples due to volatilization. The concentrations of all contaminants were observed to decrease between the time each drum was opened and until all the contaminated water was used up. In Table 3, the final concentration of contaminants in the feed after spiking is compared to the maximum groundwater concentrations reported by the US EPA. The concentrations of the various compounds in the spike solution were selected after consideration of the concentrations found in the groundwater drums once received.

The pH in both porous pots was maintained between 7.4 and 8.0, while the dissolved oxygen (DO) was controlled at ≥3 mg/l to ensure aerobic conditions in the reactors. Air was supplied to both reactors at a flow rate of 2.5 l/min. Temperature was controlled at 18 °C using a chiller with a propylene glycol/water recirculation mixture.

Table 3. Spiked contaminant concentrations and maximum reported contaminant concentrations in the groundwater

Contaminant	Maximum concentration (μg/l)	Spiked concentration (μg/l)
Naphthalene	1,900	1,000
2-Methylnaphtalene	230	200
Acenaphthylene	33	50
Acenaphthene	11	10
Carbazole	23	20
Benzene (B)	1,900	2,000
Toluene (T)	6,400	7,000
Ethyl benzene (E)	1,900	4,000
p-Xylene (X)	3,100	5,000
Methyl *tert*-butyl ether (MtBE)	2,700	1,000
tert-Butyl alcohol (tBA)	860	200

Analytical methods

Contaminated groundwater and nutrient flow rates, pH, and dissolved oxygen were monitored on a daily basis. The pH of the effluent and the

reactor contents was measured using an Orion Model 720A pH meter (Orion Research Co., Boston, MA). Dissolved oxygen in the porous pots was monitored with a Corning Checkmate II dissolved oxygen sensor (Corning, NY). For the two reactors, analysis of influent and aqueous effluent concentrations of all the contaminants, as well as the gas phase levels of MtBE, tBA, and BTEX, was performed twice a week. Gas phase concentrations of MtBE, tBA, BTEX, and PAHs were determined using a Hewlett Packard 5890 Series II gas chromatograph (GC) (Hewlett Packard, Palo Alto, CA) equipped with a flame ionization detector (FID) and a 60/80 Carbopack B/5% Carbowax 20 M glass column (Supelco, Bellefonte, PA). The detection limit was 0.10 μg/l. Gas sample volumes of 5 ml were collected from the headspace of each reactor to check for volatilization. Aqueous concentrations of MtBE, tBA, and BTEX were measured, according to US EPA Method 524, using a Hewlett Packard 6890 Series II GC/FID equipped with PTA-5 column (30 m, 0.53 mm I.D., and 3 μm film thickness) (Supelco, Bellefonte, PA) and coupled with a heated Purge and Trap (11 min at 40 °C of purging followed by 4 min at 225 °C of desorbing) consisting of a Tekmar Dohrmann 3100 Sample concentrator and a Tekmar Dohrmann AquaTek 70 Liquid Autosampler (Tekmar Dohrmann, Cincinnati, OH). The initial oven temperature of the GC was 35 °C held for 6 min, followed by a ramp of 12 °C/min to 190 °C held for an another 6 min. Method detection limits were 0.06 μg/l for MtBE and tBA and 0.01 μg/l for the BTEX compounds. Sample volumes of 40 ml were collected for this analysis.

The targeted PAHs were extracted from water by Solid Phase Extraction (US EPA Method 3535) using 47 mm ENVI^{TM}-18 DSK SPE disks (Supelco, Bellefonte, PA). The solid phase was later extracted with DCM and the extract concentrated using a TurboVap II Concentration Workstation (Zymark Corporation, Hopkinton, MA). Solid Phase Microextraction (SPME), using Carboxen/polydimethylsiloxane SPME fibers, was performed to assess the adsorption of the PAHs to the biomass flocs in the reactors (Supelco, Bellefonte, PA). PAH levels were then determined, according to US EPA Method TO-13A, by GC/MS with a Hewlett-Packard 5890 series II gas chromatograph coupled with a Hewlett-Packard 5971A mass selective detector (MSD) and a Hewlett-Packard 7673 autosampler. The MSD was operated in the selected ion monitoring (SIM) mode for quantitative analysis of targeted polynuclear aromatic hydrocarbons. An MDN-5 capillary column (30 m long, 0.25 mm i.d., and 0.25 μm film thickness) by Supelco (Bellefonte, PA) was used with the injection port operated in a splitless mode. The injection port was maintained at 290 °C and the transfer line at 320 °C. The oven temperature was initially set at 50 °C for 2 min, ramped to 280 °C at 10 °C per minute and then ramped to 310 °C at 50 °C per minute, and held at 310 °C for 10 min. Method detection limit for the PAHs was 0.01 μg/l. An effluent volume of 1 l was taken from each reactor for solid phase extraction followed by GC/MS analysis of the five PAHs of concern, while only 200 ml of the contaminated groundwater was extracted.

Volatile suspended solids (VSS) were measured weekly by drying a Whatman 934-AH Glass Microfibre filter (Clifton, NJ) at 550 °C for 1 h, filtering the sludge sample, drying at 105 °C for 2.5 h, and finally taking the difference in filter mass upon baking at 550 °C for 2 h (Clesceri et al. 1998).

Results

Throughout the study period, high degradation effectiveness of all contaminants of interest was observed in both porous pots. As shown in Table 4, over 99% of all compounds except for acenaphthylene were degraded at both flow rates studied. Despite being the pollutants with the highest levels in the contaminated groundwater, BTEX compounds were found to be best removed in both reactors ($>99.99\%$). Reactor 1, operating under the higher flow rate, was observed to perform as effectively as reactor 2, suggesting that the lower HRT did not compromise the biodegradation effectiveness of the reactor.

PAHs

Due to variations in their concentrations in the contaminated groundwater in different drums and their volatilization from the drums once opened, influent PAH concentrations were found to fluctuate significantly with time over the first 50 days. These variations were somewhat dampened during the course of the study by spiking the groundwater

Table 4. Average influent and effluent concentrations and percent removal of targeted contaminants in Reactor 1 (flow rate: 0.4 l/h) and Reactor 2 (flow rate: 0.2 l/h)

Contaminant	Average influent concentration (μg/l)	Average effluent concentration (μg/l)		Average total removal efficiency (%)	
		Reactor 1	Reactor 2	Reactor 1	Reactor 2
Naphthalene	630 (408)	0.13 (0.15)	0.16 (0.25)	99.98 (0.87)	99.97 (0.31)
2-Methylnaphthalene	128 (90)	0.03 (0.02)	0.03 (0.04)	99.98 (2.44)	99.97 (2.35)
Acenaphthylene	35.1 (18.3)	0.37 (0.17)	0.38 (0.23)	98.95 (10.06)	98.92 (8.84)
Acenaphthene	10.9 (5.9)	0.01 (0.03)	0.02 (0.06)	99.93 (0.46)	99.83 (0.69)
Carbazole	15.1 (8.9)	0.01 (0.03)	0.03 (0.17)	99.95 (7.67)	99.82 (4.91)
Benzene (B)	2,430 (1,340)	0.27 (0.72)	0.02 (0.01)	99.99 (0.03)	99.99 (0.01)
Toluene (T)	12,370 (13,420)	1.33 (5.90)	0.12 (0.10)	99.99 (0.08)	99.99 (0.01)
Ethyl benzene (E)	3,541 (3,350)	0.31 (0.58)	0.09 (0.04)	99.99 (0.41)	99.99 (0.37)
p-Xylene (X)	5,110 (4,340)	0.36 (0.85)	0.08 (0.04)	99.99 (0.09)	99.99 (0.08)
Methyl *tert*-butyl ether (MtBE)	1,130 (561)	2.86 (2.65)	2.13 (3.25)	99.75 (0.32)	99.81 (0.37)
tert-Butyl alcohol (tBA)	155 (132)	0.39 (0.93)	0.30 (0.44)	99.75 (0.51)	99.80 (0.46)

Numbers in parentheses represent ± 1 standard deviation unit.

fed to the reactors with a concentrated solution of the five PAHs dissolved in a mixture of BTEX, MtBE and tBA. Figure 2 shows the concentrations of the PAHs in the contaminated groundwater and in the effluent of reactor 1 (flow rate: 0.4 l/h). The effluent data of reactor 2 (flow rate: 0.2 l/h) are shown only in Table 4. Influent and effluent samples were not analyzed between days 50 and 75 due to a GC/MS problem that was later fixed. The collected samples were discarded since a long preservation period would result in a significant loss of the PAHs due to volatilization. Influent naphthalene levels were the highest among the PAHs; consequently naphthalene was measured outside the range of the calibration curve in most of the samples. GC/MS analysis of naphthalene in such samples was repeated after dilution.

As a result of the influent groundwater spiking that was initiated on day 136 of the study, effluent concentrations of the PAHs experienced a slight rise in both porous pots. However, the overall objective of the study was met with respect to the five PAHs studied (Figure 2). All the PAHs analyzed were low molecular weight, two-ring compounds that are inherently biodegradable. Even high influent naphthalene concentrations were biodegraded to much less than 1 μg/l. SPME of samples from the effluent as well as from inside the porous pots showed minimal biosorption effects, suggesting that biodegradation was the dominant removal process of these PAHs. All the PAHs, except acenaphthylene, were present at lower concentrations in many of the DCM extracts than the lowest standard in the calibration curve (0.05 mg/l). Concentration of such DCM extracts was required to bring the PAH levels to the calibration curve range. Surrogate recoveries (2-fluorobiphenyl and D10 1-methyl naphthalene) were low (<75%) for some of the effluent samples in both reactors. However, this does not jeopardize meeting performance objectives since the concentrations of contaminants in the effluent were so low that even using a correction factor to account for the poor recoveries does not result in effluent PAH concentrations greater than 1 μg/l.

MtBE, tBA and BTEX

BTEX, MtBE, and tBA concentrations in the influent groundwater also experienced significant fluctuations at the beginning of the study, but this problem was solved by spiking the feed with a concentrated solution of all the contaminants of interest (Figure 3). As shown in Table 4 earlier, influent MtBE and BTEX levels were the highest in the contaminated groundwater with values measured in the mg/l range. Most of the organic carbon available for microbial degradation was provided by MtBE and BTEX compounds.

Due to their high concentrations in the influent, average effluent BTEX, MtBE, and tBA concentrations in both reactors were approximately 10-fold greater than the effluent concentrations of PAHs. However, the 5-μg/l objective was also met

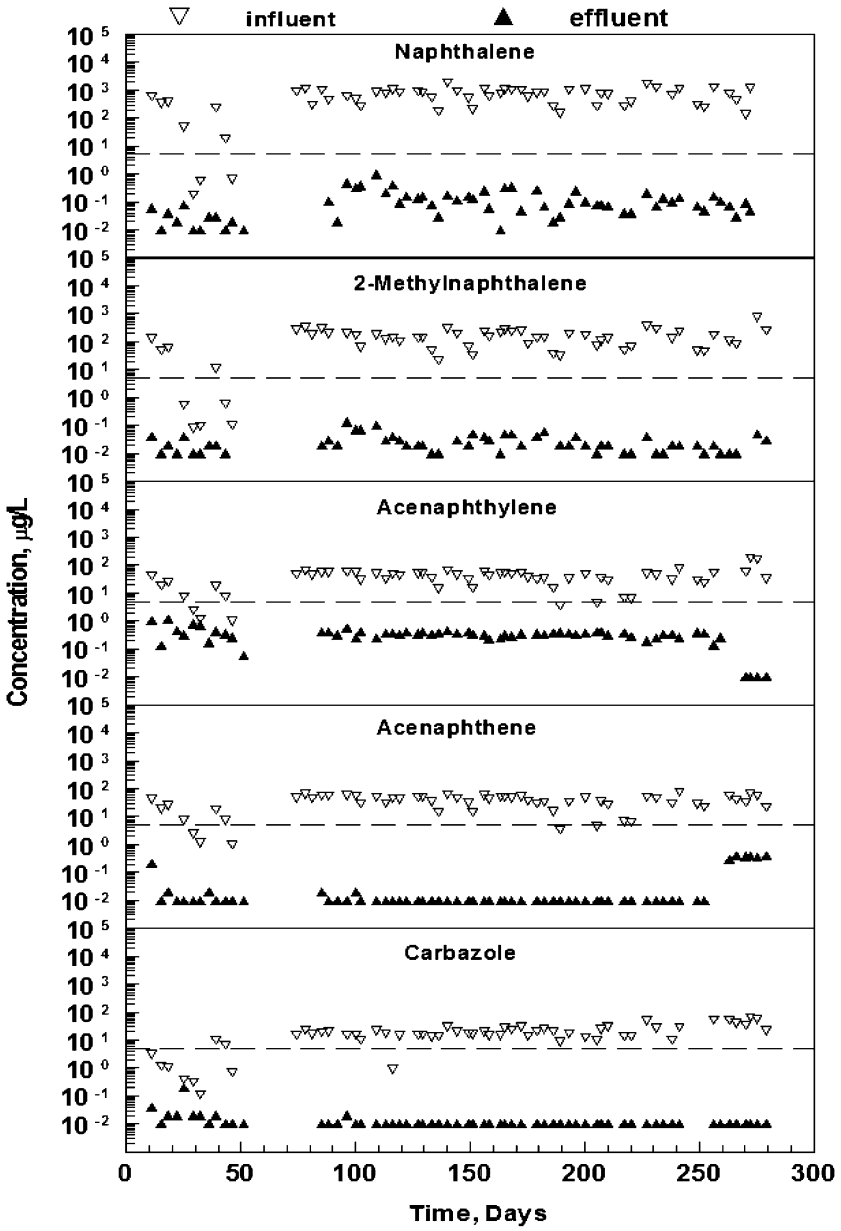

Figure 2. Concentration of PAHs in the contaminated groundwater and in the effluent of Reactor 1 (flow rate: 0.4 l/h). The dashed line corresponds to the 5 μg/l effluent goal.

for these compounds in both reactors (Figure 3). Reactor 2 (flow rate: 0.2 l/h) provided better removal for MtBE, tBA, and all BTEX compounds. Reactor 1 achieved 99.75% removal of both MtBE and tBA while reactor 2 achieved 99.81% removal of MtBE and 99.80% removal of tBA. In any case, the total removal efficiency of BTEX was greater than 99.99% for both reactors.

Finally, gas phase analysis of the contaminants of concern in the headspace of each reactor was performed to account for any volatilization that might have resulted from aeration and mixing. Only MtBE was detected in the headspace during the stable operating conditions of the bioreactors. No peaks corresponding to the BTEX compounds, tBA or the five PAHs of interest were observed in

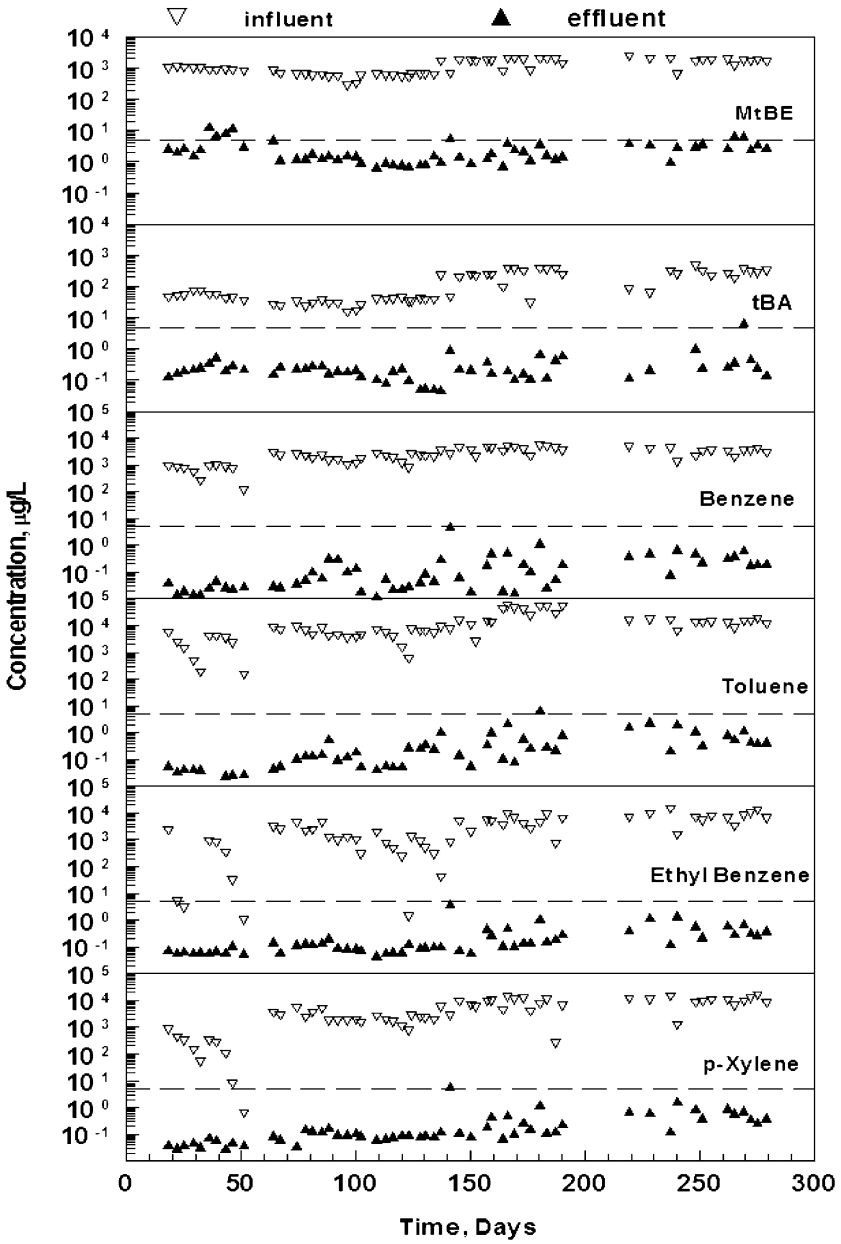

Figure 3. Concentration of MtBE, tBA, and BTEX in the contaminated groundwater and in the effluent of Reactor 1 (flow rate: 0.4 l/h). The dashed line corresponds to the 5 μg/l effluent goal.

the GC chromatograms. Gas phase MtBE concentrations averaged 0.28 and 0.11 μg/l in reactors 1 and 2, respectively. This difference can be attributed to the longer HRT in reactor 2 since a higher HRT results in better biodegradation of the contaminant. As shown in Table 5 below, air stripping accounted for 8% and approximately 5% of the influent MtBE fed to reactors 1 and 2, respectively. Consequently, the biological removal efficiency of MtBE was approximately 92% in reactor 1 and 95% in reactor 2. A similar high biological removal efficiency (>99%) was reported by Wilson et al. (2002). They operated an abiotic control reactor, in addition to the biologically active one, treating simulated contaminated water containing 150 mg/l of MtBE. MtBE mea-

Table 5. Biological removal of MtBE in Reactor 1 (flow rate: 0.4 l/h) and Reactor 2 (flow rate: 0.2 l/h)

Reactor	Gas-phase MTBE (μg/l)	MTBE stripped (%)	Total removal (%)	Biological removal (%)
1	0.28 (0.29)	8.00 (6.35)	99.75 (0.32)	91.75 (6.59)
2	0.11 (0.10)	4.74 (3.46)	99.81 (0.37)	95.07 (3.48)

sured in the head space of the abiotic reactor constituted less than 10% of the influent feed reflecting insignificant loss of MtBE due to aeration and mixing.

Microbial growth

Microbial growth was measured as total suspended solids (TSS) and volatile suspended solids (VSS) concentrations. Following an acclimation period of 2 weeks, biomass levels in both reactors trended upwards for the period of the study (Figure 4). Any decline in biomass levels in the porous pots was due to operational problems or accidents, since no sludge was wasted except for sampling purposes. A major drop in the biomass concentration in reactor 1 occurred on the 166th day of the study when the pH in reactor 2 decreased significantly. This followed the regeneration of the porous pot insert, which was done by soaking the membrane in a concentrated sulfuric acid solution for 24 h. The porous pot was rinsed with deionized water afterwards to wash off the acid; however, the rinse time was not long enough to remove all the acid from the membrane. To compensate for this loss, 1 l of biomass was transferred from reactor 1 to reactor 2, which explains the decrease in TSS and VSS in Reactor 1 at this time. TSS levels reached as high as 10,000 and 6,750 mg/l in reactors 1 and 2, respectively. The VSS were found to constitute approximately 39% of the total solids in both reactors with concentrations increasing to 3,600 and 2,350 mg/l in reactors 1 and 2, respectively. The porous pot reactors were operated at an extremely long sludge age (the only time solids were wasted was when the reactor contents were sampled). This resulted in a greater than normal biomass decay, leading to a larger fraction of inert solids in the mixed liquor. The groundwater itself might have contributed to the mineral content due to the precipitation of iron and phosphorus in the bioreactors. Higher VSS/TSS ratios were observed in membrane bioreactors that were also operated under long sludge ages. VSS/TSS ratios of 0.7 and 0.6 were measured in MBRs treating synthetic contaminated water as reported by Morrison et al. (2001) and Zein et al. (2004), respectively.

Discussion

Some researchers have designed and operated *ex-situ* biotreatment systems to degrade PAHs in the aqueous phase. Rasmussen et al. (2002) conducted a column study to assess the treatment of creosote-contaminated groundwater in a peat/sand permeable barrier. Operated at a residence time of 2 days, the barrier was able to biologically degrade 38% of naphthalene (average influent concentration of 893 μg/l) and only 4% of the 15 other 3- to 6-ring PAHs present in the contaminated groundwater at an average concentration of 170 μg/l. The major removal mechanism in their study was sorption on the peat/sand material. Our high-biomass reactors were able to degrade higher concentrations of 5 PAHs by $>99\%$ at HRTs of 15 and 32 h. One novel bioreactor that has been developed and utilized by several researchers for the biodegradation of PAHs is the Two Phase Partitioning Bioreactor (TPPB). It consists of a biologically active aqueous phase and an immiscible organic phase into which the often toxic and hydrophobic substrates are introduced. Based on cellular demand, these contaminants will partition into the aqueous phase for further microbial attack. Daugulis and Janikowski (2002) operated two TPPBs, 5 and 150 l in volume, with dodecane as the organic solvent delivering a 15-g and a 300-g mixture of naphthalene and of phenanthrene to a pure *Sphingomonas* culture with which the respective bioreactors were inoculated. The two PAHs were completely degraded in 21 h. The same culture was found to utilize acenaphthene and anthracene in another TPPB with ethanol as a co-solvent (Janikowski et al. 2002). Daugulis and McCracken (2003) conducted a similar study to degrade a mixture of low and high molecular weight PAHs (naphthalene, phenanthrene, fluoranthene, pyrene, chyrysene and benzo[*a*]pyrene) dissolved in dodecane as well by two species of *Sphingomonas*. The consortium was able to

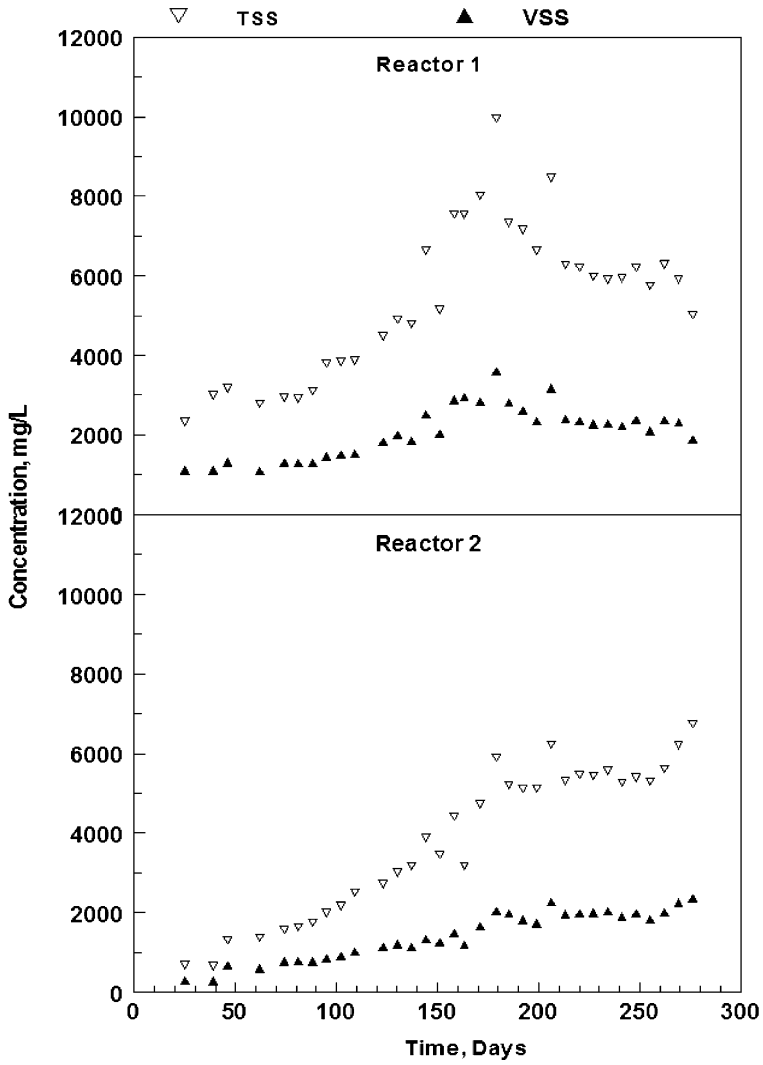

Figure 4. Biomass concentration in Reactor 1 (flow rate of 0.4 l/h) and Reactor 2 (flow rate of 0.2 l/h).

degrade all the contaminants while only the low molecular weight substrates were removed by the individual strains. Our culture was also a mixed consortium, and it was able to degrade all the contaminants to very low levels uninhibited. The biomass in the reactor was characterized as having an extremely long sludge age due to the retention of virtually all the solids by the membrane system. This is the key to achievement of a highly purified effluent from an influent containing MtBE and other VOCs.

Similarly, other studies have reported the biodegradability of MtBE and BTEX. Gasoline-contaminated groundwater was treated in two packed-bed biobarrier systems packed with either protruded stainless steel or granulated peat moss supporting microbial growth (Yerushalmi et al. 1999). Total influent BTEX concentrations ranged between approximately 1 and 21 mg/l, and the HRT was varied between 0.5 and 6 days. In both biobarrier systems, BTEX removal efficiencies were around 99.9% for all conditions examined. Guerin (2002) evaluated two bioreactor configurations, a submerged fixed film reactor (SFFR) and a fluidized bed bioreactor (FBR), for the bioremediation of contaminated groundwater containing BTEX as well as PAHs. Both treatment units proved effective in degrading approximately 0.7 mg/l of the

BTEX compounds with removal efficiencies ranging between 91.9 and 99.5% over a range of HRTs studied (3–29 h). However, the bioreactors were operated for periods not longer than 8 days. The porous pot reactors investigated in this study were operated for about 10 months and were able to effectively degrade higher total BTEX concentrations as high as 78 mg/l at HRTs of 15 and 32 h while maintaining higher removal efficiencies (>99.99%).

Porous pot reactors identical to the ones described in this paper have been reported in the literature. Wilson et al. (2001) reported MtBE removal efficiencies exceeding 99.99% when it was introduced into a porous pot reactor at an influent concentration of 150 mg/l. Pruden et al. (2003) investigated the biodegradability MtBE (7.8 mg/l) and BTEX (2 mg/l each) in an aerobic fluidized bed reactor. The reactor was able to produce effluents containing an average of 18.5 μ g/l and BTEX concentrations ranging between 1.4 and 2.2 μg/l. The <5 μg/l MtBE levels reported in the porous pot reactors were never achieved in the FBR because the shear forces in the fluidized bed precluded the full retention of biomass within the reactor. Another biotreatment system similar in principle to the porous pot reactor, an external-membrane bioreactor (MBR), was operated by Morrison et al. (2001) for more than 2 years. The microbial cultures in the MBR degraded MtBE (5 mg/l in the feed) and BTEX (1 mg/l influent concentrations) to levels less than 1 μg/l throughout the study. This is another example of the effectiveness of high biomass retention systems able to degrade MtBE to extremely low levels. The MBR achieves solids–liquid separation by forcing the water through the membrane under high pressure, whereas the porous pot relies on gravity.

Most of the aforementioned studies were conducted with spiked water, pure cultures, or in biofilm reactors at controlled temperatures and relatively long residence times. Many do not adequately reflect mixed substrate interactions, indigenous microbial communities or the environmental conditions specific to each contaminated site. It is more desirable to conduct a study using real contaminated groundwater rather than a synthetic waste feed. Groundwater plumes usually contain an array of organic pollutants in addition to natural organic matter (NOM), suspended particulates, and metals. In this study, despite the presence of MtBE, its degradation intermediate tBA, BTEX, and PAHs in the feed, both porous pot reactors demonstrated a remarkable ability to effectively treat all these compounds to levels at or near detection limits. Part of the success demonstrated in this study is attributed to the presence of a community of microorganisms in each reactor able to mineralize the contaminants either as sole carbon and energy sources or co-metabolically. As a result, no competitive inhibition was evident at any time in the porous pots from the presence of other competing compounds (PAHs and hydrocarbons). Reactors based on the porous pot configuration may prove superior to pressure- or vacuum-based membrane bioreactors, which are characterized by higher capital costs, high operation and maintenance requirements (due to the pressure or vacuum that must be used to drive the solids–liquid separation), and short service life mainly due to biofouling of the membrane. Although biofilters are relatively inexpensive, biomass clogging and support media acidification result in the deterioration of their performance and limit their applicability. The basic limitation of fluidized bed reactors lies in the availability of enough granular activated carbon (GAC) surface area for microbial attachment and, possibly more importantly, the difficulties associated with enriching bacterial strains acclimated to attached growth and high shear. Porous pot reactors do not require the use of organic solvents as in the case of the two phase partitioning reactors. This characteristic mitigates the cost of purchasing, handling, and disposing of these often toxic solvents.

In conclusion, this study evaluated and demonstrated the consistent and long-term treatability of groundwater from an MGP site contaminated with MtBE, gasoline hydrocarbons, and several PAHs to very low concentrations (<5 μg/l) in gravity-fed porous pot reactors. Based on this reactor configuration, a 1-m^3 pilot-scale bioreactor was developed to remediate synthetic MtBE-contaminated water on a larger scale. The reactor's fin-design allowed for the treatment of influent flow rates as high as 5,000 l/day while maintaining an MtBE removal efficiency of more than 99.9% (Zein et al. 2004). The field applicability of such a high biomass biotreatment system was also evaluated using a patented 8-m^3, field-scale bioreactor, which was demon-

strated to be capable of remediating groundwater contaminated with gasoline and oxygenates in Rhode Island (data from this study will be available in 2005). The HRT of this reactor was 6 h, operating at an influent flow rate of 5 gpm.

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