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Biodegradation of gasoline and BTEX in a microaerophilic biobarrier *

Laleh Yerushalmi, Michelle F. Manuel & Serge R. Guiot**
*Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Avenue, Montreal, Canada H4P 2R2 (** author for correspondence; e-mail: serge.guiot@nrc.ca)*

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Abstract

Continuous bioremediation of gasoline-contaminated water in a packed-bed biobarrier system under oxygen-limited conditions is discussed. This study was part of an extensive effort to develop an alternative technology for the *in situ* bioremediation of hydrocarbons where there is a limited supply of oxygen. Protruded stainless steel pieces and granulated peat moss were used as packing material to support microbial growth in two biobarriers. The inoculum was an enrichment culture of an indigenous microbial population from a soil sample. The biobarriers' inlet gasoline concentrations and the linear liquid velocities were similar to those commonly found at *in situ* conditions. Gasoline removal efficiencies ranged from 94% to 99.9% in the stainless steel-packed biobarrier, and from 86.6% to 99.6% in the peat moss-packed biobarrier. Effluent gasoline concentrations below 0.03 mg/l were obtained at gasoline loading rates less than 27.5 mg/l.d in the stainless steel-packed biobarrier. The remaining fraction of gasoline in the effluent consisted mainly of three aliphatic compounds and not the aromatic compounds. Both biobarrier packings supported near complete removal of the most soluble aromatic hydrocarbons of gasoline (BTEX) under all the conditions examined. The consumption of sulfate and the presence of sulfate-reducing microorganisms suggested the presence of anaerobic metabolism during the degradation of gasoline. Up to 92% gasoline was removed during the first 3 cm of the biobarriers' length.

Introduction

Contamination of groundwater by gasoline and its hydrocarbon constituents leaking from underground storage tanks, distribution systems and various industrial operations is a major environmental problem (Barbash and Roberts, 1986; Prince, 1993). Conventional treatment techniques such as pump and treat and air stripping have serious shortcomings which limit their applications and efficiencies. They include high cost and maintenance requirements, transfer of contamination from one medium to another and the duration of operation that may exceed decades to prevent continued growth of the contaminated plumes (Catwright, 1991; Hasbach, 1993).

An alternative technique for the treatment of groundwater is the use of *in situ* permeable walls or barriers placed across the flow path of a contaminated plume. There are several mechanisms by which permeable barriers remove the contaminating chemicals from a stream of water. They include sorption, precipitation, oxidation/reduction and chemical or biological degradation (Rael et al., 1995; Starr and Cherry, 1994). *In situ* remediation techniques have considerably lower costs and maintenance requirements compared to *ex situ* techniques. They also remove the contamination or immobilize the contaminants *in situ* without the need to transfer the affected material from the site, thus eliminating the risk of phase transformation of volatile compounds. *In situ* barriers using zero-valent iron (Fe^0) for the treatment of groundwater contaminated with chlorinated organic compounds have been used at pilot- or full-scale operations (Appleton, 1996; Vidic and Pohland, 1996; Focht et al., 1996). These barriers have the potential

to passively treat the contaminated plume for several years or decades (Gavaskar et al., 1998).

Biological *in situ* techniques are particularly attractive since they can completely destroy the target contaminants leaving potentially non-toxic chemicals as the products of biodegradation. *In situ* bioremediation techniques have been successfully applied in the treatment of groundwaters contaminated with petroleum hydrocarbons (Hutchins et al., 1995; Thomson et al., 1995; Starr and Cherry, 1994).

The present work discusses the development and performance of a packed-bed biobarrier system for the treatment of contaminated soil and groundwater under oxygen-limited or microaerophilic conditions. The microaerophilic condition exists when dissolved oxygen concentration is less than 2 mg/l. Remediation of groundwater under anoxic or oxygen-limited condition is very important due to the limited supply or complete lack of oxygen under *in situ* conditions. In fact, oxygen limitation is one of the major problems affecting the performance of *in situ* biological treatment systems. Also, supplying alternative electron acceptors such as sulfate and nitrate is more economical and more convenient (Hutchins et al., 1991; Brown and Norris, 1994).

The developed biobarrier system, also referred to as the permeable bioreactive barrier, uses a packed-bed permeable bioreactor to support the growth of free as well as attached microorganisms originating from soil indigenous microbial population. The developed biobarrier introduces a bioreactive zone in the subsurface environment that may be replaced during long-term bioremediation operations. The removable biobarrier can be used in groundwater treatment applications as an independent bioractive system, or it may be used as a gate in a funnel-and-gate^{TM} system. The biobarrier is based on the permeable reactive barrier (PRB) technology which is well recognized and is successfully employed for full-scale groundwater remediation (Warner, 1998; Warner et al., 1995).

The performance of the biobarrier in the bioremediation of gasoline-contaminated water was examined. The kinetics of gasoline biodegradation by the inoculum of the biobarriers was previously reported (Yerushalmi and Guiot, 1998). Granulated peat moss and protruded stainless steel were used as packing material. These two packing material provided two different removal mechanisms for gasoline: adsorption plus biodegradation when peat moss was used as the packing, and only biological degradation in the case of stainless steel packing material. The possibility of combining two different removal mechanisms in a biobarrier, i.e., physical-chemical and biological processes for the removal of contaminating compounds, demonstrates the great potential of this technology for the bioremediation of contaminated groundwater under *in situ* condition.

Materials and methods

Isolation of the microbial culture

The microbial culture used as the inoculum for the biobarriers was isolated by enrichment techniques from the top layers of a gasoline-contaminated soil sample from Montreal, Canada. The details of enrichment procedure were described earlier (Yerushalmi and Guiot, 1998). The enrichment had gone through eighteen successive transfers at the beginning of this study.

The gasoline sample used in this study contained approximately 28.9% BTEX (benzene, toluene, ethylbenzene and *m*-, *o*-, *p*-xylenes) which are the most soluble aromatic hydrocarbons in gasoline. The density of gasoline was 0.74 g/ml.

Culture medium

A minimal salts medium (MSM) was used for culture enrichment as well as the biobarriers' feed. The composition of the medium was reported before (Yerushalmi and Guiot, 1998).

Analytical techniques

The concentrations of gasoline and its constituents in the liquid phase were determined by the headspace method on a Perkin-Elmer, model Sigma 2000 chromatograph. Liquid samples received three drops of a 30% HCl solution upon withdrawal and were kept in 20-ml glass vials sealed with Teflon-lined rubber stoppers (Wheaton, Millville, NJ) at 4 °C until analyzed within a few days. The gas phase analysis of hydrocarbons used a Perkin-Elmer, model Sigma 2000 gas chromatograph equipped with a FID detector. The chromatographic conditions were reported before (Yerushalmi and Guiot, 1998).

The concentration of individual BTEX compounds was estimated from their corresponding chromatographic peaks using the appropriate calibration data. The concentration of gasoline (overall mixture) was calculated from the calibration curves using the total

area of chromatographic peaks. The method detection limits (MDL) for the individual BTEX compounds and for gasoline were 3 μg/l and 30 μg/l (0.03 mg/l), respectively. The effluent gasoline concentration reported throughout this paper refers to the concentration of the total hydrocarbons detected by chromatographic analysis. No attempt was made to separate the possible intermediate compounds from the remaining gasoline components in the effluent.

Sulfate analysis was performed on a Thermo Separation Products HPLC equipped with a ternary pump (model TSP SP-8800), an autosampler (model SP-8880) and a Waters 431 conductivity detector. Samples (100 μL) were injected into a Hamilton PRP-X100 column using a mobile phase of 4.0 mM *p*-hydroxybenzoic acid (pH = 8.5 with 2.5% methanol) with a flow rate of 2 ml/min. The analysis run time was 30 minutes.

The concentration of free cell suspension (mg/l_{liq}) was determined by dry weight analysis. The samples were filtered through a Whatman filter paper No. 1 (Fisher Scientific, Montreal, Canada) in order to remove the peat moss particles and the non-biomass colloidal material. The retrieved biomass was washed twice with distilled water and dried at 80 °C to constant weight. The estimation of the attached biomass concentration by direct methods of analysis presented serious difficulties. These methods included cell count measurement, protein analysis and biomass dry weight measurement. The encountered problem with the biofilm on peat moss was the interference from the packing itself while with the stainless steel packings, the very low concentration of attached biomass made the measurements unreliable. Finally a combination of experimental and theoretical techniques using glucose activity test was employed to indirectly estimate the attached biomass concentration.

Experiments were performed in 120-ml serum bottles using 0.5 or 2 g/l of initial glucose concentration. The dynamics of glucose degradation was followed by introducing a known number (usually 15) of stainless steel packings or peat moss granules withdrawn from the biobarriers and rinsed with distilled water, into the serum bottles. Control experiments with free biomass (10% inoculum) were performed in parallel. The final biomass concentration in each bottle was measured and the overall yield was calculated. Considering a constant biomass yield throughout the course of the process, the dynamics of biomass growth were reconstructed by using the glucose consumption data. This procedure provided an approximation for the initial biomass concentration (X_0), representing the amount of biomass introduced in the bottles by the known number of packings. These data provided an estimate for the attached biomass concentration (mg biomass/$l_{packing}$). The overall amount of biomass inside the biobarriers was estimated by integrating the curve representing the biomass concentration profile along the length of the biobarriers, using the trapezoidal integration method.

The concentration of dissolved oxygen was determined by a glass polarographic DO probe Model E05643 (Cole Parmer, Chicago, Illinois, USA) and monitored on a DO controller Model 01972 (Cole Parmer, Chicago, Illinois, USA). The dissolved oxygen concentration was measured at three points along the biobarriers: feed, first sampling port (3 cm inside the biobarrier), and effluent. The DO concentrations in feed and in the biobarriers' effluents were directly measured by inserting a DO probe in the liquid flow lines. For the measurement of dissolved oxygen concentration inside the biobarriers, a gas-tight external loop was used to redirect the liquid from the biobarriers into a 20-ml glass vial equipped with a DO probe. The accuracy of DO measurements was $\pm$1%. The detection limit of the DO controller was 0.05 mg/l.

Cultivation of sulfate-reducing bacteria

The presence of anaerobic, sulfate-reducing bacteria inside the biobarriers was examined by inoculating an anaerobic medium developed specifically for the cultivation of sulfate reducing bacteria (Widdle and Bak, 1992), with the microbial biomass withdrawn from the biobarriers. Sodium lactate or sodium acetate (20 mM) served as both the electron donor and carbon source. The medium contained Fe II (ferrous sulfate, 0.2 mM) as an indicator of sulfide production. Sterile, non-cultivated tubes served as control in these experiments.

Theoretical sulfate requirement for the oxidation of gasoline

The hydrocarbons in gasoline usually consist of 60–70% alkanes, 25–30% aromatics (mostly BTEX), and 5–10% alkenes (Mehlman, 1996). The mass of sulfate utilized per unit mass of degraded BTEX is 4.72. This value is calculated based on the requirement of 4.6 mg SO_4^{2-}/mg benzene, 4.7 mg SO_4^{2-}/mg toluene, 4.75 mg SO_4^{2-}/mg ethylbenzene, and 4.75 mg SO_4^{2-}/mg xylenes. The amount of sulfate required for the oxidation

of alkanes and alkenes is calculated from the following equations:

Alkanes:

$C_n\ H_{2n+2} + (3n + 1)/4\ SO_4^{2-} + (3n + 1)/2\ H^+ \rightarrow nCO_2 + (n + 1)\ H_2O + (3n + 1)/4\ H_2S$

mg SO_4^{2-}/mg $C_n\ H_{2n+2}$ = 5.26 ± 0.05 (for n = 4 to 14)

Alkenes:

$C_n\ H_{2n} + 3n/4\ SO_4^{2-} + 3n/2\ H^+ \rightarrow nCO_2 + n\ H_2O + 3n/4\ H_2S$

mg SO_4^{2-}/mg $C_n\ H_{2n}$ = 5.14 (for all values of n)

Based on the percentage of each group of hydrocarbon present in gasoline, the theoretical demand for sulfate as the terminal electron acceptor during the oxidation of gasoline is 5.08 mg/mg gasoline.

Biobarrier packing

The Pro-Pak, protruded stainless steel (Cannon Instrument Company, Pa., USA) and granulated peat moss (Produits Recyclable Bioforet, Quebec, Canada) were used as the packing material and support for the microbial biofilm in the biobarriers. The Pro-Pak S.S. packings were 0.6 × 0.6 cm in size and had an average particle density of 5.13 g/ml with a free space of 93%. The granulated peat moss had an average diameter of 0.4 to 0.7 cm, a particle density of 0.64 g/ml and a free space of 57%. The packings were washed thoroughly with distilled water, soaked in MSM medium and autoclaved (120 ° C, 20 minutes) in small quantities, before use.

Adsorption of hydrocarbons on packing material

The adsorption capacity of granulated peat moss and the stainless steel pieces for gasoline, benzene and toluene were determined by estimating the quantity of compounds that could be adsorbed by unit mass of the packing material. Adsorption experiments were performed in 120-ml serum bottles closed with mininert valves (Supelco, Canada), containing 10 g of dried peat moss or stainless steel packing and 100 ml of MSM. Gasoline was injected into the bottles to yield total concentrations of 10, 25, 50 and 100 μl/l. Two control bottles containing no packings also received 50 μl/l of gasoline. The bottles were shaken at 200 rpm in a wrist-action shaker (Model 75, Burrell Corporation, P.A. USA). After the establishment of equilibrium between the adsorbed and the non-adsorbed hydrocarbons in the bottles (almost 72 hours) the concentrations of gasoline as well as benzene and toluene in the gas and liquid phase of each bottle were determined. The amount of compounds adsorbed on the packings was found by difference of the final and initial amounts. The adsorption data were analyzed by the Freundlich isotherm relationship defined by the following equation:

$$X/M = K_f C_e^{1/n} \quad (1)$$

Where X/M is the amount of adsorbate adsorbed per unit weight of adsorbent (peat moss or stainless steel packings), C_e is the equilibrium solution concentration of adsorbate after adsorption, K_f is the adsorption equilibrium constant and n is an empirical constant.

The adsorption results were also used to determine the retardation factors for benzene and toluene according to the following relationship (Stuart et al., 1991):

$$R = 1 + (\rho K_f)/\eta \quad (2)$$

Where R is the retardation factor, ρ and η are the density and the porosity of the packing material, respectively.

Mineralization assay

Microcosm tests measured the extent of mineralization of ^{14}C-labeled benzene by the microbial culture. Benzene was used during the independent batch studies of hydrocarbon biodegradation because it is a known carcinogen and one of the most toxic hydrocarbon constituents in gasoline. Benzene is also the most recalcitrant aromatic hydrocarbon in gasoline and is considered as one of the significant pollutants of groundwater. The experimental procedure during the mineralization tests were reported before (Yerushalmi and Guiot, 1998).

Biobarrier operation

The biobarriers consisted of a packed-bed permeable bioreactor with a stainless steel body (25 cm × 20 cm × 10 cm), a rectangular cross sectional area and a total volume of 5 liters. They were equipped with two glass windows and several ports on the sides as well as the top for feeding, liquid recirculation during the initial batch operation and sampling. Stainless steel or Teflon tubes and connections were used throughout the experimental system in order to prevent their contribution to

the abiotic removal of hydrocarbons. The biobarriers were initially operated in batch mode in order to promote the attachment of microbial cells to the support material and to develop a microbial biofilm. The setup for the continuous operation of biobarriers is schematically presented in Figure 1. The nutrient solution (MSM) was continuously agitated and sparged with sterile air in order to provide oxygen for the process. A four-channel peristaltic pump model Gilson minipuls 2 (Gilson Medical Electronics, WI, USA) was used to deliver feed to the four inlet ports on the side of the biobarriers. Two peristaltic pumps model Masterflex (Cole Palmer Instrument Company, Illinois, USA) were used to introduce gasoline solution containing 370 mg/l gasoline in MSM medium into the biobarriers. The gasoline solution was constantly mixed throughout the course of the experiments.

Experiments were performed at room temperature ($\sim$25 $^\circ$ C). Overall gasoline removal by the biobarriers was evaluated by analyzing gas and liquid samples of the effluent. Gas samples were taken from a side tower located on the outlet port of the biobarriers. The gas phase in the side tower was in equilibrium with the liquid phase inside the biobarrier. Gasoline concentration inside the biobarriers was determined by analyzing liquid samples taken from the sampling ports along the biobarrier.

The gasoline loading rate was increased step-wise by keeping a constant hydraulic retention time (HRT) while increasing the inlet gasoline concentration. A total of 14 operating conditions were established in the stainless steel-packed biobarrier and 13 conditions in the peat moss-packed biobarrier. The packings were thoroughly saturated with liquid leaving no visible air pockets or channels inside. There were no compactions of the packings for the entire duration of the experiments which lasted over nine months.

The efficiency of biobarriers in the removal of gasoline during the continuous mode of operation was evaluated according to their removal efficiency (RE) and elimination rate (ER) as defined by the following equations:

$$\text{Removal Efficiency (RE)} = (S_i - S_e) * 100/S_i \quad (3)$$

$$\text{Elimination Rate (ER)} = Q(S_i - S_e)/V_f \quad (4)$$

The biobarriers' loading rate was expressed as:

$$\text{Loading rate (LR)} = S_i * Q/V_f \quad (5)$$

where S_i is the inlet concentration of compound (mg/l), S_e is the outlet concentration of compound (mg/l), Q is the inlet feed flow rate (l/d) and V_f is the volume of the biobarriers occupied by the packing and liquid (l_f).

Results and discussion

Overall performance of biobarriers during continuous operation

The liquid flow rate entering the biobarriers ranged from 0.8 to 9.3 l/d in the stainless steel-packed biobarrier and from 0.5 to 5.7 l/d in the peat moss-packed biobarrier, corresponding to the feed linear liquid velocities of 4.2 to 50 cm/d in both biobarriers. The inlet gasoline concentration changed from 3.7 to 74.0 mg/l. These operating parameters are similar to those normally found in a natural subsurface environment. Under these conditions the gasoline loading rate ranged from 1.1 to 68.8 mg/l_f.d in the stainless steel-packed and from 0.7 to 42.2 mg/l_f.d in the peat moss-packed biobarriers.

Both biobarrier packings supported high gasoline removal efficiencies under all the conditions examined. Removal efficiencies ranged from 94.0% $\pm$ 2.0% to 99.9% $\pm$ 0.02% in the stainless steel-packed biobarrier, and from 86.6% $\pm$ 3.5% to 99.6% $\pm$ 0.11% in the peat moss-packed biobarrier (Table 1). The effluent gasoline concentration changed from below detection limit (<0.03 mg/l) to 4.44 $\pm$ 1.5 mg/l and from 0.14 $\pm$ 0.04 to 2.46 $\pm$ 0.65 mg/l in the two respective biobarriers. The resulting overall gasoline elimination rates ranged from 1.1 to 64.8 $\pm$ 1.4 mg/l_f.d in the stainless-steel-packed biobarrier and from 0.6 $\pm$ 0.02 to 40.8 $\pm$ 0.37 mg/l_f.d in the peat moss-packed biobarrier as calculated from Equation 4.

In the stainless steel-packed biobarrier, a consistently high efficiency of more than 99% was observed except for the condition with the highest gasoline loading rate of 68.8 mg/l_f.d, where the gasoline removal efficiency decreased to less than 95%. In the peat moss-packed biobarrier, the lowest efficiency of degradation (86.6%) was obtained when HRT was 3 days and inlet gasoline concentration was 3.7 mg/l. The gasoline removal efficiencies at other operating conditions using 3.7 mg/l inlet gasoline were also relatively lower than those obtained at higher inlet gasoline concentrations. At HRT of 0.5 day, the low retention time prevented a complete biodegradation of gasoline resulting in a removal efficiency of less than 90%.

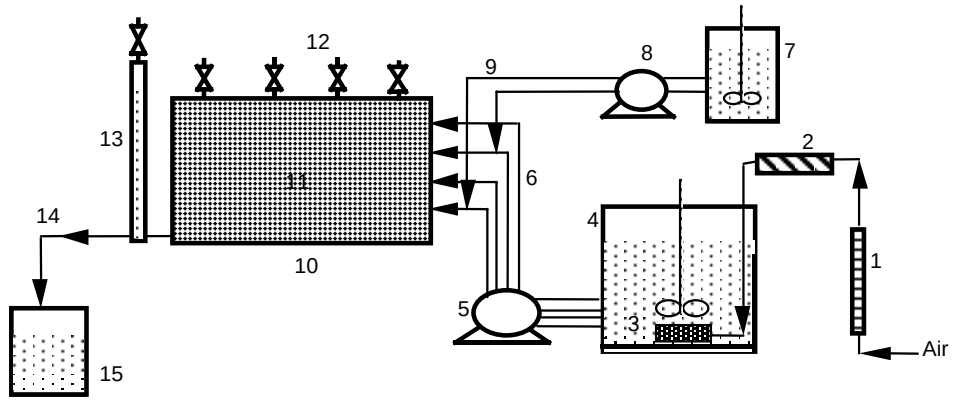

Figure 1. Schematic diagram of the biobarrier assembly during continuous operation. 1-Rotameter, 2-Air filter, 3-Air diffuser, 4-Feed tank, 5- Feed pump, 6-Feed lines, 7-Gasoline solution tank, 8-Pump, 9-Gasoline lines, 10-Biobarrier, 11-Packing, 12-Top ports, 13-Side tower, 14-Effluent line, 15-Effluent tank.

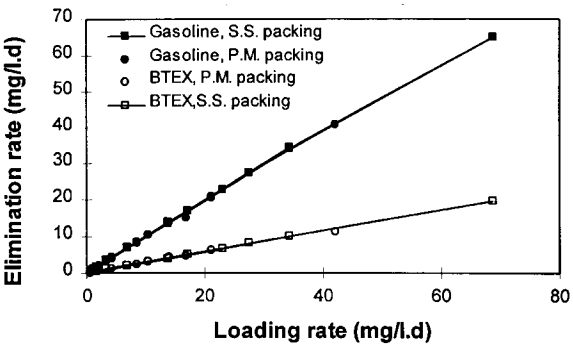

Figure 2. Overall performance of the biobarriers in removal of gasoline and BTEX.

The overall performance of the biobarriers in the elimination of gasoline and BTEX as a response to the changing loading rate of the biobarriers is presented in Figure 2. The obtained linear relationship indicates the independence of gasoline and BTEX removal efficiencies from the gasoline loading rate over the entire range of the experimental conditions (Cook and Kincannon, 1971). Overall gasoline and BTEX removal efficiencies of 98% and 99.9% were obtained from the slope of the lines, signifying the potential *in situ* applications of the biobarrier since the water-soluble BTEX is the major fraction of gasoline which causes groundwater contamination.

The different free space of the packings used in the two biobarriers (93% in the stainless steel-packed biobarrier vs. 57% in the peat moss-packed biobarrier) resulted in different HRTs under similar conditions of water flow rate or gasoline loading rate. As a result, under any given operating condition in terms of the inlet gasoline concentration and feed flow rate, the HRT of the peat moss-packed biobarrier was 1.63 times shorter than that of the stainless steel-packed biobarrier. This difference did not have any adverse impact on the performance of the biobarriers as indicated by the near complete removal of gasoline under the applied conditions. However, the faster liquid velocity (lower HRT) implies that the capacity of the peat moss-packed biobarrier for removal of gasoline may be reached before that of the stainless steel-packed biobarrier, should the loading rate continue to increase.

Granulated peat moss has been frequently used as packing in biodegradation of petroleum hydrocarbons in gas and liquid phase. Tahraoui et al. (1995) used a mixture of peat moss and chicken manure in a 50-l biofilter to remove BTX in contaminated air. Their study showed a maximum elimination capacity of 1.97 g/l.d with a BTX load of 2.2 g/l.d and inlet BTX concentrations of 1.97 to 3.6 mg/l. Forget et al., (1996) used peat moss to degrade toluene with an inlet concentration of 1.04 mg/l in a packed bed bioreactor. They reached removal efficiencies of 64.5% to 99.6% with the change of retention time from 2.1 min to 2.4 hours, respectively.

Analysis of effluent of the biobarriers revealed that the remaining fraction of gasoline consisted mainly of three compounds, identified to be of aliphatic nature, appearing before benzene during chromatographic analysis. An example of gasoline profiles in the inlet and outlet streams of the peat moss-packed biobarrier is presented in Figure 3. The exit concentration of BTEX was usually quite low and did not exceed 5.7% or 15.8% of the effluent's organic content

Table 1. Operating parameters and gasoline removal performance of the biobarriers during continuous-flow operation

		Stainless steel packing				Peat moss packing			
HRT (d)	S_i (mg/l)	LR (mg/l$_f$.d)	S_e (mg/l)	RE (%)	ER (mg/l$_f$.d)	LR (mg/l$_f$.d)	S_e (mg/l)	RE (%)	ER (mg/l$_f$.d)
6	44.4	6.9	0.31 ± 0.06	99.3 ± 0.13	6.8 ± 0.01	4.2	0.54 ± 0.06	98.8 ± 0.14	4.2±0.01
3	3.7	1.1	BDL	99.2*	1.1	0.7	0.50 ± 0.13	86.6 ± 3.5	0.6 ± 0.02
3	11.1	3.4	0.04 ± 0.01	99.6 ± 0.09	3.4	NA	NA	NA	NA
3	22.2	6.9	0.18 ± 0.06	99.2 ± 0.27	6.9 ± 0.02	4.2	1.45 ± 0.35	93.5 ± 1.57	3.9 ± 0.07
3	44.4	13.8	0.04 ± 0.01	99.9 ± 0.02	13.7 ± 0.01	8.4	0.47 ± 0.18	98.9 ± 0.40	8.3 ± 0.03
3	74.0	22.9	0.30 ± 0.15	99.6 ± 0.20	22.8 ± 0.04	14.1	0.59 ± 0.16	99.2 ± 0.22	13.9 ± 0.03
2	3.7	1.7	BDL	99.2*	1.7	1.1	0.35 ± 0.06	90.5 ± 1.62	0.9 ± 0.02
2	37.0	17.2	0.30 ± 0.13	99.2 ± 0.35	17.0 ± 0.06	10.5	0.21 ± 0.02	99.4 ± 0.05	10.5 ± 0.01
2	74.0	34.4	0.29 ± 0.1	99.6 ± 0.13	34.3 ± 0.05	21.1	2.26 ± 0.46	96.9 ± 0.62	20.4 ± 0.13
1	3.7	3.4	BDL	99.2*	3.4	2.1	0.18 ± 0.04	95.2 ± 1.08	2.0 ± 0.02
1	14.8	13.8	0.13 ± 0.03	99.1 ± 0.20	13.6 ± 0.03	8.4	0.55 ± 0.03	96.3 ± 0.20	8.1 ± 0.02
1	37.0	34.4	0.27 ± 0.16	99.3 ± 0.43	34.2 ± 0.15	21.1	0.14 ± 0.04	99.6 ± 0.11	21.0 ± 0.02
1	74.0	68.8	4.44 ± 1.5	94.0 ± 2.0	64.8 ± 1.40	42.2	2.46 ± 0.65	96.7 ± 0.88	40.8 ± 0.37
0.5	14.8	27.5	BDL	99.8∗	27.5	16.9	1.52 ± 0.22	89.7 ± 1.49	15.1 ± 0.25

The parameters (HRT, S_i, S_e, LR, RE and ER) are defined in the Materials and methods.
BDL = Below detection limit.
* = An effluent concentration of 0.03 mg/l (MDL) was assumed.
NA = Not available.

in the stainless steel-packed or the peat moss-packed biobarriers, respectively. On the other hand the concentration of the three aliphatic compounds comprised up to 63.1% or 83% of the effluent's total organic content in the two respective biobarriers as presented in Figure 4. The initial BTEX content of gasoline was approximately 28.9% while the three aliphatic compounds of interest initially comprised almost 16.4% of gasoline.

Adsorption of gasoline on packing material

In addition to microbial transformation, in the peat moss-packed biobarrier gasoline disappearance was also due to physical-chemical adsorption by the support material. As shown in Figure 5, the mass of gasoline adsorbed per unit weight of peat moss (X/M) increased proportionally with the equilibrium gasoline concentration in solution (C_e). The single-solute adsorption isotherms for benzene and toluene were evaluated according to the Freundlich relationship (Equation (1)). The isotherms for benzene and toluene were linear, yielding equilibrium constants of 10.1 and 24.1 l/kg dry peat moss, respectively. The adsorption of gasoline on the support material results in the retardation of its transport through the medium. The retardation coefficient for benzene and toluene were estimated from Equation (2) to be 6.1 and 13.3, respectively. These values indicate that the adsorption of benzene and toluene on peat moss is significant and plays a role in the overall removal of the contaminants. Unlike peat moss, the adsorption of hydrocarbons on the stainless steel packing material was negligible. The lack of adsorption properties by the stainless steel packing material, and the use of non-adsorptive material throughout the experimental system indicated that biological degradation was the mechanism responsible for the removal of hydrocarbons in the stainless steel-packed biobarrier.

Gasoline and biomass concentration gradients

The concentrations of biomass and gasoline exhibited a declining gradient along the length of the biobarriers. Typical examples of concentration profiles of biomass and gasoline are presented in Figures 6 and 7. The total concentration of biomass including free as well as attached cells was used in these graphs. In the peat moss-packed biobarrier the attached biomass accounted for 30.8% to 43.5% of the total biomass, while in the stainless steel-packed biobarrier these values changed to 4.2% to 8.1%. Both biomass and gasoline gradients were steeper in the stainless steel-packed biobarrier indicating the highest biomass accumulation and the greatest gasoline biodegradation within the first 3 cm of the biobarrier. Indeed, the major frac-

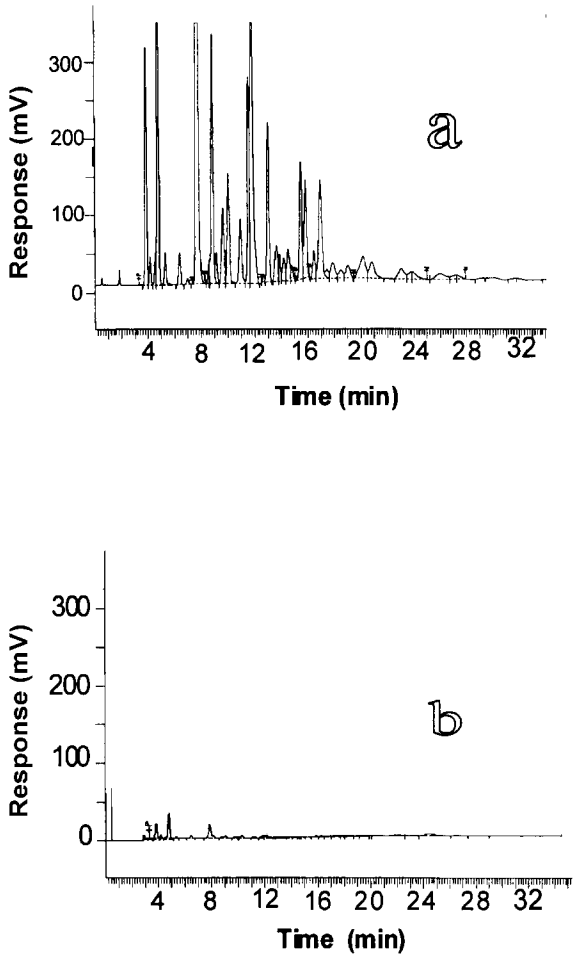

Figure 3. Comparison of the gasoline chromatograms in the inlet (a) and outlet (b) streams of the peat moss-packed biobarrier. HRT = 1 day, S_i = 37 mg/l.

tion of gasoline (up to 92%) was removed before the first port under all the conditions examined. This indicates the great capacity for gasoline removal in the stainless steel-packed biobarrier. The volumetric rate of gasoline removal (dS/dt), within the first 3 cm of the biobarrier ranged from 7.2 to 283.2 mg/l.d with the change of the overall loading rate from 1.7 to 34.4 mg/l$_f$.d. In the peat moss-packed biobarrier, biomass concentration was highest at the first port but it had a more gradual decrease with the increase of distance from the inlet port. Similarly, gasoline removal along the length of this biobarrier was slower, exhibiting removal of the major fraction of gasoline (up to 96%) in the first 6 cm of the biobarrier. The volumetric rate of gasoline removal within the first 6 cm of the peat moss-packed biobarrier ranged from 14.3 to

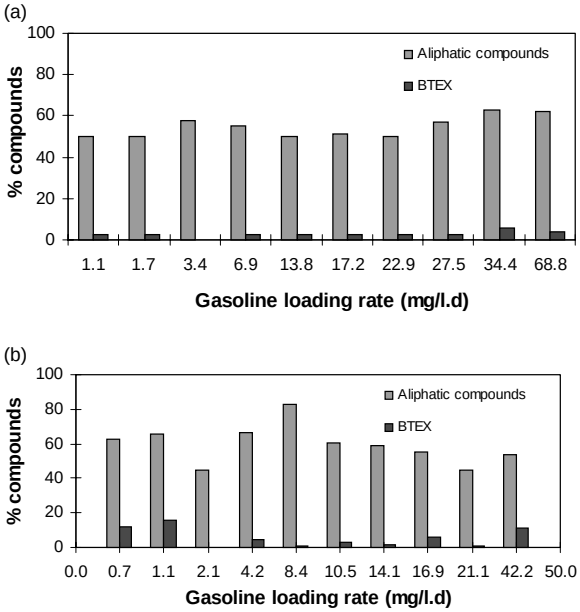

Figure 4. Fraction of BTEX and three aliphatic compounds in the effluent of the biobarriers. a: stainless steel-packed biobarrier, b: peat moss-packed biobarrier.

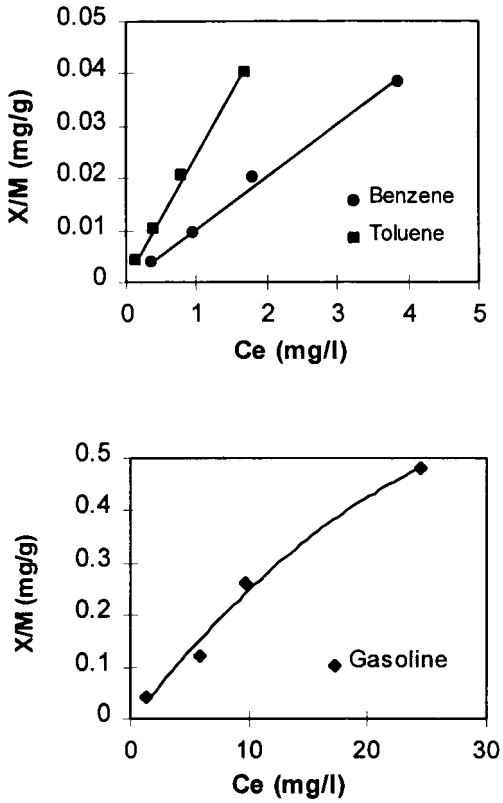

Figure 5. Adsorption of gasoline, benzene and toluene on peat moss.

120.8 mg/l.d with the change of the overall loading rate from 2.1 to 21.1 mg/l_f.d. The presence of biomass and gasoline gradients along the length of the biobarriers stems from the design of the system and the feeding technique (Figure1). Under this configuration the concentration of substrate is highest at the inlet and gradually decreases with increased distance from inlet. In the stainless steel-packed biobarrier the high biomass concentration at the first port metabolized the majority of the substrate, leaving little or no substrate to support microbial growth further along the biobarrier. However, in the peat moss-packed biobarrier the presence of biomass was observed at the other ports along the biobarrier. This could have been due to gasoline adsorption on peat moss and its subsequent desorption during the process supporting microbial growth all along the biobarrier. In a study of BTEX degradation in biofilters, Mallakin and Ward (1996) noticed a larger microbial population at the inlet of the biofilter compared to the outlet, reflecting a declining gradient of contaminant concentration along the length of the filter. Similarly, Kuhn et al., (1985) reported the presence of concentration profiles of substituted benzenes during microbial transformation whereby 70% of all xylene isomers were removed during the first 1.5 cm of the column. Complete removal was obtained during the first 4.7 cm of the column.

The overall amount of suspended biomass inside the biobarriers ranged from 173 to 495 mg biomass/l_{liq} for the stainless steel-packed biobarrier and from 246 to 631 mg/l_{liq} for the peat moss-packed biobarrier. The overall attached biomass ranged from 12 to 40 mg/$l_{packing}$ and from 82 to 220 mg/$l_{packing}$ in the two respective biobarriers. Combination of the above values yielded the total biomass content of the biobarriers which ranged from 180 to 506 mg/$l_{biobarrier}$ for the stainless steel-packed biobarrier and from 214 to 547 mg/$l_{biobarrier}$ for the peat moss-packed biobarrier. Overall biomass content of biobarriers was then used to estimate the specific rates of gasoline elimination (mg/mg biomass.d) and to further compare the performance of the two biobarriers in the elimination of gasoline. Figure 8 shows close values of specific elimination rates obtained at similar loading rates with the two types of packings suggesting similar biochemical capacities of gasoline removal with the two biobarriers. The increase of the specific gasoline elimination rate with the increase of the loading rate implies that the biobarriers operated below their maximum biodegradation potential under the conditions examined in this work. In fact, the maximum specific rate of gasoline biodegradation by the microbial culture used as the inoculum for the biobarriers was 1.06 mg/mg biomass.d which is considerably larger than the highest specific rate obtained in the biobarriers (0.13 mg/mg biomass.d). This indicates the increased potential of the biobarriers' microbial population in the degradation of gasoline, explaining the consistently high gasoline removal efficiencies obtained by the two biobarriers.

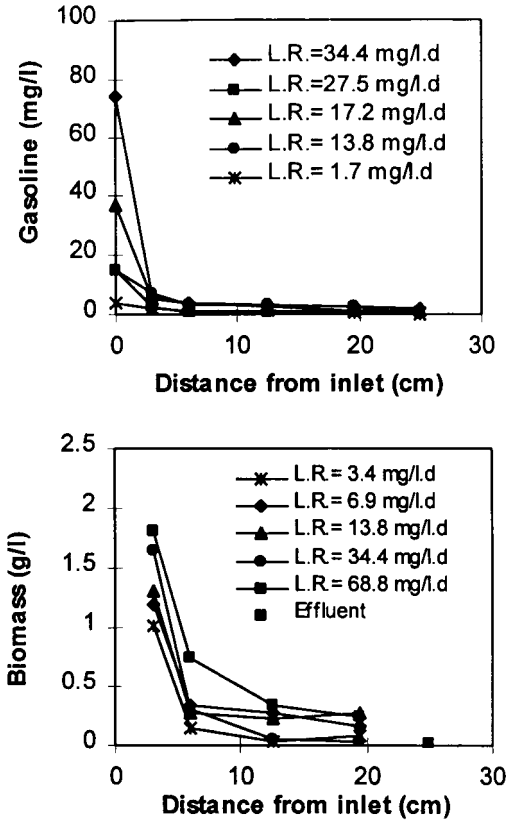

Figure 6. Concentration profiles of gasoline and biomass along the length of the stainless steel-packed biobarrier.

Mechanism of hydrocarbon biodegradation

The biomineralization capacity of the microorganisms inside the biobarriers was examined by microcosm tests using radiolabelled benzene. The tests performed under aerobic condition with two different initial benzene concentrations of 22 and 33 mg/l indicated that the biomass from both biobarriers mineralized benzene with high efficiencies ranging from 67.8% to 76.5%. The capacity of the microorganisms used as the inoculum of this biobarrier in mineralization of benzene with concentrations as high as 200 mg/l under

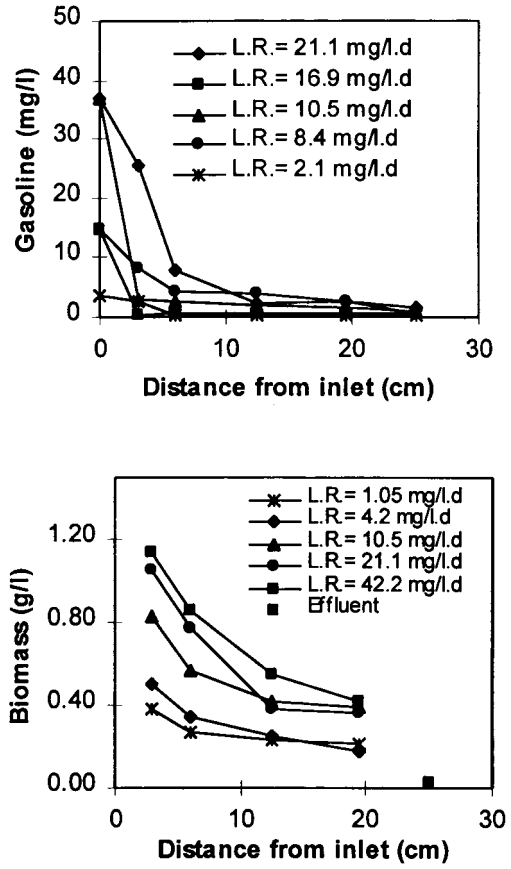

Figure 7. Concentration profiles of gasoline and biomass along the length of the peat moss-packed biobarrier.

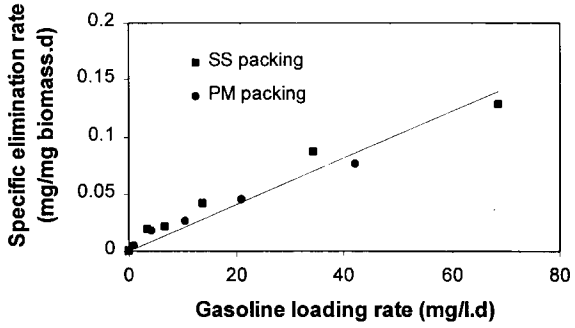

Figure 8. Variations of the specific gasoline elimination rate with the loading rate in the two biobarriers.

aerobic condition was previously shown (Yerushalmi and Guiot, 1998). The mineralization studies were performed on a regular basis using the microbial biomass withdrawn from the biobarriers in order to verify the capacity of microbial culture inside the biobarriers in biological degradation of gasoline.

In spite of a feed oxygen concentration of 8 mg/l, measurements of the dissolved oxygen (DO) concentration in the effluent as well as at the first sampling port of the biobarriers (3 cm from the inlet) showed values of less than 0.3 mg/l (frequently near zero) under the conditions examined in this work. This indicates that the supplied oxygen was quickly consumed leading to a decrease in the DO concentration. As a result, a microaerophilic condition was established along the entire length of the biobarriers. A mass balance of the degraded gasoline and the available oxygen indicated that the supplied oxygen was not sufficient to support complete aerobic biodegradation of gasoline even at the lowest concentration of gasoline employed in this study. The inlet gasoline concentration into the biobarriers ranged from 3.7 to 74.0 mg/l, while the inlet oxygen concentration was 8 mg/l. Considering 3 mg oxygen required for aerobic biodegradation of 1mg gasoline, the supplied oxygen was only sufficient to support the aerobic oxidation of 72% to 3.6% gasoline, far less than the requirements for complete aerobic biodegradation of gasoline.

The high gasoline removal efficiencies of greater than 99% obtained with limited supply of oxygen imply that the microaerophilic condition did not prevent the degradation of gasoline. This is in contrast with previous investigations which demonstrated the requirement for a minimum concentration of DO in the range of 1.0–1.5 mg/l to initiate petroleum hydrocarbon biodegradation (Wilson and Bouwer, 1997; Chiang et al., 1989). These DO concentrations are considerably higher than those observed in the biobarriers during the present study. Two mechanisms are proposed for the biodegradation of gasoline hydrocarbons: first, the contribution of anaerobic metabolism, implying that in addition to oxygen an alternative electron acceptor was present in the system, stimulating hydrocarbon biodegradation under mixed electron-acceptor condition as reported previously (Barbaro et al., 1997; Su and Kafkewitz. 1996; Miksell et al., 1993), and second, the aerobic transformation of hydrocarbons into intermediate metabolites.

The occasional analysis of remaining sulfate in the biobarriers' effluents showed the consumption of 5.5 to 111 mg/l of sulfate with the change of inlet gasoline concentration from 3.7 to 37 mg/l. Based on the theoretical 5.08 mg sulfate required for the oxidation of 1 mg gasoline, the consumed sulfate was sufficient for the anaerobic oxidation of 100% to 63.6% of the remaining gasoline. The remaining gasoline refers to the fraction that could not have been de-

graded aerobically, based on the availability of 8 mg/l of oxygen in the feed. Evidence for the presence of sulfate-reducing bacteria was provided by the growth of microorganisms, withdrawn from the biobarriers, on anaerobic media specifically designed for growth of sulfate-reducing bacteria followed by the formation of black FeS precipitation in the presence of ferrous sulfate. The microbial culture from both biobarriers produced the black FeS precipitation with the lactate- as well as the acetate-amended medium. These findings suggest that anaerobic metabolism was present in the biobarriers during the degradation of gasoline. Sulfate has been recognized as a terminal electron acceptor in the biodegradation of hydrocarbons (Edwards and Grbic-Galic, 1992; Lovley et al., 1995). As reported by Lovley et al. (1995) sulfate reduction can be linked to anaerobic biodegradation of benzene, toluene and other monocyclic aromatics with only CO_2 production and no accumulation of end products. Other electron acceptors such as oxidized metals, possibly present in the biobarriers, may have contributed to the acceptance of electrons during the biodegradation of hydrocarbons. It is also possible that some gasoline constituents, particularly those which are more recalcitrant under anaerobic condition (such as benzene), were not completely mineralized but were partly transformed to intermediate metabolites.

The high gasoline removal efficiencies of $>99\%$ obtained under low dissolved oxygen concentrations (microaerophilic condition) and with limited supply of molecular oxygen (less than the demand for complete aerobic oxidation of gasoline) is a notable advantage of the biobarrier, exhibiting its potential for *in situ* applications and under DO conditions normally found underground.

Conclusions

The developed biobarrier presents a simple and effective system for *in situ* remediation of contaminated groundwater under low dissolved oxygen concentrations commonly found in the subsurface environment. Both biobarrier packings supported high efficiencies of gasoline removal at initial concentrations of 3.7 to 74 mg/l and linear liquid velocities of 4.2 to 50 cm/d, which are close to the *in situ* conditions. Removal efficiencies of more than 99% were obtained under microaerophilic conditions. Hydrocarbon oxidation under mixed electron-acceptor condition as well as partial transformation of some gasoline constituents were postulated as possible mechanisms of gasoline removal. A declining concentration gradient of gasoline and biomass were developed along the length of the biobarriers indicating the removal of up to 92% gasoline during the first 3 cm of the biobarriers length.

Research is currently in progress to evaluate the performance of biobarriers in the bioremediation of water contaminated with polycyclic aromatic hydrocarbons. The results obtained during the present work with liquid flow velocities and gasoline concentrations commonly found underground and with two different types of packings indicate that the developed biobarrier is a powerful and effective system suitable for environmental applications. The successful performance of the biobarrier under microaerophilic and oxygen-limited conditions, an important feature of the biobarrier, demonstrates the potential of this technology for *in situ* groundwater remediation applications.

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