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Degradation of BTEX compounds in liquid media and in peat biofilters

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A mixed culture, enriched from Sphagnum peat moss, contaminated with gasoline vapours, degraded individual and mixed components of BTEX (benzene, toluene, ethylbenzene, xylene). Complete degradation of radiolabelled toluene by the mixed culture was observed in mineralisation studies. Individual isolates from a mixed culture containing *Pseudomonas maltophilia, P. testosteroni* and *P. putida* biotype A exhibited contrasting BTEX degradation patterns. While *P. putida* biotype A degraded all of the BTEX compounds, *P. maltophilia* and *P. testosteroni* appeared unable to degrade benzene and xylenes, respectively. When the peat, inoculated with the mixed culture, was used as a biofilter (6.2 cm diameter × 93 cm length) for degradation of toluene and ethylbenzene vapours, percentage removal efficiencies were 99 and 85, respectively. When the capacity of the biofilter to degrade a combination of BTEX compounds was evaluated, percentage removal efficiencies for toluene, ethylbenzene, *p*-xylene, *o*-xylene and benzene were 99, 85, 82, 80 and 78, respectively. The importance of using the mixed culture as an inoculum in the biofilter was established and also the relationship between contaminated vapour flow rate and percentage removal efficiency.

Keywords: biofilter; BTEX; biodegradation; vapours

Introduction

Biofiltration for air pollution control of volatile organic compounds (VOCs) and other toxic vapours has been used in West Germany since the early 1980s as a result of federal regulations introduced to control these emissions [18]. Interest in use of biofilters has developed more recently in North America, driven by more stringent regulatory control of toxic air emissions [9]. Treatment of contaminated vapours by biofiltration can represent an attractive alternative to other methods such as carbon adsorption, catalytic oxidation and incineration. These latter treatments require a heavy investment and continuous maintenance expenses [8,20].

During biofiltration, contaminated vapours entering the filter are adsorbed on a porous filter packing material and biologically degraded by microorganisms which become immobilised in the form of a liquid biolayer on the support material. As the contaminated vapour stream passes through the filter bed, pollutants are transferred from the vapour phase to the biolayer and are metabolised [8]. The indigenous and/or inoculated microbial population in the biofilter typically takes 1–2 weeks to develop and acclimate to a newly-introduced volatile contaminant before efficient biodegradation occurs [14]. Elimination of hazardous hydrocarbons including toluene has been achieved using acclimated activated sludge in a biofilter [15].

Packing media in biofilters range from biomaterials such as composts, wood bark, peat and heather to more amorphous materials such as sand, volcanic ash, charcoal, porous clay and polystyrene spheres. Peat provides an extensive range of nutrients which can support the development of the microbial population, for example in a biofilter [12]. It has long been recognised that some of the properties of peat are appropriate for viable maintenance of microbial cultures [10,11]. Surface-recovered peat material also contains large populations of aerobic bacteria [19]. Peat, particularly material recovered from the surface (up to 1.5 metres), contains a diverse and large aerobic bacterial population and thus provides a microbial reservoir for enrichment and selection of degraders of volatile organic carbons.

In this report, a mixed culture was selectively isolated from peat which degraded BTEX compounds (benzene, toluene, ethylbenzene, xylenes) in flask culture systems. This culture was used as a biofilter inoculum and the capacity of the biofilter to degrade BTEX compounds was characterised.

Materials and methods

Materials

Benzene, toluene, ethylbenzene and xylenes were obtained from British Drug House (BDH, Toronto, ON, Canada) and had a purity of at least 99%. Solvents used in extraction were of analytical or chromatographic grade and were also obtained from BDH. Sodium azide, mercuric chloride and ^{14}C-toluene (purity >98%, carbons universally labelled, specific activity 56.2 mCi mmol^{-1}) were obtained from Sigma (St Louis, MO, USA). Microcosm hypovials (60 and 120 ml) were purchased from Aldrich (Milwaukee, WI, USA). Gas-tight syringes equipped with a Teflon mini-inert valve (1 ml) were obtained from Supelco (Oakville, ON, Canada) and Sigma. All flasks and microcosms coming in contact with hydrocarbons and solvents were made of glass or were Teflon coated. Teflon silicone septa (20 mm) were

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obtained from Rose Scientific Ltd (Edmonton, AB, Canada). The peat moss used was relatively decomposed Sphagnum peat obtained from Plant Products (Bramalea, ON, Canada) and had the following composition, weight percent, dry: ash content, 3; C, 52; H, 6; O, 41; N, 0.5.

Mineral salts media

The mineral salt medium was a modified basal medium described by Zhang *et al* [21]. MSB medium consisted of (g L^{-1}): K_2HPO_4, 5; KH_2PO_4, 3; NH_4Cl, 3; $MgSO_4 \cdot 7H_2O$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01. To this solution, 2 ml per litre of a trace metal solution containing 200 mg L^{-1} of each of the following was added: $CaCl_2$; $CuSO_4 \cdot 5H_2O$; $MnSO_4 \cdot 5H_2O$; $ZnSO_4 \cdot 7H_2O$; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. Dilute MSB medium contained (g L^{-1}): KH_2PO_4, 1; K_2HPO_4 1; KNO_3, 1; $(NH_4)_2SO_4$, 1; $MgSO_4 \cdot 7H_2O$, 0.1; NaCl, 0.1. The pH was adjusted to 7.0.

Strain enrichment and isolation

A reactor containing 10 kg of soil contaminated with 0.5% (w/v) gasoline was aerated at 50 ml min^{-1} and the effluent gases were directed to a plexiglass biofilter containing Sphagnum peat moss having a moisture content of 70% (w/w). After 5 months, during which time the microbial population was adapted to the contaminant, 10 g (wet weight) of peat moss was added to 100 ml of MSB medium in a 250-ml Erlenmeyer flask containing a central 5-ml clear glass vial containing 2 ml of toluene. Flasks were incubated on an orbital shaker at 180 rpm and 30°C. After 3 days, when the toluene had disappeared completely from the vial, 5 ml of culture was transferred to a similar flask containing 5 g (wet weight) of peat autoclaved in the MSB medium. This culture transfer operation was repeated three more times to produce the mixed culture. This mixed culture was used for isolation of pure cultures. The culture broth was serially diluted with sterile distilled water and spread-plated on nutrient agar. The predominant colonies, after a 3-day incubation at 30°C, were isolated and purified.

The mixed culture was also harvested by centrifugation at $4080 \times g$ for 20 min and resuspended in MSB medium to give an A_{600} of 4.8–5 (1-cm light path). This culture was used in BTEX biodegradation studies or stored by mixing 0.8 ml of suspension to 0.2 ml of sterile glycerol in 1-ml sterile Eppendorf tubes. Cultures were stored at –70°C and recovered by transfer to 100 ml MSB medium in 250-ml Erlenmeyer flasks with toluene vapours supplied from the central vial as described above. Flasks were incubated on an orbital shaker at 180 rpm and 30°C for 24 h.

Preparation of bacterial suspensions was standardised by measuring suspension absorbance at 600 nm in a glass cuvette with a 1-cm light path in a spectrophotometer (Shimadzu, model UV-120-02, Kyoto, Japan). Bacterial counts were determined by standard serial dilution followed by spread plating of 0.1-ml aliquots onto nutrient agar. Plates were incubated at 30°C and plates having 30–300 colonies were counted to determine colony-forming units.

Strain identification

Predominant isolates, precultivated on nutrient agar, were identified using the API 20E system (Analytab Products, Plainsview, NY, USA) and the fatty acid Microbial Identification System (MIDI, Newark, DE, USA).

BTEX biodegradation studies in microcosm hypovials

Experiments involving degradation of BTEX compounds were carried out in 60-ml borosilicate glass hypovials containing 30 ml of diluted MSB medium inoculated with the mixed culture to A_{600} of 4.8–5. Hypovials were sealed with Teflon-backed silicon septa and aluminium crimps and were purged for 1 min with oxygen prior to addition of BTEX compounds. Control hypovials were poisoned by addition of 0.5 g L^{-1} mercuric chloride and 0.5 g L^{-1} sodium azide. At each sampling point, triplicate microcosms were withdrawn for analysis.

Mineralisation studies were conducted using 120-ml serum hypovial microcosms sealed with Teflon septum and aluminium crimp. Each microcosm was equipped with central toluene vial and linked to a series of traps for volatile organics (methylene chloride) and CO_2 (0.5 M KOH), as illustrated in Figure 1. Each serum bottle contained 50 ml of the MSB medium inoculated with the mixed culture and each head space was flushed with oxygen for 1 min. The MSB medium was spiked with toluene (containing ^{14}C-toluene) to produce initial toluene concentrations of 50, 100, and 200 mg L^{-1}.

Biofilter apparatus

Biofilters were constructed from plexiglass tubing with a path length of 93 cm and internal diameter of 6.2 cm with 0.6 cm wall thickness (Figure 2). Materials used in construction were stainless steel, Teflon, plexiglass with minor amounts of Nalgene tubing. Sampling ports were located at the entry and exit ports and at three intervals along the length of each column, as illustrated. Column temperatures were maintained at 23–25°C.

Sphagnum peat moss was sieved through a 1.657-mm mesh to remove fibres and other large particles which might affect hydrocarbon flow through the filter. Peat was amended with 3 g each of NH_4Cl, K_2HPO_4, KH_2PO_4 per kg dry peat and the pH of the peat was adjusted to 7.0 with NaOH, 8 g kg^{-1} dry peat. Two litres of mixed culture, A_{600} 4.8–5, were centrifuged, resuspended in 500 ml of the cul-

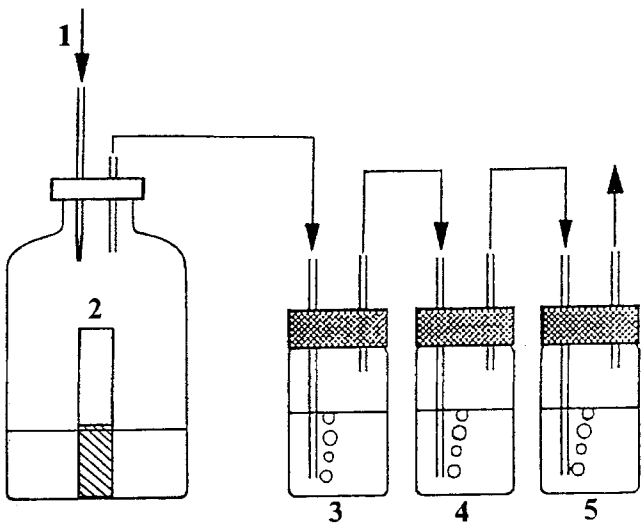

Figure 1 Microcosm and associated vapour traps. Components: 1, purge of nitrogen gas; 2, microcosm; 3, trap containing 10 ml methylene chloride; 4 and 5, traps containing 10 ml of 0.5 M KOH.

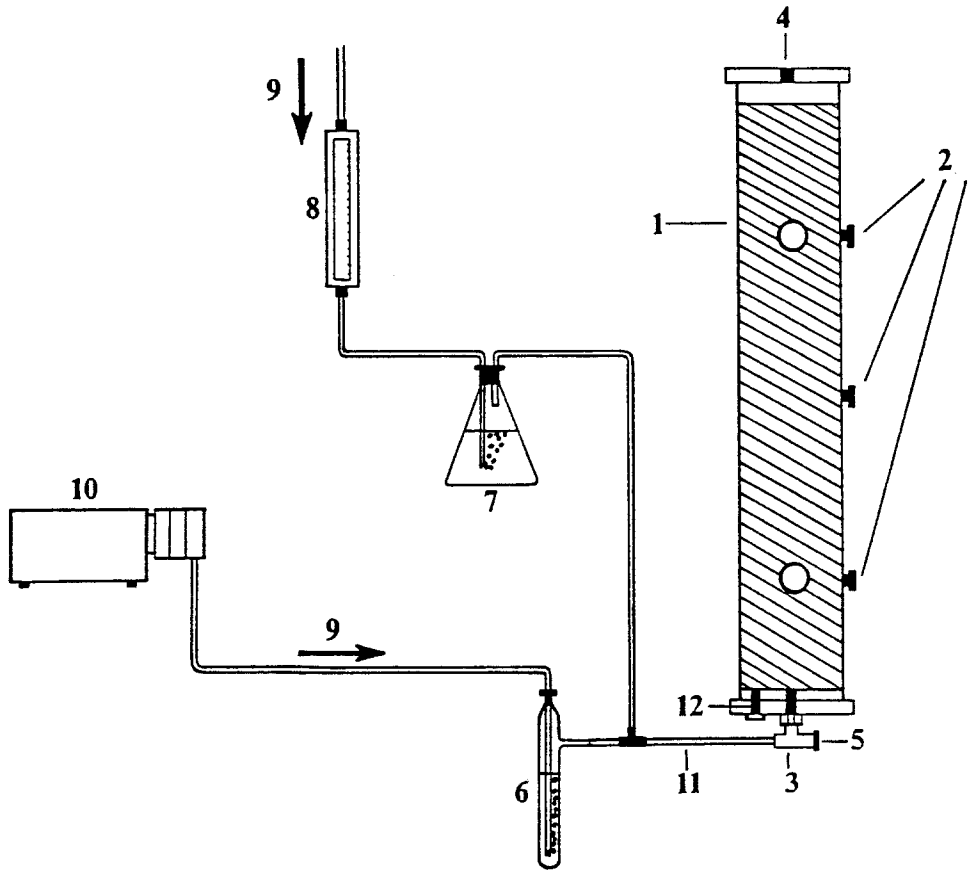

Figure 2 Schematic of laboratory biofilter apparatus. Components: 1, column; 2, sampling ports; 3, inlet; 4, outlet; 5, gas sampling device; 6, solution of hydrocarbon; 7, humidifier; 8, flowmeter; 9, air stream; 10, peristaltic pump; 11, mixing tube.

ture supernatant fluid and added to 1 kg of peat. The biofilter column was packed with 1150 g of inoculated moist peat (70% w/w water) and the surface tapped gently with a 3-cm diameter rod to produce an active filter bed of 3 litres in volume. Contaminated vapour streams were generated by purging air through a test tube containing the desired hydrocarbon. Moist air was generated separately by passing atmospheric air through a humidifier. Both vapour and moist air streams were combined and directed into the biofilter. Concentrations of BTEX compounds in influent and effluent vapours were monitored by GC analysis of samples taken with a 1-ml gas-tight syringe (Hamilton Co, Reno, NV, USA). Control filters contained uninoculated peat moss and $HgCl_2$ (0.1%).

The biofilter containing nutrients and inoculum was purged with air and toluene vapours under standard conditions (inlet flow rate, 10 ml min^{-1}) for 15 days until a steady state was reached. The inlet flow rate was then set at 5 ml min^{-1} and increased in a stepwise manner at 24-h intervals. Concentrations of toluene vapours at the inlet and outlet were sampled six times daily and percentage contaminant removal efficiencies were plotted against flow rate. Residence times of the gas stream within the biofilter were determined from the flow rate and the biofilter open pore volume.

In order to evaluate the importance of using an inoculum in the biofilter, three glass columns of path length 40 cm and internal diameter 3.5 cm, containing nutrient-supplemented peat moss, were set up. The control filter was poisoned with mercuric chloride and sodium azide. The second filter was inoculated with the mixed culture and the third filter was left uninoculated.

Determination of BTEX sorbtion capacity of peat moss

Hydrocarbon sorbtion capacities of peat moss were determined using methods described by Cohen *et al* [4].

The capacity of peat moss to extract free phase hydrocarbons was evaluated in calibrated centrifuge tubes. The tubes, filled with a 1:2 peat:water slurry, were centrifuged at $1470 \times g$ for 5 min. Excess water was poured off. The tubes were further filled with slurry and the centrifuge process was repeated until a wet-packed volume of 10 ml was achieved. Water (10 ml) was added to each tube and, in separate experiments, 0.1 ml of BTEX solvents were added. The tubes were sealed and shaken for 15 min. Tubes were then centrifuged for 3 min at $1470 \times g$. The amount of hydrocarbon absorbed by the peat was determined by measuring the volume of free phase hydrocarbon remaining on the surface of the water. The procedure was repeated with additional increments of 0.2 ml hydrocarbon until no further absorbtion occurred.

The potential of peat moss to extract hydrocarbons from a hydrocarbon-saturated aqueous solution was determined by centrifuging an aqueous peat slurry at $5900 \times g$ for 15 min in 60-ml hypovials (equipped with Teflon-coated sili-

cone septa) to obtain a packed volume of 10 ml. Each vial was then filled with a saturated hydrocarbon solution and 1 g L^{-1} sodium azide. Vials were sealed and shaken to allow adsorbtion of hydrocarbon. Vials were centrifuged at $5900 \times g$ for 15 min and residual hydrocarbon in the supernatant phase was determined by gas chromatography.

Scintillation counting experiments

To recover radiolabelled material, liquid media in microcosms were acidified by adding 0.5 ml of 1 M H_2SO_4. Microcosms were hand-shaken and the head space was flushed with pre-purified nitrogen gas at a rate of 15 ml min^{-1} for 30 min to purge residual toluene and CO_2 through to the series of trapping devices. $^{14}CO_2$ trapped in the microcosm KOH trap (1 ml) and in units B1 and B2 (10 ml each) was withdrawn and added to 8 ml of Ecolite scintillation cocktail (ICN Biomedicals, Irvine, CA, USA) and the radioactivity was counted in a liquid scintillation counter (Beckman LS 1701, Fullerton, CA, USA). To quantify radiolabel in liquid media and cells, biomass was separated by centrifugation at $10444 \times g$ for 10 min. Recovered cells were transferred to scintillation vials containing 8 ml of cocktail. Volatile organics and residual toluene, trapped in unit A, was analyzed by transferring 2 ml of the methylene chloride to 8 ml of cytoscint scintillation cocktail (ICN Biomedicals) followed by scintillation counting.

Gas chromatographic analysis

BTEX compounds were analyzed using a 30-m × 0.32-mm fused silica megabore DB-624 capillary column coated with a 1.8-μm thickness of cyanopropyl, phenyl, dimethyl polysiloxane polymer (Chromatographic Specialities, Brockville, ON, Canada). The gas chromatograph unit (GC-14A, Shimadzu, Kyoto, Japan) was equipped with a splitless injector and flame ionization detector (FID) and was connected to a data integrator (Chromatopac CR501, Shimadzu, Japan). Column injection and detection temperatures were 200°C and 210°C, respectively. Helium at 5 ml min^{-1} was the carrier gas. The temperature programme was 100°C, 2 min; temperature increase rate, 5°C min^{-1}; 200°C, 8 min.

BTEX components were recovered for GC analysis by hexane liquid–liquid extraction using a micro extraction method similar to that of Glaze *et al* [7] and Patrick *et al* [16]. A 2-μl volume of extract was injected into the gas chromatograph. Gas chromatographs were calibrated on a daily basis using three replicate standards.

BTEX concentrations in the vapour phase were injected directly into the GC which was calibrated using standards prepared by adding accurate amounts of BTEX compounds to serum bottles with Teflon-faced silicon septa and allowing complete volatilisation. A 500-μl volume was taken from the serum bottles using a Hamilton gas-tight syringe and injected into the gas chromatograph.

Results

A mixed culture having a capacity to degrade BTEX compounds was developed in Sphagnum peat moss by exposure to gasoline vapours for 5 months. The mixed culture was recovered from the peat moss by serial transfer in toluene-enriched mineral salts medium. The predominant microbial isolates in the mixed culture were identified as *Pseudomonas maltophilia*, *P. testosteroni*, *P. putida* biotype A, and three enteric bacteria, using the MIDI system, which is based on a fatty acid similarity index. The similarity index of the microbial identification system is a numerical value which expresses how closely the fatty acid composition of an unknown organism compares with the mean fatty acid composition of the strain used to create the library entry listed as its match. The database search presents the best matches and associated similarity indices. An exact match gives a similarity index of 1.000 and strains with indices ≥ 0.500 and with a separation between first and second choice of 0.100 are good matches. The three *Pseudomonas* strains had very good matches with the database, namely 0.827, 0.803 and 0.775 for *P. putida* biotype A, *P. maltophilia* and *P. testosteroni*, respectively. The enteric bacteria exhibited weak matches with the database and thus remain unidentified.

The capacity of the mixed culture to degrade individual BTEX components at initial concentrations of 30, 50 and 70 mg L^{-1} in aqueous shaken microcosms was investigated. Average rates and extent of degradation were corrected for abiotic losses. The results are presented in Table 1. In all cases, average degradation rates were low with high initial concentrations of hydrocarbon. With an initial concentration of 30 mg L^{-1} of hydrocarbon, average degradation rates ranged from 0.5 (benzene) to 6.0 (toluene) mg L^{-1} per day over the first 5 days of the experiment. For ethylbenzene and toluene, the average degradation rate from day 5 to day 15 was low, reflecting substrate depletion, but significantly increased with other substrates, probably due to culture acclimation. Overall percentage degradation after 20 days ranged from 90–100% in all cultures except with benzene at initial concentrations of 50 and 70 mg L^{-1}.

The ability of the mixed culture to mineralise toluene was determined in microcosms incubated on an orbital shaker at 180 rpm and 25°C for 14 days. Poisoned microcosm controls containing 0.5 g L^{-1} sodium azide and mercuric chloride were included in the experiment. Residual volatilised products and CO_2 were then collected in the traps by flushing the microcosm with nitrogen gas and the percent distribution of radiolabel for the six triplicate microcosms was determined (Table 2). On average, 92.6% of the radiolabelled material was recovered. In microcosms having initial concentrations of 50 and 100 mg L^{-1} toluene, 66–69% of radiolabelled substrate was converted to CO_2. The low percentage of $^{14}CO_2$ recovered in microcosms with 200 mg L^{-1} substrate suggests that this substrate concentration was toxic for the mixed culture.

The capacity of the mixed culture and three of the individual isolated strains to degrade a mixture of BTEX compounds was also investigated in microcosms containing mineral salts medium supplemented with 70 mg L^{-1} each of toluene, ethylbenzene, *p*-xylene and *o*-xylene and 40 mg L^{-1} of benzene. Cultures were incubated on an orbital shaker at 180 mg L^{-1} at 25°C for 30 days and depletion of BTEX compounds was monitored (Figure 3 a–d). In the case of the mixed culture, high rates of BTEX degradation were observed over the first 15 days and concentrations of

Table 1 Effects of initial concentrations on degradation of benzene, toluene, ethylbenzene, *p*-xylene and *o*-xylene. Degradation was performed in an oxygenated, sealed serum bottle at 25°C for 20 days. Values represent means for triplicate microcosms

Hydrocarbon	Initial concentration (mg L^{-1})	Total hydrocarbon degraded (mg L^{-1}) after		Average degradation rates (mg L^{-1} day^{-1})		% Degradation after 20 days
		5 days	15 days	0–5 days	5–15 days	
Benzene	30	2.5	27.5	0.5	2.5	98
	50	2.0	15.0	0.4	1.3	34
	70	1.0	5.0	0.2	0.4	7
Toluene	30	30.0	0.0	6.0	0.0	100
	50	26.0	46.0	5.2	2.0	100
	70	20.0	47.0	4.0	2.7	100
Ethylbenzene	30	20.0	25.0	4.0	0.5	100
	50	13.5	43.5	2.7	3.0	100
	70	10.0	40.0	2.0	3.0	95
p-Xylene	30	13.0	28.0	2.6	1.5	99
	50	10.0	38.0	2.0	2.8	97
	70	10.0	37.0	2.0	2.7	92
o-Xylene	30	7.5	22.5	1.5	1.5	98
	50	5.0	27.0	1.0	2.2	93
	70	5.0	25.0	1.0	2.0	90

Table 2 Results of the mass balance experiment. Distribution of radiolabels is expressed in percent

Type of hydrocarbon	Radiolabel distribution (%)							
	Biomass	Supernatant medium	Activity in methylene chloride trap (3)[a]	Activity in KOH traps (4,5)[a]	CO_2 in microcosom trap (2)[a]	Total CO_2 recovered	Total radiolabel recovered	Radiolabelled CO2 recovered as % of total radiolabel recovered
Toluene (50 mg L^{-1})								
Test	11.5	16.04	0.40	0.20	62.37	62.57	90.51	69.13
Control	24.6	56.39	7.66	0.09	1.43	1.52	90.17	1.69
Toluene (100 mg L^{-1})								
Test	11.37	21.2	0.25	0.66	63.10	63.80	96.58	66.06
Control	32.80	38.99	15.90	0.20	0.52	0.72	88.41	0.81
Toluene (200 mg L^{-1})								
Test	15.77	47.72	18.40	1.14	8.50	9.64	91.53	10.53
Control	20.35	53.70	16.74	0.05	5.45	5.45	96.24	5.66

[a] Numbers refer to Figure 1.

each contaminant approached zero by the end of the experiment.

In the case of the individual cultures, *P. maltophilia*, *P. testosteroni* and *P. putida* biotype A, the different strains exhibited contrasting BTEX degradation patterns. *P. maltophilia* did not degrade benzene but exhibited high degradation rates on all other BTEX compounds. *P. testosteroni* had a relative inability to degrade xylenes but appeared effective in degradation of ethylbenzene, toluene and benzene. *P. putida* biotype A degraded all of the BTEX compounds but during the initial 7 days, when degradation of toluene and benzene was occurring, there was a lag in the degradation of xylenes and ethylbenzene.

The capacity of peat biofilters, inoculated with the mixed culture and supplemented with nutrients, was investigated. The moisture content of the peat was maintained at 70% w/w. Biofilters poisoned with mercuric chloride (0.5 g L^{-1}) and sodium azide (0.5 g L^{-1}) were run as controls. Humidified air containing hydrocarbon vapours was passed through the biofilters and concentrations of hydrocarbons at the filter inlet and outlet were monitored over the course of each experiment. The pattern of removal of toluene by the biofilter is illustrated in Figure 4 for both the inoculated filter and the poisoned control. Inlet toluene concentration in the air was maintained at 70 mg L^{-1}. During the first 15 days in both filters, some toluene was observed in the outlet while some toluene was also removed by the filter. Because of the lack of microbial degraders in the poisoned filter, it is assumed that the initial toluene vapours were adsorbed by the peat material which resulted in saturation of the filter

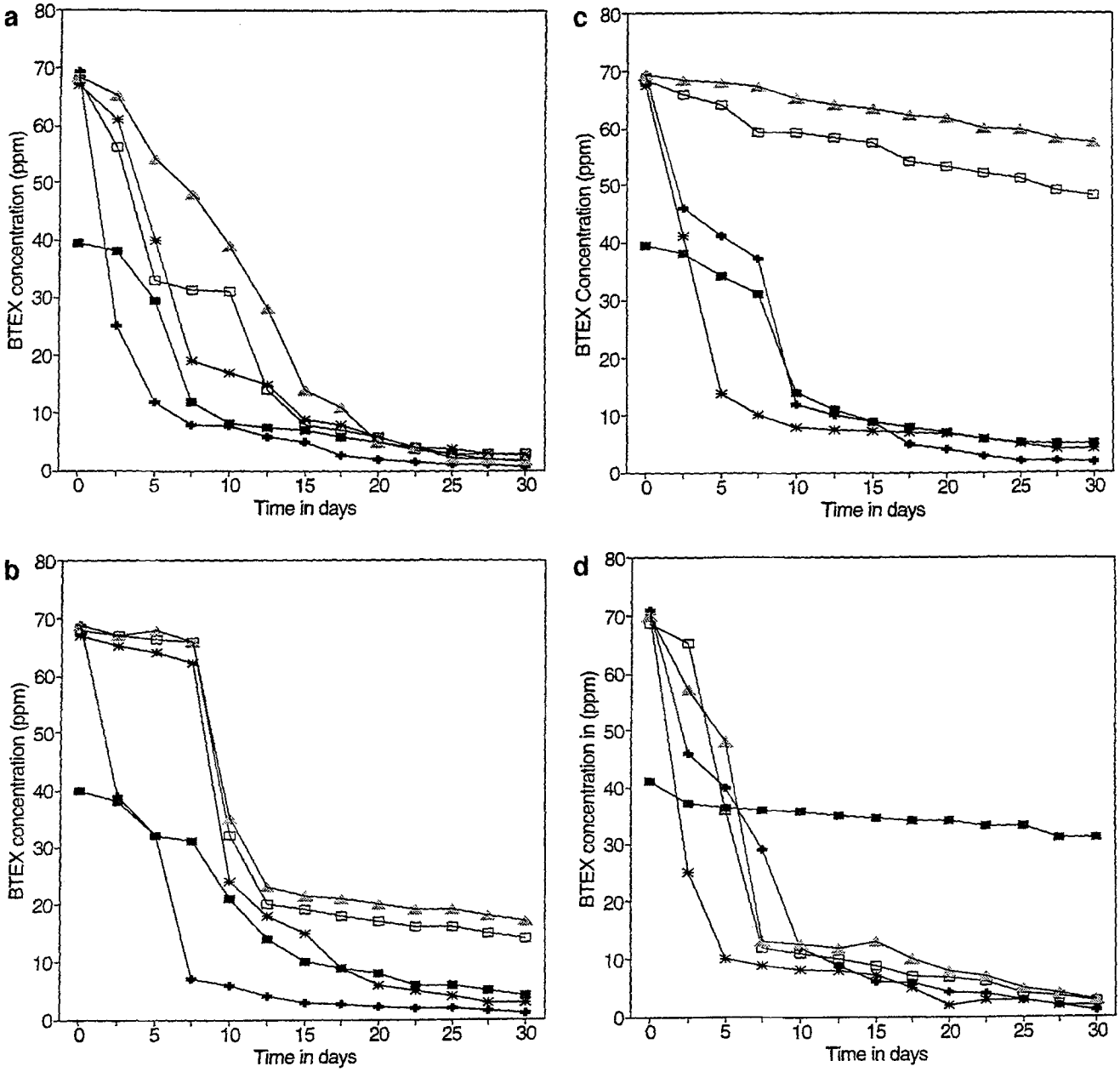

Figure 3 Removal of BTEX compounds from aqueous microcosms inoculated with: a) the mixed culture; b) *Pseudomonas putida* biotype A; c) *Pseudomonas testosteroni*; d) *Pseudomonas maltophilia*. Microcosms were incubated on an orbital shaker at 180 rpm at 25°C. Symbols: ■ benzene; ✚ toluene; ✳ ethylbenzene; ⊟ *p*-xylene; △ *o*-xylene.

after about 2 weeks. Thereafter, the inlet and outlet toluene concentrations were approximately the same. The unpoisoned filter showed a lag period of about 2 weeks during which time outlet toluene vapour concentrations began to decline, probably due to a combination of some toluene absorption to peat and the development of the microbial population. After the acclimation periods, the outlet toluene concentration was 0.45 mg L^{-1} air, representing a removal efficiency of $> 99\%$. Effective toluene removal was observed between days 12 and 23. After 25 days, a reduction in toluene removal efficiency was observed. A second inoculum of the mixed culture and nutrient solution was prepared and injected through the filter sampling devices, which returned the toluene removal efficiency to 99%. Numbers of bacteria capable of utilising toluene in both the active biofilter and the poisoned control were monitored from sampling ports at 15 and 65 cm from the filter inlet over the course of the experiment (Table 3). Low bacterial counts were observed in the poisoned control filter. In the active biofilter, microbial counts increased dramatically during the 15-day acclimation period. The microbial population was much larger at the inlet end of the filter compared to the outlet end, most likely reflecting a declining gradient of contaminant concentration along the length of the filter due to biodegradation.

The time course of degradation of ethylbenzene in the active and poisoned biofilters is presented in Figure 5. The degradation pattern was similar to that observed for toluene,

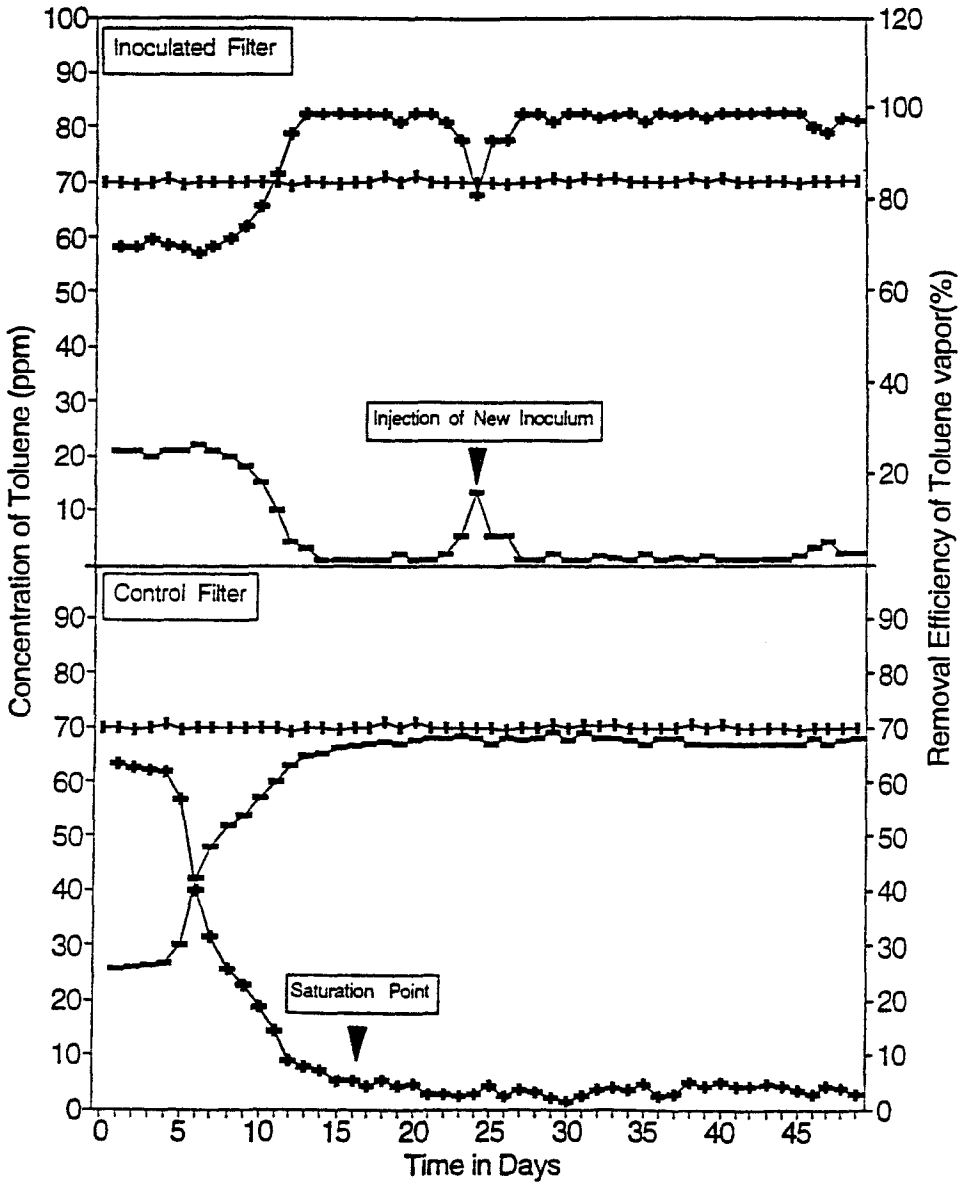

Figure 4 Degradation of toluene vapour in a peat biofilter. The filter contained 3 L of Sphagnum peat moss with a moisture content of 70% w/w. Sphagnum peat moss was neutralized by 8 g NaOH kg^{-1} dry peat and amended with nutrients. The flow rate of gas stream for both filters was 10 ml min^{-1}. Filters were operated at room temperature. Symbols: ■ inlet concentration; ▬ outlet concentration; ✚ removal efficiency.

Table 3 Plate counts of aerobic bacteria for the peat filter column inoculated with a mixed culture of bacteria

Filter column	Sampling port[a]	Plate count (CFU g^{-1})[b]				
		Time (days)[c]				
		0	10	15	25	50
Active filter	6	4.4 E5	2.0 E8	3.4 E9	5.0 E9	2.2 E5
	26	9.0 E5	4.2 E6	1.0 E7	3.1 E8	3.0 E4
Poisoned filter	6	1.2 E1	2.5 E1	3.1 E1	6.73 E2	1.0 E3
	26	2.2 E1	1.3 E1	2.6 E1	9.65 E2	1.2 E3

[a] Distance between bottom of the filter and sampling port (inch).
[b] Microbial numbers reported per gram of peat.
[c] Time of sampling during a 50-day incubation period.

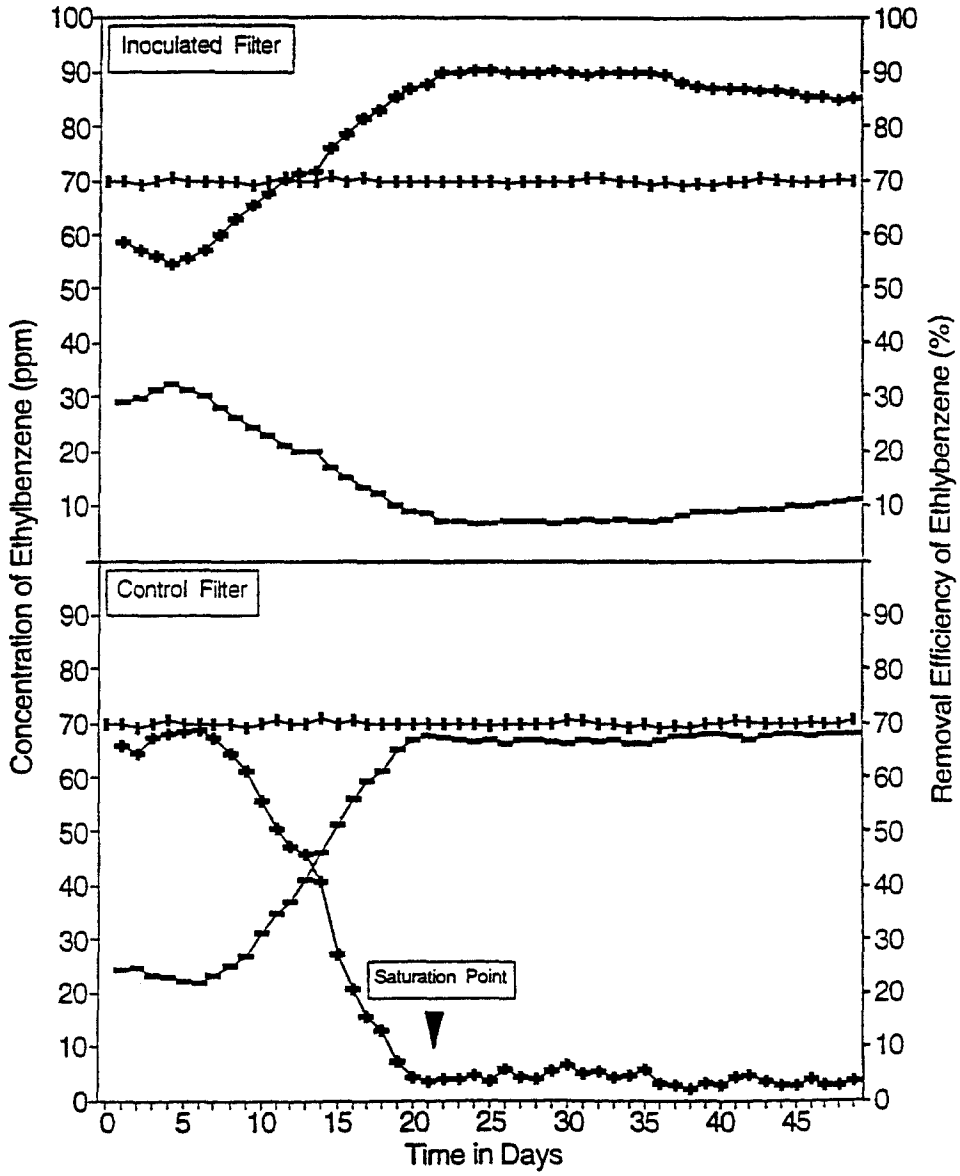

Figure 5 Degradation of ethylbenzene vapours in a peat biofilter. The filter contained 3 L of Sphagnum peat moss with a moisture content of 70% w/w. Sphagnum peat moss was neutralized by 8 g NaOH kg^{-1} dry peat and amended with nutrients. The flow rate of gas stream for both filters was 10 ml min^{-1}. Filters were operated at room temperature. Symbols: ■ inlet concentration; ▬ outlet concentration; ✚ removal efficiency.

manifesting an initial acclimation period during which the poisoned filter became saturated as the outlet ethylbenzene concentration approached the inlet level. After 18 days, the active biofilter had stabilised and manifested an ethylbenzene removal efficiency of about 85%.

The capacity of the biofilter to degrade a combination of BTEX compounds was then investigated using the same biofilter, nutrient addition and inoculum method. The inlet concentration of each BTEX component in the poisoned control and inoculated filter was 70 mg L^{-1}. Hydrocarbon vapours were sampled at the inlet and outlet of the biofilter at 10-day intervals. The results are presented in Table 4. Declines in outlet vapour concentrations were apparent at the 20-day sampling point and stabilised after 30 days. The percentage removal efficiencies are illustrated in Figure 6. After 30 days, percentage removal of toluene, ethylben-

Table 4 Concentration of hydrocarbons in the inlet and outlet of inoculated peat biofilter for degradation of BTEX compounds. Peat moss had a moisture content of 70% w/w and the flow rate of gas stream was 10 ml min^{-1}

Type of hydrocarbons	Hydrocarbon concentration (mg L^{-1})						
	Inlet	Outlet[a]					
Sampling time(days)	0–50	0	10	20	30	40	50
Benzene	70	26	26	17	16	15	15
Toluene	70	28	22	6	1	0.5	0.5
Ethylbenzene	70	30	26	11	10	9	9
p-Xylene	70	28	27	15	13	12	11
o-Xylene	70	27	26	16	15	14	13

[a] Time point of gas sampling.

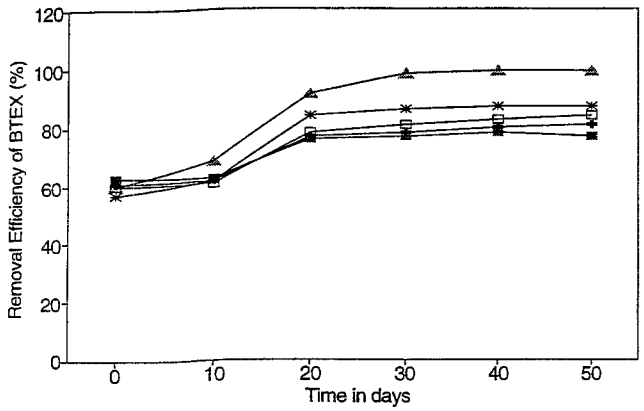

Figure 6 Removal efficiency of a biofilter for a combination of BTEX vapours. The filter contained 3 L of Sphagnum peat moss with a moisture content of 70% w/w. The flow rate of the gas stream was 10 ml min^{-1} and filters were operated at room temperature. Symbols: benzene; toluene; ethylbenzene; *p*-xylene; *o*-xylene.

zene, *p*-xylene, *o*-xylene and benzene was 99, 85, 82, 80 and 78, respectively.

The effect of flow rate/residence time of toluene-contaminated vapours through the plexiglass biofilter was also investigated. Percentage removal efficiency ranged from 55–99.5% for the vapour flow range 80–5 ml min^{-1}. This represents residence times ranging from 0.03–0.5 h (Figure 7).

The patterns of toluene removal from an uninoculated and inocualted filter and also from an abiotic control filter are compared in Figure 8. During the first 10 days, outlet concentrations of 15 mg L^{-1} were observed in all three filters, assumed to be due to toluene absorbance by the peat material. After 10 days the concentration of toluene in the outlet vapours of the poisoned filter gradually increased to approach inlet concentrations. Concentration of toluene in the uninoculated filter also increased but at a more gradual

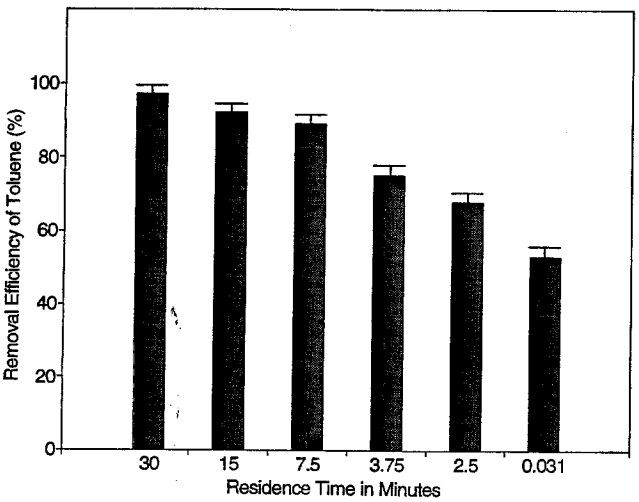

Figure 7 Relation between residence time and removal efficiency of toluene in the peat biofilter. The volume of the filter was 3 L, packed with 1.1 kg of inoculated Sphagnum peat moss. The pH of the support material was brought to neutrality and the moisture content adjusted to 70%. The support material was inoculated with the mixed culture. Change in the flow rate and gas sampling of inlet and outlet of the filter was conducted every 24 h.

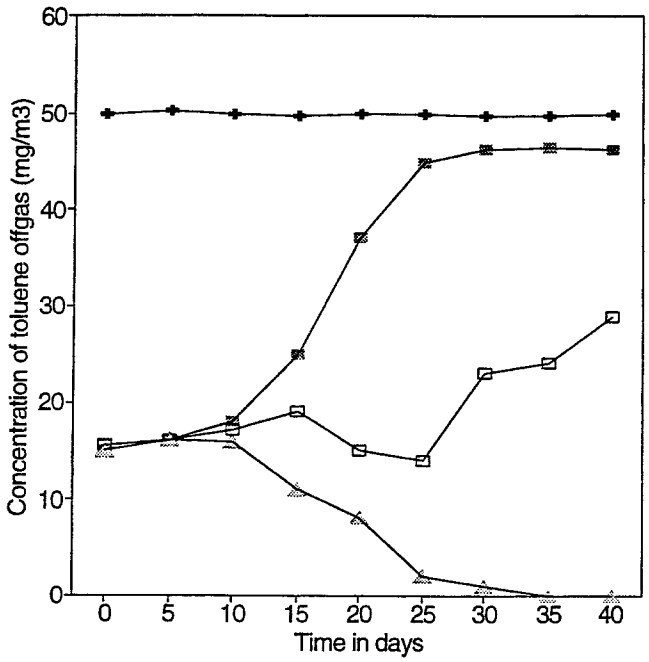

Figure 8 Effect of inoculum in degradation of toluene in peat filters. Three glass filters were used in this experiment. The first filter (control) was poisoned with mercuric chloride and sodium azide. The second filter was inoculated with the mixed culture. The third filter was uninoculated. Symbols: inlet concentration of three filters; outlet concentration of control filter; outlet concentration of uninoculated filter; outlet concentration of inoculated filter.

rate, reaching 29 mg L^{-1} after 40 days. The difference in outlet vapour concentrations between the poisoned control and the uninoculated biofilter was ascribed to biodegradation of contaminant vapours by the indigenous population. In the case of the inoculated biofilter, outlet concentration gradually decreased to zero after 35 days, clearly illustrating the beneficial effect of the inoculum in efficient operation of the biofilter.

The BTEX sorbtion capacity of Sphagnum peat moss was determined by examining contaminant removal from the free phase and from solution. In examining the free phase, where various volumes of each contaminant were mixed with water-saturated peat moss for 15 min, 1 g of dry peat moss absorbed 0.33, 0.30, 0.29 and 0.24 ml of benzene, ethylbenzene, xylenes and toluene. In examining the solution, where vials containing a 10-ml packed volume of water-saturated peat were filled to their 60-ml capacity with water, presaturated with individual BTEX components, the peat moss adsorbed 96, 89, 68, and 84% of available B, T, E and X, respectively.

Discussion

Biodegradation in flask cultures demonstrated the capacity of the mixed culture and three of the individual isolates to degrade BTEX compounds. Greater overall degradation of BTEX compounds was observed with the mixed culture and indeed use of mixed cultures is recognised as important for complete degradation of mixtures of organic hydrocarbons [3]. *P. testosteroni* had a limited ability to degrade *p*- and *o*-xylene while *P. maltophilia* did not appear to

degrade benzene. In general, benzene and xylene isomers are more recalcitrant than toluene. *Pseudomonas* sp strain CFS-215, for example, degraded toluene preferentially over benzene and *p*-xylene [1].

Efficient degradation of toluene and ethylbenzene was observed in the peat biofilter after a lag of approximately 2 weeks. The lag period can be attributed to sorbtion of hydrocarbons by the peat material and concomitant acclimation of bacteria. Contaminant removal from control filters during the first few days of operation clearly demonstrates the sorbtion effect, after which time there was a sharp rise in contaminant concentration in the effluent gas stream. Studies involving contaminant sorbtion onto peat from free phase and solution further illustrate the capacity of peat moss to sorb BTEX components. The sorbtion capacity of peat for a variety of petroleum products is widely recognised [2,5,13]. A review of the literature indicates that the guaiacyl lignin is the major component of peat contributing to sorbtion capacity [4]. Examination of the structure of guaiacyl lignin reveals aromatic rings similar in structure to BTEX compounds. Initial acclimation of an inoculated biofilter to the waste gas stream was reported by Zilli *et al* [22] for degradation of phenol by *P. putida* and by Peters *et al* [17] for VOC treatment.

When a mixture of BTEX compounds was treated in a biofilter, all five components: benzene, toluene, ethylbenzene, *p*-xylene and *o*-xylene were removed, although removal of benzene was least efficient. This is consistent with the degradation observed in flask cultures. Degradation of benzene in vapours is slow when it is the only contaminant present and its biodegradability is significantly increased in the presence of other organic contaminants [9].

Efficient degradation of toluene and ethylbenzene was observed in the biofilters. Eitner [6] reported organic carbon removal efficiencies ranging from 51–94% and odour reductions ranging from 82–99% in studies on biofilter performance.

Flow rate of toluene vapours in the range 5–30 ml min^{-1}, as expected had a dramatic effect on the extent of toluene removal. Average toluene removal increased from 55% to 99.5% for air flow rates ranging from 80–5 ml min^{-1}. The results reported here describe removal of high concentrations of BTEX vapour components using a biofilter. These studies now need to be extended to investigate the effect of vapour contaminant concentration on biofilter removal and relationships between flow rate and contaminant concentration on removal efficiency.

Acknowledgements

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