Microbial treatment of a styrene-contaminated air stream in a biofilter with high elimination capacities

C Juneson, OP Ward and A Singh

Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

A styrene-utilizing mixed microbial culture was isolated and utilized in a biofilter for the biological treatment of a contaminated air stream. Biofilter media consisted of composted wood bark and yard waste. The biofilters were acclimated at 120 s residence time and further evaluated at 60 and 30 s gas residence times. The biofilters received organic loading rates of up to 350 g/m³ h. The styrene volumetric removal rate was a function of the organic loading rate and increased with increasing loading rates. Average volumetric removal rates of 69–118 g/m³ h observed in our studies were higher than reported values for styrene biofilters. Average styrene removal efficiencies ranged from 65% to 75% (maximum 100%). Axial analysis of styrene concentration along the column indicated that the bulk of the styrene removal occurred in the first section of the biofilter. Analyses of the media indicated that the moisture content of the first section (50–55% w/w) was significantly lower than in the second and third sections (65–70% w/w). The pressure drops across the biofilter were low due to the high concentration of large media particles. The total pressure drops were 1–3, 4–6, and 10–16 mm for the 120-, 60-, and 30-s residence time periods, respectively. *Journal of Industrial Microbiology & Biotechnology* (2001) **26,** 196–202.

Introduction

Styrene is widely used as a starting material for the production of synthetic polymers such as polystyrene and as a solvent in the polymer processing industry. As a colorless, volatile, and strong-smelling compound, styrene causes occupational exposure hazards as well as atmospheric pollution. The World Health Organization (WHO) issued guidelines for the maximum permissible concentrations of styrene: 800 μg/m³ for a 24-h average and 70 μg/m³ for a 30-min average [28]. High concentrations of styrene vapors irritate the eyes and nose, and can affect the nervous system, liver, lungs, and kidneys [4,25]. Styrene is one of 189 hazardous air pollutants listed under Title III of the 1990 Clean Air Act Amendments (CAAA) which required a 90% reduction in hazardous air pollutant releases by year 2000 [12]. In general, the concentration of styrene in industrial waste gases is up to 1 g/m³, with an average range of 150–400 mg/m³ [5].

Biofilters have been used for many years for the purification of waste gases and removal of nuisance odorous compounds [13,14,17]. Biological treatment of volatile organic compounds in biofilters offers an inexpensive alternative to conventional technologies such as catalytic and thermal oxidizers, wet scrubbing, ozonation, and activated carbon adsorption [17,24]. The principle of biofiltration is based on the ability of microorganisms to convert, under aerobic conditions, organic pollutants to water, carbon dioxide, and biomass. Reports on the biofiltration of styrene are relatively scarce. In laboratory-scale compost biofilters for the treatment of styrene-contaminated gases, elimination capacities of 2.7–5.9 kg COD/m³ day have been reported [19,22,23]. Recent investigations using bacteria in a peat biofilter [2] and a

biotrickling filter [20] reported styrene elimination capacities of 12–32 g/m³ h. The performance of biofiltration using styrene-degrading fungi has also been evaluated with reported elimination capacities of 62–79 g/m³ h [7,8].

This paper presents the results of biofiltration treatment of a styrene-contaminated airstream with high elimination capacities. A three-stage biofilter, packed with a mixture of composted wood bark and yard waste, was used to investigate the effect of loading rates on styrene removal. The interrelationships between pressure drop, moisture content, biofilter configuration, and styrene removal efficiency are discussed.

Materials and methods

Enrichment of styrene-utilizing microorganisms

The enrichment of microorganisms capable of degrading styrene was performed using a sequential subculture technique in which the sole carbon source was styrene. The culture was grown in 250-ml Erlenmeyer flasks containing 3.5 ml of inoculum and 50 ml of phosphate–ammonium medium containing trace elements. Phosphate–ammonium medium contained (per litre): K_2HPO_4 2 g; KH_2PO_4 2 g; $NaNO_3$ 2 g; NH_4Cl 1 g; $MgSO_4 \cdot 7H_2O$ 0.2 g; $CaCl_2 \cdot 2H_2O$ 0.05 g; $MnSO_4$ 0.02 g; and trace metal solution 3 ml. Trace metal solution contained (per litre): $FeCl_3 \cdot 6H_2O$ 0.162 g; $ZnCl_2 \cdot 4H_2O$ 0.0144; $CoCl_2 \cdot 6H_2O$ 0.012 g; $Na_2MoO_4 \cdot 2H_2O$ 0.012 g; $CaCl_2 \cdot 2H_2O$ 0.006 g; $CuSO_4 \cdot 5H_2O$ 1.9 g; H_3BO_4 0.5 g; and HCl 37 ml. The pH of the medium was adjusted to 7.0.

The inoculum source used was activated sludge from the local wastewater treatment plant. Styrene, as a sole carbon and energy source, was supplied in a gaseous phase through the use of small reservoirs located in the headspace of the flasks. The flasks were stoppered with standard foam plugs and covered with aluminum foil with a moderately tight seal. The shake flasks were incubated
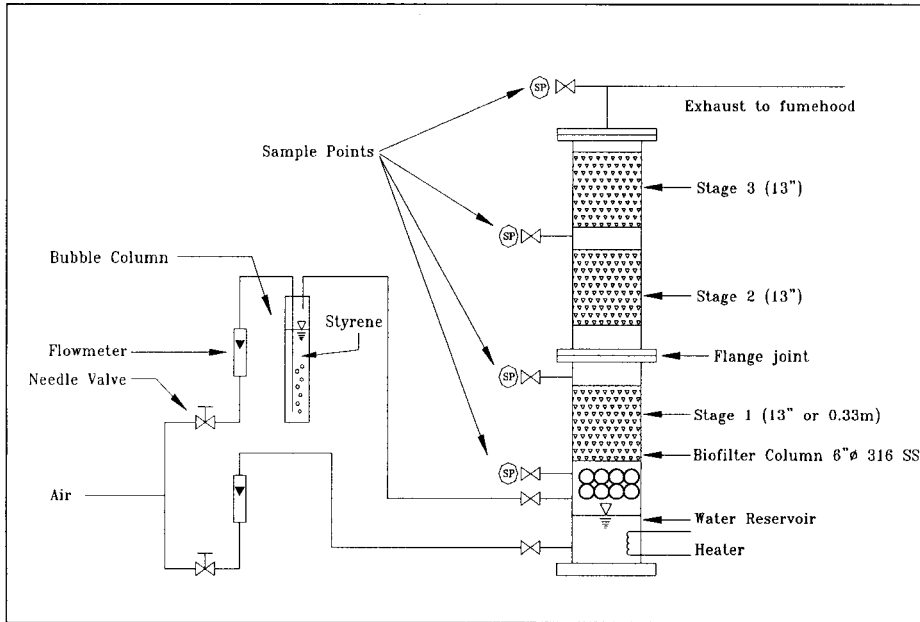
Figure 1 Styrene biofiltration system.

on an orbital shaker (200 rpm) at room temperature (20–22°C) for 5 days. All subsequent subcultures contained 50 ml of the phosphate–ammonium medium and 1 ml of the previous culture, and were incubated for 3 days.

Culture isolation and identification

Pure cultures were isolated from the mixed culture by serially diluting the culture broth and spread-plating on nutrient agar. The nutrient agar (Difco, Detroit, MI) medium contained (per litre): beef extract 3 g; peptone 5 g; and agar 15 g. The predominant colonies after a 3-day incubation at 30°C were isolated and purified. The component strains of the styrene-utilizing culture were isolated and identified using the fatty acid microbial identification (MIDI) system at Ontario Ministry of Agriculture and Food (Guelph, Ontario).

Production of cultures for biofilters

A larger volume of culture was developed in a 1.5-l fermenter. The fermenter was constructed using a 1.5-l Bellco flask where two styrene reservoirs were suspended in the headspace of the flask by galvanized wire, each containing 5 ml of pure styrene. Aeration was provided by 6-mm Tygon tubing and flow rate was monitored and controlled by a 65-mm variable area rotameter and needle valve (Cole Parmer Instrument, Vernon Hills, IL). The liquid culture medium was not sparged directly, but the air stream was provided at a point just above the styrene reservoirs which served to improve the volatilization of the styrene. A magnetic stirrer/heating plate (Cole Parmer, Vernon Hills, IL) and a 25-mm stirrer bar provided mixing at the rate of approximately 75 rpm.

The 10-ml inoculum was provided by the most recent subculture for the fermenter containing 500 ml of phosphate–ammonium-enriched medium. The culture was incubated for 5 days while maintaining the pH of the culture at 7.0 with 5 N HCl or 5 N NaOH. At the end of the growth cycle, 450 ml of the culture was decanted and 450 ml of fresh medium was added to the

fermenter. The fermenter was run for an additional 5 days. This extended growth of the culture resulted in further selection of
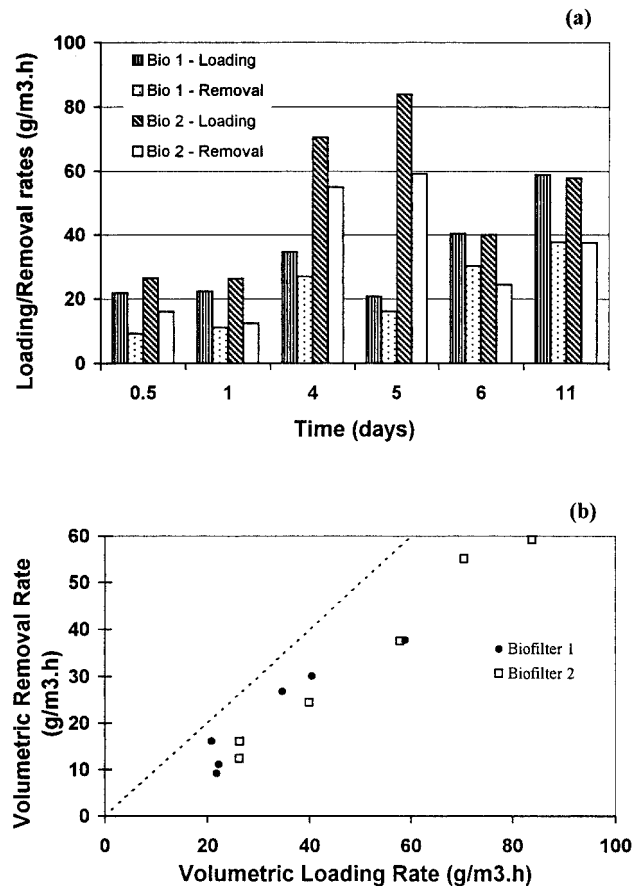

Figure 2 Styrene biofiltration at 120 s residence time. (a) Organic loading rate and volumetric removal rates as a function of time. (b) Volumetric removal rate as a function of organic loading late.

Figure 3 Styrene biofiltration at 60 s residence time. (a) Organic loading rate and volumetric removal rates as a function of time. (b) Volumetric removal rate as a function of organic loading rate.
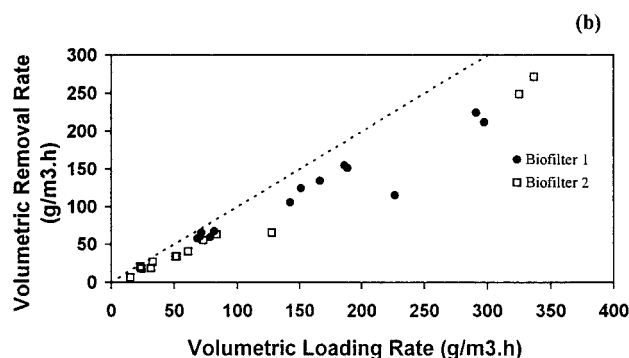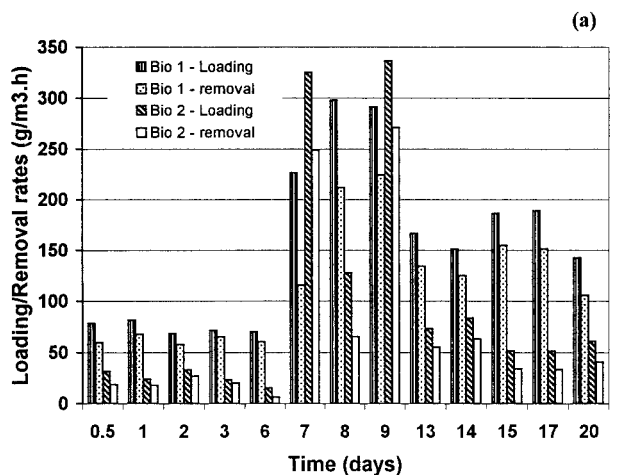
styrene utilizers. The cell concentration at the end of this period was greater than 8 g/l. This culture was used at 1% v/v to inoculate the filter media.

Culture growth was monitored by measuring optical density at 600 nm in a 1-cm light path in a UV spectrophotometer, UV-120-02 (Shimadzu, Kyoto, Japan). Total microbial count (TMC) was determined by spread-plating on nutrient agar. All counts were the averages of three determinations. In general, variations between triplicate plates were <20%.

Biofilter equipment
The biofilter column apparatus used for the experiments is illustrated in Figure 1. The biofiltration column was constructed of 15-cm stainless steel pipe. The height of the column was 1.2 m and the column was separated into four sections. The bottom section served as a water reservoir for humidification of the influent gas stream. The second, third, and fourth sections contained biofilter media. Each active stage was 33 cm high and was packed to a depth of 30 cm with a composted wood product biofilter medium. Media restraints constructed of perforated stainless steel plate held the media in place. A sample port (6 mm) was located on top of each stage. The influent air stream was separated into two components. The slip stream was directed through a styrene bubble column consisting of a modified 500-ml Nalgene graduated cylinder. The bulk component of the air stream entered the biofilter at the bottom port located below the surface of the water. The styrene-contaminated air stream entered the
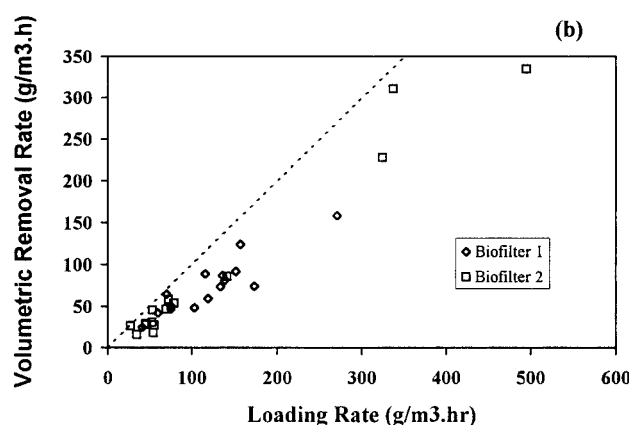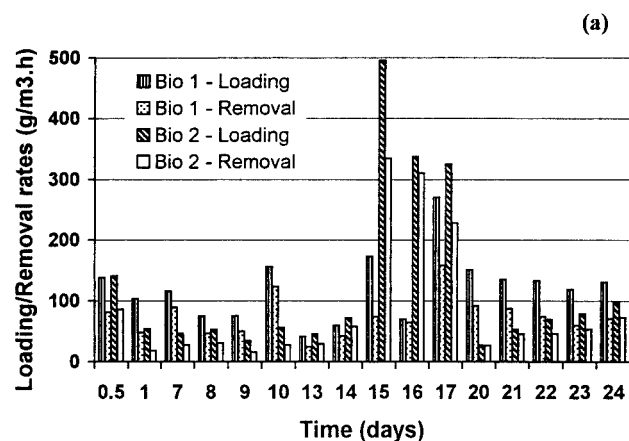

Figure 4 Styrene biofiltration at 30 s residence time. (a) Organic loading rate and volumetric removal rates as a function of time. (b) Volumetric removal rate as a function of organic loading rate.

biofilter column at a point above the surface of the water and mixed with the humidified air stream in a mixing zone packed with 25-mm random polypropylene spheres. The water reservoir
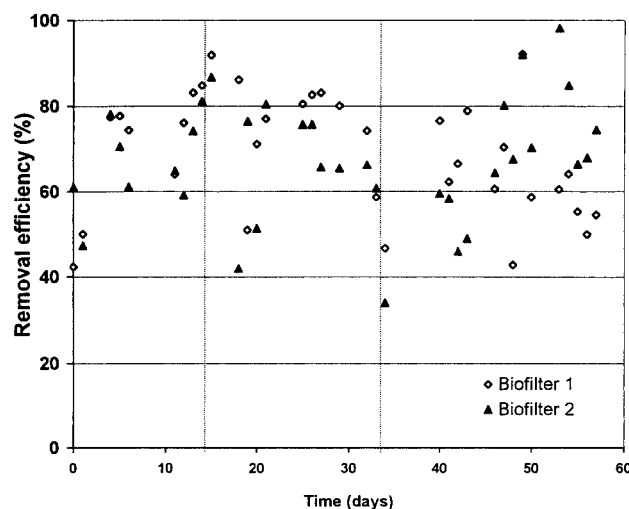

Figure 5 Styrene removal efficiency as a function of time in biofilters at different residence times.

Table 1 Summary of the performance (average values) of two biofilters

Parameters	Residence time (s)		
	120	60	30
Biofilter 1			
Inlet styrene concentration (g/m^3)	0.7 (0.2–1.5)	1.0 (0.2–4.9)	0.7 (0.1–2.4)
Organic loading rate (g/m^3 h)	33 (21–59)	155 (68–298)	122 (41–270)
Volumetric removal rate (g/m^3 h)	22 (9–38)	118 (58–224)	74 (25–158)
Removal efficiency (%)	67 (42–78)	76 (50–91)	61 (42–92)
Biofilter 2			
Inlet styrene concentration (g/m^3)	0.8 (0.2–2.8)	1.0 (0.2–5.6)	0.8 (0.1–4.3)
Organic loading rate (g/m^3 h)	50 (26–84)	95 (15–336)	124 (27–495)
Volumetric removal rate (g/m^3 h)	34 (12–59)	69 (7–271)	88 (15–334)
Removal efficiency (%)	68 (47–78)	73 (42–87)	71 (34–98)

was heated with electric coils to maintain the water temperature at 30°C. The air flow rates of the slip stream and the bulk air stream were measured by stainless steel float rotameters and controlled with needle valves. A Plexi glass sight glass mounted on the side of the column facilitated monitoring of the water level in the biofilter.

Biofilter media

The biofilter medium consisted of a commercial product obtained from CVT America (Lawrenceville, NJ). The medium consisted of a composted wood bark mixed with other yard wastes. Particle size distribution was determined through a sieving process using sieves of sizes 4, 6, and 10 mm. The particle size distribution of the media was <4 mm, 45% w/w; 4–6 mm, 20%; 6–10 mm, 25%; and >10 mm, 10%.

Biofilter operation

Prior to loading the biofilters, the moisture content of the medium was adjusted to 70% w/w by spraying water onto it and mixing thoroughly between water additions. The biofilter medium (16.7 l) was mixed thoroughly with 167 ml of styrene-utilizing microbial culture (1% v/v) in aluminum foil trays prior to loading it in the biofilters. The gas residence time and the flow rate of styrene for start-up of biofiltration were set at 120 s and 15 ml/min, respectively. The duration of the start-up period was 12 days, at the end of which the gas residence time of the biofilters was reduced to 60 s. The flow rate of air through the styrene bubble column ranged from 20 to 45 ml/min. Loading rates were maintained for 4 days, at the end of which air flow rate through the styrene slip stream was varied to change the loading rate. The biofilter run lasted another 4 days and this process was repeated for a period of 4 weeks. Thereafter, the gas residence time of the biofilters was reduced to 30 s and run for 6 weeks. During this period, the flow rate of air through the styrene bubble column was varied between 20 and 60 ml/min and the run time for each flow rate setting was 4 days.

The pressure drop across the biofilter beds was also monitored twice a week. The pressure drop across each section and across the entire bed was measured with a water manometer. The manometer was connected to the sample ports with 6-mm Tygon tubing (Cole Parmer, Vernon Hills, IL).

To evaluate non-biological losses, an abiotic control column was prepared. The moisture content of the biofilter medium was adjusted to about 70% and the medium was sterilized at 121°C for
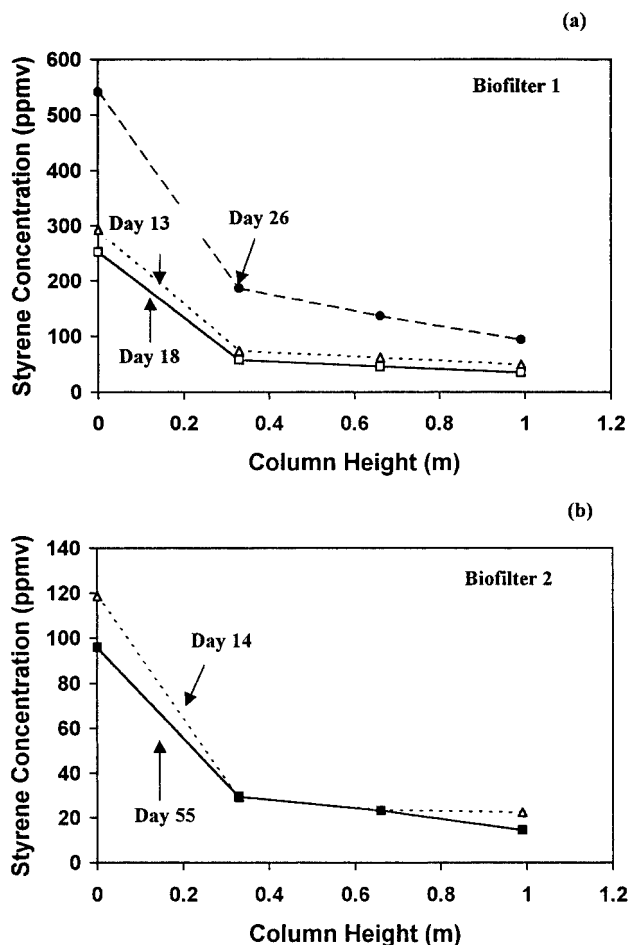

Figure 6 Styrene concentration as a function of column height at different stages of reactor. (a) Axial analysis of biofilter 1. (b) Axial analysis of biofilter 2.

30 min. Thereafter, an aqueous solution of mercuric chloride was mixed thoroughly with the sterilized medium at a final concentration of 4.5%. The styrene removal efficiency of the abiotic control was negligible.

Sampling and gas chromatographic analysis

Samples were taken from sample ports 1 and 4 of the biofilter for influent and effluent gas analyses, respectively. In addition, ports 3 and 4 were sampled to determine styrene concentration at different heights of the filter bed. Styrene analyses were performed in duplicate. The samples were taken with 20-ml gas-tight syringes that also served as temporary storage reservoirs. The syringe mouth was sealed with a septum after the sample was obtained and the sample was analyzed immediately.

For gas chromatography (GC) analysis, 0.1 ml of air was withdrawn from the sample container with a gas-tight syringe (Chromatographic Specialties, Brockville, Ontario) and injected into a gas chromatograph GC-14A (Shimadzu) equipped with a flame ionization detector (FID) and Shimadzu 504 Integrator. The column used for the analysis was DB-624X with internal diameter 0.317 mm and length 30 m (Chromatographic Specialties). Helium was the carrier gas, column temperature was programmed from an initial temperature of 40°C to a final temperature of 150°C, injector temperature was 250°C, and FID was at 275°C. Different masses of styrene were injected into the GC and the peak areas were recorded. Two standards (10 and 50 ppmv in air) used were supplied by Matheson Specialty Gases (Barrie, Ontario).

Results

The main focus of the biofiltration experiments was to investigate and characterize the effect of styrene loading rate and contaminant residence time in the column on styrene removal. Two columns were packed with culture-inoculated biofilter media and set up in the configuration indicated in Figure 1.

At a gas residence time set at 120 s, both biofilter columns were initially operated at a styrene loading rate of 22–26 g/m^3 h for a period of 3 days and then adjusted to 35–84 g/m^3 h for a period of 8 days. The maximum removal rates for loadings of 58, 70, and 84 g/m^3 h were 38, 55, and 60 g/m^3 h, respectively (Figure 2).

The column gas residence time was adjusted to 60 s and the effect of styrene loading rate on contaminant removal was again evaluated (Figure 3). At a residence time of 60 s, maximum removal rates for loading of 291, 325, and 337 g/m^3 h were 224, 248, and 271 g/m^3 h, respectively (Figure 3). At a gas residence time of 30 s, maximum styrene removal rates for loadings of 324,
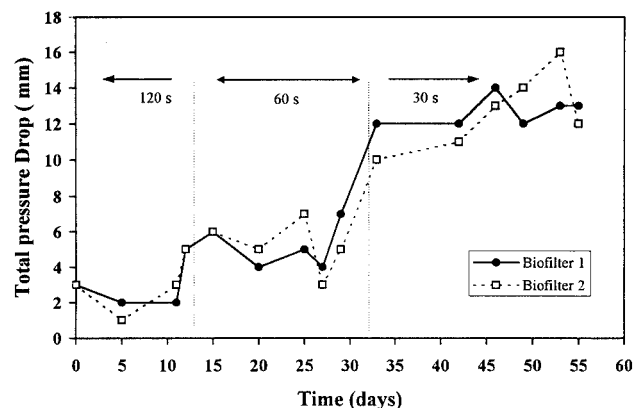
Table 2 Environmental conditions of the biofilters

Column and stage	Moisture content (%)	Total microbial count (cfu/g dry wt)	Pressure drop (mm H_2O)
Biofilter 1-1	55.4	6.1×10^7	0–2
Biofilter 1-2	66.7	3.8×10^7	1–3
Biofilter 1-3	70.2	5.0×10^7	2–8
Biofilter 2-1	60.9	2.3×10^7	0–2
Biofilter 2-2	67.8	4.8×10^7	1–3
Biofilter 2-3	73.9	5.5×10^7	2–7

Figure 7 Total pressure drop as a function of time in styrene biofilters.

337, and 495 g/m^3 h were 228, 310, and 334 g/m^3 h, respectively (Figure 4).

Volumetric removal efficiencies observed in the columns were plotted as a function of organic loading for the three residence times. The data are presented in Figure 5. Removal efficiencies fluctuated during daily operation of the biofilters. Removal rates ranged from a low of 45% to a high of 91% (average 75%) for the 60-s residence time runs. The removal efficiency for the 30-s experiment ranged from a low of 35% to a high of 90% (average 65%). A summary of performance of the two biofilters is provided in Table 1.

The styrene concentration was monitored along the column height and the results for biofilters 1 and 2 are summarized in Figure 6. The biofilters were sampled between each stage at the 0.33-, 0.66-, and 0.99-m heights. The bulk of the styrene (80% of total) removal occurred in the first stage of both biofilter columns.

At the conclusion of the 30-s residence time biofilter runs, the media from each stage were sampled and analyzed for moisture content, TMC, and pressure drops. The results are presented in Table 2. The moisture content of the bottom stage for both biofilters was significantly lower than the other stages. TMC ranged from 2.3×10^7 to 6.1×10^7 in different sections of the biofilters. The pressure drop across the first stage was the lowest. The moisture analysis performed on media from the three stages correlated well with the pressure drop observations. The first stage exhibited the lowest moisture content and pressure drop and the third stage had the highest moisture content and pressure drop.

The pressure drop data across the biofilter columns over the course of the experiments are summarized in Figure 7. The total pressure drop across the biofilter column ranged from 1 to 3, 4 to 6, and 10 to 16 mm for the 120-, 60-, and 30-s residence time periods, respectively.

Discussion

Six predominant pure cultures, isolated from the liquid enrichment cultures and identified, were *Stenotrophomonas maltophilia*, *Flavobacterium mizntali*, *Nocardia globerula*, *Rhodococcus erythropolis*, *Micrococcus varians*, and *Xanthobacter agilis*. These could potentially be the organisms present in the styrene-degrading mixed culture inoculated, but there is no evidence that they survived. Styrene degradation by strains from four of the genera, *Pseudomonas* [16], *Nocardia* [19], *Rhodococcus* [26], and

Xanthobacter [9], has been commonly reported in the literature [25].

The gas residence time during the start-up period was 120 s while the concentration of styrene ranged from 0.2 to 2.8 g/m³. The initial adsorption of styrene onto the surface area of the biofilter media was completed during the first hour of operation. This observation suggested that physical removal mechanisms of styrene did not contribute significantly to overall removal of styrene. The removal efficiencies were low during the first 2 days of start-up, and thereafter increased. The increase in volumetric removal efficiencies of the two biofilters indicated the successful acclimatization of the styrene-utilizing microbial culture to the biofilter conditions in 11 days. Biofiltration with compost, soil, or activated sludge inocula requires long acclimation periods ranging from several weeks to months to obtain good elimination capacities [3,24]. However, inoculation of the biofilter media with specific and adapted microorganisms reduced the start-up time to achieve stable elimination capacities [1,21].

The loading rate at the 60- and 30-s residence times was 25–500 g/m³ h and styrene concentration was 0.2–5.6 g/m³. Maximum styrene removal rates of 271 and 334 g/m³ h were observed at 60 and 30 s residence times, respectively. The relationship between loading rate and removal rate observed for the 30-s residence time was the same as that observed for the 60-s residence time. It is evident from the results that as the organic loading rate increased, the volumetric removal rates increased as well up to a certain threshold. Above that critical loading rate, any further increases in loading rate resulted in the same removal rate [18,23].

Volumetric removal rates up to 62 g/m³ h in a styrene-degrading biofilter containing the yeast *Exophilia jeanselmei* have been reported [8]. Under optimum moisture and oxygen conditions, the yeast biofilter exhibited removal rates up to 91 g/m³ h [7]. In a bacterial peat biofilter, mean styrene reduction and volumetric removal rates were 70% and 12 g/m³ h, respectively [2]. The commercial composted wood medium used in the present investigation has been optimized by the manufacturer for use in air contaminant biodegradation. Peat biofilters tend to compress, giving a high-pressure drop. These two aspects may explain the better styrene degradation performance of our system. The lower rates of styrene degradation for a yeast culture likely reflect the fact that mixed cultures have greater potential to optimally adapt to the biofilter conditions.

Analysis of styrene concentration along the column indicated that the bulk of the styrene removal occurred in the first stage of both biofilter columns. Higher removal rates in the zones of the biofilter closest to the influent port were probably due to the high concentration gradient resulting in higher mass transfer rates into the biofilm. In most of the biofiltration studies, most of the contaminant removals have been reported near the inlet at the first stage of the biofilter [3,8].

A moisture content of 40–65% is required for optimal performance of compost biofilters [6,15,27]. Low water content results in a decreased biodegradation activity and the formation of preferential flow channels in the filter bed [10,13], whereas too much moisture can lead to clogging, compaction, and formation of anaerobic zones [6].

The TMC values were relatively high in stage 1 of biofilter 1 as compared with the stage 1 of biofilter 2. Since the medium was a composted wood and yard waste mixture, the composting process would have continued due to highly aerobic, wet, ambient biofiltration conditions. This may have contributed to the higher TMC values observed for stages 1 and 2. During the sampling of the medium, the top biofilter section (stage 3) did not have a distinct styrene aroma, but rather a strong pungent odor of organic acids and aldehydes typically associated with compost piles.

The pressure drop is a function of the superficial gas velocity through the biofilter column and as the velocity or flow rate increases, the pressure drop increases proportionally. The pressure drop observed across the biofilters was low, suggesting the absence of both compacting problems and excess biomass growth into the packing [11,13]. The particle size distribution did not change much during the course of the biofilter experiments and the high concentration of large particles probably resulted in high porosity. The lower pressure drop reduces energy requirements to pass air through the biofilter and reduces the need to have a more pressure-resistant biofilter housing. With a higher pressure drop, there is potential for greater liquid retention in the biofilter medium, thereby reducing the oxygen transfer efficiency of the system.

Conclusion

Biological treatment of a styrene-contaminated air stream was successfully performed with high removal rates in a biofilter containing a commercial medium consisting of composted wood bark and yard waste.

References

1 Acuna ME, F Perez, R Auria and S Revah. 1999. Microbiological and kinetic aspects of a biofilter for the removal of toluene from waste gases. *Biotechnol Bioeng* 63: 175–184.
2 Arnold M, A Reittu, A von Wright, PJ Martikainen and M-L Suihko. 1997. Bacterial degradation of styrene in waste gases using a peat filter. *Appl Microbiol Biotechnol* 48: 738–744.
3 Baltzis BC. 1998. Biofiltration of VOC vapors. In: Lewandowski GA and LJ Defilippi (Eds.), Biological Treatment of Hazardous Wastes. John Wiley and Sons, New York, pp. 119–150.
4 Bond JA. 1989. Review of the toxicology of styrene. *Crit Rev Toxicol* 19: 227–249.
5 Chou M-S and C-C Hsiao. 1998. Treatment of styrene-contaminated airstream in biotrickling filter packed with slags. *J Environ Eng* 124: 844–850.
6 Corsi RL and L Seed. 1995. Biofiltration of BTEX: media, substrate, and loading effects. *Environ Prog* 14: 151–158.
7 Cox HHJ, FJ Magielson, HJ Doddema and W Harder. 1996. Influence of the water content and water activity on styrene degradation by *Exophiala jeanselmei* in biofilters. *Appl Microbiol Biotechnol* 45: 851–856.
8 Cox HHJ, RE Moerman, S van Baalen, WNM van Heiningen, HJ Doddema and W Harder. 1997. Performance of a styrene-degrading biofilter containing the yeast *Exophiala jeanselmei*. *Biotechnol Bioeng* 53: 259–266.
9 Hartsman S, JP Smits, MJ van der Werf, F Volkering and JAM de Bont. 1989. Metabolism of styrene oxide and 2-phenylethanol in the styrene-degrading *Xanthobacter* strain 124X. *Appl Environ Microbiol* 55: 2850–2855.
10 Heslinga DC. 1994. Biofiltration Technology. VDI Berichte 1104. VDI Verlag, Dusseldorf, pp. 13–18.
11 Kiared K, L Bibeau, R Brzezinski, G Viel and M Heitz. 1996. Biological elimination of VOCs in biofilter. *Environ Prog* 15: 148–152.
12 Lee B. 1991. Highlights of the Clean Air Act Amendments of 1990. *J Air Waste Manage Assoc* 41: 16–19.
13 Leson G and AM Winer. 1991. Biofiltration: innovative air pollution control technology for VOC emissions. *J Air Waste Manage Assoc* 41: 1045–1054.
14 Mallakin A and OP Ward. 1996. Degradation of BTEX compounds in liquid media and in peat biofilters. *J Ind Microbiol* 16: 309–318.

202

15 Medina VF, T Webster, M Ramaratnam and J Devinny. 1995. Treatment of gasoline residuals by granular activated carbon based biological filtration. *J Environ Sci Health* A30: 407–422.

16 O'Connor K, CM Buckley, S Hartmans and ADW Dobson. 1995. Possible regulatory role for non-aromatic carbon sources in styrene degradation by *Pseudomonas putida* CA-3. *Appl Environ Microbiol* 61: 544–548.

17 Ottengraf SPP. 1986. Exhaust gas purification. In: Rehm H-J and G Reeds (Eds.), Biotechnology, Vol. 8. VCH, Weinheim, pp. 425–452.

18 Ottengraf SPP and AHC van den Oever. 1983. Kinetics of organic compound removal from waste gases with a biological filter. *Biotechnol Bioeng* 25: 3089–3102.

19 Ottengraf SPP, JPP Meesters, AHC van den Oever and HR Rozema. 1986. Biological elimination of volatile xenobiotic compounds in biofilters. *Bioproc Eng* 1: 61–69.

20 Pol A, FJJ van Haren, HJM Op den Camp and C van der Drift. 1998. Styrene removal from waste gas with a bacterial biotrickling filter. *Biotechnol Lett* 20: 407–410.

21 Shareefdeen Z and BC Baltzis. 1994. Biofiltration of toluene vapor under steady-state and transient conditions: theory and experimental results. *Chem Eng Sci* 49: 4347–4360.

22 Sorial GA, FL Smith, MT Suidan, A Pandit, P Biswas and RC Brenner. 1998. Evaluation of trickle-bed air biofilter performance for styrene removal. *Water Res* 32: 1593–1603.

23 Togna AP, GJ Sklandany and JM Caratura. 1994. Treatment of BTEX and petroleum hydrocarbon vapors using a field pilot bioreactor. Proceedings of the 49th Purdue Industrial Waste Conference. Lewis Publishers, Chelsea, pp. 437–448.

24 Van Groenestijn JW and PGM Hesselink. 1993. Biotechniques for air pollution control. *Biodegradation* 4: 283–301.

25 Warhurst AM and CA Fewson. 1994. Microbial metabolism and biotransformations of styrene. *J Appl Bacteriol* 77: 597–606.

26 Warhurst AM, KF Clarke, RA Hill, RA Holt and CA Fewson. 1994. Metabolism of styrene by *Rhodococcus rohodochrous* NCIMB 13259. *Appl Envrion Microbiol* 60: 1137–1145.

27 Webster TS, JS Devinny, EM Torres and SS Basrai. 1996. Biofiltration of odors, toxics and volatile organic compounds from publicly owned treatment works. *Environ Prog* 15: 141–147.

28 WHO. 1987. Air quality guidelines for Europe. *WHO Reg Publ Eur Ser* 23: 118–127.