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Biodegradation of BTEX vapors in a silicone membrane bioreactor system

H Attaway[1], CH Gooding[2] and MG Schmidt[1]

[1]*Department of Microbiology and Immunology, Medical University of South Carolina, 173 Ashley Avenue, P.O. Box 250504 Charleston, SC 29425-0504, USA;* [2]*Department of Chemical Engineering, Clemson University, Clemson, SC 29634-0909, USA*

The biotreatment of complex mixtures of volatile organic compounds (VOCs) such as benzene, toluene, ethylbenzene, and xylene isomers (BTEX) has been investigated by many workers. However, the majority of the work has dealt with the treatment of aqueous or soil phase contamination. The biological treatment of gas and vapor phase sources of VOC wastes has recently received attention with increased usage of biofilters and bioscrubbers. Although these systems are relatively inexpensive, performance problems associated with biomass plugging, gas channeling, and support media acidification have limited their adoption. In this report we describe the development and evaluation of an alternative biotreatment system that allows rapid diffusion of both BTEX and oxygen through a silicone membrane to an active biofilm. The bioreactor system has a rapid liquid recycle, which facilitates nutrient medium mixing over the biofilm and allows for removal of sloughing cell mass. The system removed BTEX at rates up to 30 μg h^{-1} cm^{-2} of membrane area. BTEX removal efficiencies ranged from 75% to 99% depending on the BTEX concentration and vapor flowrate. Consequently, the system can be used for continuous removal and destruction of BTEX and other potential target VOCs in vapor phase streams. *Journal of Industrial Microbiology & Biotechnology* (2001) **26,** 316–325.

Keywords: bioreactor; silicone membrane; BTEX; vapor-phase; biodegradation

Introduction

Volatile organic compounds (VOCs) constitute a significant portion of the hazardous wastes being treated globally today. Many of these compounds are readily biodegraded under aerobic conditions. Although the biotreatment of VOCs in soils, groundwater and liquid impoundments have been heavily studied, it has only been within the last decade that biotreatment has become an accepted and mature technique for treating hazardous vapor streams [19,27]. Typical treatment processes include biofiltration, bioscrubbers and trickling bed filters. Each of these processes has limitations including overgrowth of biomass within the filtration unit, backpressure fluctuations, channeling of the vapor stream, acidification of the filter bed over time, and moisture control due to evaporation at high vapor flow rates [5,18,27].

When VOCs are treated aerobically in stirred-tank or liquid-recycle reactors, oxygen transfer to the biodegrading microorganisms usually becomes a limiting factor. Typically, most bioreactor systems use an air sparge or bubbling method to deliver oxygen to a contaminated aqueous wastestream. This method is problematic in that the retention time of the air bubbles in the reactor systems is usually short and does not allow for complete transfer of oxygen to the liquid phase, and as the air bubbles pass through the system they may strip VOCs from the liquid before they can be completely degraded [28]. The use of pure oxygen in these systems can reduce sparging requirements but adds significant expense to the process.

Correspondence: MG Schmidt, Department of Microbiology and Immunology, Medical University of South Carolina, 173 Ashley Avenue, P.O. Box 250504, Charleston, SC 29425-2230, USA
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To get around these problems researchers have investigated the use of "bubbleless" aeration membrane systems [6,22,27]. Some high-transfer microporous membranes have shown good results for low biomass systems such as secondary nitrification treatment [6], but in high organic loading situations they tend to require constant backflushing to keep the micropores free of biomass plugging (Refs. [19,22,27]; unpublished research in this laboratory) as well as being expensive to purchase.

Nonporous diffusive materials such as silicone rubbers have been used to aerate liquid wastestreams because they are highly permeable to oxygen [27,28]. Debus [9] and Debus and Wanner [10] used silicone tubing as a bubbleless source of pure oxygen to treat ethylbenzene and xylenes in aqueous wastestreams.

Silicone rubber membranes have also been used as the basis for extractive membrane bioreactors (EMB) in which a liquid or gas VOC stream is passed along one side of the membrane and an oxygenated liquid biomedium is passed along the other side [13–16,20,25]. Hydrophobic organics in the wastestream diffuse through the membrane to an active biofilm on the biomedium side where they are metabolized. In both the aeration system and EMB scenarios, oxygen and the waste substrates were supplied on opposite sides of the membrane to a metabolizing biofilm. Biofilm thickness was of major importance in terms of biodegradative efficiency [9,10,14–16,20]. The biofilm, which grew in thickness throughout the testing periods, was able to minimize transfer of the organics from the waste phase through the silicone membrane to the clean oxygen or aqueous biomedium phase. However, in both cases deterioration was reported when biofilm thickness became large enough that mass transfer of oxygen and/or substrate was impeded. This should not be a constraint in a system in which both oxygen and the organic substrates are delivered from one

side of the membrane to the surface of the biofilm. As the biofilm thickness increases, there is no physical impediment and the surface organisms still receive the same quantity of substrate and oxygen.

Benzene, ethylbenzene, toluene and xylenes are among the most often cited VOC environmental contaminants due to their ubiquitous presence in commonly used fuel and petroleum products. Because they are both toxic and relatively water soluble compared to other petroleum constituents, their entry into surface and drinking water is of concern. Much of the early work with volatile hydrocarbons focused on the biodegradation of single compounds by isolated bacterial strains [3,8,11,17,23]. However, with the increasing use of groundwater, soil, and vapor-phase bioremediation systems to treat petroleum-based contamination, investigations of multicomponent waste streams have become more common [1,2,4,7,10,13,26].

We have investigated the use of a gas-permeable silicone membrane system to treat contaminated vapor streams optimally by providing a multisubstrate blend of benzene, ethylbenzene, toluene, *m*-xylene, *p*-xylene and *o*-xylene (BTEX) coincident with oxygen to an actively metabolizing dual-strain biofilm.

Materials and methods

BTEX-degrading strains

The organisms selected as the inoculum for the membrane bioreactor were two *Pseudomonas putida* isolates, designated TX1 and BTE1, obtained from a mixed culture enrichment that readily degraded BTEX. BTEX in this work refers to a six-component blend of benzene, toluene, ethylbenzene, *m*-xylene, *p*-xylene, and *o*-xylene. The isolates were enriched from river sediment acquired at an industrial portage site in Charleston, South Carolina by serially transferring portions of sediment in basal salts medium containing BTEX. After three successive transfers over 3 weeks, two distinct BTEX-degrading isolates were defined by plating them on basal salts agar exposed to BTEX vapors. The two isolates demonstrated distinct aromatic substrate utilization patterns with BTE1 using benzene, toluene, and ethylbenzene as growth substrates whereas TX1 used toluene, *m*-xylene, and *p*-xylene. Growth was defined by the increase in culture turbidity concurrent with loss of substrate. TX1 also grew on the xylene metabolic pathway intermediates *m*- and *p*-toluate whereas BTE1 could not. Neither isolate utilized *o*-xylene as a growth substrate but both exhibited co-oxidative degradation of it in the presence of the other BTEX compounds.

Media

The basal mineral salts medium used for reactor operation contained (per liter of distilled water): K_2HPO_4, 5.0 g; NaH_2PO_4, 2.5 g; $(NH_4)_2SO_4$, 1 g; disodium nitrilotriacetate, 15 mg; $MgSO_4{\cdot}7H_2O$, 30 mg; $CaCl_2$, 10 mg; $MnSO_4{\cdot}H_2O$, 5 mg; $FeSO_4{\cdot}7H_2O$, 1 mg; $CoCl_2$, 1 mg; $ZnSO_4{\cdot}7H_2O$, 1 mg; $CuSO_4{\cdot}5H_2O$, 0.1 mg; H_3BO_3, 0.1 mg; $Na_2MoO_4{\cdot}2H_2O$, 0.1 mg. The pH was adjusted to 7.0 with 1 N NaOH.

Typical heterotrophic plate counts were made on tryptic soy agar (TSA; Difco, Detroit, MI). BTE1 could be differentiated easily from TX1 on TSA because it produced a whiter and more raised colony. The ratios of BTE1 to TX1 during reactor operation were calculated from cell counts made on TSA. The concentration of TX1 was further confirmed using a differential plating medium for TX1 made by adding 3 mM *p*-toluate to 1 l of basal salts medium solidified with 16 g of Bacto-Agar (Difco).

Analytical procedures

BTEX vapor samples from reactor runs were analyzed with a Hewlett-Packard 5890A gas chromatograph (GC) equipped with a 30-m Hewlett-Packard HP-624 column and FID detector (Hewlett-Packard, Wilmington, DE). The concentration of BTEX components in the liquid were not measured directly but were assumed to be present in proportion to their vapor concentration as defined by their Henry's Law coefficients. BTEX gas standards were made by adding known amounts of neat BTEX components to serum bottles that had been volumetrically measured and were capped with Teflon-lined rubber septa. The BTEX components were allowed to volatilize completely in the bottle for approximately 1 h at 25°C, at which time they were sampled with a 50 μl gastight syringe. BTEX concentrations were measured as ppm_v (micromoles substrate per mole of air at 25°C) and reported as the sum of the individual component concentrations.

Culture optical density (OD) was measured spectrophotometrically at 660 nm. Protein concentrations were determined by the bicinchoninic acid enhanced method (BCA protein assay kit instructions, Pierce Chemical, Rockford, IL) using bovine serum albumin as the standard.

Membrane bioreactor design

The system was designed for the passage of BTEX-laden air through a dry silicone tube that in turn would allow diffusion of BTEX out to an active aqueous biofilm as diagrammed in Figure 1. The system provided both the substrate (BTEX) and the electron

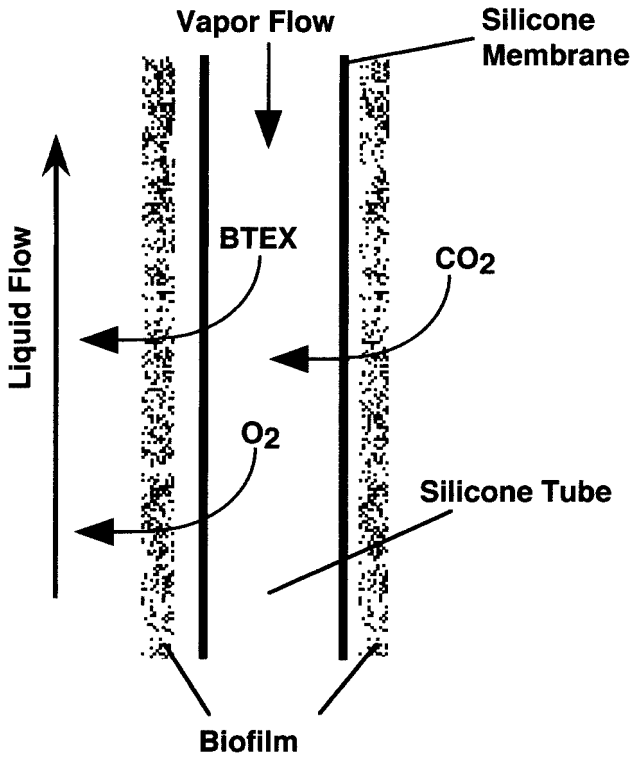

Figure 1 Cross-sectional representation of a silicone tube biofilm system.

acceptor (O_2) concurrently to the biofilm rather than requiring aqueous air sparging on the biofilm side as reported with other systems [14,15,20,25,28]. The system also allowed for the diffusion of the metabolic by-product CO_2 back into the airstream for removal. Figure 2 shows a schematic of the system design. The silicone membrane module was constructed by inserting two 15.24-m lengths of Silastic tubing (1.02 mm I.D., 2.16 mm O.D.) (Dow Corning, Midland, MI) in spiral fashion inside a polypropylene cylinder with internal dimensions of 6.5×18.5 cm. Polypropylene end caps with one liquid and one gas transit hole, each equipped with a 0.25-in. stainless steel (SS) fitting, were clamped to each end of the cylinder. The silastic tubing was fed through a gastight septum at the gas transit hole on each end cap. The inlet gas transit fitting was connected with SS tubing to a 500-ml stoppered glass flask filled with approximately 100 ml of an equimolar solution of BTEX. The equilibrated headspace in the flask served as the source of BTEX vapor. A stainless steel compressed air line with a control flowmeter (Cole-Parmer, Vernon Hills, IL) was directed through a 0.3-μm sterile Hepa filter (Whatman, Maidstone, England) into the BTEX vapor flask while a second flowmeter-controlled line was directed through a tee to the inlet gas line. This allowed blending of BTEX vapor with clean air to provide desired concentrations and flow rates. Waste gas was directed to a carbon trap to sequester any nonmetabolized BTEX components. Both inlet and exit lines on the membrane module were outfitted with septum sampling ports for GC analysis.

Liquid transit holes on the membrane module were connected with 0.25-in. stainless steel tubing to a commercial fermentor system (LH Fermentation series 210, Berkshire, England) containing a gastight 4.5-l (working volume) vessel. A variable-flow micropump with an autoclavable head (Cole-Parmer) was incorporated to recycle culture liquid between the fermentor and the membrane module thereby providing complete mixing for the system. Rapid recycle ($1\ l\ min^{-1}$) of culture medium between the module and the fermentor also allowed for representative monitoring of the environmental parameters of the membrane module in the fermentor vessel. The density of the biofilm on the silicone tubing during routine system operation was not monitored, as that would have required dismantling the system. However, the cell density recycling through the system was monitored and was considered as an indirect measure of the health of the biofilm at the vapor/aqueous interface. Temperature in the system was maintained at 25°C. Dissolved oxygen (DO) was monitored with an autoclavable DO electrode (Ingold, Wilmington, MA). The pH was monitored in the fermentor vessel with an autoclavable pH electrode (Ingold) and was maintained between 6.8 and 7.0 by the buffering capacity of the basal salts medium. Sterile basal salts medium was introduced into the fermentor vessel and waste liquor was removed through a dual peristaltic pump (Cole-Parmer) at a total system dilution rate of $0.143\ d^{-1}$. Liquid within the fermentor vessel was mixed at an agitation rate of 300 rpm. The sealed fermentor vessel was equipped with a Teflon-lined rubber septum port to sample

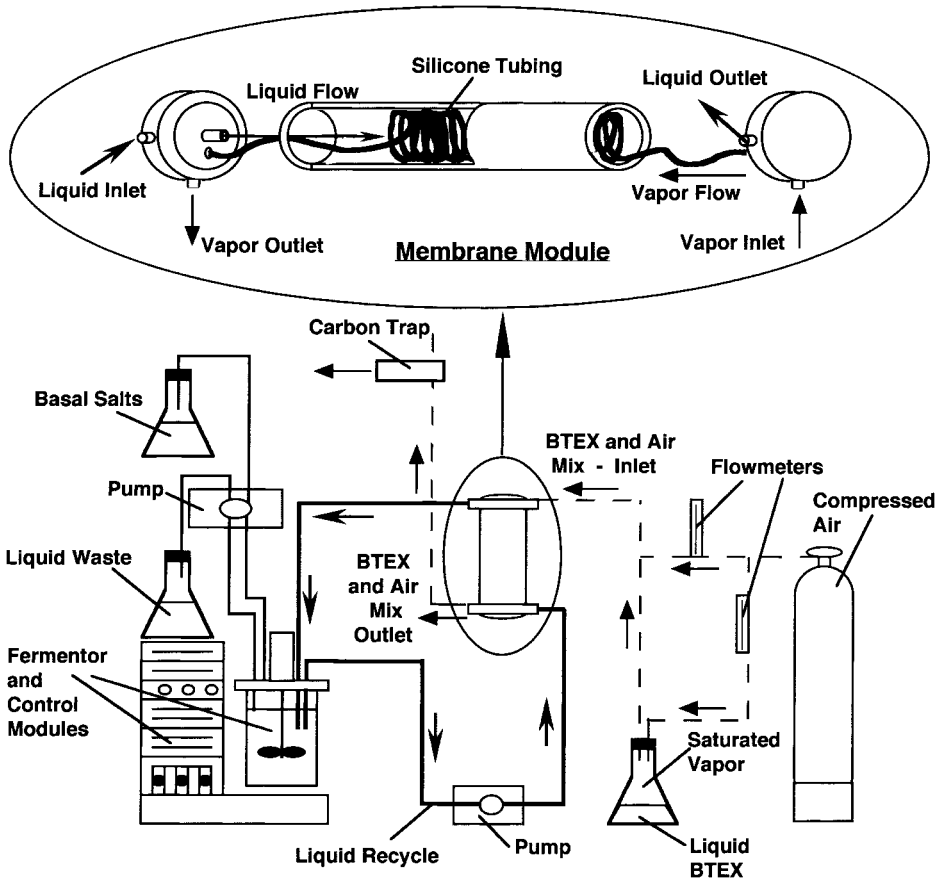

Figure 2 Membrane reactor system and silicone module schematic diagram. Vapor flow is represented by dashed lines and liquid flow is represented by solid lines.

Table 1 Typical BTEX component makeup in inlet vapor stream[a]

Compound	Average ppm_v	SD
Benzene	513	44
Ethylbenzene	78	21
Toluene	269	49
m- and *p*-Xylene	121	35
o-Xylene	56	16
Total BTEX	1037	166

[a]Data averaged from six samplings over 100 h of continuous vapor flow with inlet BTEX concentration set to approximately 1000 ppm_v.

vapor headspace. All heat-stable system components making liquid and vapor contact were sterilized by autoclaving them prior to use. Pressure gauges were treated with sterilizing concentrations of ethanol prior to system assembly.

At the initiation of the run the fermentor portion of the bioreactor was filled with 4.5 l of sterile basal salts medium and was inoculated with 100 ml of an active mixed culture (OD 0.01) of TX1 and BTE1 in roughly equal proportions. Liquid BTEX (1 mmol of each component) was added to the fermentor and impeller mixing was initiated. Liquid recycle through the entire system was initiated 24 h later when the culture had demonstrated active growth on the BTEX.

Bioreactor run termination sampling

At the end of the reactor operation, samples of fermentor liquor and silicone tube biofilm were compared for their activity against BTEX. Duplicate 10-ml samples of fermentor liquor were pelleted and resuspended in 30 ml of sterile basal salts medium in 160 ml septum-sealed serum bottles. BTEX was added (3 μmol of each component) to each bottle, whereupon they were inverted and shaken at 25°C. The bottle headspace concentrations were around 1600 ppm_v, which were similar to the higher vapor concentrations tested during the bioreactor run. Vapor phase removal was monitored *via* GC and protein determinations were made at timed intervals. Two separate samples of biofilm were aseptically scraped from the silicone tubing and treated identically to the fermentor liquor samples.

To determine the biomass loading attached to the silicone tubing at system termination, the module was carefully dismantled and the tubing with attached biomass was removed. The tubing was digested for 72 h in 2 l of 2 N NaOH. The tubing was then removed and rinsed with 1 l of distilled water. The 2 l of NaOH digestate and 1 l of water rinse were combined, diluted appropriately, and the protein concentration was determined.

Results

Membrane bioreactor system operation

Following inoculation, growth in the fermentor vessel (which had an OD below 0.01 following inoculation) demonstrated rapid consumption of the added BTEX as seen by the reduction of DO from 100% to 9% in the sealed system after 4 h. To prevent oxygen depletion during this initial growth interval, air was periodically sparged through the liquid for several minutes. Sparging was kept to a minimum to prevent volatilization and loss of BTEX. The concentrations of the BTEX components in the fermentor vessel were not measured during this startup period. After 24 h, the OD increased to 0.05 and liquid recycle from the fermentor portion of

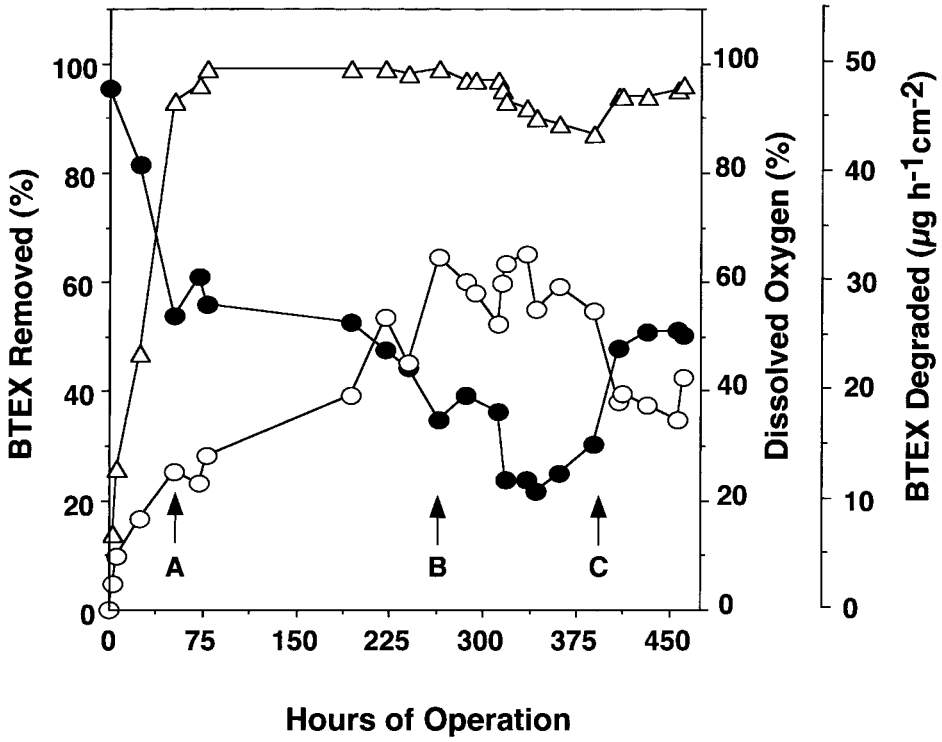

Figure 3 Bioreactor run startup: Operational hours 0–461. The inlet BTEX concentration was slowly increased from about 600 ppm_v at time point A to about 1100 ppm_v at time point B. The inlet BTEX concentration was reduced to about 750 ppm_v at time point C. (△) BTEX removed (%); (●) dissolved oxygen (%); (○) BTEX degraded ($\mu g\ h^{-1}\ cm^{-2}$).

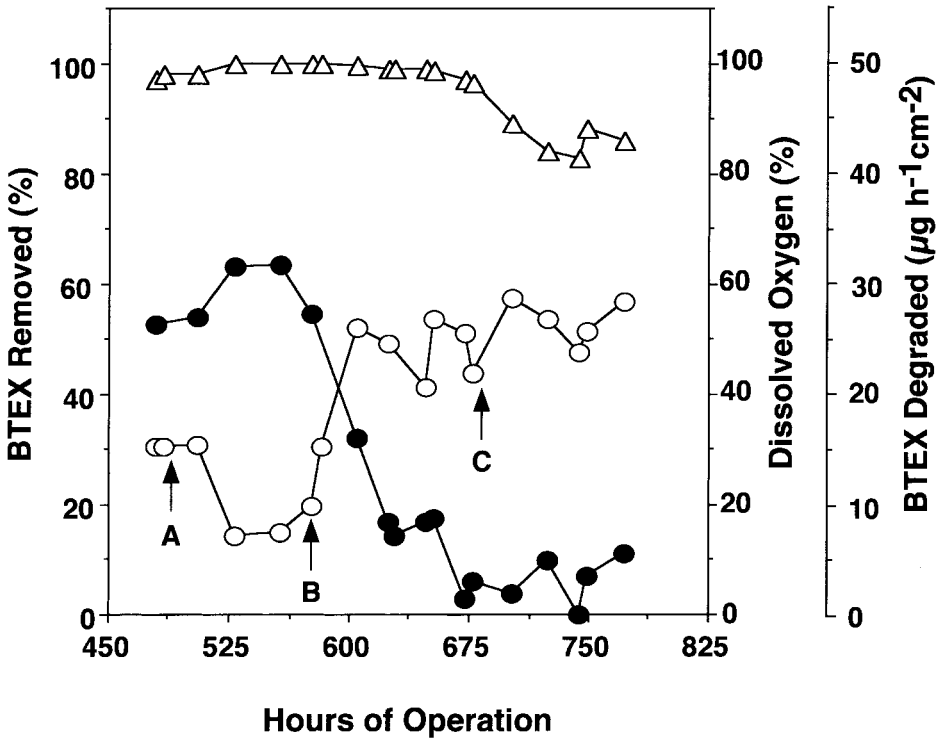

Figure 4 Bioreactor operated with reduced vapor flow rates and increasing BTEX concentrations: Operational hours 479–772. The vapor flow rate was reduced from 250 to 100 ml min^{-1} at time point A. The BTEX concentration was increased from about 750 to about 2000 ppm_v at time point B. The BTEX concentration was further increased to about 2500 ppm_v at time point C. (△) BTEX removed (%); (●) dissolved oxygen (%); (○) BTEX degraded (μg h^{-1} cm^{-2}).

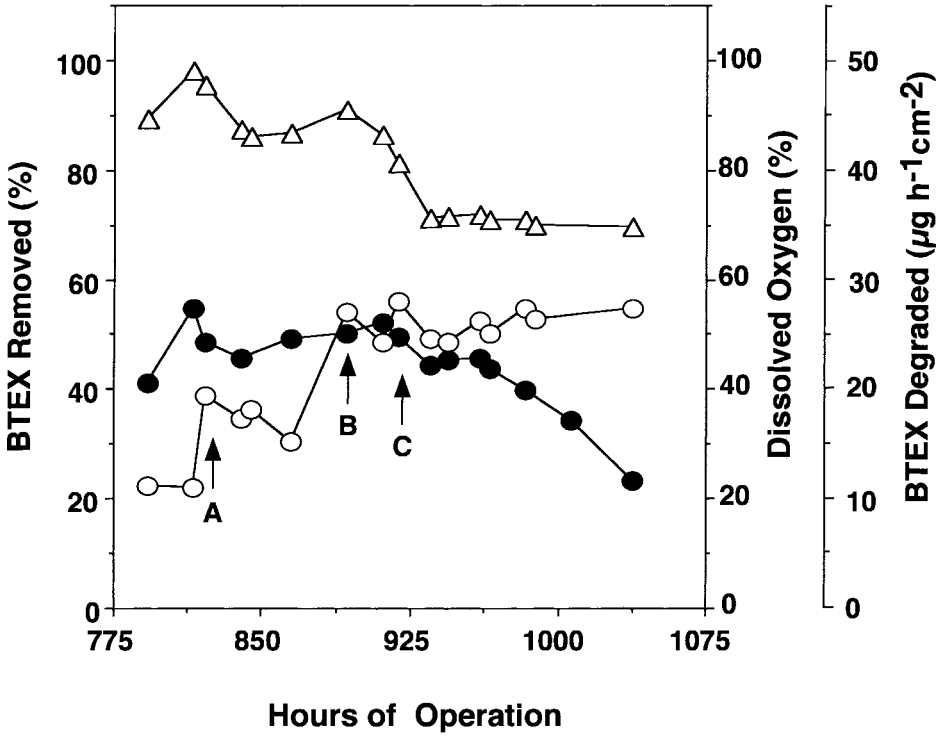

Figure 5 Bioreactor operated with increasing vapor flow rates: Operational hours 791–1038. Sequential increases in the vapor flow rate from an initial 100 ml min^{-1} to 250, 300 and 350 ml min^{-1} at time points A, B and C, respectively. (△), BTEX removed (%); (●) dissolved oxygen (%); (○) BTEX degraded (μg h^{-1} cm^{-2}).

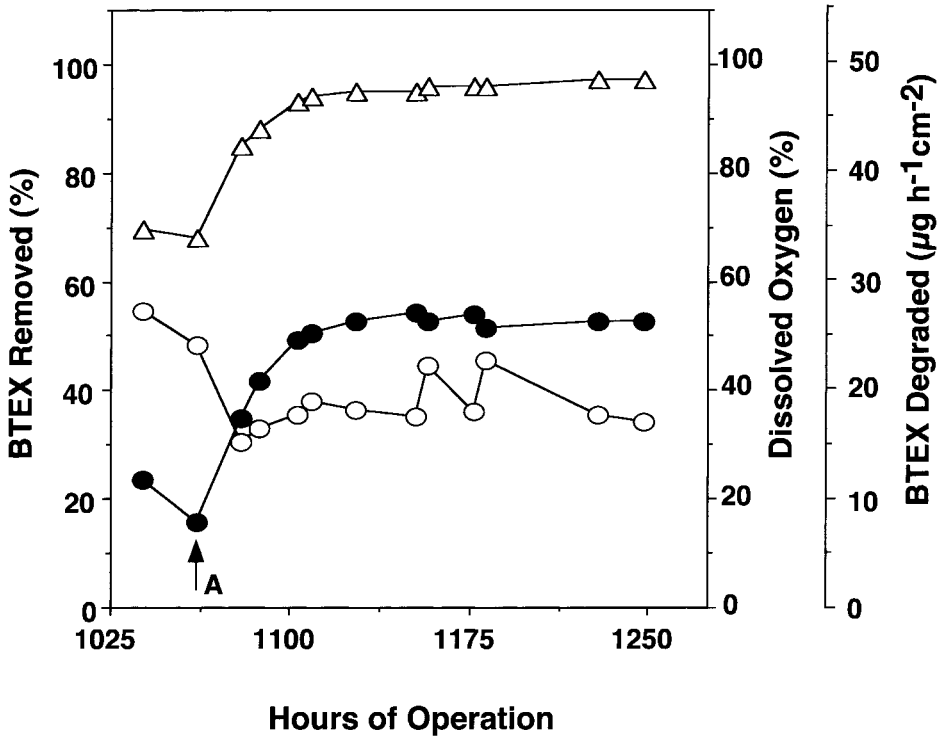

Figure 6 Bioreactor operated with reduced vapor flow rates and reduced BTEX concentrations: Operational hours 1038–1250. The vapor flow rate was returned to 250 ml min^{-1} and the BTEX concentration was reset to about 700 ppm_v at time point A. (△) BTEX removed (%); (●) dissolved oxygen (%); (○) BTEX degraded (μg h^{-1} cm^{-2}).

the bioreactor to the membrane module system was initiated. Additional sterile basal salts medium (725 ml) was added to the system to compensate for filling of the dry module and connecting plumbing. Vapor flow containing BTEX at approximately 600 ppm_v had been initiated at a rate of 250 ml min^{-1} 24 h prior to introduction of the culture in order to equilibrate the vapor portion of the system. The BTEX outlet concentration was within 5% of the inlet concentration at startup. Following the start of liquid recycle through the system there was no longer any requirement for periodic air sparging. Oxygen was supplied to the culture medium in tandem with BTEX through the membrane tubing at a concentration sufficient for growth. During the operation of the system the ratios of BTEX components to each other in the vapor stream varied slightly due to their individual vapor pressures, as well as subtle changes in flow rates, ambient temperature, and air pressure. A representative BTEX vapor makeup and sample deviation are described in Table 1 for samples taken with an inlet BTEX concentration of 1000 ppm_v. Similar proportional results were seen when the inlet BTEX concentration was adjusted up or down during the experimental run. The average ratio of the BTEX components in the vapor stream for the entire 1250-h experimental run was (benzene) 6.9:(toluene) 4.3:(ethylbenzene) 1.4:(*m*-xylene) 1.1:(*p*-xylene) 1.1:(*o*-xylene) 1.0.

To facilitate ease of analysis, the overall performance data from the system run was divided into four consecutive time periods and is described in the four consecutive figures (Figures 3–6). Table 2

Table 2 Key flow and concentration parameter changes made during system run

Hour(s) of operation	Parameter change	Vapor flow (ml min^{-1})	BTEX concentration (ppm_v)[a]	Vapor retention (s)	Corresponding figure
53→286	Incrementally increased BTEX concentration	250	600→1100	5.98	point A, Figure 3
286→388		260	1200	5.75	points BC, Figure 3
388	Reduced BTEX concentration	260	1200→750	5.7	point C, Figure 3
505	Reduced vapor flow rate	260→100	750	5.75→14.94	point A, Figure 4
529→557		110	750	13.58	points AB, Figure 4
577→677	Increased BTEX concentration	110	750→2600	13.58	point BC, Figure 4
792	Reduced BTEX concentration	110	2600→800	13.58	Figure 5
820	Increased vapor flow rate	250	800	5.98	point A, Figure 5
893	Increased vapor flow rate	300	800	4.98	point B, Figure 5
919	Increased vapor flow rate	350	800	4.27	point C, Figure 5
1079	Reduced vapor flow rate	250	700	5.98	point A, Figure 6

[a]The average concentration rounded to the nearest 50 ppm_v.

highlights the key flow rate and concentration changes made during these time periods. For performance representation purposes in these figures, the elimination of BTEX by the system is described in two forms. First, data were graphed as an absolute percentage of vapor phase BTEX removed. Each of the values was derived from a comparison of the vapor concentration observed at the outlet compared to that present at the inlet. Secondly, the data were graphed as the weight of BTEX degraded per surface area of the membrane/biofilm interface. This second method allowed for visualization of effects from modifications in BTEX loading to the system through concentration and flow rate adjustments.

Performance of the system during startup is shown in Figure 3. From time point A to point B the BTEX vapor concentration was slowly increased from approximately 600 ppm_v to about 1100 ppm_v. Under these conditions a steady increase of biofilm growth on the silicone tubing was observed through the semitransparent polypropylene module simultaneous with removal of BTEX from the vapor stream. Concurrent with an increase in the vapor feed concentration of BTEX was a decrease in dissolved oxygen within the liquid or fermentor portion of the membrane bioreactor. During this initial startup OD in the fermentor increased from 0.05 to 0.40 by hour 264 consistent with growth on BTEX. The headspace concentration of BTEX in the sealed fermentor portion of the system dropped from a concentration of 400 ppm_v at startup to undetectable levels by hour 76.

From time point B to time point C in Figure 3, the system BTEX inlet concentration stabilized to a concentration of approximately 1200 ppm_v. Removal efficiency of the system for BTEX dropped from 99% to around 87% suggesting the degradation limits of the system under these conditions had been exceeded. As can be seen in the interval from B to C the maximal removal rates for BTEX averaged 30 $\mu g\ h^{-1}\ cm^{-2}$. DO dropped to around 20% during this period. At time point C the BTEX concentration was reduced to around 750 ppm_v and the system returned to a removal efficiency of 98% and simultaneously showed an increase in DO to around 50%.

The next perturbation tested was the effect of lowering the vapor flow rate while increasing the BTEX concentration shown in Figure 4. The vapor flow rate was incrementally reduced from 250 ml min^{-1} at time point A to 100 ml min^{-1} at time point B. The vapor concentration was incrementally increased from about 750 ppm_v at time point B to about 2600 ppm_v at time point C. This low flow/high concentration loading resulted in a reduction of BTEX removal efficiency to around 85% and lowered the DO to below 5%.

Following an initial reduction in BTEX concentration from about 2600 ppm_v to approximately 800 ppm_v and subsequent increase in BTEX removal efficiency to around 98%, sequential increases in the vapor flow rate at time points A (250 ml min^{-1}), B (300 ml min^{-1}), and C (350 ml min^{-1}) were instituted as shown in Figure 5. The flow rate of 250 ml min^{-1} resulted in a slight decrease in DO and a stable decrease in BTEX removal efficiency to about 87%. A flow rate of 350 ml min^{-1} lowered the BTEX removal efficiency to about 71% and further decreased the level of dissolved oxygen. The latter portion of the DO decline was attributed to a unintentional increase in the BTEX inlet concentration up to about 1000 ppm_v due to variations generated by replacement of the compressed air supply at hour 965.

The system was returned to a vapor flow rate of 250 ml min^{-1} and a BTEX concentration of around 700 ppm_v as shown in time point A Figure 6. At this loading the system mimicked its previous performance at hours 408–462 in Figure 3 with BTEX removal efficiencies around 98% and DO concentrations around 50%. Throughout the bioreactor run the removal of each of the BTEX components relative to each other was consistent and a preferential elimination pattern was not observed during the various operational perturbations.

During this series of experiments the OD of the culture, as measured in the sealed fermentor, rose from 0.05 to a maximum of 0.40 around hour 286 and then steadily declined to 0.09 at run termination. We suspect this was not an actual decline in the overall population of the system because BTEX removal performance did not deteriorate accordingly. Rather we speculate that it was the result of a slow accumulation of the total system biomass in the module's fixed biofilm over time as well as the subsequent lack of substrate and nutrients available to the unattached recycling cells.

The ratio of BTE1:TX1 in the recycling fermentor liquor was quantified over time by counting the two distinct colony types on TSA plates. The concentrations of TX1 in the samples were further verified by counting colonies on selective *p*-toluate plates. Following the initial startup period, the population ratio averaged 2.17 ± 0.94 to system termination and was consistent regardless of the operational changes administered. No other bacterial isolates were detected during the reactor run. Fungal spore growth was observed on TSA plates after operational hour 677 but it did not appear to impact the system's performance.

At hour 1249 the system run was terminated and the membrane module was drained, removed, and dismantled. Upon inspection a thick tan biofilm was observed on the tubing. It completely covered the entire silicone surface and large open channels were present in the center of the biomass-laden tubing spiral. The channels were probably created as liquid medium recycled through the system. Samples of both the recycled fermentor liquor and the biofilm layer were then tested for BTEX activity in sealed serum bottle tests. The specific activity of the recycled fermentor liquor was 27.14 ± 1.90 nmol $min^{-1}\ mg^{-1}$ whereas the biofilm samples yielded a specific activity of 47.08 ± 4.24 nmol $min^{-1}\ mg^{-1}$. By calculating a specific activity from the total protein present on the module tubing biofilm at termination and the BTEX removal at that point the module was degrading 1.86 nmol $min^{-1}\ mg^{-1}$.

Discussion

Characteristics and substrate range of TX1 and BTE1

Our goal at the start of this work was to develop a practical system for removal of BTEX vapors from air streams. An essential component of the system was a biocatalyst that was both robust and efficient. TX1 and BTE1 met the selection criteria in that they readily consumed five of the six components of BTEX as sources of carbon and energy. The remaining *o*-xylene isomer was co-oxidized by the mixed culture. Our enriched coculture appears to be similar in activity and makeup to the *P. putida* consortium constructed by Oh and Bartha [21] for biofiltration treatment of a three-component vapor stream containing benzene, toluene, and *p*-xylene. Our work with six blended BTEX components correlated well with their observations in that using this particular mixture of organisms, with complimentary aromatic pathway activities, functioned in an advantageous manner for efficient removal of mixed aromatic hydrocarbons.

Bioreactor membrane system performance

When the bioreactor system was first initiated there was excellent diffusion of BTEX through the tubing into the sealed system.

Evidence of this was a noticeable increase in BTEX concentration in the headspace of the fermentor. Subsequent elimination of BTEX in the headspace of the fermentor after 72 h of system operation and lack of significant levels detected over the remainder of the bioreactor run were consistent with overall growth of the culture and biofilm development. We speculate that as the biofilm formed on the tubing the majority of BTEX that had diffused across the membrane was consumed by the attached organisms. Residual BTEX that passed through uncolonized portions of the membrane was then metabolized by unattached or sloughed cells.

Following startup of the reactor, the concentration of unattached cells recycling through the system rose initially and then declined through the end of the run even though BTEX removal remained stable. This is consistent with observations obtained by Freitas dos Santos and Livington [14], using an extractive membrane system, that as the unattached cell population declined the biofilm thickness increased, resulting in the majority of the biomass becoming attached to the silicone membrane.

Biofilm formation in liquid/liquid extractive membrane reactors is effective in reducing loss of VOCs due to air stripping. However, performance concensions are made when using this design in that reduced oxygen and VOC fluxes will result as the biofilm thickness increases [9,10,28]. By using a novel membrane measurement technique a film thickness of 200–400 μm was shown to be optimal when treating DCE-contaminated wastewater [14]. Oxygen diffusion through the biofilm to the DCE/membrane interface was the limiting factor [15]. As a result, physical shearing was required to maintain the biofilm at an optimal thickness. In the reported EMB systems, oxygen and the organic substrates were diffusing from opposite sides across the biofilm. In our system, the thickness of the biofilm should not pose a serious problem because both oxygen and substrate are fed on the same side of the biofilm.

Oxygen is readily permeable through silicone rubber but not to the same extent as BTEX compounds [9,10,20]. Silicone permeability values (K_a) for the BTEX components used in this study ranged from 6.2×10^{-7} to 6.9×10^{-7} mol m^{-1} s^{-1} atm^{-1} whereas the permeability of oxygen was 2×10^{-8} mol m^{-1} s^{-1} atm^{-1} [24]. The BTEX:oxygen permeability contrast in this system is partially compensated for by the fact that oxygen is at a much higher concentration in the vapor phase than BTEX. We can calculate the mole fraction permeability ratio across our silicone tubing membrane from the molar flux of each component, expressed as:

$$M_a = \frac{K_a(Pi_a - Po_a)}{T}$$

where M_a is the molar flux of component a through the membrane (mol m^{-2} s^{-1} atm^{-1}), K_a is the permeability of the membrane to component a (mol m^{-1} s^{-1} atm^{-1}), Pi_a is the partial pressure of component a inside the tube (atm), Po_a is the partial pressure of component a outside the tube (atm), and T is the thickness of the membrane (m). In the reactor tests we undertook there was theoretically sufficient oxygen in the BTEX:air vapor stream to allow for complete oxidation of the substrates to CO_2 and water. Therefore the only limiting factor should be the permeation ratio of oxygen to BTEX across the membrane.

Clearly, the permeating molar ratio of oxygen to BTEX declines as the vapor concentration of BTEX increases, and likewise this ratio increases as BTEX is consumed by the biofilm along the length of the tubing. Therefore, if the ratio is insufficient to allow for complete metabolism of BTEX by the biofilm at the inlet portion of the tubing, as BTEX is consumed along the full length of tubing the ratio will eventually become sufficient and oxygen will no longer be a limiting factor. This presumes that the tubing or available membrane surface area is large enough for the ratio to be achieved. In our system compressed air was used resulting in an oxygen concentration of 21%. In future designs higher oxygenation values would be possible if pure O_2 was used as the vapor carrier as in other reports [9,10,28] but safety and cost issues will need to be evaluated.

Oxygen limitation was observed upon comparing high vapor flow/low BTEX concentration segments of the bioreactor run against low vapor flow/high BTEX concentration segments. From time points B to C in Figure 3, the average removal of BTEX was 30 μg h^{-1} cm^{-2} and the DO concentration was around 20%. This indicated that under these circumstances oxygen was not limiting within the biofilm as significant dissolved oxygen was observed. However, following time point C in Figure 4 the average removal rate was less (25 μg h^{-1} cm^{-2}) and DO approached zero. Although the net loading of BTEX was very similar between these time points, the vapor flow rate in the Figure 4 time points were reduced 2.5-fold relative to the Figure 3 time points and the concentration of BTEX presented to the biofilm was 2.2-fold higher. Thus, the ratio of available oxygen relative to BTEX presented to the biofilm was lower resulting in a depletion of oxygen at the biofilm surface. The efficiency of BTEX removal was also a function of the contact time of the vapor with the membrane. When the retention was less than 5.98 s, using the concentrations we tested, the percent removal of BTEX dropped below 90% as seen at time points B to C in Figure 3 (5.75 s) and following time point B in Figure 5 (4.15–5.98 s).

A comparison of the data presented suggest that the system behaved consistently and that the maximum removal rate for BTEX was approximately 30 μg h^{-1} cm^{-2} under a variety of conditions. The exception to this was when there was a sufficiently high ratio of BTEX to oxygen in the vapor stream. In order to obtain an optimal removal of BTEX the surface area of the system must be increased or the BTEX loading decreased such that the overall concentration of oxygen permeating through the system membrane is not limiting. To obtain consistent performance greater than 98% for BETX removal a loading rate of approximately 23 μg h^{-1} cm^{-2} or less was required.

The results of specific activity testing at the system's termination indicated that the unattached organisms recycling through the system had less activity against BTEX than the attached biofilm organisms. This is understandable because those unattached organisms were probably in a starvation or death phase due to lack of substrate. The attached organisms, particularly those in direct contact with the silicone membrane, were probably in a much healthier and/or active state. When the overall activity of the module's biofilm was calculated based on the amount of total cell protein present the activity was much lower than the values obtained utilizing sealed serum bottles. We attribute this to the fact that a large portion of the attached biofilm was probably not participating in the biodegradation. We suspect that a thin layer of cells directly at the surface of the membrane was responsible for the majority of the metabolism. As the layer or biofilm grew, cells were displaced outward. Measurements to define or assay the cells within the biofilm responsible for the metabolism or the BETX were not performed. However, it is anticipated that the layer of cells in immediate contact with the membrane would have specific activities similar to those obtained from the sealed serum bottle

studies. The large amount of biomass attached to the silicone tubing was also an indication that the cell mass over time was becoming sequestered in the module. The system had a high liquid recycle rate in part to allow for sloughing biomass from the module to be removed. However, the OD drop in the fermentor portion of the system over time combined with continued good performance and the large cell mass removed from the module suggest that the tubing and biofilm began to act as a physical filter. The module matrix then simply reaccumulated the biomass during the latter portion of the run.

Although true quantitation and qualitative determinations of the biomass in the system was not possible without dismantling the system, we feel that quantitative ratio assessments of the BTE1 and TX1 population in the fermentor samples taken over time were reflective of the biofilm makeup. If we assume that sloughing of each of the strains from the biofilm was equivalent and thus cells recycled through the fermentor are representative of the attached biofilm, then the biofilm BTE1:TX1 ratio over time was about 2:1. Due to their individual vapor pressures, benzene and toluene were the highest concentration components in the test vapor stream as shown in Table 1. Because benzene is consumed only by BTE1 and toluene consumption is probably split between BTE1 and TX1, it is logical that BTE1 would represent a larger percentage of the biofilm population. If a vapor stream were to contain much lower concentrations of benzene and toluene and higher xylene isomer concentrations we would expect TX1 to dominate.

Comparison of this type of membrane reactor to other types of vapor treatment systems such as biofilters, bioscrubbers and trickling bed reactors is somewhat discretionary because their reported elimination capacities are typically based on reactor bed volume whereas membrane systems are based on surface area or vapor volume throughput. Biofilters and bioscrubbers have demonstrated elimination capacities of BTEX components in the range of 20–80 g substrate m^{-3} h^{-1} [19]. If we calculate removal rates for our system based on the total volume of the silicone membrane module its maximum elimination capacity was 105 g substrate m^{-3} h^{-1}. When comparing our system to more similar microporous membrane systems, removal rates are based on tubing volume rather than total module volume. Using this method, Ergas *et al* [12] reported a maximum elimination capacity for toluene of 42 g m^{-3} min^{-1} using a hollow fiber membrane system to treat contaminated air. Our system yielded a similar maximum rate of 43 g m^{-3} min^{-1} for the combined removal of all six BTEX compounds. Therefore, our system performance was similar to or somewhat better than reports for other aromatic vapor treatment systems.

In summary, utilizing a selected dual culture as the catalyst in a silicone membrane reactor system, consistent removal of BTEX vapors with high efficiency for over 1250 h was possible, without a significant decrease in performance, as long as loading parameters and vapor retention time limitations are not exceeded. The system provides both good oxygen and BTEX transfer coincident to an active biofilm, thus minimizing biofilm thickness as a transport-limiting factor as reported by other investigators. The nonporous silicone system has advantages over similar microporous membrane systems in that it has no pores to plug and limit transfer. Therefore, the system does not require periodic backflushing. Further, the system described here also has advantages over other biofilter systems commonly in use in that channeling and/or increased bed backpressure is not an issue, moisture maintenance and other evaporative problems are absent, and pH control is easily monitored and maintained. Finally, the modular nature of the system enables easy expansion simply by adding more tubing as further catalytic activity or biofilm surface area is required. With the removal of volatile organics in vapor streams fast becoming a significant regulatory issue globally, the use of simple nonporous membrane systems such as this show promise for biotreatment of hazardous vapor streams.

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