

Development of a Membrane-Based Vapor-Phase Bioreactor

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ABSTRACT

A vapor-phase bioreactor has been developed utilizing porous metal membranes in a cylindrical design employing radial flow as opposed to traditional axial flow for the vapor stream. The system was evaluated for the biodegradation of *p*-xylene (*p*-xylene) from a water-saturated air stream by *Pseudomonas putida* ATCC 23973 immobilized onto sand. The biocatalyst was placed in the annular space between two cylindrical, porous stainless-steel membranes. Details of the reactor system are presented along with biological data verifying system performance. The feed flow rate and *p*-xylene concentration were varied between 60 and 130 cm³/min and 15–150 ppm, respectively. Continuous reactor operation was maintained for 80–200 h with removal efficiencies (based on *p*-xylene disappearance) between 80 and 95%. The effluent concentration histories were compared to determine the operating range of the bioreactor.

Index Entries: Bioreactor; biocatalytic reactor; biofilter; biodegradation; bioremediation; volatile organic hydrocarbon; *p*-xylene.

INTRODUCTION

Vapor-phase bioreactors have been used for the degradation of gaseous trichloroethylene (1), for hydrocarbon removal (2), and for *p*-xylene degradation (3,4). Currently, over 200 vapor-phase bioreactors are in operation in Holland for the removal of organic contaminants from air in industries, such as ceramics for the treatment of ethanol and isopropanol; resin production facilities to treat phenol; plywood production

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facilities to treat formaldehyde; and paint production facilities to treat various solvents (5). Since the US is primarily focused on site-specific remediation, vapor-phase bioreactors have not yet gained as widespread use there as they have in Europe. Clearly, expanding bioremediation technology and effectively employing vapor-phase bioreactors require innovation and research to establish the proper conditions under which these types of bioreactors can function.

The principle on which this research is based is that an organic vapor stream of known composition can be degraded through a biodegradative process by an immobilized microbial phase in a radial-flow, packed-bed reactor. This research was motivated by pollution control standards mandated by the Environmental Protection Agency (EPA) with the aim of environmental protection, specifically, air pollution control. The EPA dictates the emission standards of hazardous air pollutants, of which organic compounds comprise a majority. *Para*-xylene (*p*-xylene) was chosen for the test system, because it is on the EPA's list of hazardous chemicals as given by section 112(b) of the Clean Air Act and because from a microbial kinetic standpoint, the system is relatively simple with known kinetics for ATCC 23973 (6). The primary objective of the first phase of this research was to validate the performance of the new reactor design.

Apel *et al.* (4) used a packed-bed bioreactor for *p*-xylene degradation with *Pseudomonas putida* immobilized onto Pall rings. The reactor configuration consisted of a liquid nutrient feed stream flowing in a countercurrent flow to the inlet gas stream. The *p*-xylene feed rate was 16 mL/min with a concentration of 140 mg/min of organic. This system achieved 46% degradation. However, 75 mg/min of organic was detected in the liquid stream leaving the reactor. Vaughn *et al.* (3) employed a similar system, except glass beads and diatomaceous earth were used as packing materials. Nearly 100% removal rates were reported. However, *p*-xylene was detected in the liquid stream from the reactor. The membrane reactor developed in this work does not have a liquid outlet stream.

To provide a contribution to the growing vapor phase bioreactor technology, among the objectives of this research were (1) to build and test a novel packed-bed vapor-phase reactor for the biodegradation of *p*-xylene with an immobilized microbial phase and (2) to evaluate the bioreactor's performance by generating data at different process conditions, i.e., influent concentration and flow rate. The radial-flow reactor was developed with the aim of increasing mass-transfer rates of the organic hydrocarbon to the immobilized cells, or biofilm, which is an important factor when treating pollutants whose water solubilities are low. Radial-flow operation provides a high surface area for contacting of the feed stream and maintains a low pressure drop (2–4 psi), which ensures a uniform distribution of the feed stream along the entire length of the reactor. Additionally, the occurrence of dry spots within the reactor packing is prevented as the flow is uniformly distributed across the bed.

REACTOR DESCRIPTION AND OPERATION

Figure 1 is a schematic of the reactor showing two different cross-sectional views. The top view represents a cross-section in the longitudinal direction (xy -plane), and the lower view a cross-section in the transverse direction (xz -plane). The core of the reactor consists of two concentric cylinders made of 316 microporous stainless steel (Pall PMM Metal Membrane Filters, New York), each of which was tungsten-inert gas welded to form the cylinders. The outer diameter of the inner cylinder is 1.63 cm, and the inner diameter of the outer cylinder is 2.54 cm, which gives a reactor thickness of 0.91 cm. This entire assembly is fitted and sealed on top and bottom with o-rings into an outer cylindrical casing made of 304 stainless steel. The reactor plumbing is made from 304 stainless-steel tubing with a diameter of 0.125 in. The reactor was tested for operation up to 60 psi, though the experiments did not produce pressures above 20 psi.

The packing material, which also served as the support medium for the biofilm, consisted of white quartz sand particles (Sigma Chemical Company, St. Louis, MO), which are nonporous and have an average diameter of 0.025 cm. The average crystalline density of the sand was measured to be 2.7 g/mL, and the void fraction of the bed was determined to be 0.30. The reactor volume for this bench-scale model is approx 169 cm³.

The *p*-xylene contaminant stream is introduced into the reactor through the inner cylinder core (Fig. 1). The concentration and humidity of the contaminant stream are regulated using an air tank and a three-stage bubbler, as indicated by Fig. 2. Because the two inner cylinders are capped, the vapor mixture flows radially outward through the microporous metal to the inner cylindrical shell containing the sand and biofilm where the *p*-xylene is enzymatically degraded by the microorganism. The gaseous effluent stream then flows to the outer cylindrical shell by convection and leaves the reactor through the exit port located at the top of the reactor.

The essential elements of the experimental setup are depicted in Figure 2, in which the reactor is integrated into a flow system for proper control of flow rates and effluent analysis. The 700–800 ppm (the units of ppm in this work are on a molar basis) *p*-xylene/air mixture was obtained premixed in a compressed gas tank (Airco, Port Allen, LA). The air used as the carrying medium was supplied from a second cylinder and was metered through a mass flow controller into a three-stage bubbler, where it became saturated. Mass flow controllers from Brooks Instrument Division (models 5850C, Emerson Electric, Hatfield, PA) were used to maintain volumetric gas flow rates at specified values up to 200 mL/min. The mass flow meters were calibrated using a bubble flow meter and permitted operational ranges of 0–200 and 0–100 cm³/min, respectively, for the *p*-xylene/air mixture and the air stream. The effluent from the reactor flowed to a Vista 6000 series gas chromatograph (GC) (Palo Alto, CA) connected to a Hewlett-Packard integrator (Avondale, PA) where the *p*-xylene composition of the effluent gas was

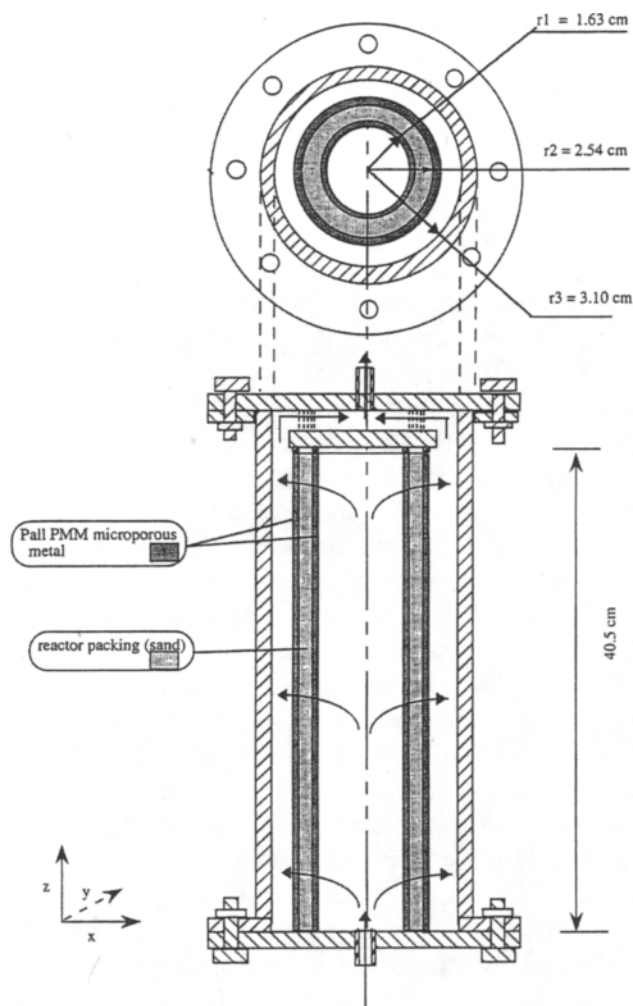


Fig. 1. Schematic of reactor configuration showing concentric cylindrical shell arrangement. Height of filled reactor is 40.5 cm (16 in.).

determined. The GC was equipped with a flame ionization detector for the quantitative analysis of the *p*-xylene. Two 1-mL sample loops installed in the GC permitted direct sampling of both the introduced gas and the effluent gas. The estimated combined error for control and measurement of the *p*-xylene flow into and out of the reactor is $\pm 5\%$.

MATERIALS AND METHODS

Microorganism and Cultivation

The microorganism used in the experiments was *P. putida* ATCC 23973, which was ordered freeze-dried from American Type Culture Collection (Rockville, MD). The medium that was used to culture the cells

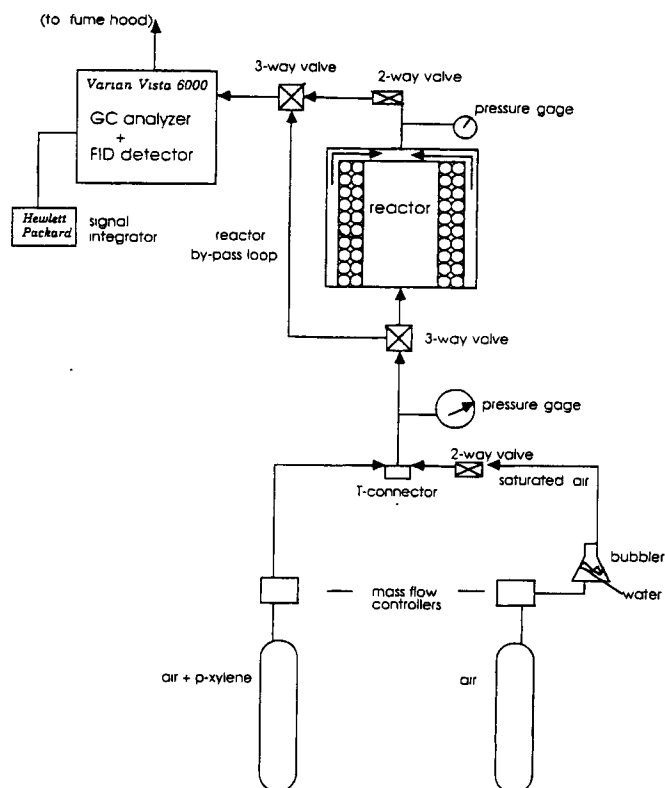


Fig. 2. Schematic line diagram of experimental setup.

was a benzoate medium (medium 1271), the suggested choice of medium for *P. putida* 23973 from ATCC. The composition of the medium on a 1-L solution basis was as follows: NaCl (5.0 g), $(\text{NH}_4)_2\text{HPO}_4$ (3.0 g), $\text{C}_7\text{H}_5\text{O}_2\text{Na}$ (3.0 g), KH_2PO_4 (1.2 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.20 g), all from Mallinckrodt (Paris, KY); and yeast extract (0.50 g), from Difco Labs (Detroit, MI). The cells were cultivated in four 1-L flasks with 250 mL of growth medium and 250 g of white quartz sand, which had been autoclaved. (The sodium benzoate was prepared separately as a filter-sterilized solution and added to the autoclaved growth solution prior to inoculation.) The cell solutions were grown in a shaking flask incubator at 25°C.

The growth state of the cells was monitored by sampling approx 2 mL of the cell-culture solution at various times and measuring the absorbances at a wavelength of 600 nm. Absorbance being directly proportional to the mass of cells in the sample, the growth curve was initially obtained from a graph of the natural logarithm of absorbance as a function of time from a culture grown in a sand-free medium. From this graph, the exponential growth phase of the cells was complete between the fifth and sixth days of growth, after which the onset of the stationary phase was observed. Based on the discussion by Dunbar (7), cells should be harvested during the stationary phase of growth for an active biofilm.

Between the fifth and sixth day of growth, the cell cultures were ready to be transferred to the vapor-phase bioreactor reactor.

Biodegradation Experiments

A controlled-release method of micronutrient delivery in the form of nutrient beads (provided by Grace-Sierra Horticultural Products Company, Milpitas, CA) was used for the cells during the biodegradation experiments. The nutrient breakdown of these beads is as follows on a weight percentage basis: ammonium (10.9%), nitrate (9.1%), urea (4.0%), phosphorus (5.5%), sulfur (0.7%), potassium (0.5%), and micronutrients (3.1%), with the balance being a resin coating made from linseed or soybean oil reacted with a cyclic diene. This provided controlled release of nutrients on exposure to moisture. When the cells had reached steady-state growth, nutrient beads in an amount approximately equal to the mass of the nutrients in the original growth medium were added to each of the four flasks containing the cell cultures.

Prior to packing the reactor, the microporous metal membrane cylinders between which the packing was to be situated were autoclaved for 40 min. On cooling of the metal, the cell culture/sand solutions were poured between the two metal membrane cylinders. The solution was drained through the base, the assembly placed within the reactor's metal casing, and the top and bottom of the reactor attached. The biodegradation experiments commenced as the flows of the saturated air and *p*-xylene/air mixture were passed through the reactor. The flows were adjusted to achieve influent *p*-xylene concentrations between 15 and 150 ppm at flow rates between 60 and 130 cm³/min.

Analysis

GC analysis of *p*-xylene in both the feed and the effluent gas streams was performed at various time intervals during reactor operation. The operating conditions of the GC were: oven temperature set at 130°C, injector temperature set at 140°C, FID temperature set at 150°C, sensitivity set at 10–12 mV, carrier flow rate (He) set at 25 mL/min, with sample loops of 1 mL each. The column was 5% didecyl phthalate plus 5% Bentone 34 on Chromosorb W-HP 80/100 with an inner diameter of 2 mm and a length of 2 m.

To determine the amount of cell mass on the biofilm, several mixed samples of approx 1 g of sand were taken from the packed reactor before and after each experiment. A known volume of a 10% solution of sodium pyrophosphate (7) was added to each sand sample to shear the cells off of the sand. The samples were then vortexed, the sand allowed to settle, and the absorbance of the solution at 600 nm measured. Quantifying cell mass based on absorbance measurements was accomplished by prior dry cell weight measurements taken from a culture grown in a solids-free medium. From the correlation of optical density with cell mass obtained, the cell mass removed

from the sand was determined. By drying and weighing the sand, estimates of cell mass/g of sand were made and resulted in 9.4×10^{-4} – 1.2×10^{-3} g of biomass/g of sand as the initial concentration of biomass within the reactor.

RESULTS AND DISCUSSION

The parameters that were investigated and allowed to vary were the influent *p*-xylene concentration and the flow rates of the influent streams. The relative values of the *p*-xylene/air stream and the saturated air stream were chosen such that a range of different *p*-xylene concentrations would be studied at total flow rates within two ranges: 60–63 and 121–130 cm³/min. The vapor-phase *p*-xylene concentration that would cause inhibition was estimated from the liquid-phase inhibition constant for the suspended-culture microbial kinetics of the *P. putida* strain used (6) and Henry's law. The influent substrate concentration was chosen to be less than this estimated value. Microbial kinetics developed for suspended growth systems have been shown to apply equally to fixed-film systems (8–10).

The ordinate of the graphs shown in Figs. 3–7 is a normalized concentration representing the ratio of *p*-xylene in the outlet stream to that in the inlet stream. These ratios were determined from the integrated response areas of the GC signals for the *p*-xylene streams entering and exiting the reactor.

To determine the adsorption breakthrough time of *p*-xylene on the biofilm/sand support, experiments were performed to determine the effects of any possible adsorption phenomena in the absence of biodegradation reactions. A representative result is shown in Fig. 3, which shows the elution curve of 284 ppm of *p*-xylene at a flow rate of 60 cm³/min in an experiment performed with cells that had been autoclaved prior to packing of the reactor. This experiment was performed at a reactor pressure of 15 psi with only the saturated air flowing through the system prior to starting the *p*-xylene/air mixture flow at time $t = 0$. Breakthrough occurs within 1 h with an inlet feed of 284 ppm. Thus, any decrease in the outlet concentration of *p*-xylene would subsequently be attributed to biodegradation kinetics.

As Fig. 4 and its inset show, steep variations in the outlet concentration of *p*-xylene occurred when sampling of the outlet stream was done during the first 25 h of operation. During the first 10–25 h of operation, several factors are simultaneously occurring:

1. The pressure inside the reactor is stabilizing as flow to the reactor is begun. Although the pressure drop through the reactor is not >3 psi, the pressure inside the reactor reaches a stable value of 10–14 psi during its first hour of operation.
2. Adsorption and breakthrough of *p*-xylene are occurring during the first hour of operation.
3. Acclimation of the cells to the new carbon substrate is occurring. During packing of the reactor, cells that have been growing from 5–6 d in a growth medium with benzoate as their sole carbon

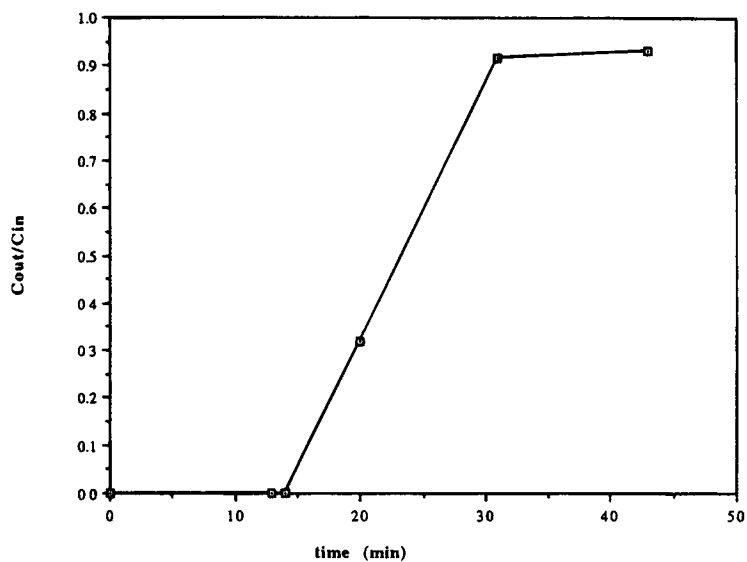


Fig. 3. Elution curve of 284 ppm feed of *p*-xylene at a flow rate of 60 cm³/min in packed bed experiment performed with immobilized cells that had been autoclaved.

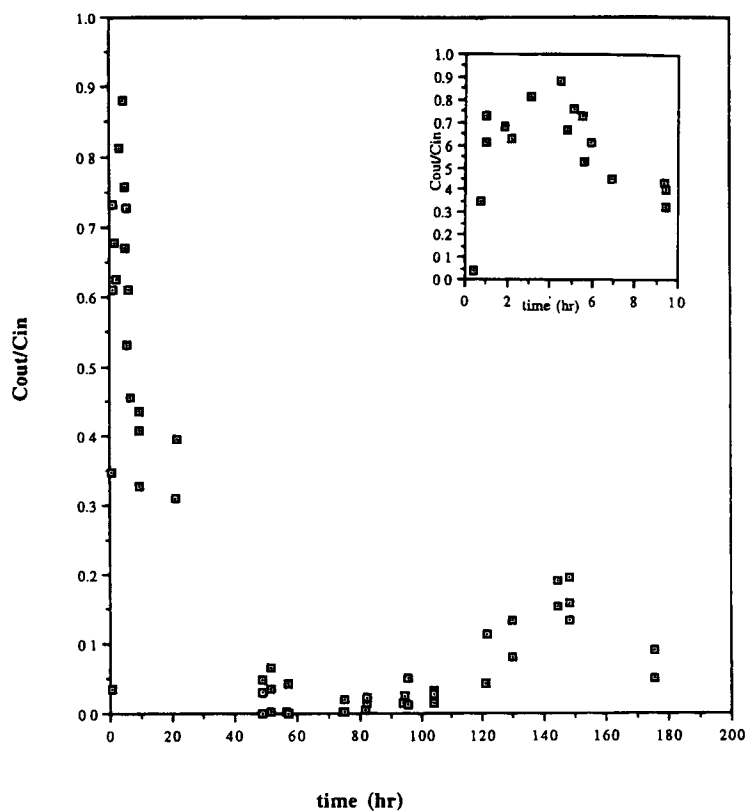


Fig. 4. Normalized concentration of *p*-xylene in the exit stream of the reactor as a function of time at the following conditions: C₀ = 36 ppm, flow rate = 121 cm³/min. The exit concentration of *p*-xylene is normalized with respect to its feed concentration. The inset is blow-up of the initial region of the effluent curve of *p*-xylene.

source were abruptly immersed into an environment initially containing only micronutrient beads; *p*-xylene as the substrate is introduced to the packed, immobilized cells only after transport to and final assembly of the reactor are completed 1–2 h later. During these initial stages of reactor operation, the cells are then becoming accustomed to a new environment.

Factors 1–3 all occur during the initial stages of reactor operation and have an effect on the effluent profile obtained during the first 25 h, as shown by Figs. 5 and 6.

As time progresses beyond this initial region characterized by high variations, the effluent concentration curves are seen to vary in a more continuous manner (Figs. 4–7). Figure 5 shows the effluent curves obtained with a feed concentration of 15 ppm at flow rates of 62 and 125 cm³/min. The bioreactor was run for 180 h (7.5 d) at a flow rate of 62 cm³/min with only 8–16% of the *p*-xylene feed detected in the reactor outlet. This corresponds to a removal of 84–94% of the *p*-xylene being fed to the reactor. In a separate experiment with an inlet *p*-xylene concentration of 15 ppm at a higher flow rate of 125 cm³/min, 60–88% removal of *p*-xylene was observed during the 120 h (5 d) of operation. Although there is relatively more scatter in the exit concentrations than in the latter case, the reactor does seem to approach a steady state after 60 h of operation.

The results for the experiments conducted within the 28–36 ppm concentration range at flow rates of 121 and 60 cm³/min are shown in Figs. 4 and 6, respectively. Both of these experiments produced comparable overall results with the bioreactor reaching 80–90% removal efficiencies after the first 25 h of operation. These removal efficiencies were maintained for 128 h (5.3 d) for the experiment run at 60 cm³/min, and for 178 h (7.4 d) at a flow rate of 121 cm³/min.

The experiment run at the lower flow rate of 60 cm³/min with an inlet feed of 28 ppm of *p*-xylene (Fig. 6) seemed to reach a steady-state value with the normalized output concentration of *p*-xylene varying between 0.06 and 0.15. After 60 h of operation, <10% change in the effluent concentration of *p*-xylene was observed. The experiment run at the higher flow rate of 121 cm³/min with an inlet *p*-xylene feed of 36 ppm (Fig. 4) produced removal efficiencies above 95% after the 57th h of operation. This performance continued for the next 48 h, after which the normalized effluent concentration of *p*-xylene began a slight increase reaching a relative maximum of 0.2. Approximately 30 h after this relative peak, the normalized *p*-xylene effluent concentration decreased to 0.05–0.1. The slight rise in the effluent profile corresponded to an experimental observation of an increase in pressure to 18 psi beyond the 105th h of reactor operation. This increase in pressure may have been caused by an increase in biomass within the packed bed, because removal efficiencies were in the range of 90–95% directly preceding this pressure increase.

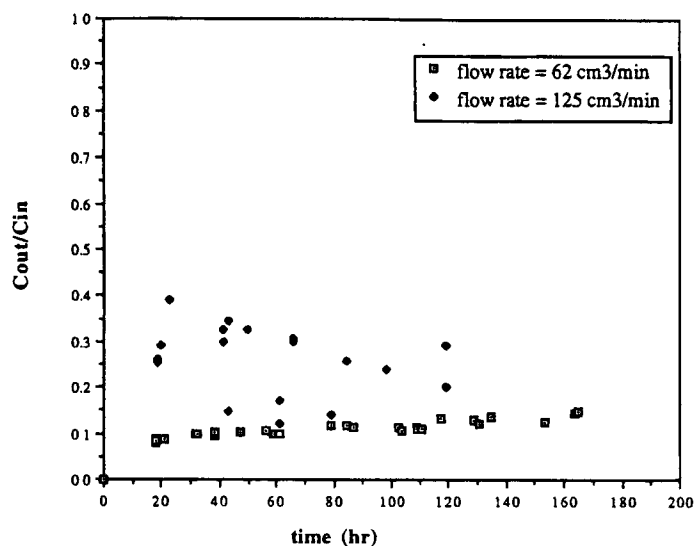


Fig. 5. Normalized concentration of *p*-xylene in the exit stream of the reactor as a function of time at an influent *p*-xylene concentration of 15 ppm.

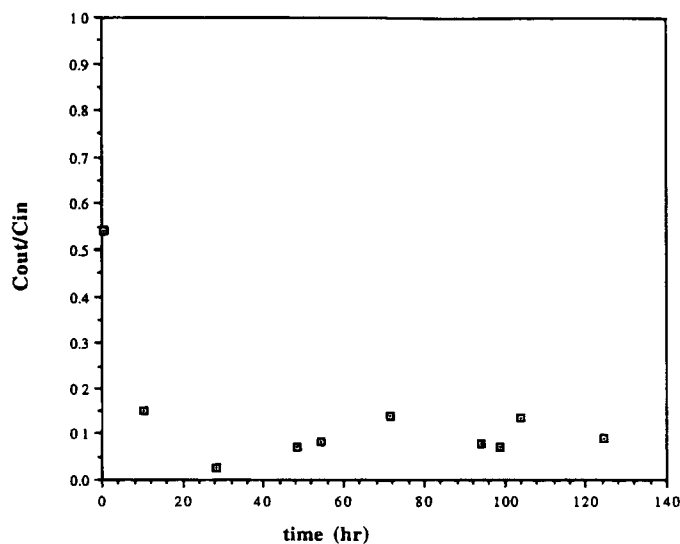


Fig. 6. Normalized concentration of *p*-xylene in the exit stream of the reactor as a function of time at an influent *p*-xylene concentration of 28 ppm and a flow rate of 60 cm³/min. The exit concentration of *p*-xylene is normalized with respect to its feed concentration.

A graphical compilation of other results is shown in Fig. 7, in which the results of the experiments performed with *p*-xylene feed concentrations of 52, 100, and 150 ppm at comparable flow rates are shown. With increasing levels in *p*-xylene feed concentrations, the removal efficiencies decrease. The experiment conducted with the 52 ppm feed is seen to reach a steady state based on the exit concentrations of *p*-xylene. The other two cases run

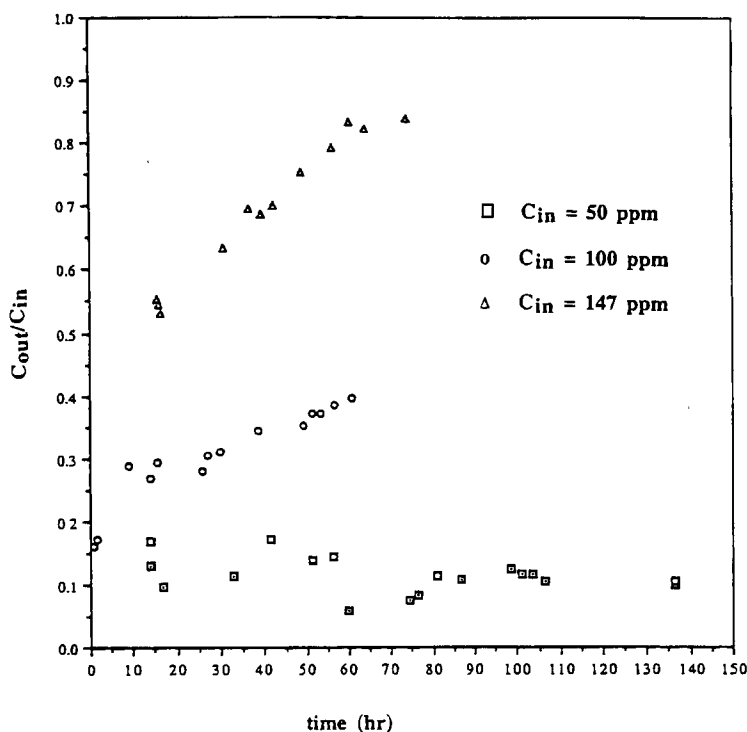


Fig. 7. Normalized concentration of *p*-xylene in the exit stream of the reactor as a function of time at an influent *p*-xylene concentrations of 50 ppm (squares), 100 ppm (circles), and 147 ppm (triangles) at flow rates of 63, 62, and 62 cm³/min, respectively. The exit concentration of *p*-xylene is normalized with respect to its feed concentration.

at feed concentrations of 100 and 150 ppm show a steady increase in the amount of *p*-xylene in the effluent stream over time. Per Fig. 7, overall *p*-xylene removal efficiencies in this range successively decreased as the influent concentration increased. This was probably owing to organism toxicity resulting from increased *p*-xylene concentrations in the reactor bed.

Figure 8 is a plot of the average removal rate of *p*-xylene in mg/h vs the mass flow rate to the reactor. As the mass flow rate increases, the removal rate increases up to an observed maximum at 0.02215 mg/min corresponding to an inlet concentration of 50 ppm and a flow rate of 125 cm³/min. Higher mass flow rates of *p*-xylene result in a decrease in productivity of the reactor, which is attributed to organism inhibition at the higher liquid-phase organic concentrations. The reactor productivity ranged from 0.0021 mg/(h cm³) up to 0.013 mg/(h cm³)

CONCLUSIONS

The radial-flow vapor-phase bioreactor functioned very well for the biodegradation of *p*-xylene and could achieve high removal rates (based on *p*-xylene disappearance). The region of operation for the defined reactor

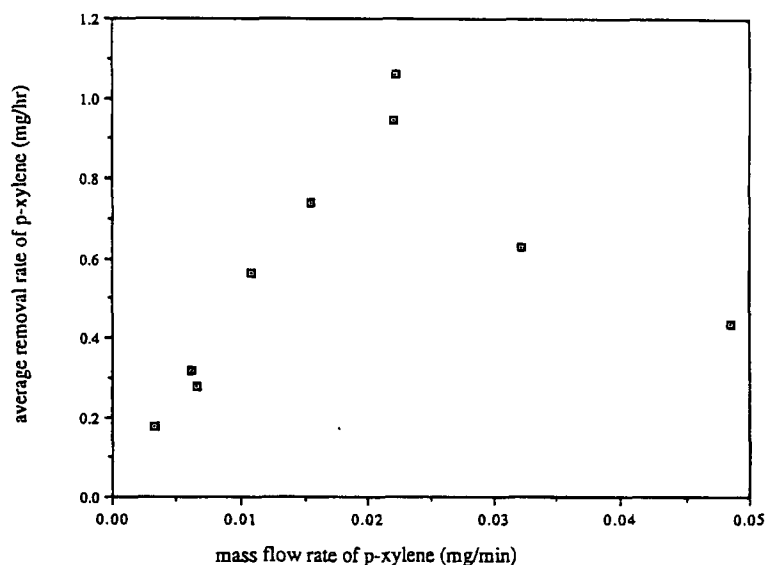


Fig. 8. Average removal rate of *p*-xylene vs influent mass flow rate of *p*-xylene for all nine experimental runs.

system/immobilized *P. putida* microorganism was found to be within the range of 15–150 ppm for the influent *p*-xylene concentration and flow rates between 60 and 130 cm³/min. Continuous biodegradation was demonstrated within this defined operating region, which resulted in removal efficiencies of 80–90% of the influent *p*-xylene for operating times up to 200 h.

Mass-transfer limitations did not appear to be significant for this reactor system within the 15–36 ppm range, once removal efficiencies were comparable and in both cases remained above 60% when the total feed flow rate was doubled. As the influent feed concentration increased from 50–100–147 ppm, the removal efficiencies successively decreased, while still remaining as high as 60 for the 100-ppm case. The system did appear to become saturated with *p*-xylene after 60 h of operation at the 147 ppm and 62 cm³/min conditions.

The gas-phase bioreactor developed in this study does support and verify the novel design for the vapor-phase biodegradation of *p*-xylene with the particular microbial system utilized. Modeling and numerical validation of the reactor system studied are a currently ongoing endeavor.

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