ELSEVIER

Biochemical Engineering Journal 5 (2000) 83–88

Biochemical Engineering Journal

www.elsevier.com/locate/bej

Short communication

A membrane bioreactor with novel modules for effective biodegradation of toluene

Zeyi Xiao[a], M. Hatta Dahlan[b], X.-H. Xing[b,*], Yumi Yoshikawa[b], K. Matsumoto[b]

[a]*Department of Chemical Engineering Machinery, School of Applied Chemistry and Chemical Engineering, Sichuan University, 610065 Chengdu, Sichuan, China*

[b]*Department of Chemical System Engineering, Division of Material Science and Chemical Engineering, Yokohama National University, 79-5 Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan*

Received 7 June 1999; accepted 24 November 1999

Abstract

Two novel membrane modules, tubular and spiral type with numerous hollow fibers of silicone rubber were incorporated into a bioreactor system for effective degradation of volatile organic chemicals (VOC) in wastewater. Biodegradation and transfer of toluene as a model VOC were studied in the membrane bioreactors using *Pesudmonas putida* mt-2 as the degrader. The overall mass-transfer coefficient of toluene across the membranes was estimated to be 4.8×10^{-7} m/s for the tubular type module and 4.6×10^{-7} m/s for the spiral one. Both bacterial growth and toluene degradation in the two types of membrane bioreactors were the same as those obtained from the culture under an ideal condition. The mass transfer of toluene across the membranes was not a rate-limiting factor for the bacterial degradation of toluene.

Keywords: Biodegradation; Mass transfer; Membrane bioreactor; Volatile organic chemicals; Wastewater treatment

1. Introduction

Remediation of wastewater containing aromatic VOCs such as benzene, toluene, xylene, and phenol has being paid much attention in recent years. Aerobic microbiological treatment is effective, but the aeration not only reduces the biodegradation of the VOCs due to their air stripping [1] but also causes a secondary air pollution. An effective biological treatment process to avoid or minimize the air stripping is thus required. In our previous study, a simple biodegradation system consisting of a gas stripping tank followed by a bioreactor in series (ASTB) was proposed for the enhanced removal of toluene using *Pesudmonas putida* mt-2 as the degrader [2]. The principle of toluene degradation in this system is air stripping of toluene from the stripping tank and degradation of the air-stripped toluene in the bioreactor.

Moreover, a silicone rubber membrane has been found to be useful in separation of VOCs due to its selective permeability for VOCs with the rejection to other ions, chemicals and water even at normal pressure [3]. The silicon rubber membrane has been successfully integrated into a bioreactor and shown to be useful for the separation and degradation of phenol and BTX [4,5]. Like in our previous ASTB system, in the membrane-integrated bioreactor where the targeted pollutants separated from the original wastewater can be degraded under a well-controlled condition, without the effect of extreme conditions in pH, salt concentrations and/or the other components coexisting in the original wastewater, microbes in a pure or mixed culture selected by acclimation to the targeted chemicals can be used.

The key-factors affecting the performance of VOCs removal in a silicone-rubber membrane bioreactor are considered to be the mass transfer and biodegradation rates. The degradation rate can be improved by the selection or use of microbes with high degrading capability, and the optimization of cultivation conditions such as cell density, DO and medium compositions. On the other hand, design of a membrane module is extremely important for improving the mass transfer of VOCs across the membrane. However, all the researches on silicone-rubber membrane bioreactor reported so far used a single silicone tube of large diameter and thick wall [4,5]. From a viewpoint of practical application, it is necessary to investigate further on the feasibility of types of membrane module. The present study was aimed at

* Corresponding author. Tel.: +81-45-339-4025; fax: +81-45-339-4025.
E-mail address: xhxing@mail.chemeng.bsk.ynu.ac.jp (X.-H. Xing).

1369-703X/00/$ – see front matter
PII: S1369-703X(99)00071-6

examining the behavior of mass transfer and degradation of toluene as a model VOC in a silicone-rubber membrane bioreactor with novel modules. Two types of membrane modules with numerous hollow fibers of silicone rubber, i.e. tubular and spiral, were used. This hollow-fiber membrane has much smaller diameter and thinner wall than those employed in other researches.

2. Materials and methods

2.1. Membrane modules

A manufacturing company (Nagayanagi Industrial, Tokyo, Japan) offered the membrane modules of tubular and spiral types for this study. These modules consist of numerous hollow fiber membranes of silicone rubber and have the same membrane area. The tubular type module was manufactured by packing the hollow fibers into a bunch form, and the spiral module was prepared by knitting the fibers into a bamboo-blind-like form which was further wound into spiral shape. The principal specifications of the membrane modules were summarized in Table 1.

2.2. Microorganism and medium

In this experiment, *Pseudomonas putida* mt-2 (ATCC33015) purchased from Japan Collection of Microorganisms was used as the toluene degrader. This bacterium holds the well-known TOL plasmid that encodes the enzymes required for the degradation of toluene and xylene. Composition of the basal medium for cultivation was as follows (in kg/m^3): $(NH_4)_2SO_4$, 2.5; K_2HPO_4, 1.4; $Na_2HPO_4{\cdot}12H_2O$, 3.6; $MgSO_4{\cdot}7H_2O$, 2.5; $CaCl_2{\cdot}2H_2O$, 1.6×10^{-2}; $FeSO_4{\cdot}7H_2O$, 2.0×10^{-3}, which was the same as used in our previous study [2]. An aqueous solution of toluene with concentration of about 0.6 kg/m^3 was used as the toluene–containing wastewater. Medium used for bacterial preculture was prepared by mixing the toluene aqueous solution and the basal medium at a volume ratio of 1:1.

Table 1
Principal specifications of two membrane modules

Membrane module	M60-75S-71031-2	M60-75S
Type of membrane tubes	Tubular	Spiral
Membrane material	Silicon rubber	
Housing material	Polycarbonate	
Membrane tube O.D. (m)	3.2×10^{4}	
Membrane tube I.D. (m)	2.0×10^{4}	
Tube wall thickness (m)	0.6×10^{4}	
Membrane area (m^2)	0.1	
Tube length (m)	0.20	0.14
Number of tubes	750	750

2.3. Chemical reagents

All the chemicals used in our experiments are of commercial grade and purchased from WAKO Chemical, Japan.

2.4. Preculture

Preculture procedure for the bacterium was the same as that reported in our previous study [2]. In brief, after inoculating the bacterial cells in 0.5×10^{-6} m^3 aliquots into a 1.0×10^{-4} m^3 vial containing 1.0×10^{-5} m^3 preculture medium, the preculture was done in an incubator under 301 K and at 130 rpm by shaking for 3 days. The final cell concentration in terms of OD_{660} by the preculture reached 0.40–0.45.

2.5. Experiments with membrane bioreactors

The membrane bioreactor system shown in Fig. 1 consisted of a membrane module ①, a bioreactor ② and a wastewater tank ③. Working volumes of both the bioreactor and wastewater tank were 1.0×10^{-3} m^3. The toluene-containing wastewater placed in the wastewater tank was recycled through the lumen side of membrane, and the basal medium placed in the bioreactor was circulated through the shell side of membrane. A sparger installed in the bioreactor was used for aeration to maintain the bioreactor under aerobic condition. In all experiments, the membrane module, the wastewater tank and the bioreactor were immersed into a temperature-controlled bath so as to keep a constant temperature of 301 K. Both the wastewater tank and the bioreactor were agitated by a magnetic stirrer at 150 rpm.

For bacterial cultivation using the membrane bioreactor, the precultured bacterial cells were inoculated in the bioreactor containing the basal medium at an inoculum size of 1/20 (v/v). The cultivation was started by circulating both

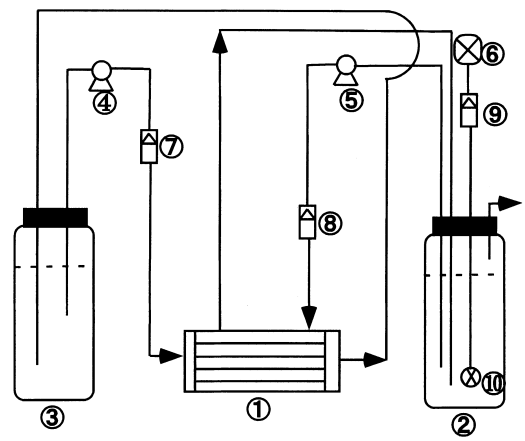

Fig. 1. Scheme of the experimental apparatus. ① membrane module, ② bioreactor, ③ wastewater tank, ④ ⑤ pumps, ⑥ compressor, ⑦ ⑧ ⑨ flow meters, ⑩ sparger.

the medium and the wastewater containing toluene at the flow rates of 5.0 and 3.0×10^{-5} m^3/h, respectively. The air flow rate in the bioreactor was set at 1.8×10^{-3} m^3/h (0.03 vvm). During the cultivation, toluene concentration in liquid phase of the wastewater tank, cell concentration, DO and pH in the bioreactor, and toluene concentrations in the liquid phase and the exit gas phase of the bioreactor were measured. Samples were taken from the inlets of the lumen side of membrane and the shell side, representing the liquid phases of both the wastewater tank and the bioreactor.

The same experiment except inoculation of bacterial cells into the bioreactor was carried out for estimating the overall mass-transfer coefficient of toluene across the membrane.

2.6. Analysis

Toluene concentrations in liquid and gas phases were measured by gas chromatography (GC) equipped with a flame ionization detector [2].

Bacterial cell concentration was measured by optical density at 660 nm with a spectrometer (UV-1200, Shimadzu, Japan).

DO concentration in the bioreactor was monitored by a DO sensor (Ingold type, Biott, Japan) installed in the bioreactor.

3. Results and discussion

3.1. Overall mass-transfer coefficient

In general, transfer of toluene across the membrane occurs as follows: fluid-film mass transfer near two sides of the membrane surface and diffusional mass transfer through the membrane itself. The overall mass-transfer coefficient k_a can be expressed by a resistances-in-series model as follows:

$$\frac{1}{k_a} = \frac{1}{k_w} + \frac{r_i \ln(r_o/r_i)}{D_p K_p} + \frac{r_o/r_i}{k_b} \quad (1)$$

where k_w and k_b are the mass-transfer coefficients in fluid films of the wastewater side and the culture side, respectively, K_p is the partition coefficient of toluene between membrane and aqueous phase, D_p is the diffusion coefficient of toluene through the membrane, and r_i and r_o are the inner and outer radii of the membrane tube, respectively. Under the present experimental conditions, fluid flow inside the membrane was estimated to be completely laminar flow (the Reynolds number inside the membrane tube being estimated to be 53.9), and the toluene transfer across the membrane is principally governed by the diffusion of toluene molecules through the membrane. It is reasonable to deal with the mass transfer using the overall mass-transfer coefficient comprehensively. Flux of toluene across the membrane can be correlated to the toluene concentration change in the wastewater as indicated by Eq. (2).

$$J = -\frac{dC}{dt}\frac{V_w}{A} \quad (2)$$

In accordance with the general mass transfer model, the flux can also be expressed as:

$$J = k_a(C - C_1) \quad (3)$$

The following equation for estimating the overall mass-transfer coefficient can be derived by incorporating Eqs. (2) and (3) and conducting a simple integration if C_1 can reach equilibrium,

$$\ln\frac{(C_0 - C_1)}{(C - C_1)} = \frac{A}{V_w}k_a(t - t_o) \quad (4)$$

where C_0 and C are the toluene concentrations in the wastewater at time t_0 and t, respectively, C_1 is the toluene concentration in the bioreactor, A is the membrane area (0.1 m^2), and V_w is the volume of the wastewater (1.0×10^{-3} m^3). Incidentally, because toluene concentration in the bioreactor increased significantly in an initial period and reached a plateau as will be shown later, t_0 means the time when C_1 reaches a constant level.

An experiment on mass transfer of toluene under the absence of bacterial cells was carried out. The experimental results are shown in Fig. 2. Taking the difficulty in the precise measurement of toluene concentration into consideration, C_1 was judged to reach equilibrium after about 1 h for tubular type module and 0.5 h for spiral one. Toluene concentration in the exit gas from the bioreactor with the

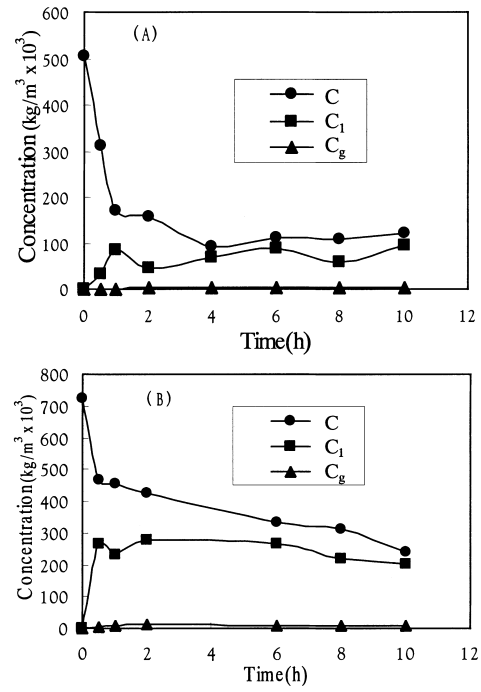

Fig. 2. Time course profiles of toluene concentration in liquid phases of wastewater tank and bioreactor and in exit gas phase of bioreactor for examination of mass transfer. (A), tubular type module; (B), spiral type module.

spiral type module reached the constant value of about 10.0×10^{-3} kg/m^3 after 0.5 sh, which was higher than that in the tubular type module system (around 3.0×10^{-3} kg/m^3). Since there was no bioreaction occurring in this experiment, total amount of toluene decreased in the wastewater tank was equal to the sum of toluene mass partitioned into the silicon membrane, the increment of toluene in the bioreactor and the cumulative amount of toluene discharged by gas stripping from it. From the initial toluene concentration changes in the wastewater, the bioreactor and the exit gas (Fig. 2), the toluene partitioned into the silicon membrane was roughly estimated as 250 mg in the tubular type module and less than 10 mg in the spiral one. Livingston has reported that partitions of nitrobenzen and 3-chloronitrobenzen between a silicon tube and an aqueous phase reached equilibrium after 1 h for a 1-mm wall thickness tube and 0.5 h for a 0.5-mm one, and that the amount of the organic solvents partitioned into the tubes is markedly dependent upon the wall thickness of the silicon tubes [6]. The difference in the amount of toluene partitioned into two types of silicon-rubber membranes still remains unclear, but it was considered to be caused by the different membrane structures and will be examined in details later. On the other hand, after C_1 reached constant, the toluene decreased in the wastewater tank was almost equal to the cumulative amount discharged with the exit gas. The higher C_g in the spiral type module system thus explained why the decline in C in the spiral type module system was more remarkable than that in the tubular one after C_1 reached equilibrium.

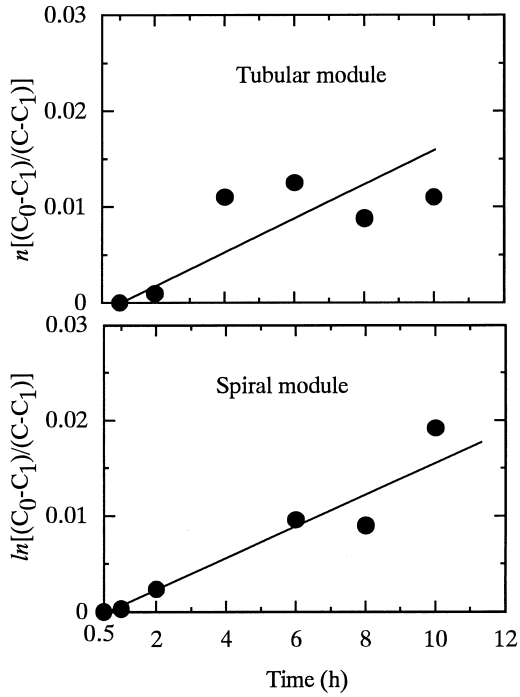

Fig. 3. Estimation of overall mass-transfer coefficients in the two types of membrane modules.

According to Eq. (4), the overall mass-transfer coefficients of toluene across the silicone-rubber membrane after C_1 reached equilibrium were calculated to be 4.8×10^{-7} m/s for the tubular type module and 4.6×10^{-7} m/s for the spiral type module from Fig. 3 by plotting the experimental data in Fig. 2 after $t\geq1$ h (the tubular type module) and $t\geq0.5$ h (the spiral one).

3.2. Toluene degradation and cell growth

Fig. 4 shows time course profiles of toluene degradation and cell growth in the membrane bioreactors with the tubular and spiral type modules. The two types of membrane modules showed a similar trend in both the toluene degradation and cell growth. From this figure, it was obvious that the toluene concentration in the wastewater dramatically declined and the cell concentration increased exponentially during the initial several hours. The decreased toluene was accordingly transported into the culture medium and was consumed by the bacterial cells except a small amount of toluene discharged with the exit gas (Fig. 5). Using the data obtained in the exponential growth phase, the specific growth rate constant in the two types of membrane bioreactors was evaluated to be roughly 0.45 h^{-1}, which was equal to the maximum specific growth rate of the same

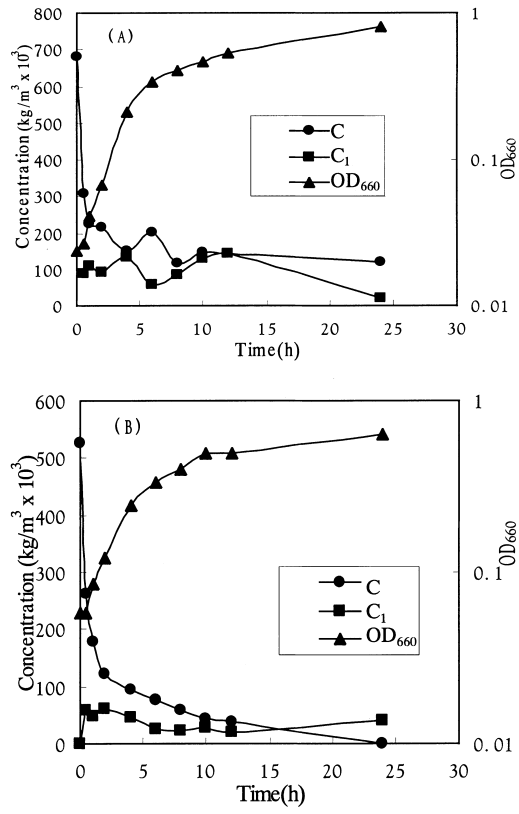

Fig. 4. Time course profiles of toluene concentration in wastewater tank and bioreactor and cell growth in bioreactor. (A), tubular type module; (B), spiral type module.

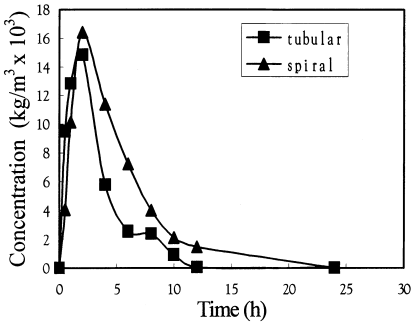

Fig. 5. Time course profile of toluene concentration in exit gas of bioreactor.

bacterium in an ideal culture with closed vials reported by Hatta et al. [2]. Judging from the high residual toluene concentration in the two types of membrane bioreactors, it was obvious that the mass transfer of toluene across the membrane was sufficient to supply the carbon source to the microorganism and that the bacterial growth was the limiting-step for toluene biodegradation.

After the exponential growth phase, the bacterial concentration increased slowly with the cultivation time which corresponded to the slow decrease of toluene concentration in the wastewater. This was presumably caused by the other factor(s) such as depletion of DO and/or nitrogen sources which limited the bacterial growth and toluene degradation. A typical time course profile of DO during the culture using tubular type module is shown in Fig. 6. The concentration of DO decreased rapidly soon after the start of cultivation, and became almost zero after 10 h cultivation, which supported that the low DO level would have limited the cell growth. However, the DO concentration began to increase gradually after 12 h cultivation. Because a DO profile in a bioreactor is closely related to the microbial metabolic activity, the reason of DO increase after 12 h cultivation was supposed to be a change in the bacterial cell activity which might have been caused by the depletion of nitrogen source and/or other component(s) in the medium. Lee et al. [5] have reported the importance of nitrogen source for the growth of *P. sp* and for toluene degradation.

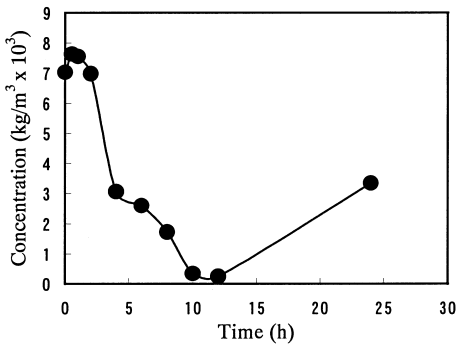

Fig. 6. Time course profile of dissolved oxygen concentration in bioreactor with tubular type module.

To evaluate the removal efficiency of toluene in the bioreactors, R_t, by neglecting the toluene amount partitioned into the membrane, the following equation can be used:

$$R_t = \frac{(C_{total} - C_t)V_w \int Q_g C_g dt}{C_{total} V_w} \quad (5)$$

where C_{total} and C_t are the concentrations of toluene in the wastewater at the start and end of cultivation, respectively, C_g is the toluene concentration in the exit gas from the bioreactor at time t, and Q_g is the air flow rate. The integration term in Eq. (5) sums the total amount of toluene discharged with the exit gas. The R_t achieved in the membrane bioreactors with tubular and spiral type modules after 24 h was calculated to be 83 and 93%, respectively. Both types of membrane modules exhibited much higher removal efficiency compared with that obtained in our ASTB system [2], which was about 30%.

From the present study, it can be concluded that a bioreactor with a membrane module consisting of numerous hollow fibers of silicone rubber is feasible for the effective treatment of VOCs. A detailed analysis for such a system is now being performed.

4. Nomenclature

A	membrane area, m^2
C	transient toluene concentration in the wastewater, kg/m^3
C_g	toluene concentration in the exit gas, kg/m^3
C_0	toluene concentration in the wastewater at $t=t_0$, kg/m^3
C_1	toluene concentration in the bioreactor, kg/m^3
C_t	toluene concentration in the wastewater at the end of cultivation, kg/m^3
C_{total}	initial toluene concentration in the wastewater, kg/m^3
D_p	diffusion coefficient of toluene through the membrane, m^2/s
K_p	partition number of toluene into the membrane
k_a	overall mass-transfer coefficient, m/s
k_b	mass-transfer coefficients in the fluid film of medium side, m/s
k_w	mass-transfer coefficients in the fluid film of wastewater side, m/s
Q_g	flow rate of air, m^3/h
R_t	removal efficiency of toluene in the bioreactor
t	time, h
t_o	time when C_1 becomes a constant value, h
V_w	volume of wastewater, m^3

Acknowledgements

The authors thank Mr. Shizuo Furukawa and Mr. Takakuni Tanaka of Yokohama National University for their technical help with the experiment.

References

[1] K.N. Truong, J.W. Blackburn, The stripping of organic chemicals in biological treatment processes, Environ. Prog. 3 (1984) 143–152.
[2] M. Hatta, X.-H. Xing, Y. Yoshikawa, K. Matsumoto, A novel and simple biodegradation process of volatile organic chemicals from waste water based on gas-stripping principle, J. Biosci. Bioeng. 87 (1999) 519–524.
[3] J.M. Watson, P.A. Payne, A study of organic compound pervaporation through silicone rubber, J. Membrane Sci. 49 (1990) 171–205.
[4] A.G. Livinston, A novel of membrane bioreactor for detoxifying industrial wastewater I. Biodegradation of phenol in a synthetically concocted wastewater, Biotechnol. Bioeng. 41 (1993) 915–926.
[5] J.Y. Lee, Y.-B. Choi, H.-S. Kim, Simultaneous biodegradation of toluene and *p*-xylene in a novel bioreactor: experimental results and mathematical analysis, Biotechnol. Prog. 9 (1993) 46–53.
[6] A.G. Livinston, A novel membrane bioreactor for detoxifying industrial wastewater: II. Biodegradation of 3-chloronitrobenzen in an industrially produced, Biotechnol. Bioeng. 41 (1993) 927–936.