Development of Polysulfone Membranes for Bacteria Immobilization to Remove Phenol

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ABSTRACT: We have investigated the feasibility of developing polysulfone (PS) membranes to partially immobilize *Pseudomonas* and to evaluate the inhibitory effect of phenol on immobilized *Pseudomonas* by monitoring their growths in partially immobilized cell and free-suspension systems. The polysulfone membranes used in this study were wet spun from 20 wt % of PS in 1-methyl-2-pyrrolidone (NMP) solvent using water as the bore fluid as well as the external coagulant. Scanning electron microscopy (SEM) characterization of the newly developed PS hollow fibers suggests that fiber cross-section consists of multilayer microporous structures useful for cell immobilization. Experiments were conducted using P*seudomonas* bacteria to remove phenol with initial phenol concentrations of 300 mg/L and 1000 mg/L. In a free suspension (no membrane) system, it was observed that the bacteria were able to grow optimally at 300 mg/L of phenol and degraded phenol almost completely in about 26 h. However, neither cell growth nor phenol degradation occurred when initial concentration of phenol was increased to 1000 mg/L. In a cell-immobilized membrane system, the cell growth and phenol concentration profile in the medium were very similar to those obtained in a free-suspension culture if phenol concentration was 300 mg/L. However, when the initial phenol concentration was increased to 1000 mg/L, data obtained in a cell-immobilized membrane system was discernibly different from that obtained in the suspension culture. In the former case, phenol concentration decreased in the beginning of the test, indicating that the carbon source has been consumed and immobilized cells within the membrane had begun to multiply. As soon as the phenol concentration decreased to about 600 mg/L (at which concentration, substrate inhibition was not as severe as 1000 mg/L), partial immobilization occurred when some cells diffused out of the membrane into the medium and optical density became measurable in the medium. It was found that cell growth continued for the next 28 h, reaching a maximum optical density in the medium of 0.610 absorbance units, and phenol was also completely degraded. J Appl Polym Sci 70: 2585–2594, 1998

Keywords: polysulfone membrane; partial immobilization; biodegradation; *Pseudomonas*; removal of phenol

INTRODUCTION

Advanced biocatalytic systems using immobilized cells have potential in various applications ranging from wastewater treatment to the production of therapeutics. Many advanced bioreactor systems such as packed-bed and membrane bioreactors use biocatalysts, such as micro-organisms, plant, or animal cells, immobilized into or onto solid substrate to reduce cell washout and improve the biocatalyst concentration.[1]

The immobilization of micro-organisms can be defined as any technique that limits the free migration of cells. Cell mobility can be restricted by

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aggregating the cells or by confining them into, or attaching them to, a solid support. The two broad types of immobilization commonly used are attachment and entrapment. The main advantage of cell immobilization is that it simplifies separation and recovery of the immobilized cells as well as any specific binding partner. The immobilized preparation can then be reused either in batch or in continuous systems and, hence, diminishes process cost. In addition, immobilization often enhances the stability of cells through multipoint attachment, resulting in conformational fixation or by protecting the immobilized cells from adverse conditions.[2,3] As a result, immobilized cells survive longer storage time and can be reactivated more easily when supplied with suitable nutrients. However, mass transfer limitation is often exhibited for immobilized systems, particularly those involving entrapment. In some cases, this may be advantageous when substrate inhibition occurs. This advantage can be exploited in the field of biological degradation of industrial effluent where often high concentration of toxic organics, such as phenol, inhibit cell growth, and leads to ineffective treatment of industrial wastewater. In other words, the entrapment technique of cell immobilization can, therefore, serve as a means of protecting the cells behind a barrier in which immediate direct contact between high phenol concentration and the cells is minimized. Phenol degradation can still be effected without the effect of substrate inhibition on the cells. However, for some cases, immobilization can also have detrimental effects. If the mass transfer limitation becomes the rate-determine step, it reduces the rate of phenol degradation.

Much research has been done on cell immobilization using entrapment barriers such as activated carbon and calcium alginate.[4–7] Research is not sufficiently extensive in areas of cell immobilization where entrapment barriers like semipermeable membranes are used.[8–10] This research, therefore, extends previous research and deals with the development of polysulfone hollow fiber membrane as an entrapment barrier for immobilizing *Pseudomonas* bacteria for exposure and subsequent degradation of high phenol concentration. Here, polysulfone hollow-fiber membrane will serve both as the barrier and porous matrix into which cells will preferentially diffuse and be immobilized. As a result, the cells are not directly exposed to the toxicity of high phenol concentration and thus prevented from substrate inhibition.

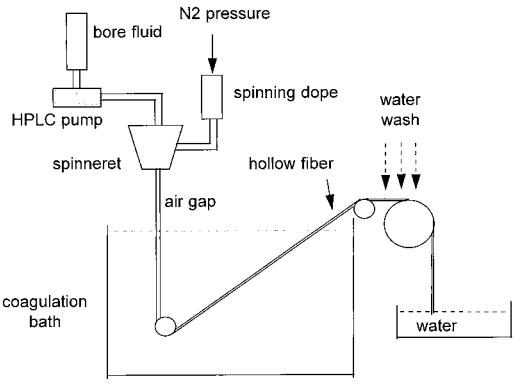

Figure 1 The schematic diagram of the hollow-fiber spinning process.

LITERATURE REVIEW

Membranes

Membrane is a thin barrier that prevents mass movement but allows restricted and or regulated passage of one or more species through it. Many review articles have been published on various membrane formations and structures.[11–15] Generally, asymmetric membrane is the important one. It has a dense skin layer that integrally bonded in series with a thick porous substructure. The skin and the substructure are composed of the same material. The skin layer, which contains the effective separating layer, is one of the key elements in determining the membrane permeability and selectivity.

The phase inversion process is one of the most important means to prepare asymmetric membranes. In this technique, a polymer is dissolved in a suitable solvent or solvent mixtures and spun into a coagulation bath, as illustrated in Figure 1. Upon contact with the internal and external coagulants, solvent exchange occurs between the nascent fiber and coagulants. A hollow fiber is formed when the as-spun fiber is fully precipitated. The resultant membrane morphology is dependent on the kinetics and thermodynamics of solvent exchange mechanisms as well as the elongational stresses, solid concentration, and air-gap distance during the fiber spinning process.[11,14,16]

Cell Immobilization

The use of immobilized cells began in the 19th century when microbial immobilization was taken advantage of in a trickle-filter system with a biological film for producing acetic acid.[17] Since then, similar approaches have been adopted for wastewater

treatment, and many new techniques have been developed in which cells were immobilized by two broad methods, namely attachment and entrapment.[1] Attachment means that the micro-organisms adhere to a surface or other organisms by self-adhesion or chemical bonding. Entrapment means that the organisms are caught in the interstices of porous materials or physically restrained within membranes. Self-adhesion or adsorption is primarily achieved through ionic or hydrophilic interactions between the cells and the support. An advantage of adsorption method of immobilization is that the support is often regenerable because binding is reversible. However, this reversibility also limits this method, as the cell may detach from the support, particularly if the optimal conditions during operation are significantly different from those used during adsorption. A particular form of adsorption is the affinity binding between a protein or cell and its support. In this case, the binding forces usually involves different noncovalent forces including both ionic or hydrophobic interactions as well as hydrogen bonding and Van der Waals interactions. Reviews on recent developments in affinity adsorption and affinity chromatography can be found in the literature.[7,18,19]

Whereas covalent coupling is more often used for protein immobilization, entrapment is more usual for cell immobilization. Entrapment of cells represents a more definite means of immobilization that does not depend significantly on cellular properties. This approach is by far the most popular for cellular immobilization.[1,8–10] In entrapment, the cells are held within the interstices of porous materials, such as a sponge or fibrous substances. The main advantage is that pre-formed support material are more resistant to disintegration, and entrapment is usually not harmful to cells. However, it may be difficult to reach a high cell concentration due to limited pore volume usually available for entrapment within typical support materials.

Cell entrapment in calcium alginate is a well-developed technique.[6,7,20] Ethanol production with calcium alginate-immobilized cells is one of the popular subjects in the literature. Physical confinement may also be achieved through the use of membrane encapsulation where cells are entrapped and immobilized by the physical restraints of membranes and encapsulating gel matrices. The advantage of encapsulation is the large surface area for substrate and cell contact. Typical examples of this method include the use of pre-formed membranes either in the form of hollow fibers or in the form of ultrafiltration devices.[21,22]

Membranes Applications in Immobilization

Because membranes achieve the separation of molecular mixtures by restricting the permeation of certain components while others may pass unhindered, cells can be restrained by semipermeable membrane materials that isolate the organism from the bulk liquid. The cells can be immobilized into the membrane (a technique frequently used for the fabrication of biosensors),[23] or they can be allowed to propagate into a void that is enclosed by the membrane (a technique used with membrane reactor system).[21,22] Cells can be entrapped by inclusion within membrane filter devices like hollow fiber, flat plate, or spiral-wound units. Hollow-fiber membranes form a tubular structure that is usually arrayed as a parallel fiber bundle within a cylindrical module. In one configuration, cells are trapped on the shell side of the hollow fiber while aerated nutrient medium is rapidly recirculated through the fibers. The membrane, therefore, allows the molecular transport of soluble material to and from the immobilized cells while confining and protecting the enclosed organisms. This type of membrane support also provides extra protection against contamination.

Research studies[8–10,21,22] have shown that hollow-fiber bioreactors, with the organisms confined to one side of the porous fiber and the soluble substrate and products on the other side, seem to be the most practical. However, the major disadvantages of membrane systems are high cost and membrane fouling, resulting in added mass transfer resistance and aeration difficulties.

Cell Immobilization for Phenol Degradation

Biological degradation of toxic organics is a promising technique for treating industrial effluents. By degrading phenol (a toxic organic chemical commonly found in industrial effluents) and utilizing phenol as a source of carbon, numerous bacteria grow optimally at a phenol concentration ranging from about 100 to 200 mg/L. At higher phenol concentrations of about 1000 mg/L and more (which is usually the case in industrial effluents), bacterial cells exhibit substrate inhibition and growth is affected. It is, therefore, necessary to construct a “membrane barrier” between high phenol concentration and the cells so

that phenol degradation can still be effected without the effect of substrate inhibition on the cells.

Although microorganisms like *Pseudomonas* and *Candida* did not tolerate more than 1500 mg/L of phenol, the cells survived temporary high phenol concentrations up to 15,000 mg/L, and degraded about 90% of the adsorbed phenol when they were adsorbed on activated carbon.[6] Bettmann and Rehm[24] studied the degradation of phenol by *Pseudomonas* immobilized in calcium alginate and found that the cells showed better degradation rates than free cells. They also reported that the immobilized bacteria could be exposed to higher phenol concentrations without loss of cell viability. These results suggest that the advantage of using membranes in cell immobilization is that the membrane "protects" the cells from direct contact with the toxic high concentration of phenol. The "protection" is achieved by diffusion of cells into the pores of the membrane. The cells in the pores are then not in direct exposure to the high concentration of phenol and, therefore, the continued growth of cells even at such high concentrations can occur.

As opposed to the immobilized system, the free suspension cells are directly exposed to high phenol concentration, which causes cell damage and loss of cell viability. The free suspension cells are thus able to grow at an optimum rate only at a particular substrate concentration. Beyond this critical concentration, there is substrate inhibition and the cells are inhibited in their growth and metabolic activities.

It is, therefore, concluded that using membranes in cell immobilization for waste water treatment may be a better process than a free-suspension cell system. However, it is important to point out that when cells are fully immobilized in membranes, the growth of the cells, though prolonged, becomes slower. This might be due to mass transfer limitation in the hollow-fiber membrane system. Therefore, partial immobilization, which combines the advantages of free-suspension cell and immobilization systems, may be an alternative to improve the overall performance.

EXPERIMENTS

Development of Polysulfone Hollow-Fiber Membranes

Polysulfone (PS) was purchased from Amoco and 1-methyl-2-pyrrolidon (NMP) was obtained from Merck. A 20/80 (weight ratio) PS/NMP solution was prepared as the spinning solution. We chose this composition because the previous data[11,14] on PS membranes suggested that a solid concentration higher than 30% may created membranes with a tight structure, while a concentration low than 18% may create very porous fibers. The viscosity of this spinning solution was 320 cp, measured with a Haake viscometer at room temperature. Water was used as the internal and external coagulants.

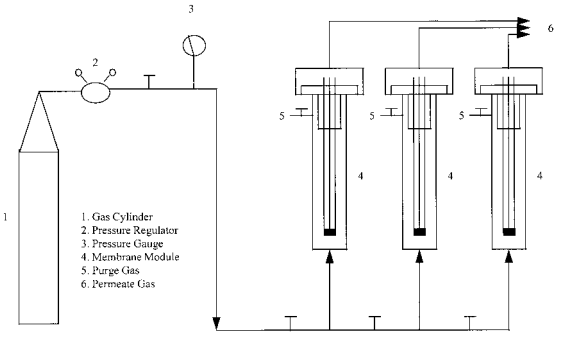

Figure 2 The schematic diagram of the testing apparatus for gas permeation measurements.

Figure 1 illustrates the schematic diagram of the hollow fiber-spinning apparatus.[13,16] The formulated dope was fed under nitrogen pressure and bore fluid was fed by 500D Syringe Pumps, made by ISCO. The accuracy of this ISCO precision pump was $\pm 0.5\%$ of flow rate. The spinning dope and the bore fluid met at the tip of the spinneret, which was immersed in the coagulation bath. After the formation of hollow fibers, fibers were stored in a water bath for at least 1 day and then transfered to a tank containing fresh methanol for at least 1 h to remove the residual NMP completely. Hollow fibers thus treated were used for further test and study.

SEM and Permeance Test

Membrane samples for SEM study were first immersed in liquid nitrogen and fractured, and then sputtered with gold using a JEOL JFC-1100E Ion-Sputtering Device. We employed a JEOL™ JSM U3 electron microscope to investigate fiber morphology.

To characterize the porosity of the newly developed membranes, we measure the gas permeance through a hollow-fiber module. Figure 2 shows a fabricated module for permeance test. The permeances of gases through the hollow-fiber module

Table I Composition of FI, FII, FIII, and FIV (Standard Nutrition for *Pseudomonas* Bacteria)

ID	Chemical Compositions	gL^{-1}
FI	K_2HPO_4	65
	KH_2PO_4	19
	$NaNO_3$	50
FII	$MgSO_4 \cdot 7H_2O$	10
	$FeSO_4 \cdot 7H_2O$	0.556
FIII	$(NH_4)_2SO_4$	50
FIV	Nitrilotriacetic acid	1.5
	$MnSO_4 \cdot H_2O$	0.5
	$CoCl_2 \cdot 6H_2O$	0.1
	$CaCl_2$	0.1
	$ZnSO_4 \cdot 7H_2O$	0.1
	$CuSO_4 \cdot 5H_2O$	0.01
	H_3BO_3	0.01
	$Na_2MoO_4 \cdot 2H_2O$	0.01

were determined using a bubble-flow meter and calculated using the following equation:

$$\text{Permeance} = \frac{Q}{\Delta PA} = \frac{Q}{n\pi DL\Delta P}$$

where L is the effective length of the fibers (15 cm); ΔP is the transmembrane pressure drop (1 bar); A is the membrane effective surface area; Q is the the gas flux reading; n is the number of tested fibers (15 filaments); and D is the outer diameter of the fibers (0.3 cm). We use GPU as the gas permeation units, and one GPU is equal to 1×10^{-6} cm^3 (STP)/cm^2-s-cmHg.

Immobilization

Micro-Organisms

Cultures of *Pseudomonas putida* ATCC 49451 were used throughout the experiment. *Pseudomonas putida* has been studied extensively[25,26] and has been in biodegradation of chemicals.[27–30] A medium stock solution was prepared as the source of minerals and nutrients for the growth of *Pseudomonas putida*. The medium employed in this work was made up of portions of FI, FII, FIII, and FIV, and their detailed compositions are shown in Table I.

Cells were activated before the experiments were carried out. Activation was performed to decrease the lag phase of the cell-growth cycle during biodegradation. To prepare for activation, 2.5 mL each of FI, FII, FIII, and FIV was measured into a flask and autoclaved at 121°C. A fixed amount (y volume) of phenol was added into the flask and then made up to 250 mL with deionized (DI) water. Cells were added from agar slant into the 250 mL medium. The flask was placed in a gyratory shaker for agitation and aeration of the contents. It took 14 h for the activation. When cells in the activation process had grown to the late exponential phase, an originally colorless solution changed to a greenish yellow solution. For phenol biodegradation study, 2 mL of the culture was inoculated to a new fresh medium (prepared similarly as in the activation process), and the phenol biodegradation was monitored by measuring optical density, pH, and phenol concentration at regular time intervals. Table II summarizes the medium compositions for activation and biodegradation. Optical density was measured using a UV-spectrophotometer (Shimadzu 1601) at an absorbance of 600 nm, while change of pH value was measured using a pH electrode and meter (Model HI 9201, Hanna Instruments, USA).

For quantitative analysis of phenol, a further 3-mL sample was taken and acidified to pH 2 with concentrated sulphuric acid to quench the biodegradation reaction. The acidified samples were ex-

Table II Medium Compositions for Activation and Biodegradation

	Activation	Biodegradation
Phenol stock concentration, mg/L	20,000	20,000
Phenol concentration in the medium, mg/L	200	200
Volume of phenol added, mL	2.5	2.5
Volume of FI, mL	2.5	2.5
Volume of FII, mL	2.5	2.5
Volume of FIII, mL	2.5	2.5
Volume of FIV, mL	2.5	2.5
Volume of activated cells added, mL	—	2
Volume of DI water used, mL	237.5	235.5
Total medium solution volume, mL	250	250

Table III Effect of Bore Fluid Flow Rate on Hollow-Fiber Spinning (Pressure is 6 psi)

Flow Rate (mL/min)	Results
0.2	membranes were not hollow
0.3	membranes were not hollow
0.4	hollow portion was oval
0.5	hollow portion was more circular

tracted with 3 mL methylene chloride, which contained 100 mg/L *o*-cresol as an internal standard. Phenol concentration was then determined using capillary gas chromatography (GC) (Perkin-Elmer, Model 8700) equipped with a split injector and flame ionization detector. The injector and detector temperatures were both at 300°C. Two microliters were injected and the oven-temperature profile started with maintaining at 100°C for 1 min before ramping at 10°C/min to 130°C.

Cell Harvesting

To collect and obtain cells at the desired concentration (at late exponential phase), the cell suspension was centrifuged and the biomass was collected from the bottom of the centrifuging tubes. In other words, when the cells had grown to the exponential phase (observed by color change and approximated by time), the suspension was distributed into 10-mL centrifuging tubes and centrifuged at 20,000 rpm for about 15 min. The supernatant was discarded, and the yellow pellets (biomass) at the bottom of the tubes was washed with mineral solution and centrifuged again. We rinsed and centrifuged three times to ensure complete removal of phenol. The collected biomass was added with mineral solutions to about 80 mL and stored in a bottle for later experimental uses. For quality control, the prepared cell solution was measured by optical density.

Batch Immobilization

Immobilization was carried out immediately after cell harvesting. A bundle of 15 fibers was placed into a flask containing the medium and autoclaved. Thirty-five milliliters of the harvested cells were then added to the flasks containing the fibers. An initial optical density of the medium was measured and the flasks placed in a gyratory shaker for agitation and aeration. We provided 16 h to allowed the cells diffusing into the membranes. When the solution changed from colorless to greenish yellow, we remeasured the optical density of the spent medium. In addition, small sections of the fibers were cut out for SEM study to ensure the occurrence of immobilization. The fibers were then removed and their surfaces was slightly rinsed with DI water to dislodge surface cells. The rinsed fibers were replaced into a fresh medium (200 mL) containing phenol. We monitored its optical density, pH, and phenol concentration as a function of time.

To compare the effect of immobilization on phenol degradation, a free-suspension culture was carried out simultaneously. The free-suspension culture was also conducted using a 200-mL phenol medium. Two phenol concentrations were utilized in these comparative studies: 300 mg/L and 1000 mg/L.

RESULTS AND DISCUSSION

Hollow-Fiber Morphology

Various attempts were taken to fabricate hollow fibers during the fiber spinning. As illustrated in Tables III and IV, fiber exterior quality depends on several factors. Two of them are bore fluid flow rate and dope pressure. When bore fluid flow rate was low (0.2–0.3 mL/min), the membranes produced were not hollow. This might be due to the fact that there was no enough coagulant to induce precipitation at the inner surface to form the inner membrane skin. When the flow rate was increased, a desired precipitation took place at the inner surface and resulted in the hollow center. In case of dope pressure, we observed unstable spinning if the dope pressure was low (3 psi). The spinning dope could not be extruded continuously

Table IV Process and Spinning Conditions for PS Hollow Fibers

Process and Spinning Conditions	Value
Spinning solution	PS/NMP
Polymer concentration (by weight)	20%
Viscosity at 25°C, cp	320
Internal coagulant used (bore fluid)	tap water
External coagulant used	tap water
Bore fluid flow rate, mL/min	0.5
Dope pressure, psi	6
Take-up velocity, rpm	20
Air-gap, cm	0

into the coagulation bath. However, when the dope pressure was too high (8 psi), the spinning process became unstable again because the motor of the current setup could not manage the high-speed spinning, thus leaving a proportion of entangled and very thin fibers in the coagulation bath. A pressure of 6 psi was found to be the optimal condition to yield a stable process and stronger fibers. The permeance of this new developed as-spun fiber is 28.5 GPU, which indicates that the porosity of this membrane may be similar to microfiltration and ultrafiltration membranes.

Figure 3 shows a detailed structure of a wet-spun PS fibers as taken by the SEM. The fiber has two arrays of finger voids close to the inner and outer surface, which is consistent with previous reports.[12] In addition, a five-layer structure is evident; namely, (1) inner skin, (2) inner void, (3) central sponge, (4) outer void, and (5) outer skin. Measurements of the pore sizes in the dense layer showed that the pore sizes ranged between 0.2 to 0.7 μm, suggesting that microfiltration rather than ultrafiltration membranes have been developed.

Characteristics of layers (1) and (5) were a double-skin structure composed of small polymer particle aggregates. One advantage of the double-skin surface is that even if one surface is damaged, the other will continue to reject solutes and prevent contaminant leakage. Generally, the inner and outer skin layers should have the same pore size to fully utilize the advantage of the double skin. The highly reliable permselectivity of the double skin is valuable in any application, especially in bioseparation where leakage must least occur.

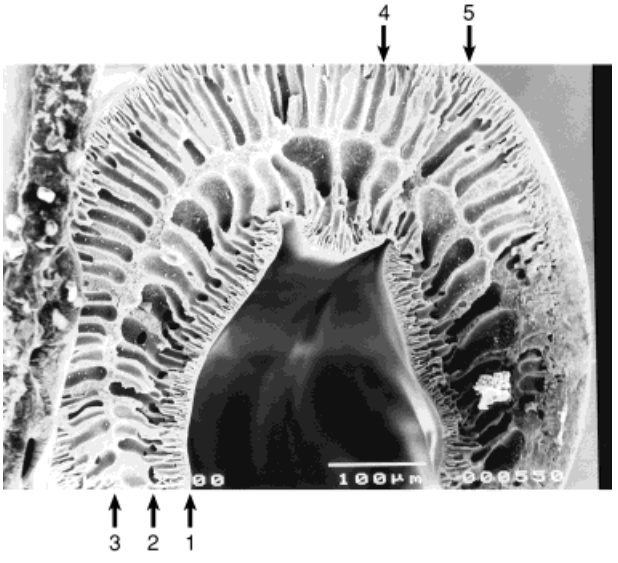

Figure 3 Cross-section of the hollow-fiber membrane produced (five-layer structure).

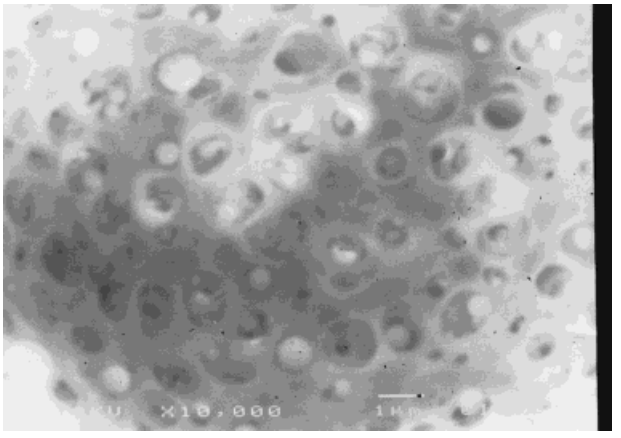

Figure 4 SEM photo of microvoids and bacteria distribution in the membrane external surface in the beginning of biodegradation.

The major characteristic of layers (2) and (4) is the finger-like void structure. These layers have low hydrodynamic resistance to liquid flow, and have high membrane permeability. Pore sizes range from 0.2 μm to 0.4 μm. Characteristics of layer (3) is a central open-cell sponge layer that contributes to the strength of membrane with skin layers. Pore sizes in this support layer range between 0.5–0.7 μm.

Bacteria Immobilization in Hollow-Fiber Membranes

Figure 4 shows the SEM photo of the hollow-fiber membrane just after immobilization and at the beginning of phenol degradation, while Figure 5 show the SEM photos end of the phenol degradation experiments. As illustrated in Figure 5, the cells in the suspension had diffused through the microporous membrane skin and *Pseudomonas* bacterial cells (size of 0.5–1 μm) were trapped. Cells confined in the pores continued to multiply during the phenol degradation experiments as the membrane allowed the molecular transport of nutrients to the immobilized cells, sustaining cell metabolism. Cell growth is clearly evident by comparing Figures 4 and 5. It is important to note that an increase in cell population in the hollow fiber and the production of small-sized cells might result in cell leakage from the hollow-fiber membrane. However, based on the data of the optical

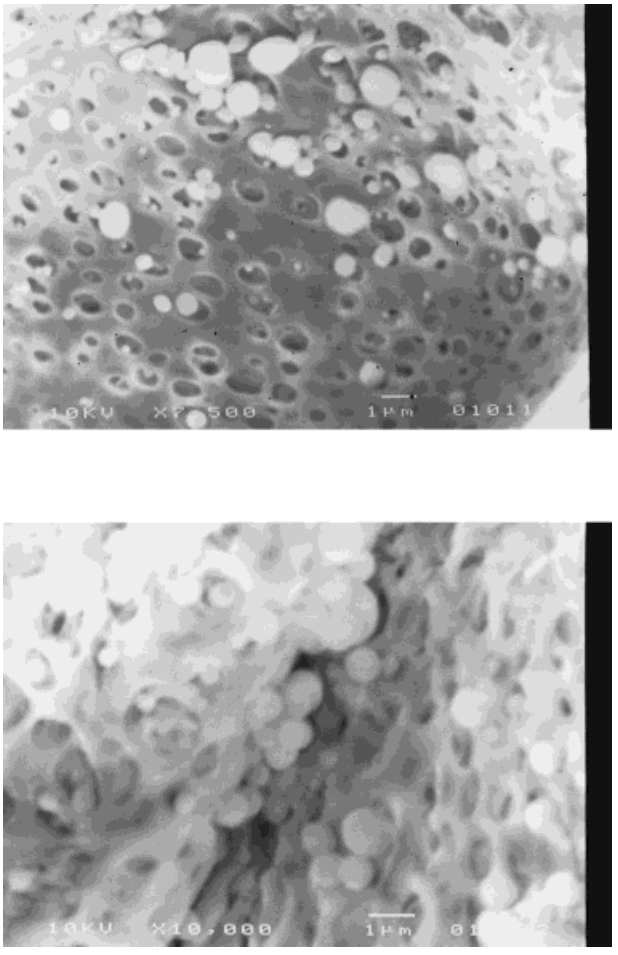

Figure 5 SEM photo of microvoids and bacteria distribution at the end of biodegradation (top: in the membrane external surface ×4875, bottom: in the finger-like voids ×6500).

density in suspension during experiments, it was found that the majority of cells were still immobilized.

Comparison of Suspension and Immobilized Cell System

Figures 6 and 7 show cell growth and phenol concentration profile in typical suspension and immobilized systems, respectively. For both systems, experiments were conducted for initial concentrations of 300 and 1000 mg/L phenol. For cell growth in suspension, it is observed that the growth at 300 mg/L follows the batch growth curve of a lag phase, an exponential phase and a stationary phase, as illustrated in Figure 6(a). In other words, *Pseudomonas* bacteria were able to grow optimally at 300 mg/L of phenol and degraded phenol almost completely in about 26 h, as shown in Figure 6(b). After 32 h, the optical density of *Pseudomonas* bacteria in the suspension started to decrease, shown in Figure 6(a), because of the lack of nutrition (phenol). When initial concentration of phenol was changed to 1000 mg/L, neither cell growth nor phenol degradation occurred to any measurable extent. Substrate inhibition was severe at 1000 mg/L phenol in the medium.

Figure 7 shows the respective typical data obtained when cells were cultured in the polysulfone hollow-fiber system at initial concentrations of 300 and 1000 mg/L phenol. In the case of 300 mg/L phenol, both the cell growth and phenol concentration profile in the medium were very similar to those obtained in the suspension culture (compare Figs. 6 and 7 at 300 mg/L phenol). In addition, similar to the previous suspension

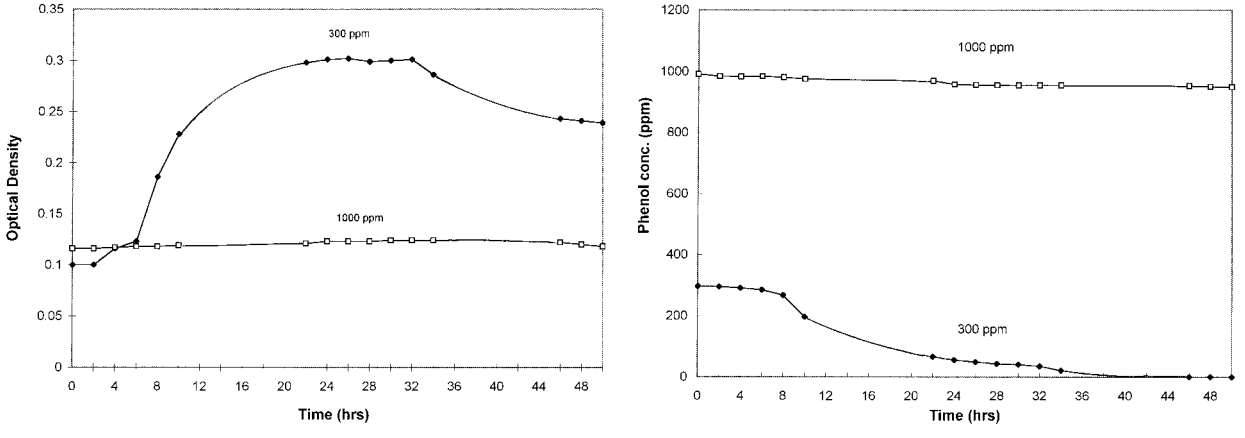

Figure 6 (a) Cell growth in suspension at 300 mg/L and 1000 mg/L phenol. (b) Phenol concentration profile of medium at 300 mg/L and 1000 mg/L phenol.

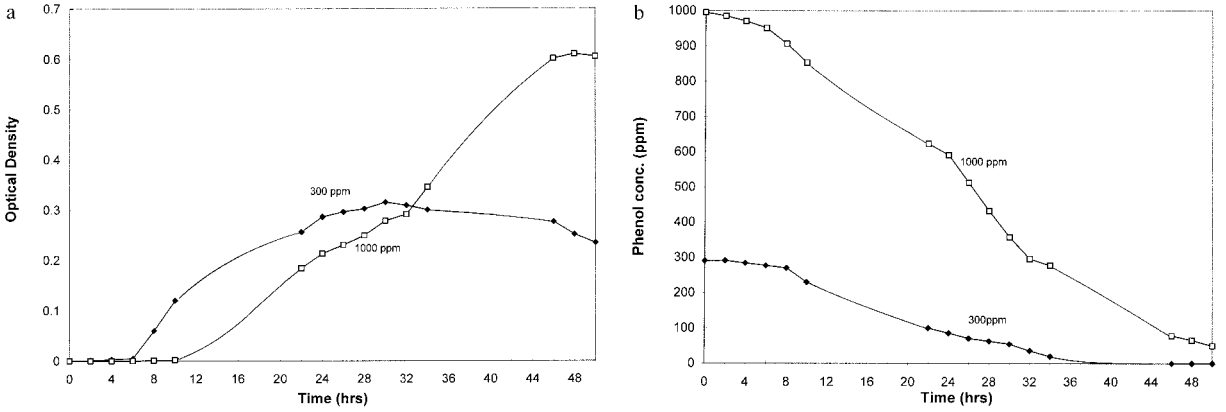

Figure 7 (a) Cell growth in immobilized membranes at 300 mg/L and 1000 mg/L phenol. (b) Phenol concentration profile of medium at 300 mg/L and 1000 mg/L phenol.

case, the optical density of *Pseudomonas* bacteria in this partial immobilized system started to decrease after 32 h of the test, shown in Figure 7(a). This is due to the same reason that most nutrition (phenol) has been consumed in the first 32 h. These results indicate that cell growth as well as phenol degradation occurred only outside the membrane due to the reasonably high mobility of cells in this partially immobilized system. However, the situation changes significantly if 1000 mg/L phenol was used initially. The data obtained in the immobilized system was discernibly different from that obtained in the suspension culture. Although there was no cell growth in the suspension culture at 1000 mg/L phenol, cell growth in the partially immobilized system was evident from the start of the experiment.

As observed from Figure 7(b), phenol concentration decreased from the start, indicating that the carbon source has been consumed and the cells had begun to multiply within in the membrane. During this time, the bacteria were completely immobilized in the membrane matrix. It was not until the phenol concentration has decreased to about 600 mg/L (at which concentration, substrate inhibition was not as severe as 1000 mg/L) that cell mobility increased and optical density became measurable in the medium. It was found that cell growth continued for the next 28 h reaching a maximum optical density in the medium of 0.610 absorbance units, and phenol was also completely degraded. Based on a material balance, cell growth must have occurred in the hollow-fiber membranes, because about 900 mg/L phenol was consumed and an optical density of only 0.610 absorbence units was recorded in the medium.

These results suggest that partial cell immobilization in hollow-fiber membranes provides an effective shield for cells from the toxicity of high phenol concentrations, and maintains cell growth with a concomitant degradation (consumption) of phenol. In this stage, phenol degradation occurs mainly within the membrane, and the rate of degradation depends on the mass transfer of phenol from the steam to the membrane and the total amount of immobilized cells. When phenol concentration is decreased to a tolerable concentration (about 600 mg/L), cell mobility increases, and some cells suspense in the medium. Phenol degradation occurs in both medium and membrane.

CONCLUSION

We have demonstrated that partially immobilized *Pseudomonas* in polysulfone (PS) membranes can degrade phenol. For comparison, we observed that P*seudomonas* bacteria in a free suspension (no membrane) system first. We found that these bacteria were able to grow optimally at 300 mg/L of phenol, and phenol was degraded almost completely in about 26 h. Neither cell growth nor phenol degradation occurred when initial concentration of phenol became 1000 mg/L. The cell growth and phenol concentration profile in a partially immobilized membrane system were very similar to those obtained in a free-suspension culture if phenol concentration was 300 mg/L. However, when the initial phenol concentration was increased to 1000 mg/L, data obtained in a cell-immobilized membrane system was discernibly different from that obtained in the suspension culture. In the former case, phenol concentration

decreased in the beginning of test, indicating that the carbon source has been consumed and the immobilized cells had begun to multiply within the membrane, behaving like a fully cell-immobilized system. Once phenol concentration was decreased to about 600 mg/L (at which concentration, substrate inhibition was not as severe as 1000 mg/L), partial immobilization occurred and optical density became measurable in the medium. It was found that cell growth continued for the next 28 h, reaching a maximum optical density in the medium of 0.610 absorbance units, and phenol was also completely degraded.

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