Development of Cellulose Acetate Membranes for Bacteria Immobilization to Remove Phenol

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ABSTRACT: The objective of this study is to investigate the feasibility of developing cellulose acetate (CA) membranes to partially immobilize *Pseudomonas putida* (ATCC 49451) and to evaluate the inhibitory effect of phenol on the immobilized bacteria by monitoring their growth in partially immobilized and free-suspension systems. The cellulose acetate membranes used in this study were wet spun from 20 wt % of CA in 1-methyl-2-pyrrolidone (NMP)/acetone (30 : 70) solvent using water as the bore fluid as well as the external coagulant. Scanning-electron microscopy (SEM) characterization of the newly developed CA hollow fibers suggests that the fiber cross section consists of multilayer microporous structures useful for cell immobilization. Experiments were conducted using the bacteria to degrade phenol at 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 profiles 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 were 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 immobilized cells within the membrane had begun to multiply. As soon as the phenol concentration decreased to about 700 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 32 h, reaching an optical density in the medium of 0.42 absorbance units and a significant amount of phenol was degraded. J Appl Polym Sci 68: 1677–1688, 1998

Key words: cellulose acetate membrane; partial immobilization; biodegradation; pseudomonas; removal of phenol

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INTRODUCTION

Industrial waste water often contains high concentrations of phenol and phenolics. Inadequate handling of these toxic organics has led to their release into the environment. Because of their

toxicity and persistence, they represent a serious pollution problem. Therefore, biological degradation of industrial effluents has received great attention in both academia and industry. Numerous bacteria have been identified to use phenol as a carbon and energy source.[1–5] These bacteria may utilize phenol in waste streams as a substrate for growth. However, it has been found that these bacteria only grow optimally at a phenol concentration of about 50–100 mg/L. At higher concentration of phenol, their growth is inhibited. To protect bacteria from being damaged as well as to maintain a continuous cell growth, it is necessary to construct a barrier between the high concentration of phenol and the bacteria. In this case, phenol degradation can still be effected without the effect of substrate inhibition. Immobilization of micro-organisms is one of the techniques to meet this requirement.

In principle, immobilization of micro-organisms can be defined as any technique that limits the free migration of cells. Cell mobility can be restricted by 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.[6–12] 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. 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 the case of removing phenol, mass transfer limitation may be advantageous when substrate inhibition occurs. 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. In addition, the immobilization procedure used may destroy or

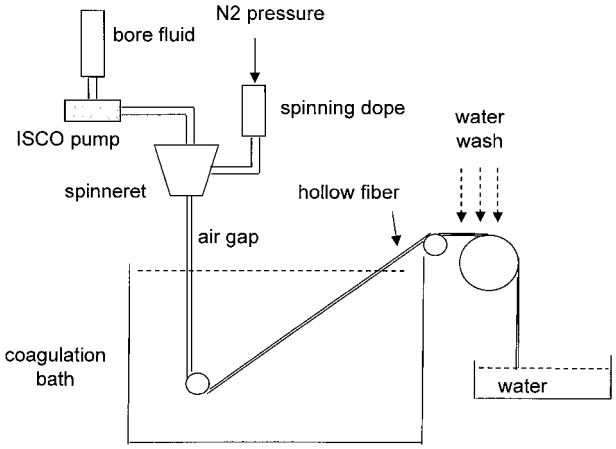

Figure 1 Schematic diagram of the hollow-fiber spinning process.

significantly alter cell functionality. Therefore, the material chemistry, surface morphology, and bulk structure of a membrane plays an important role on its suitability for cell immobilization.

LITERATURE REVIEW

Membranes

A membrane is a thin barrier that prevents mass movement but allows restricted and or regulated passage of one or more species through it. Membranes can be symmetric (isotropic), asymmetric (anisotropic), and composite (multilayer). Symmetric membranes have a uniform pore size throughout the whole cross section of the membrane, while asymmetric membranes have a graded pore size. Composite membranes consists of multilayer structures. Many review articles have been published on various membrane formations and structures.[13–17] Generally, asymmetric membranes have 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.

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. For example, Kesting et. al.[13,16] reported that spinning solutions containing 17 wt % of polysulfone could be used to fabricate an ultrafiltration hollow-fiber membrane with pore sizes ranging from 0.01 to 0.1 μm, while 37 wt % polysulfone was required to produce an air-separation membrane with pore sizes less than 5 Å. In addition, fibers spun from 17 wt % of polysulfone have a multilayer finger-like void structure, while fibers from 37 wt % of polysulfone have not.

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.[18] 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.[19] Attachment means that the microorganisms 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. The method is usually simple, merely mixing the two under appropriate conditions, and is generally mild. 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.[12,20,21]

In the case where adsorption is not sufficiently adequate for immobilization, it may be advisable to covalently couple the cell to the support. This is the method of chemical or covalent bonding. Several methods for the formation of covalent bonds between protein or cell and support exist and can be found in the literature,[20,21] protein or cells may even be crosslinked using a bifunctional reagent such as glutaraldehye. Care must be taken at choosing a particular method as the reactive conditions used may damage the functionality of the immobilized entity.

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.[19,22,23] 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.[11,12] Alginic acid is a uronic polysaccharide that forms gels in the presence of certain cations such as calcium, strontium, and barium. Calcium alginate beads are formed by dripping a sodium alginate-cell mixture into a calcium chloride solution. 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 preformed membranes either in the form of hollow fibers or in the form of ultrafiltration devices.[24,25]

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)[25,26] 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. The membrane therefore allows the molecular transport of soluble material to and from the immobilized cells while confining and protecting the enclosed organisms.

Research studies[22–24] 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. These drawbacks may be reduced by dynamic clean processes (such as high-pressure backwash) or if one can develop membrane systems with "partial" immobilized cells. This is due to the fact that the cell growth in a partial immobilized membrane system is very similar to that obtained in a free-suspension culture if phenol concentration is low. Membrane fouling and limited mass transfer are no more critical for cell growths. When the initial phenol concentration is high, bacteria stay within membranes and are protected by thin membrane barriers. Once phenol was consumed and its concentration decreased to a certain concentration that substrate inhibition was not severe, partial immobilization occurred and some cells diffused out of the membrane into the medium and degraded phenol.

Cell Immobilization for Phenol Degradation

Although microorganisms like *Pseudomonas* and *Candida* do not normally 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.[10] Bettmann and Rehm[1] 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 apparently "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 that 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 is 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.

In this project, our objective is to develop a cellulose acetate membrane for partial immobilizing bacteria to remove phenol. The feasibility of shielding bacteria by immobilizing them in a hollow-fiber membrane is investigated. The morphology of cellulose acetate hollow-fiber membrane and its use as an entrapment barrier for immobilizing *Pseudomonas* bacteria are studied. The inhibitory effect of different concentrations of phenol on *Pseudomonas* bacteria growth is also examined.

EXPERIMENTS

Materials and Spinning Devices

Cellulose acetate (CA) is chosen as the membrane-forming polymer because of its good chemical and mechanical properties.[27,28] For example, CA is resistant to sterilizing agents, is easy to fabricate into objectives, and is inexpensive. In addition, CA has a moderately polar nature, possessing both hydrophilic and hydrophobic properties, and is soluble in a wide variety of solvents. All these features have made CA an attractive material for gas/liquid/biomaterial separation membranes.[13,14,17] In this study, CA was generously supplied by Hoechst Celanese Corporation and came in the form of white flakes.

The spinning dope was made of 20 wt % CA in

Table I Spinning Conditions for Partial Immobilized CA Membranes

Length of air gap (cm)	0.0
Extrusion pressure (psi)	22.0
Internal coagulant flowrate (ml/min)	0.55
Average viscosity of dope solution (cP.)	5350
Speed of take-up roller (m/min)	1.14

an *N*-methyl-2-pyrrolidone (NMP)/acetone mixture. The weight ratio NMP and acetone was 30:70. Acetone is a common solvent for CA. Because it was highly volatile, a second solvent NMP was added to suppress its vaporization and to maintain the stability and shelf life of spinning solutions. Both solvents are miscible with water, which was used as the precipitation coagulant. This is the dissolving procedure: CA flakes was first dispersed in a cold NMP/acetone solvent mixture at 0–3°C with a high-speed stirrer. The chilled solvent reduced the dissolving rate of CA and thus prevented powders from agglomeration. The dope container was then agitated in an ice bath for 1.5 h and at room temperature until it was fully dissolved. Viscosity was measured using a HAAKE VISCOSITY MV Sensor System at room temperature. The average viscosity of this dope is about 53 poise.

Wet spinning was used as the technique to spin the hollow fibers. Figure 1 illustrates the schematic diagram of the hollow-fiber spinning apparatus.[15] The spinneret was placed below the liquid level in the coagulation bath, and the emerging dope solution was immediately exposed to the effects of the precipitation liquid. Water, a nonsolvent, was used as both the internal and external coagulant. 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 (0.5% of flow rate. The speed of the take-up roller could be adjusted in response to the speed of the nascent fiber formed. Extra care was taken to ensure that the take-up speed was nearly the same as the fiber free-falling velocity to ensure that no external elongational stresses were applied to the nascent fibers. Table I summarizes optimal spinning conditions we employed in the study. After the formation of hollow fibers, fibers were stored in a water bath for at least 1 day and then transfer 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.

Membrane Characterization

Membrane samples for Scanning Electron Microscopy (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 study membrane cross section. The physical dimensions of CA hollow-fiber membrane were measured on microtomed sections using a microscope (Olympus Vanox). To avoid distortion, a bundle of wet hollow-fiber membranes were inserted through a piece of plastic plate. The protruded ends were cut using a very sharp razor blade. The membrane inner and outer diameters (i.d. and o.d.) and its wall thickness were measured under the microscope. The average of o.d. and i.d. are 0.24 and 0.089 mm, respectively.

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

$$\text{Permeance} = \frac{Q}{\Delta PA} = \frac{Q}{n\pi DL\Delta P} \qquad (1)$$

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 (0.24 cm). We use GPU as the gas permeation units, and one GPU is equal to 1×10^{-6} cm^3 (STP) cm^{-2} s^{-1} cmHg^{-1}. The average permeance of this new developed as-spun fiber is 23.2 GPU, which indicates that the porosity of

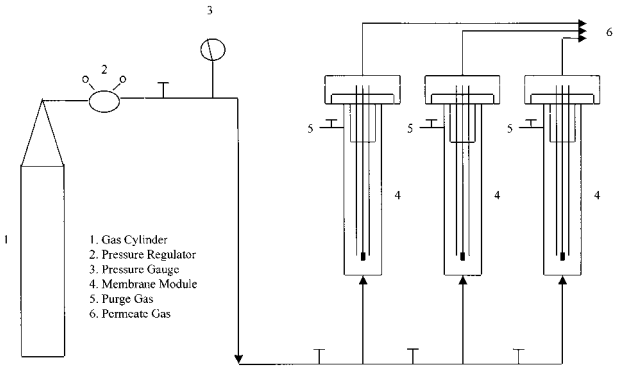

Figure 2 Schematic diagram of the testing apparatus for gas permeation measurements.

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

ID	Chemical Compositions	g/L
FI	K_2HPO_4	65
	KH2 PO_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

this membrane may be similar to microfiltration and ultrafiltration membranes.

Harvesting of Cells and Preparation of Feed Stock Solutions

Cultures of *Pseudomonas putida* ATCC 49451 (ATCC is a cell bank organization that stands for American Type Cell Collection) were used throughout the experiment. *Pseudomonas putida* has been studied extensively[24,25] for the biodegradation of chemicals.[29–33] 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 II.

Cell cultivation using *Pseudomonas putida* (ATCC 49451) was carried out in two steps: (1) growth of an inoculum from a seed culture, and (2) cultivation using the inoculum obtained from the first step. The first process is known as cell activation. It was carried out before cell cultivation to shorten the lag phase of the cell growth cycle during biodegradation. The second process is the degradation experiment where the activated cells were resuspended in a fresh medium. The growth curve of the cells was obtained by monitoring the optical density and pH of the broth experiment.

Pseudomonas putida was first activated in 250 mL of medium solution. In this process, cells from agar slants stored in the refrigerator were used as the inoculum and added to the medium solution. This medium solution was prepared by adding 2.5 mL each of the feed stock solutions prepared, phenol was also added at 200 mg/L to provide a carbon source for cell growth. The color change of this activation medium solution was then observed. After about 14 h, the color of this solution changed from colorless to yellowish-green. The cells had reached exponential growth phase and 2 mL of this solution was then withdrawn. This 2 mL of solution containing the activated cells from the first medium solution was resuspended into a second fresh medium solution prepared the same way. The compositions of both medium solutions are tabulated in Table III.

In all these processes, steam sterilization of the culture vessel and the media at 121°C is an essential step before inoculation. The culture vessels were then placed in a gyratory shaker with a water bath that vigorously agitates and aerates the contents, facilitates maintenance of aseptic operation, and provides close temperature control. The growth of the cells in this medium was closely monitored. The pH, optical density, and phenol concentration of the medium solution were measured at regular intervals. Phenol concentration was determined using gas chromatography.

For cell density and pH analysis, 6 mL of sample from the biodegradation flask was taken for determination of pH and biomass. pH was measured using a pH electrode and pH meter (Model HI 9021, Hanna Instruments, USA). Cell growth was monitored spectrophotometrically by measuring the absorbance at 600 nm. The 600 nm was chosen as the reference because it was the optimal wave length indicating cell concentration. For the analysis of phenol, a further 3 mL of sample was taken and immediately acidified with 6 *N* sulfuric acid to quench the biodegradation reaction. The acidified samples were extracted with 3 mL of methylene chloride (Merck, Darmstadt, Germany), which contained 100 mg/L *o*-cresol (Merck, Darmstadt, Germany) as internal standard. The extract was analyzed for phenol using GC. GC analysis was carried out using a capillary GC (Perkin-Elmer, Model 8700) equipped with a split injector and flame ionization detection (FID). The injector and detector temperatures were both 300°C. Injection volume was 2 μL, and the sample split ratio was 20:1. The oven-temperature profile started with maintaining at 100°C for 1 min before ramping at 10°C/min to 130°C, after which the program was halted.

The cells were activated and cultured as mentioned earlier and harvested at the point when its growth was in the late exponential phase. From the growth curve obtained from the previous ex-

Table III Medium Compositions for Activation and Biodegradation

	Activation	Biodegradation
Phenol stock solution concentration (mg/L)	40,000	20,000
Phenol concentration as carbon source (mg/L)	200	200
Volume of phenol added (mL)	1.25	2.5
Volume of FI added (mL)	2.5	2.5
Volume of FII added (mL)	2.5	2.5
Volume of FIII added (mL)	2.5	2.5
Volume of FIV added (mL)	2.5	2.5
Volume of activated cells solution added (mL)	—	2.0
Volume of deionised water added (mL)	238.75	235.5
Total volume of medium solution (mL)	250.0	250.0

periments, the time taken for the cells to reach this phase was approximated. The medium solution was then emptied into 10-mL centrifuging tubes and centrifuged at 20,000 rpm for 20 min to spin down the biomass to the bottom of the centrifuge tube. The spent medium at the top was discarded and the biomass pellets at the bottom were rinsed with a mineral solution. This mineral solution contained the same composition as the medium solution used for fermentation but without phenol and cells. The mineral solution together with the biomass was then centrifuged again. This procedure was repeated three times to wash away any residual phenol. After the last centrifugation, the biomass pellets were resuspended in 80 mL of mineral solution and stored in a bottle. The optical density of the solution was then measured. A summary of the conditions used is given in Table III below.

Immobilization and Monitoring of Phenol Degradation

Immobilization of the cells using hollow-fiber membranes was carried out after the cells were harvested. A medium solution comprising of the same stock solutions was first prepared. Fifteen fibers of membrane, each 15 cm long, were bundled tightly together to prevent diffusion of phenol and the cells through both ends. The fibers were then soaked in the culture vessel containing medium. These were autoclaved to sterilize them. After autoclaving, 2 mL of phenol (to make 300 mg/L) and 35 mL of the cell solution were added into the medium solution. Optical density of the solution was measured and the solution was left overnight in a gyratory shaker for the cells to diffuse into the membranes. After about 16 h of diffusion, the optical density of the medium solution was measured again. Small sections of some of the fibers were cut and prepared for SEM observations. SEM photographs were taken of the cross sections to check whether cells were immobilized in the fibers. After immobilization, the fibers were removed from the spent medium solution and their surfaces rinsed with deionized water to dislodge loosely bound cells. These fibers were then immersed in another 200 mL of medium solutions containing 300 mg/L of phenol. The degradation of the phenol was then monitored using gas chromatography (GC). The same procedure was simultaneously carried out for the degradation of 1000 mg/L of phenol. Each set of experiments was repeated to check for reproducibility.

Free Suspension Cells Experiments

To investigate the effectiveness of cellulose acetate hollow-fiber membranes as entrapment barriers, a set of free-suspended cell experiments was also carried out to compare the inhibitory effect of phenol on cell growth. The procedures for these experiments were similar to that of the immobilized cells, except that the cells were added to the medium solution together with the phenol solution and no fibers were added. The degradation of phenol in this solution was monitored in the same way. The experiment was carried out for both 300 mg/L and 1000 mg/L of phenol, and each set was performed twice.

RESULTS AND DISCUSSION

Membrane Morphology

Figure 3(a,b) illustrates the cross-section morphology of the newly developed hollow-fiber membranes, and shows that it is comprised of three layers with different pore sizes. The membrane

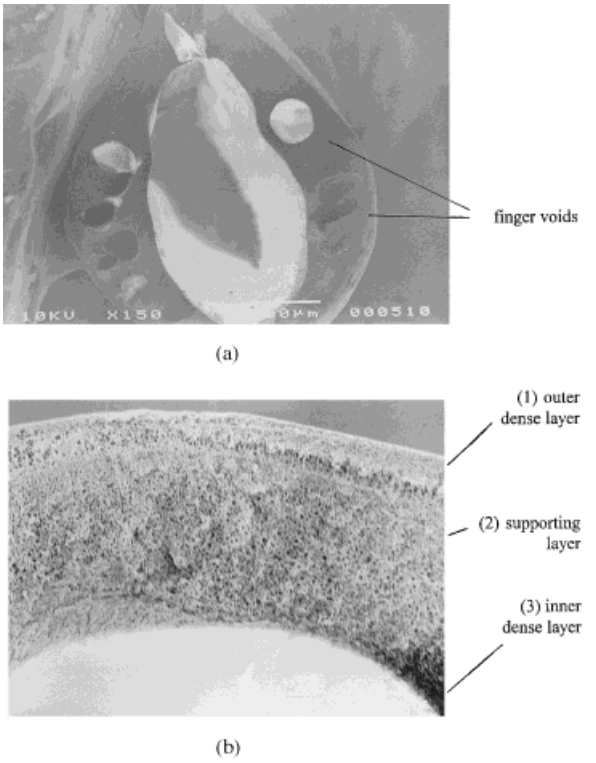

Figure 3 Cross section of the hollow-fiber membrane produced (three-layer structure): (a) a cross-sectional view (×58.5 magnification); (b) an enlarged view (×292.5 magnification).

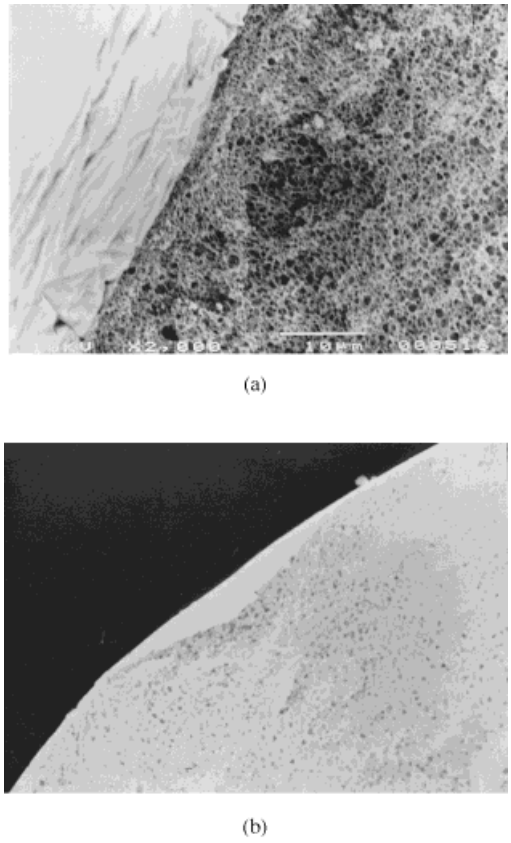

Figure 4 SEM pictures of inner skin and outer skin morphology: (a) inner skin (×1200 magnification); (b) outer skin (×600 magnification).

produced is anisotropic: outer layer (1) and inner layer (3) have smaller pores, and the central layer (2) has slightly larger pores. Both inner and outer skin layers are thin when comparing to the thickness of the central supporting layer. Figure 4(a,b) gives closer looks at both inner and outer skin layers and show that the membrane has a very thin outer layer. The pore size next to the inner skin layer was estimated to be approximately 0.5 μm. The high concentration of pores next to the skin is desirable, as it increases the permeability of the membrane.

Because the spinning solution contains 20% of CA, a large amount of solvent has to be leached out. This results in tremendous shrinkage and local imbalance during the solvent exchange stage. These stresses are parts of driving forces to produce finger voids.[34,35] Figure 5 shows close-up views of the shape of the voids. The estimated length of one void is approximately 80 μm.

Polymer precipitation rate in the nascent membrane also has an effect on membrane performance and morphology. During the spinning of CA hollow-fiber membranes, we observed that full external precipitation of the nascent fiber was not instantaneous as it entered the external coagulant bath. The inner skin layer had already begun to form on the inside surface of membrane before the fiber was immersed in the external coagulant bath. In the external coagulant bath, precipitation of the polymer in the fibers became complete. The emerging as-spun fiber remains weak and translucent in appearance for around 10 s before it become white and strong enough to be pulled.

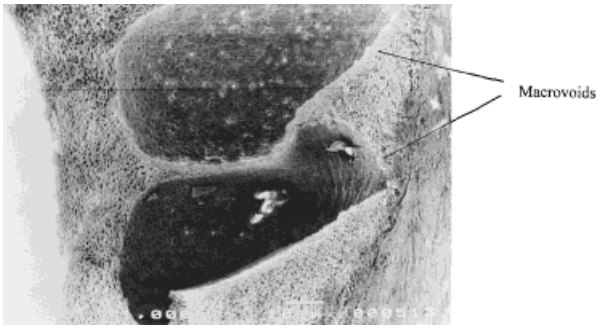

Figure 5 SEM pictures of microvoids (×550 magnification).

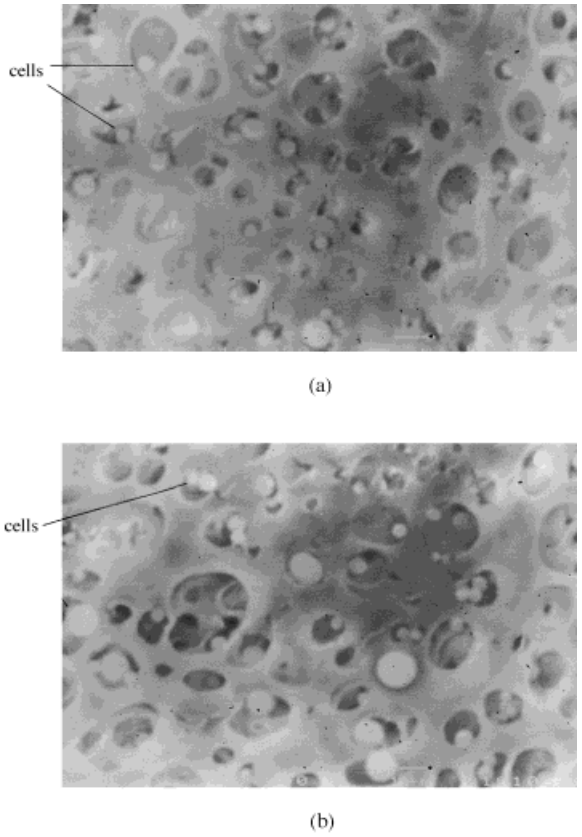

Figure 6 SEM photos of bacteria distribution at the beginning of biodegradation: a cross-sectional view of a CA fiber near the outer skin; (b) near the middle layer (×6000 magnification).

From Figure 4(a,b), we notice that the membrane produced has a very thin inner and outer skin layer. As mentioned before, a thin skin layer is desirable, as it presents low resistance to the separation process.

Feasibility of Cell Immobilization

The key to have successfully immobilized *Pseudomonas putida* cells in CA hollow fibers is to make sure that the immobilized cells can survive and grow when trapped inside the matrix of the membrane. This is essential for their degradation capability. SEM observations were chosen to study the immobilized *Pseudomonas* bacteria within CA hollow-fiber membrane. SEM pictures of the cross sections of the fibers at two different stages of the degradation experiment were prepared. Figure 6(a,b) shows the fiber's cross sections after the cells had diffused into the fiber after 1 day. Figure 7, on the other hand, shows the fiber's cross sections at the end of the degradation of 300 mg/L of phenol.

Cell growth is clearly evident by comparing Figures 6 and 7. 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 density in suspension during experiments, it was found that the majority of cells were still immobilized. Figure 6(a,b) also shows that the cells can be entrapped inside the pores of CA membrane structure. The size of the cells before degradation was approximately 0.5 μm. The entrapped cells were uniformly distributed in both layers 1 and 2 of the membrane structure. Figure 7 shows that after degradation of phenol, the cells grew and increased in size as well as number. The approximated size of the cells at this stage was about 1 μm. The cells found outside the pores in these two photographs could be due to the dislodgment caused during the fracturing of the fiber for SEM observation. Comparing the two sets of photographs, we can see evidence of cell growth during the degradation process. Furthermore, Figure 7 shows the change in the cell size distribution, appearance of buds, and cell division. All these provide a direct visual evidence of the presence of viable and active cells. Thus, we can say that the immobilization has not altered the cells' physical appearance and their growth much, and the newly developed CA membrane also allowed the molecular transport of nutrients to the immobilized cells, sustaining cell metabolism.

Comparing Phenol Degradation by Suspension Cells and Immobilized Cells

Degradation of Phenol Using Free-Suspension Cells

Batch experiments were performed for the degradation of 300 mg/L and 1000 mg/L of phenol by

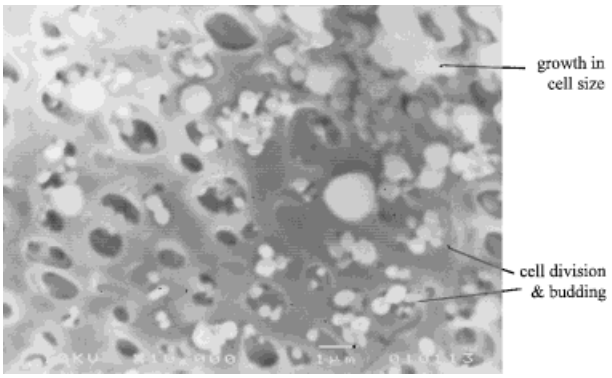

Figure 7 SEM photo of bacteria distribution at a cross section of a CA fiber near the outer skin at the end of biodegradation (after degrading 300 mg/L of phenol) (×5500 magnification).

free-suspension culture. For each concentration, duplicate experiments were conducted. Figure 8(a,b) shows the typical results for cell growth and phenol concentration profiles for cells grow in suspension at initial concentrations of 300 mg/L and 1000 mg/L phenol. From Figure 8(a), it can be seen that cell growth at 300 mg/L followed the batch growth curve of a lag phase, an exponential phase and a stationary phase. The bacteria were able to grow optimally at 300 mg/L of phenol and degraded phenol completely in about 28 h [Fig. 8(b)]. However, when the initial concentration of phenol was 1000 mg/L, neither cell growth nor phenol degradation occurred to any measurable extent when cells were cultured in suspension. Substrate inhibition was severe at 1000 mg/L phenol in the medium.

In suspension culture at 1000 mg/L phenol, the bacteria were exposed to a higher toxicity caused by the high phenol concentration. This toxicity of the microenvironment surrounding the cells made cell growth unfavorable, and the cell eventually died as a result. The slight decrease in the optical density of the suspension culture indicated a reduction in the cell density or the presence of cell lysis. These were signs of a lack of cell viability, suggesting that the cells had died over the 26-h process.

Degradation of Phenol Using Immobilized Cells

Batch experiments for phenol biodegradation were also performed for immobilized cells. Duplicate experiments were conducted for initial concentrations of 300 mg/L and 1000 mg/L phenol. Figure 9(a,b) shows the typical data obtained when cells were cultured in the immobilized hollow-fiber system at initial concentrations of phenol of 300 mg/L and 1000 mg/L. In the case of 300 mg/L phenol, both the cell growth and phenol concentration in the medium were very similar to those obtained in the suspension culture (comparing the two sets of results at 300 mg/L). This result indicates 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 9(b), phenol concentration decreased from the start, indicating that the carbon source had 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 900 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

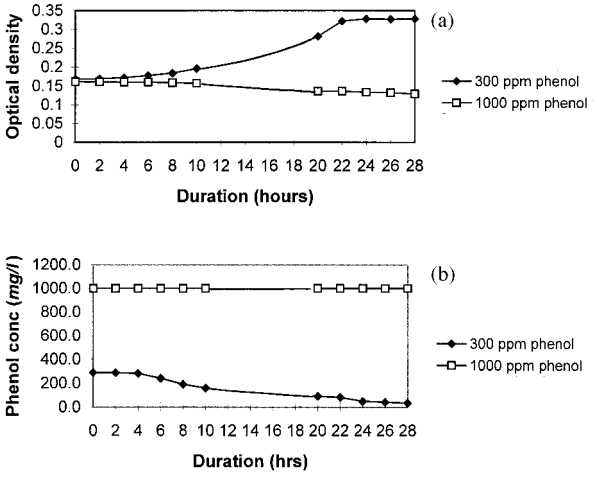

Figure 8 Cell growth and phenol concentration profile in suspension culture for 300 and 1000 mL/g (ppm) initial concentration of phenol: (a) cell growth; (b) phenol concentration.

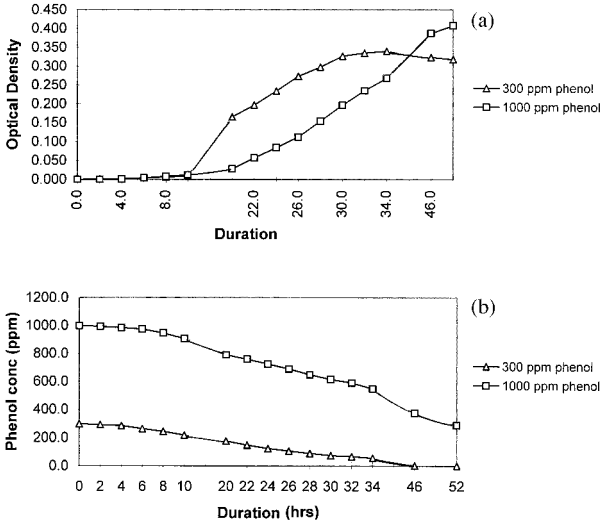

Figure 9 Cell growth and phenol concentration in immobilized culture for 300 and 1000 mg/L (ppm) initial concentration of phenol: (a) cell growth; (b) phenol concentration.

40 h, reaching a maximum optical density in the medium of 0.408 absorbance units. During that time, phenol was degraded to 374.6 mg/L.

Based on a material balance, cell growth must have also occurred in the hollow-fiber membrane, because 625.4 mg/L phenol was consumed and only 0.408 optical density units were found in the medium. Cells in the membrane were able to grow because the membrane surface created a diffusional barrier for the phenol to reach the cells. The cells were protected from the harmful effect of the high toxicity of phenol in the bulk liquid and were able to grow and multiply within the pores (as evident from Fig. 7).

These results suggest that partial cell immobilization in CA 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 900 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 putida* (ATCC 49451) in newly developed cellulose acetate (CA) membranes can degrade phenol. The CA membranes were wet spun from spinning dopes made of 20 wt % CA in a 1-methyl-2-pyrrolidin (NMP)/acetone (30 : 70) mixture and water was used an internal and external coagulants.

To investigate the effectiveness of cellulose acetate hollow-fiber membranes as entrapment barriers, a set of experiments was carried out to compare the inhibitory effect of phenol on cell growth. We observed that *Pseudomonas* bacteria in a free-suspension (no membrane) system were able to grow optimally at 300 mg/L of phenol and phenol was degraded almost completely in about 28 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 900 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 40 h, reaching a maximum optical density in the medium of 0.408 absorbance units. During that time, phenol was degraded to 374.6 mg/L.

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