Bull. Environ. Contam. Toxicol. (2005) 75:783–788

DOI: 10.1007/s00128-005-0819-4

Bulletin of Environmental Contamination and Toxicology

Phenol Degradation in a Hybrid Membrane System

Y. Li, C. Wang

College of Environmental Science and Engineering, Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, Hohai University, Xikang Road #1, Nanjing, 210098, People's Republic of China

Received: 24 November 2004/Accepted: 26 July 2005

Many industrial processes produce phenolic wastewater in which phenol may be present at a concentration as high as several grams per liter (Ahmed et al., 1995; Razo-Flores et al., 2003). The fate of these compounds is of serious environmental concern since they are toxic and persist. Usually biological treatment of industrial effluents and bioremediation of the contaminated environment have been considered the most promising approaches since they are effective, and the end products produced are innocuous (Juarez-Ramirez et al., 2001).

While many bacteria can degrade phenol at a concentration of about 50-100 mg/L, difficulties arise in the treatment of higher concentrations due to the increased toxicity of phenol to the microbial population (Zaisev, 1995) and cellular lysis also occurs (Juarez-Ramirez et al., 2001). At high concentrations, cells immobilization was shown to be an effective way to maintain a continuous phenol degradation and cell growth (Tope et al., 2001; Lin & Wang, 1991). In comparison with suspension cells, the main advantages in the use of immobilized cells include the retention in the reactor of higher concentrations of microorganisms and protection of cells against toxic substances.

Biological process can be enhanced by the addition of granular activated carbon (GAC) into the treatment system. Introducing absorbents (e.g. GAC) into a polymer membrane-forming system also offers the opportunity for the development of complex polymer composite. Taking this into account, the objective of this work is to develop the activated carbon-filled polyethersulfone composite hollow fiber membranes for immobilizing bacteria to enhance phenol tolerance of bacteria. The morphology property of the developed hollow fiber was firstly investigated. Then the sorption behavior of phenol on hollow fiber membranes was measured. Finally the degradation of high concentrations of phenol was studied by cells immobilized in the hollow fiber membranes.

MATERIALS AND METHODS

A phenol-degrading bacterium was isolated from phenol-contaminated soil and was tentatively identified as *Pseudomonas. Putida.* Stock cultures were

Correspondence to: Y. Li

maintained on nutrient agar slants and stored at 4°C The activation and preparation of cells before immobilization was the same as described by Chung et al. (1998). The granular activated carbon (GAC) used in this study was a coconut-shell-based type. The physical properties of the activated carbon are as shown in Table 1. The average size of the carbon particles was about 5 μm. The GAC was washed with Milli Q water to remove the fine particles and then it was dried at 110°C for 24 hours, and finally stored in a desiccator for subsequent use.

Table 1. Physical properties of granular activated carbon.

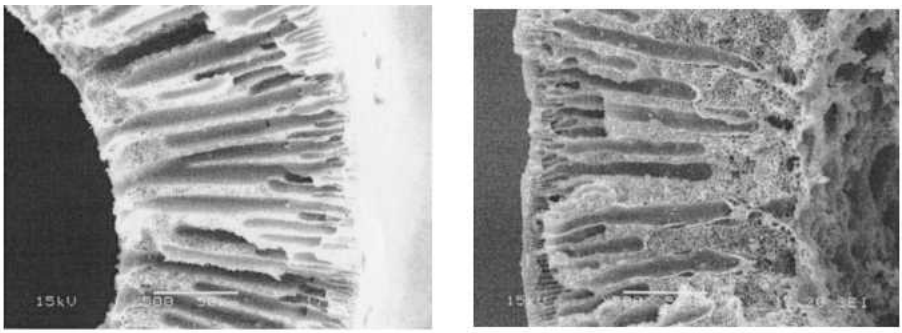
Adsorbent	Activation method	Raw material	Particle size (μm)	Surface Area (m^2 g^{-1})	Pore volume (cm^3 g^{-1})
Activated carbon	Chemical	Coconut shell	< 10	912	0.9

Asymmetric hollow fiber membranes were obtained from the lab of Membrane Technology of Nanjing Industrial University. It was fabricated according to the method described by Wang et al. (2000). The dope solution was prepared by dissolving polyethersulfone (PES) in 1-methyl-2-pyrrolidone (NMP) to give a concentration of about 20 weight percent PES. GAC was added to the dope solution according to a weight ratio of 1:4 of GAC to PS. Cell concentration was determined by measuring the optical density (OD) at an absorbance of 600 nm using a Shimadzu UV-Visible Spectrophotometer UV-1601. Phenol was analyzed by using a capillary gas chromatograph (Chung et al. 1998).

An abiotic study was performed to study the sorption of phenol in mineral solution by different hollow fiber membranes. Chopped fibers of 2.0 cm were prepared and placed into a stopped 250 mL concial flask, after which the flask was autoclaved. Phenol and autoclaved Milli Q water were added to the flask, which was then placed on rotary shaker at 120 rmp and 30 °C for at least 1 day to reach adsorption equilibrium. Phenol concentrations were then analyzed.

When the activated cells had reached their late exponential growth phase (evident from the change in the medium color to a distinctive yellowish-green as well as an optical density (OD_{600}) of 0.40-0.43 absorbance units), the suspension was distributed into 10 mL centrifuge 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. The process was repeated for three times to ensure complete removal of phenol. The collected biomass was resuspended in mineral solution for later experimental uses.

Immobilization was carried out immediately after cell harvesting. Four boundless of nine 9cm long autoclaved fibers were placed into a flask containing sterilized medium, where 35 mL of the harvested cells were added. The flask was then placed on the gyratory shaker for 18 hours to allow diffusion of cells into the fibers. After immobilization, the fibers were removed from the solution and rinsed with autoclaved deionised water to remove surface cells, after which the fibers were used for phenol degradation experiments. Experiments were performed for



A B

Figure 1. SEM pictures of the cross section of hollow fiber membranes: A, GAC free hollow fiber membrane; B, GAC incorporated hollow fiber membrane.

culture medium containing 500 mg L^{-1} and 1200 mg L^{-1} phenol solution in each immobilized system. All phenol degradation experiments were conducted in duplicate.

RESULTS AND DISCUSSION

The scanning electronic microscope (SEM) observation was performed to study the structure and morphology of hollow fiber membranes. Figure 1 show the detailed structures of the wet spun fibers taken by the SEM. A three-layer structure was observed in both cases.

Because pure solvent (NMP) was used as internal coagulant, polymer molecules at the fiber inner surface were dissolved into the internal coagulant. When the nascent hollow fiber contacted with the external coagulation bath, instantaneous demixing also took place on the fiber outer surface and a dense outer skin was formed and the precipitation front advanced further from the fiber outer surface.

As shown in Fig.1, starting immediately under the fiber outer skin, the macro voids extended across the fiber wall and opened up at the fiber inner surface. No macro voids originating from the inner surface were observed. There was no nodular structure beneath the inner surface. Instead, cavities of large size are found in the inner surface layer. The fiber inner surface of this kind of fiber was covered by numerous big pores (around 10 um in diameter). Size of these cavities decreased towards the fiber outer surface. The internal coagulant tended to penetrate into the nascent fiber. So hollow fiber with a selective outer skin layer, a highly porous substructure and a more open inner surface can be formed under these spinning conditions.

With GAC present in the fiber, the fiber structure became more rough and jagged as observed from Fig.1. The layers observed were not as clearly defined compared to that of GAC free hollow fibers. The three-layer structure when GAC was used is likely to facilitate adsorption (due to a larger surface area) and cell immobilization (due to larger space for entrapment) since the void layer is porous.

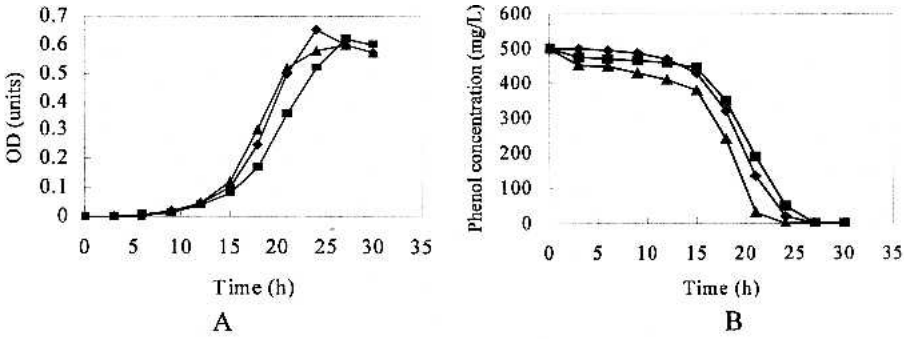


Figure 2. Cell growth and phenol degradation profile at phenol initial concentration of 500 mg/L: (A) cell growth; (B) phenol degradation; (♦) free suspension; (■) pure PES membrane; (▲) activated carbon filled membrane.

Another difference noted when GAC was used is that the color of the membranes obtained was black compared to the white color obtained if no GAC was added.

Freundlich adsorption isostherm, which was defined by Equation 1 is used to describe the sorption of phenol on hollow fiber membranes.

$$Q_{ads} = K_{ads} C_e^{1/n} \quad (1)$$

Where Q_{ads} is the amount of a compound adsorbed per unit weight of hollow fiber membrane (mg g^{-1}), and C_e is the concentration of adsorbate remaining in solution after reached equilibrium (mg L^{-1}). K_{ads} and 1/n are Freundlich constants, whereby K_{ads} is a measure for the adsorption capacity, and 1/n is a measure for the adsorption intensity. Freundlich adsorption parameters obtained in two systems with different hollow fiber membranes are summarized in Table 2.

Table 2. Freundlich parameters for hollow fiber membrane adsorption.

Substrates	PES Membrane without GAC			PES membrane with GAC (1:4)		
	K_{ads}	1/n	R^2	K_{ads}	1/n	R^2
Phenol	3.29	0.37	0.98	4.60	0.35	0.99

It was found that the adsorption capacity of phenol was enhanced when GAC was incorporated in the hollow fiber membranes. Since GAC has a stronger adsorption of phenol than PES, the results are anticipated and would be useful during the biotransformation study.

To compare the effectiveness of different hollow fiber membranes as the immobilization substrates, batch experiments for phenol degradation were conducted both in free cells and immobilized cells systems with initial concentrations of 500 and 1200 mg/L phenol. Figure 2 and 3 show cell growth and phenol concentration profile in typical suspension and two immobilized systems, respectively. For cell growth in suspension, it was observed that the growth at 500 mg/L follows the batch growth curve of a lag phase, an exponential phase and a stationary phase (Fig. 2). Cell concentration was as high as 0.62 absorbance units and phenol was completely degraded within 28 hours.

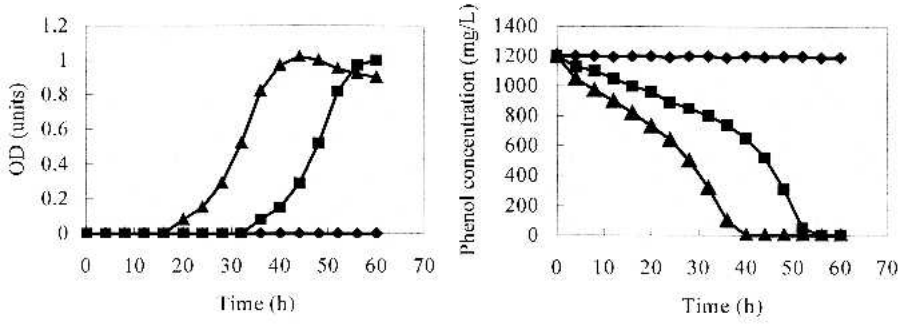


Figure 3. Cell growth and phenol degradation profile at phenol initial concentration of 1200 mg/L: (A) cell growth; (B) phenol degradation; (♦) free suspension; (■) pure PES membrane; (▲) activated carbon filled membrane.

When initial phenol concentration was increased to 1200mg/L, however, neither cell growth nor phenol degradation occurred to any measurable extent since phenol inhibition was severe at high concentration.

For the two immobilized systems, in the case of 500 mg/L phenol, both the cell growth and phenol concentration profile in the medium were similar to those obtained in the suspension culture (comparing the three sets of results at 500 mg/L). This results shows 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 systems. However, the situation changed significantly when 1200 mg/L phenol was used initially. The data obtained in the immobilized systems was discernibly different from that obtained in the suspension culture. Although there was no cell growth in the suspension culture at 1200 mg/L phenol, cell growth in the partially immobilized systems was evident from the start of the experiment.

As observed from Fig. 3, phenol concentration decreased from the start, indicating that phenol had been consumed and the cells had begun to multiply within 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 that suspension cells became measurable in the medium. At this concentration, substrate inhibition was not as severe as 1200 mg/L. Cell concentrations continued to increase, reaching a maximum cell density in the medium of about 1.0 absorbance units.

It was also found that phenol was completely degraded within 40h by GAC incorporated hollow fiber immobilized system, whereas 52h were needed to completely remove phenol when pure PES hollow fiber was used. This may be due to the following facts: on the one hand, compared with PES membrane, GAC filled hollow fiber membranes can adsorb more phenol from the solution to the surface of the membranes, resulting a lower phenol concentration solution for suspension cells to grow; on the other hand, with the addition of GAC, the higher porosity and macrovoid structure of filled fibers make the cell growth more

favorable within the membranes and phenol easier to diffuse into membranes, and therefore, increase the degradation rate of phenol.

REFERENCES

Ahmed AM, Nakhla GF, Farooq SJ (1995) Phenol degradation by Pseudomonas aeruginosa. J Environ Sci Health 30:99-107

Chung TS, Loh KC, Goh SK (1998) Development of cellulose acetate membranes for bacteria immobilization to remove phenol. J Appl Polymer Sci 68:1677-1688

Juarez-Ramirez C, Ruiz-Ordaz N, Cristiani-Urbina E, Galindez-Mayer J (2001) Degradation kinetics of phenol by immobilized cells of Candida tropicalis in a fluidized bed reactor. World J Microbiol Biotechnol 17:697-705

Lin JE, Wang HY (1991) Degradation of pentachlorophenol by non-immobilized, immobilized and co-immobilized Arthrobacter cells. J Ferment Bioeng 72:311-314

Razo-Flores E, Iniestra-Gonzalez M, Field JA, Olguin-Lora P, Puig-Grajales L (2003) Simultaneous biodegradation of a phenol and 3,4-dimethylphenol mixture under denitrifying conditions. J Environ Eng 129:999-1006

Tope AM, Srinivas N, Kulkarni SJ, Jamil K (2001) Mesoporous molecular seize (MCM-41) as support material for cell immobilization and transformation of 2,4,6-trinitrotoluene (TNT): a novel system for cell immobilization. J Mol Catal B 16:19-27

Wang DL, Li Y, Teo WK (2000) Highly permeable polyethersulfone hollow fiber gas separation membranes prepared using water as non-solvent additive. J Mem Sci 176:147-158

Zaisev GM (1995) Utilization of halogenated benzenes, phenols and benzoates by Rhodococcus opacus GM-14. Appl Environ Microbiol 61:4191-4193