

Continuous Phenol Biodegradation at High Concentrations in an Immobilized-Cell Hollow Fiber Membrane Bioreactor

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Received 31 May 2006; accepted 18 January 2007

DOI 10.1002/app.26416

Published online 25 April 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Phenol degradation at high concentrations was investigated under continuous operation in an immobilized-cell hollow fiber membrane bioreactor. *Pseudomonas putida* ATCC49451 was immobilized in asymmetric polysulfone hollow fiber membranes through entrapment within the porous regions and through attachment on the membrane surfaces. Bioreactor performance was assessed based on the startup period, the effect of feed rate (ranging from 21 to 120 mL/h), the relative contribution of the lumen and the shell sides to phenol degradation, the effect of feed phenol concentration (1000–2000 mg/L) and the long-term operation of the bioreactor. The bioreactor startup was very short, and steady state was accomplished within 160 h. An optimum degradation capacity with respect to phenol loading rate was observed because of the tradeoff in the amount

of phenol degraded against the increase in feed rate. It was also found that at higher feed rate, the shell side contributed to a larger proportion of the total phenol degraded compared with the lumen. On the basis of these results, it was found that options abound for the operating conditions of the bioreactor. These can be chosen depending on whether complete phenol degradation or high degradation capacity is desirable. Finally, long-term sustainable continuous operation of the bioreactor was demonstrated without significant biofilm fouling on the membranes. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 105: 1732–1739, 2007

Key words: waste; hollow fiber; poly(ether sulfone); membrane bioreactor; phenol; biodegradation; microencapsulation

INTRODUCTION

Phenol and phenolic compounds are well-known components of many industrial effluents, such as those from coal gasification, coking plants, petroleum refineries, and many chemical industries (pharmaceuticals, resin, fertilizer, and dye manufacturers).^{1,2} The fate of these compounds is of major environmental concern since these compounds are widely used, are toxic to aquatic life, and impart objectionable tastes to drinking water even at very low concentrations.³

Different treatment methods, including physical, chemical, and biological methods, are available for the removal of phenol and its homologues from waste waters. Among these, biological methods are generally preferred since they are effective, and the end products are innocuous.⁴

Biological degradation of phenol has been extensively studied using different pure or mixed bacterial cultures. At low concentrations, phenol can be biodegraded and removed from the waste water effec-

tively. However, difficulties arise in the treatment of higher concentrations due to the increased toxicity of phenol to the microbial population^{5–7} and cellular lysis sometimes occurs.⁴

At high concentrations, to protect the cells from being damaged, it is necessary to construct a barrier between the toxic high phenol concentration and the cells. Cell immobilization in hollow fiber membranes, an established technique to meet this requirement, has been proven effective.^{5,6,8–11}

In earlier studies, we have investigated different kinds of hollow fiber membranes^{8–10} for immobilizing *Pseudomonas putida* ATCC49451 to degrade phenol at concentrations higher than 1000 mg/L. It was found that the immobilized cells could degrade high phenol concentrations to a low enough level before suspension cells started to leak from the membranes and grew in suspension. In a subsequent study, an immobilized-cell hollow fiber membrane bioreactor was fabricated to study the feasibility of degrading phenol at high concentrations under batch operation. It was found that phenol at concentrations above 1000 mg/L could be degraded by the cells immobilized through the lumen of the hollow fiber membranes. In an independent study, Chung and et al.⁵ have also analyzed process development for

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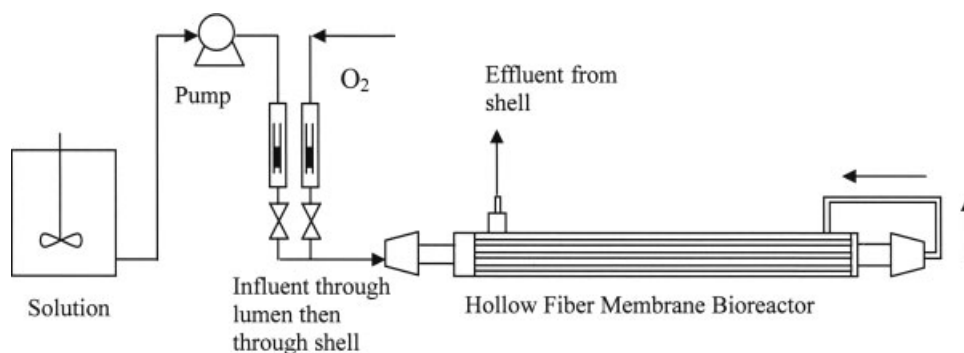


Figure 1 Schematic diagram of the experimental setup.

phenol degradation in a hollow fiber membrane bioreactor in which a dispersive agent was added to the growth medium to minimize biofilm fouling on the membranes. In our most recent research, we have used the immobilized-cell hollow fiber membrane bioreactor for the cometabolic biotransformation of 4-chlorophenol in the presence of phenol at high concentrations.¹² In all of these reports, ours as well as those in the recent literature,^{9,11} operation of the bioreactor was evaluated in the batch mode.

In considering the practical application of engineered systems for waste water treatment, it is prudent to investigate continuous operation of the hollow fiber membrane bioreactor system under different operating conditions. This is not straightforward as continuous biological waste water treatment operations often suffer from problems such as oxygen limitation, low degradation rate, and biofouling from uncontrolled biomass growth. Furthermore, an understanding between biodegradation efficiency and operating parameters under continuous operation tended to be more heuristic than deterministic. In this research, the performance of the immobilized-cell hollow fiber membrane bioreactor for biodegrading high concentrations of phenol under continuous operation was examined. Among the various pertinent issues investigated were the startup period, the effect of feed rate, the relative contribution of the lumen and the shell sides to phenol degradation, the effect of feed phenol concentration and the long-term performance of the bioreactor.

MATERIALS AND METHODS

Microorganism, culture conditions, and chemicals

Pseudomonas putida ATCC49451 was used throughout this study. Stock cultures were maintained on nutrient agar (Oxoid, Hampshire, UK) slants and stored at 4°C. A chemically defined culture medium, which consisted of a mineral salt medium and a trace mineral solution, was used in this study. The composition of the mineral salt medium and trace mineral solution

has been described by Loh and Wang.¹³ All chemicals used in this study were of analytical grade. Phenol (Merck, Darmstadt, Germany) was dissolved in 0.02N NaOH solutions to make 10,000 mg/L stock solutions.

Hollow fiber membrane fabrication

Asymmetric polysulfone (PS) hollow fiber membranes were fabricated based on the wet spinning technique described by Chung et al.¹⁰ PS was purchased from Amoco (Ohio) and 1-methyl-2-pyrrolidone (NMP) from Merck (Darmstadt, Germany).

Hollow fiber membrane bioreactor

The hollow fiber membrane bioreactor used in this study was constructed to resemble shell-and-tube heat exchangers (Fig. 1). Each glass module (0.8 cm inner diameter; 50 cm in length) consisted of 25 asymmetric PS hollow fiber membranes (0.4 and 0.8 mm inner and outer diameters, respectively; 50 cm in length). The hollow fibers were arranged in a cluster and held in place in the glass module at both ends, using quick-drying Araldite High Performance Epoxy Adhesive Resin (Ciba Specialty Chemicals, England). The resin sealed the spaces between the individual hollow fibers. The modules were subject to heat treatment by placing them overnight in the oven at 80°C to harden the resin at both ends. After solidifying the epoxy resin, the modules were then ready for use.

Analytical methods

Three milliliter sample was periodically withdrawn from the effluent outlet for analysis (Fig. 1). Cell concentration was determined by measuring the optical density (OD) at an absorbance of 600 nm using a Shimadzu UV-Visible Spectrophotometer UV-1601. Cell density was obtained from the formula DCW (mg/L) = $314.5 \cdot OD_{600}$.¹³ For analysis of phenol concentration, the sample was acidified to pH 2 with 6N sulfuric acid to quench the bioreaction. Extraction of

phenol from the acidified samples was achieved with an equivolume of methylene chloride (GC grade, Merck, Darmstadt, Germany), which contained 100 mg/L *o*-cresol (Merck, Darmstadt, Germany) as internal standard. A 1 μ L extract was then analyzed using a capillary gas chromatograph (Perkin–Elmer, Model 8700).¹³ The sensitivity of the gas chromatograph (GC) was within 1 mg/L. GC measurements were performed in duplicates and the data were identical ascertaining good reproducibility of the GC technique.

EXPERIMENTAL

Experimental setup

Figure 1 shows the schematic diagram of the experimental setup. A peristaltic pump (EYELA Micro-Tube Pump MP-3, Tokoy, Rikakikai) was used to pump the solution (e.g., activated *Pseudomonas putida*, phenol) from the Erlenmeyer flask through the lumen and then through the shell side of the hollow fiber membrane bioreactor. The flow rate was maintained at 360 mL/h⁶ during cell immobilization and changed accordingly for the relevant experimental runs.

Sterility

All media (except phenol), pipette tips and Erlenmeyer flasks fitted with cotton plugs were autoclaved at 121°C for 20 min before use. Culture transfers were conducted aseptically around a Bunsen flame to minimize contamination. Experiments were conducted in the laminar flow hood. The equipment used in the experimental setup was sterilized by ultra-violet irradiation. Prior to the start of each experiment, the hollow fiber membrane bioreactor module was sterilized by pumping 70% ethanol through it at a flow rate of 360 mL/h.

Activation

Pseudomonas putida was first induced by transferring a loop of stock culture maintained on nutrient agar slant to the mineral medium containing 200 mg/L of phenol as the sole carbon source. Two milliliter of the induced cells in the late exponential growth phase was then inoculated into 250 mL of the culture medium. After inoculation, 200 mg/L phenol was then added directly to the flask. Cultures were well mixed in a water bath rotary shaker (Gyatory Water Bath Shaker Model GFL 1092) at 30°C and 160 rpm. When the activated cells had reached their late exponential growth phase, cells were ready to be used for immobilization in the hollow fiber membranes. The late exponential growth phase was evident from the

change in the medium color to a distinctive yellowish-green as well as an OD₆₀₀ of 0.40–0.43 absorbance units.

Immobilization

Prior to immobilization of the cells in the hollow fiber membranes, Millipore ultra-pure water that had been autoclaved at 121°C for 20 min was used to rinse the bioreactor for 2 h to remove the remaining traces of 70% ethanol that was used for sterilization. Two hundred and fifty milliliter of cell culture grown in 200 mg/L of phenol was used for immobilization. The activated cells in their late exponential growth phase were used directly from the flask for immobilization. The cells were immobilized by introducing them at a flow rate of 360 mL/h through both the lumen and the shell side of the hollow fiber membrane bioreactor. This immobilization process took about 5–6 h.

Reactor operation

Following immobilization, the bioreactor was operated under continuous feeding through start-up, various influent concentrations and flow rates, and long-term operation conditions. Samples were taken from the effluent at appropriate times to determine the suspension cell density and phenol concentrations. The experiment was conducted by aerating the feed line to the bioreactor with purified oxygen (180 mL/h) continuously. An abiotic experiment was carried out to verify that phenol was not otherwise removed by volatilization. Influent phenol concentration was kept constant at 1000 mg/L, while the flow rate was kept at 21 mL/h during start up. The pH of the influent was adjusted to 7. During biodegradation, pH was not controlled but the effluent pH never dropped below 6.8 in all the experiments. The reactor was operated at room temperature, which was controlled at 26°C. After achieving steady state, the system was investigated for sustained continuous operation at gradually increasing feed rate (21, 30, 45, 60, and 120 mL/h) with influent containing 1000 mg/L phenol. Afterwards, the bioreactor was operated with influent concentration ranging from 1000 to 2000 mg/L at each of the different flow rates. Finally, the bioreactor was examined for long-term operation.

RESULTS AND DISCUSSION

Bioreactor startup

There are remarkably few research reports that detailed the startup phase during operation of hollow fiber membrane bioreactors. There could be a number of reasons for this: (1) the operation of hollow fiber membrane bioreactors has mainly been conducted in

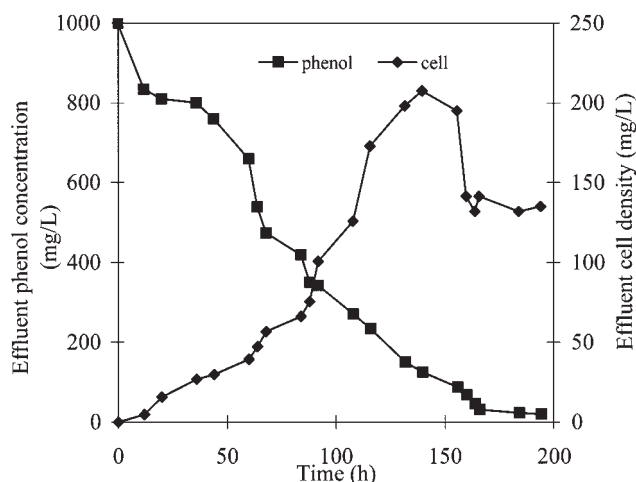


Figure 2 Effluent phenol and cell concentrations during startup phase (influent phenol at 1000 mg/L, feed flow rate at 21 mL/h).

the batch mode,^{5,6,11} (2) that the startup phase is thought to be relatively irrelevant compared with the long-term operation period of a bioreactor and (3) that the startup phase has no direct influence on the future performance of the bioreactor.¹⁴ When a bioreactor is operated continuously, it is however very important to investigate its startup phase. This is especially important during acclimatization of a new bioreactor system, as well as the re-start of a bioreactor system after cleanup, maintenance and/or a process upset. Our first investigation in this research involved an analysis of the startup period of our hollow fiber membrane bioreactor.

After the bacteria had been immobilized in the membranes, the bioreactor was fed continuously with 1000 mg/L phenol at a flow rate maintained at 21 mL/h. The cell and phenol concentrations taken at the exit of the bioreactor are depicted in Figure 2. The cell concentration increased exponentially initially, concomitant with the exponential degradation of phenol. During this period (up to 150 h), there was also a discernible buildup of biomass within the membranes (from SEM observations - not shown). After 160 h, the phenol concentration in the effluent was less than 10 mg/L; the removal efficacy was higher than 99% while suspended cell concentration was steady at about 140 mg/L. The system was deemed operating at steady state at this time. The bioreactor was operated at this feed flow rate for another 40 h before the feed flow was increased to the next flow rate.

The current startup phase was fairly short compared with start up times that have been reported for other immobilized cell biodegradation systems. Whaley et al.¹⁵ reported that 2 weeks elapsed before the cell population established itself in a bioscrubber column system in which cells were immobilized on packings in the column while Ergas et al.¹⁶ reported

several weeks before dichloromethane degradation occurred in an immobilized cell column biofiltration system. In a tubular membrane bioreactor, complete colonization of the membrane was observed only after 10 days of colonization with the cells growing in the stationary phase.¹⁷

The brevity of the startup period of our bioreactor system could be due to a number of reasons. First of all, the dissolved oxygen level in the feed was always maintained at saturation through sparging with pure oxygen before the feed was introduced into the bioreactor. By operating in this manner, oxygen was never a limiting factor. Cell immobilization was also maximized by pumping the activated cell suspension during immobilization first through the lumen followed by circulation through the shell. In this case, bacteria could find their ways into the porous regions in the membranes both from the lumen, and the external surfaces of the shell sides. Lastly, the influent was circulated through the lumen and then through the shell allowing maximum hydraulic retention time in the bioreactor for phenol to be degraded.

Effect of hydraulic retention time on bioreactor performance

After the initial startup, the bioreactor system was operated at varying feed rate to examine the effect of influent flow rate (or hydraulic retention time) on the bioreactor performance. 1000 mg/L phenol was fed at feed rates ranging from 30 to 120 mL/h. Figures 3 and 4 show the results of these experiments.

As can be seen in Figure 3, generally the increase in the influent flow rate was felt in the bioreactor effluent within the first 2 h—the phenol concentration increased while the cell concentration decreased. A new steady state was re-established within 20 h of each increase in the feed flow rate. With the increase

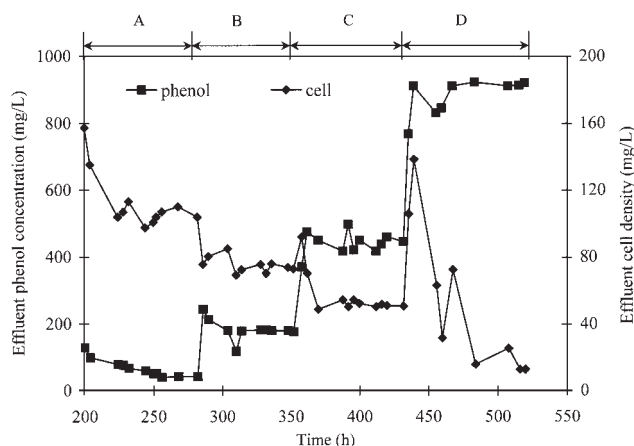


Figure 3 Temporal profiles of phenol and cell concentrations under different influent flow rates (phase A: 30 mL/h; B: 45 mL/h; C: 60 mL/h; D: 120 mL/h).

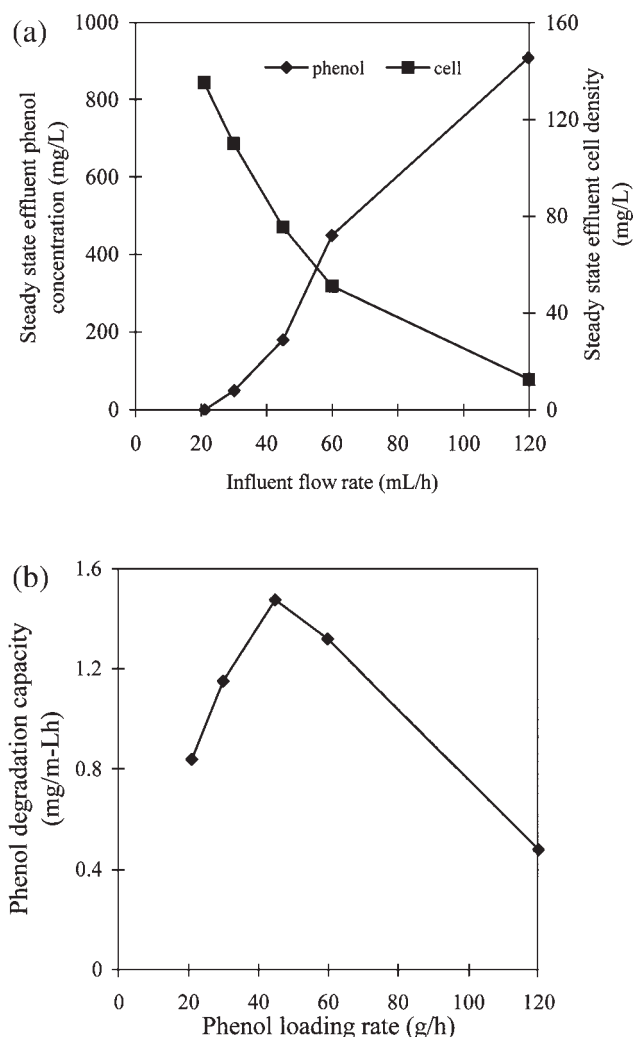


Figure 4 Effect of influent flow rate on reactor performance: (a) phenol and cell concentrations in effluent; (b) phenol degradation capacity as function of phenol loading rate.

in feed rate, it was observed that the immobilized cells were dislodged from the membranes and were washed out with the effluent. These appeared as yellowish flocs in the effluent. This was most pronounced at the highest feed rate investigated (120 mL/h). At that feed rate, significant fluctuations in the effluent cell concentrations were observed, and the suspended cell concentration eventually decreased to a negligible amount. Very little of the phenol fed was degraded; the steady state effluent phenol concentration was 900 mg/L, a mere 10% degradation. Phenol at 900 mg/L exerted substrate inhibition on freely suspended cells, and it was no surprise that the suspended cell concentration in the effluent was so low. Figure 4(a) summarizes the steady state phenol and cell concentrations in the effluent at the various feed rates studied.

Degradation capacity (in mg/mL phenol degraded per hour) was determined based on the product of

the amount of phenol degraded and the bioreactor retention time to assess the performance of the bioreactor. Although the specific degradation rate (in mg phenol degraded per mg cell per hour) would have been a better indication of the efficiency of the immobilized cells in the bioreactor, it was not used because of the difficulty in ascertaining the immobilized cell density. This was further complicated by the fact that the immobilized cells could have different specific activity in the lumen and the shell side of the bioreactor, as we would later show when we compare the relative differences in the contributions of the two sides to the overall phenol degradation. Nevertheless, we were sure that the immobilized cells grew within the membranes through SEM visualizations, and also, as we shall describe later, from our observations of membrane clogging during long-term operation of the bioreactor.

Figure 4(b) plots the degradation capacity as a function of phenol loading rate. As the feed flow rate increased (hence the phenol loading rate), the steady state amount of phenol degraded decreased. The tradeoff between these two parameters resulted in an optimal degradation capacity. The maximum degradation capacity was 1.48 mg/(mL/h), which resulted at the phenol loading rate of 45 g/h. Similar results have also been observed when the feed phenol concentration was varied at other feed flow rates (data presented in Table I). What this means is that continuous operation of the hollow fiber membrane bioreactor system can be conducted at either the optimal phenol degradation capacity (in this case a feed rate of 45 mL/h) or the highest feed rate permissible for a predetermined minimum amount of phenol degradation, for example 30 mL/h feed rate for a minimum 90% degradation of the 1000 mg/L phenol feed. This is in contrast to a batch operation, in which the bioreactor was operated until complete depletion of the phenol in the feed.^{5,6}

Relative contribution of the lumen and the shell to phenol degradation

In most waste water treatment studies involving hollow fiber membrane bioreactors, the bacteria were only immobilized either from the shell^{5,11} or the lumen.⁶ The waste water was also fed into the bioreactor in different fashions in these batch demonstrations. The waste water to be treated was pumped through the lumen, while bacteria solution¹¹ or growth medium⁵ were circulated through the shell side. In Loh et al.,⁶ the synthetic waste water containing the growth nutrients required was delivered through the shell only. We recognized in our fabricated membranes, based on their porous structures^{6,9,10} that it was possible to immobilize the bacteria both from the lumen and the shell, and conse-

TABLE I
Effect of Phenol Concentration and Influent Flow Rate on Effluent Concentration and Degradation Capacity

Influent flow rate (mL/h)	Feed concentration (mg/L)	Steady state effluent concentration (mg/L)	Degradation capacity [mg/(mL/h)]
21	1000	10	0.83
21	1200	140	0.89
21	1400	290	0.93
21	1600	450	0.97
21	1800	560	1.04
21	2000	720	1.08
30	1000	50	1.14
30	1200	240	1.15
30	1400	410	1.19
30	1600	530	1.28
30	1800	690	1.33
30	2000	1150	1.02
45	1400	620	1.40
45	1600	1020	1.05
60	1200	590	1.46
60	1400	980	1.01

quently there could be significant advantage in circulating the waste water through both the lumen and the shell; their relative contributions to the phenol degradation was hence investigated.

For this analysis, when the bioreactor was operating at steady state, samples were taken for cell and phenol concentrations measurements from both the exit from the lumen, and the effluent from the shell. The amounts of phenol degraded are plotted in the histogram shown in Figure 5. It can be seen that phenol was degraded in both the lumen and the shell for all the feed flow rates investigated. At the lowest flow rate (21 mL/h), the lumen and the shell contributed equally to the phenol degradation. This seemed contradictory at first glance since the shell has three times larger volume compared with the total lumen space, and hence a longer retention time. What happened here was that almost half of the 1000 mg/L phenol was effectively degraded in the lumen, and the balance was therefore left to the immobilized and freely suspended cells in the shell. While there was much more degradation capacity in the shell, this was not fully utilized. At higher feed flow rates, the difference in the relative contributions of the lumen and the shell became more discernible. Given that the retention time in the shell was longer, and that phenol degradation was accomplished by both immobilized and freely suspended cells, much more of the phenol was degraded in the shell at the higher feed rates. Furthermore, the cells immobilized on the shell side, as well as the freely suspended cells, also experienced lesser substrate inhibition effects since they were exposed to lower phenol concentrations.

At this point, it may be instructive to compare the mode of waste water circulation (through both the

lumen and the shell) and the operation mode (batch versus continuous). We take our current experimental results obtained at the feed rate of 21 mL/h to compare with those reported in the literature by Loh et al.⁶ and Chung et al.⁵ In the case of Loh et al., the cells were immobilized only on the shell side of the hollow fiber membranes, and the bioreactor was operated batchwise with the waste water circulated through the shell side only. For a feed of 1200 mg/L phenol (data to be discussed later in Table I), accounting for the total surface area of the membranes, the degradation rate for the current configuration was 4.72×10^{-4} mg/(mm²/h), while it was 4.41×10^{-4} mg/(mm²/h) for that of Loh et al.⁶ In the case of Chung et al., the cells were immobilized on the lumen side of the membranes, the bioreactor was operated batchwise, and waste water was only circulated through the lumen. For a feed of 2000 mg/L phenol, accounting for the total surface area of the membranes, the degradation rate for the current configuration was 5.70×10^{-4} mg/(mm²/h) while that reported by Chung et al.⁵ was 1.23×10^{-4} mg/(mm²/h). It is therefore apparent that our current mode of waste water circulation offered a much higher degradation capacity than either of the earlier reports. These results also ascertained the advantage of continuous operation for treating pollutants which exert substrate inhibition on the degrading microorganisms. In the case of batch operations, the immobilized bacteria were initially exposed to high substrate concentrations (with consequent substrate inhibition effects), which gradually decreased as operation continued. As such, the bacteria were challenged with varying substrate conditions during the operation. This contrasted with continuous operation in which the bacteria were acclimatized and exposed to a stable

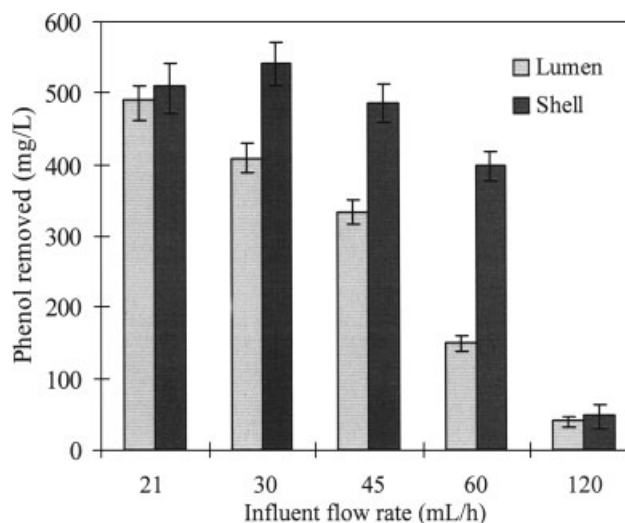


Figure 5 Relative contributions of lumen and shell to overall phenol degradation.

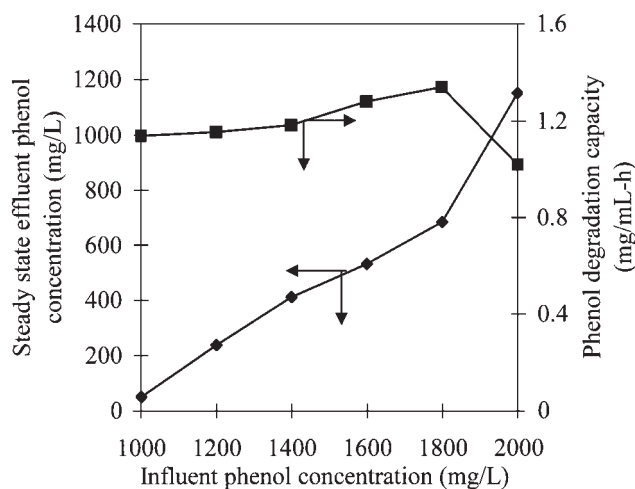


Figure 6 Effect of feed phenol concentration on reactor performance (feed flow rate at 30 mL/h).

waste water environment, hence supporting their optimized degradation behavior.

Effect of influent concentrations

At each feed flow rate investigated (21, 30, 45, 60, and 120 mL/h), the effect of feed phenol concentration was studied by increasing the phenol concentration stepwise after each steady state had been achieved (Fig. 6). This was increased until such point when the steady state phenol concentration in the effluent was above 800 mg/L. This effluent concentration of 800 mg/L phenol was chosen because this was the highest phenol concentration that the *P. putida* cells were able to degrade in suspension. When the phenol in solution is brought to this level, subsequent treatment of the effluent can be effected in a separate suspension cell bioreactor when necessary.

The steady state phenol concentration in the effluent and the consequent degradation capacities are summarized in Table I. As expected, with the increase in the feed flow rate, the maximum phenol concentration that could be degraded to less than 800 mg/L also decreased. At a single feed flow rate, the degradation capacity was found to increase slowly up to the maximum phenol concentration that could be treated. For illustration, consider the results obtained at feed flow rate of 30 mL/h: when phenol concentration was increased from 1000 to 1800 mg/L (the maximum phenol concentration at which steady state effluent concentration was less than 800 mg/L), the degradation capacity increased from 1.14 to 1.34 mg/(mL-h). This can be rationalized as follows. As the phenol concentration increased, the mass transfer of phenol from the feed through the membrane is expected to increase. This has two major effects on the cells. While the larger mass transfer of phenol allows more cells to grow, and hence generates higher

cell mass, the higher phenol concentration within the membranes also exerts higher substrate inhibition on the cells. At 30 mL/h, these two effects negated each other at the medium to high phenol concentration range (e.g., 1000–1800 mg/L), but at 2000 mg/L phenol, substrate inhibition dominated, and the degradation capacity took a plunge.

The results presented in this investigation demonstrate that operating conditions can be selected based on the desired degradation amount or the desired degradation capacity although in some cases necessitating the use of an additional suspension cell bioreactor.

Long-term operation

Biofilm fouling is a major problem in immobilized cells bioreactor systems.^{11,18} It is therefore necessary to investigate the performance of the hollow fiber membrane bioreactor during long-term continuous operation. The objective here was to find out if there were uncontrolled biomass growth, and if so, how it could be mitigated.

In this investigation, the bioreactor was initially operated continuously at 21 mL/h feed rate with a fixed influent concentration of 1000 mg/L for a period of 15 days. During this extended period, there was practically no phenol detected in the effluent. However, it was observed that gradually increasing cell densities occurred inside the bioreactor (immobilized as well as suspension) and the influent to the lumen was partially blocked after 15 days of operation. Aziz et al.¹¹ and Chung et al.⁵ have reported the use of tetrasodium pyrophosphate as a dispersive agent to curb biofilm fouling, but this involved the addition of an extra chemical which not only added cost, but also uncertainty to the state of the treated water. To remove the excess biomass in our case, the bioreactor was transiently operated for about 6 h at a higher influent flow rate (45 mL/h) and higher gas flow rate (240 mL/h). These provided increased fluid shear so that the excess biomass could be dislodged. During this time, it was observed that cell flocs were flushed out of the bioreactor. The bioreactor was subsequently operated at 45 mL/h and gas flow rate of 180 mL/h for the next 20 days without cell blockage due to the higher fluid shear. Cell growth within the bioreactor could also have been restricted because high concentrations of phenol exert substrate inhibition on the cells. Furthermore, metabolic intermediates of phenol are also known to be toxic to cell growth.¹⁹ The phenol concentration in the effluent during that long-term operation was less than 80 mg/L, indicating a 92% phenol removal and a phenol degradation rate of 1.64 mg/(mL-h) at steady state. For long-term operation without significant biofouling, it

is therefore recommended that the bioreactor be operated under these conditions.

CONCLUSIONS

Continuous phenol degradation was accomplished in an immobilized-cell hollow fiber membrane bioreactor. Among the various issues investigated were the startup period, the effect of hydraulic retention time, the relative contribution of the lumen and the shell sides to phenol degradation, the effect of feed phenol concentration and the long-term performance of the bioreactor. The bioreactor startup phase was very short. This was accomplished through a unique way of circulating the waste water through the bioreactor, first through the lumen and then through the shell. The immobilization of the cells from both the lumen and the shell sides of the membranes also aided in shortening the startup phase. Degradation capacity based on the amount of phenol degraded per unit time was used to assess the performance of the bioreactor, and it was found that there exists an optimum degradation capacity with respect to hydraulic retention time. It was also found that at higher feed flow rate, the shell side contributed to a larger proportion of the total phenol degraded compared with the lumen. This has been rationalized based on the relative dominance of the effect of mass transfer against substrate inhibition on the immobilized cells. Taken altogether, the results confirmed that continuous operation of the bioreactor can be conducted through a number of operating conditions, depending on whether complete phenol degradation or high degradation capacity is desirable. This opens up more opportunities for waste water treatment compared with operating the bioreactor in the batch mode. Finally, it has been demonstrated that long-term con-

tinuous operation of the bioreactor can be sustained without significant biofilm fouling on the membranes.

The authors thank Professor Tai-Shung Chung for his technical expertise and for providing his facilities in spinning the hollow fiber membranes. Yi Li is also appreciative of the PhD scholarship provided to him by the National University of Singapore.

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