Chemical Engineering Journal 160 (2010) 565–570

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Continuous biodegradation of phenol in a spouted bed bioreactor (SBBR)

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ARTICLE INFO

Article history:
Received 22 October 2009
Received in revised form 25 March 2010
Accepted 26 March 2010

Keywords:
Spouted bed
Bioreactor
Biodegradation
Immobilization
Phenol

ABSTRACT

Experiments were carried out to evaluate the continuous biodegradation of phenol using *Pseudomonas putida*, immobilized in polyvinyl alcohol (PVA) gel matrices in a specially designed spouted bed bioreactor (SBBR) at different conditions. The plexiglas reactor had a total volume of 1.1 l and was equipped with a surrounding jacket for temperature control. The mean residence time in the SBBR was determined experimentally by tracking the concentration of a tracer compound in a continuous effluent stream. The effects of initial phenol concentration, air flow rate, liquid flow rate and PVA particle size, on the rate of phenol biodegradation, were investigated. It was found that the rate of continuous biodegradation increased with increasing the initial phenol concentration and decreased with increasing the liquid flow rate. Mass transfer and hence the accessibility of the biomass to phenol was enhanced by decreasing the PVA particle size and increasing the air flow rate, which had a positive effect on the rate of biodegradation.

1. Introduction

Phenol is an aromatic compound that is widely used in processes involving petrochemical, chemical, pulp, paper, tannery and coal refining industries. It is often present, therefore, in wastewater generated by these industries. Various treatment alternatives such as activated carbon adsorption, ion exchange, liquid–liquid extraction, and chemical oxidation have been reported. However, these techniques often suffer from serious drawbacks including high cost and the formation of by-products (secondary pollution). On the other hand, biodegradation is an environmental friendly and cost effective alternative that proved to be efficient in the removal of phenol. However, during biological treatment, the bacteria must first adapt to the phenol, which is known to be toxic to microorganisms. It has been reported that phenol is inhibitory to bacteria growth at concentrations above 0.05 g/l and bactericidal at concentrations of about 2 g/l, if the bacteria is not adapted to phenol [1]. Therefore, in order to obtain efficient biodegradation, microbial acclimatization to phenol is necessary. Most studies on phenol degradation have been carried out with bacteria, mainly from the *Pseudomonas* genus. Specifically, *Pseudomonas putida*, which is a rod-shaped, gram-negative bacterium, has commonly been used, due to its ability to degrade organic solvents in general and its high removal efficiency of phenol in particular [2]. Nevertheless, numerous other types of bacteria and biosorbents were also utilized for biodegradation or removal of phenol, such as: *Rhodococcus erythropolis* [3]; *Bacillius* sp. [4]; *Alcaligenes faecalis* [5]; *rhizobium Ralstonia taiwanensis* [6]; *Nocardia hydrocarbonoxydans* [7]; *Candida tropicalis* [8–10]; *Cupriavidus metallidurans* [11] and activated sludge [12].

Biomass immobilization is an important and effective technique that is usually employed to protect the bacteria from high phenol concentrations and allow reutilization [13]. *P. putida* has been studied by many researchers in free and immobilized forms in different types of bioreactors. Gonzalez et al. [14] investigated the biodegradation of phenolic industrial wastewaters by a pure culture of *P. putida* immobilized by entrapment in calcium-alginate gel beads hardened with Al^{3+}. In general, the efficiency of biodegradation may be affected by many factors such as phenol concentration [15], temperature [16], sunlight [17,18], the presence of other nutrients [19], the presence of other pollutants [20] and bacterial abundance [21]. The treatment of wastewater containing phenol has been focusing on employing and exploring new types of bioreactors with high performance for practical utilization. These included the use of hollow fiber membrane contactors [22,23]; fluidized bed bioreactor [13,24]; microbial fuel cells [25] and fixed-biofilm process [26]. Other novel bioreactors that have been developed for other biotreatment applications include rotating rope bioreactor [27]; two phase partitioning bioreactor [28] and foam emulsion bioreactor [29]. However, most of these reactors have difficulty in long-term operation and scale-up which limit their practical application in any industrial process.

The spouted bed bioreactor (SBBR) is characterized by a systematic intense mixing due to the cyclic motion of particles within the bed, which is generated by a single air jet injected through an orifice in the bottom of the reactor. It has many advantages over the

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doi:10.1016/j.cej.2010.03.068

conventional bubble column and other flow bioreactors, including better mixing and contact between substrate and cells, and faster oxygen transfer rate, which lead to higher rates of phenol removal. The reactor proved to be effective in the batch biodegradation of phenol [30]. The main objective of the present study, therefore, is to evaluate the continuous biodegradation of phenol by *P. putida* immobilized in PVA gel in a SBBR at different operating conditions.

2. Materials and methods

Detailed description of the bacteria preparation, acclimatization and immobilization as well as the analytical techniques can be found elsewhere [13]. However, for the sake of clarity and completeness, brief descriptions are repeated here.

2.1. Reagents

Analytical grade phenol was purchased from BDH Chemicals, UK. Synthetic phenol solutions were prepared in the desired concentrations by dissolving predetermined amount of phenol in distilled water. The prepared solutions were always kept in a brown flask to avoid light oxidation of the phenol. All other chemicals and PVA powder were of analytical grade and were also obtained from BDH, UK.

2.2. Preparation of microbial culture

A special strain of the bacterium *P. putida* (P300) was obtained in an AMNITE cereal form from Cleveland Biotech Ltd., UK. A 100 g of the cereal is mixed in a 1000 ml of 0.22% sodium hexameta phosphate buffered with Na_2CO_3 to a pH of 8.5. The mixture was homogenized in a blender for about one hour, decanted and kept in the refrigerator at 4 °C for 24 h. Bacteria slurry was prepared by four consecutive steps of low speed centrifugation at 6000 rpm for 15 min. The supernatants were collected and centrifuged again at 10,000 rpm for 20 min. The biomass attached to the walls of tubes was re-suspended in the solvent.

2.3. Immobilization

Polyvinyl alcohol (PVA) gel was used for immobilizing the bacteria cells. A homogenous 10 wt% PVA viscous solution was prepared by mixing 100 g of PVA powder with 900 ml of distilled water at about 70–80 °C. The 10% mixture is known to result in good quality polymer matrix with high porosity [31]. PVA is a synthetic polymer that has better mechanical properties, and it is more durable than Ca-alginate which is biodegradable and can be subject to abrasion [32]. The formed mixture was allowed to cool to room temperature before adding 10 ml of the bacterial suspension prepared as in Section 2.2, then well stirred for 10–15 min to ensure homogeneity of the solution. The solution was then poured into special molds and kept in a freezer at −20 °C for 24 h, then transferred to the refrigerator and allowed to thaw at about 4 °C. The freezing–thawing process was repeated 3–4 times, with 5 h for each cycle. The frozen molds were cut into the specified sizes, washed with distilled water to remove any uncross-linked chains, and sent for acclimatization.

2.4. Acclimatization of bacteria

The immobilized bacteria, prepared as in Section 2.3, were suspended in a 1 l solution containing 1000 mg/l of glucose as an easy biodegradable source of organic carbon in addition to 825 mg/l of other essential mineral nutrients with concentrations shown in Table 1. The activation of the bacteria is confirmed through microscopic analysis and further confirmed by reduction in the glucose concentration. Once activated, the bacteria were then slowly acclimatized to phenol concentrations by increasing the phenol concentration from zero to up to 300 mg/l over a period of 5 days. At the same time, glucose concentration was gradually reduced from 1000 mg/l to zero.

Table 1
Composition of mineral salt medium.

Component	Concentration (mg/l)
$MgSO_4{\cdot}7H_2O$	300
K_2HPO_4	250
$CaCl_2{\cdot}2H_2O$	150
$(NH_4)_2CO_3$	120
$FeSO_4{\cdot}7H_2O$	3.5
$ZnSO_4{\cdot}7H_2O$	1.3
$MnCl_2{\cdot}4H_2O$	0.13
$CuSO_4{\cdot}5H_2O$	0.018
$CoCl_2{\cdot}6H_2O$	0.015
$Na_2MoO_4{\cdot}2H_2O$	0.013
Total	824.98

2.5. Analytical methods

Phenol concentration in the biomass free samples was determined quantitatively using Chrompack Gas Chromatograph, Model CP9001. The accuracy of the analyzer was checked to be within ±0.5 ppm and confirmed for low concentrations (less than 50 mg/l) using a Shimadzu UV Spectrophotometer, Model UV-2450. Measurements for each phenol sample were carried out in duplicates and a standard solution was used to recheck the accuracy of the GC after every 4 h of continuous operation. All experimental results reported in the study were based on averaging results of repeated experimental runs (duplicates), with the standard deviation ranging from 2 to 5% of the reported average.

2.6. Spouted bed bioreactor

A SBBR was specially designed and fabricated to evaluate the continuous biodegradation of phenol. The Plexiglas reactor had a total volume of 1.1 l and was equipped with a surrounding jacket for temperature control. A water bath was used to circulate water into the reactor jacket at the desired temperature. The spouted bed bioreactor is characterized by a systematic intense mixing due to the cyclic motion of particles within the bed, which is generated by a single air jet injected through an orifice in the bottom of the reactor. A schematic diagram of the spouted bed reactor is shown in Fig. 1. The reactor was used to assess the effects of mixing, PVA particle size, liquid flow rate and initial phenol concentration on the continuous biodegradation rate. In all experiments the amount of inorganic medium (nutrients) was kept constant at 825 mg/l.

3. Results and discussion

3.1. Effect of air flow rate

Air flow rate into the bioreactor plays an important role in providing enough oxygen for the biodegradations as well as sufficient mixing through particle movement within the reactor. The effect of air flow rate on the continuous biodegradation of phenol was assessed for three different flow rates, namely, 1, 3 and 5 ml/min. The liquid flow rate, initial phenol concentration and temperature were fixed at 10 ml/min, 30 mg/l and 30 °C, respectively. At regular intervals, samples from the output stream were collected and analyzed for their phenol concentration. The reduction of phenol concentration as a function of time for different air flow rates is

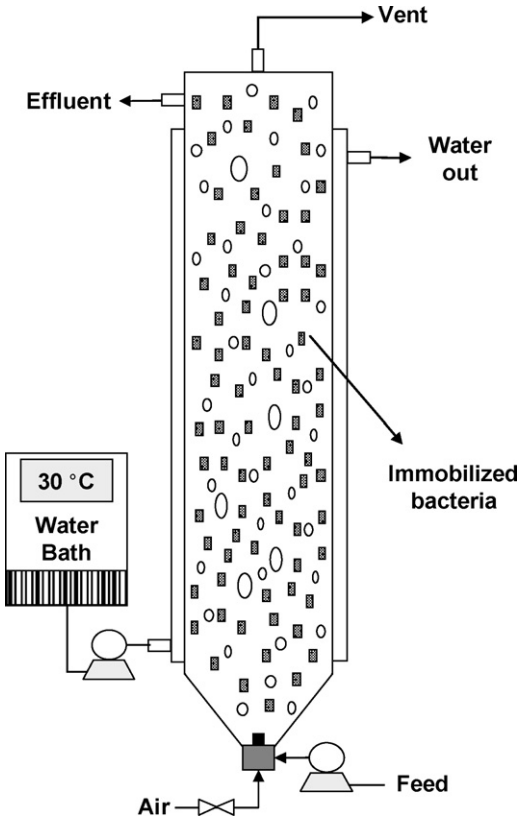

Fig. 1. A schematic diagram of the spouted bed bioreactor (SBBR).

shown in Fig. 2. The results clearly show that the reduction rate of phenol concentration (or the biodegradation rate) depends on the air flow rate. This dependency, however, seems to diminish for flow rates higher than 3 l/min. It is believed that the air flow rate affects the biodegradation of phenol through two main factors: mixing and aeration (or providing the necessary oxygen). To assess the effect of these two factors separately, experiments were carried out using air–nitrogen mixture at a total flow rate of 3 ml/min, but with different ratios, and the results are shown in Fig. 3. As expected, when pure nitrogen was used, there was no biodegradation of phenol, due to the absence of aeration. The slight biodegradation observed when using nitrogen for mixing is due to the dissolved oxygen already existing in the feed solution. Compared to degradation

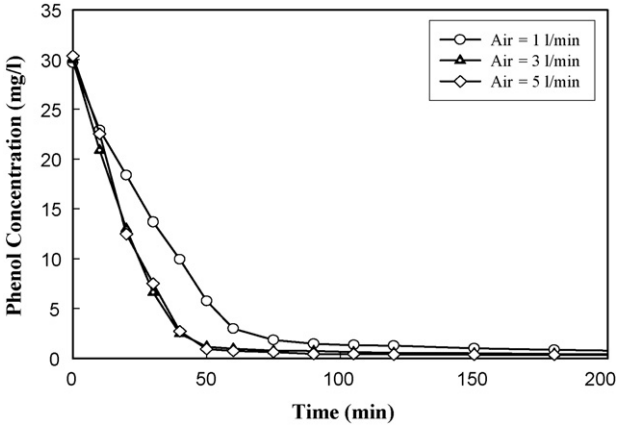

Fig. 2. Concentration of phenol in the reactor as a function of time for different air flow rates. Initial phenol concentration = 30 mg/l; PVA volume = 300 ml; reactor temperature = 30 °C; liquid flow rate = 10 ml/min.

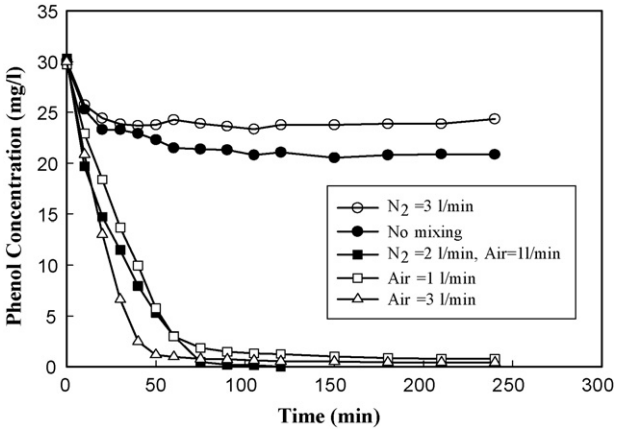

Fig. 3. Concentration of phenol in the reactor as a function of time for different air/nitrogen flow rates. Initial phenol concentration = 30 mg/l; PVA volume = 300 ml; reactor temperature = 30 °C; liquid flow rate = 10 ml/min.

without mixing, the degradation using nitrogen mixing is lower, because of the stripping of the dissolved oxygen by the nitrogen stream. However, when air was introduced, the rate of degradation significantly increased, which is a clear indication that the removal of phenol is mainly due to biodegradation and that other factors such as evaporation and adsorption on PVA had insignificant contribution [13]. When the air ratio was increased to 33%, the reduction of phenol concentration seemed to follow the same trend as that of 1 l/min of air as shown in Fig. 2. This clearly indicates that at air flow rates higher than 1 l/min, the main factor for enhancing phenol biodegradation rate is the availability of sufficient oxygen for the biodegradation process and that the effect of mixing at these conditions is negligible. This also implies that the optimum air flow rate for the continuous biodegradation process is 3 l/min. It is important to note here that the effect of mixing may prove to be more important for smaller PVA particle size or very low initial phenol concentration. These factors are examined further in Sections 3.3 and 3.4.

3.2. Effect of liquid flow rate

In all experimental runs, the synthetic phenol solution was continuously fed to the bioreactor using a peristaltic pump at a constant flow rate with an accuracy of ±1 ml/min. The effect of the liquid feed flow rate on the biodegradation of phenol was evaluated for three different flow rates, namely 10, 20 and 40 ml/min. The variation of the phenol concentration with time for the three flow rates is presented in Fig. 4, which obviously shows that the initial biodegradation rate decreased with increasing the liquid flow rate. This is expected, since increasing the liquid flow rate results in lowering the residence time in the bioreactor and consequently, gives the immobilized bacteria less time for biodegradation. The residence time for the first flow rate (10 ml/min) was estimated to be 60 min based on the residence time distribution analysis. Therefore the residence time for the other two flow rates, namely 20 and 40 ml/min, can be estimated to be 30 and 15 min, respectively. A plot of the initial biodegradation rate as a function of residence time is shown in Fig. 5. Clearly, the initial biodegradation rate increases linearly with increasing the residence time. The plot is a near perfect straight line as illustrated by the least square fitting with coefficient of determination, R^2, of 0.998.

3.3. Effect of initial phenol concentration

Initial phenol concentration plays an important role in the continuous biodegradation process, since some hydrocarbon con-

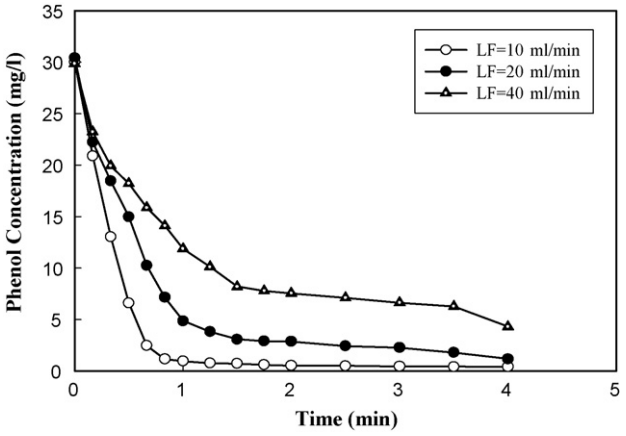

Fig. 4. Concentration of phenol in the reactor as a function of time for different liquid flow rates (LF). Initial phenol concentration = 30 mg/l; PVA volume = 300 ml; reactor temperature = 30 °C; air flow rate 3 l/min.

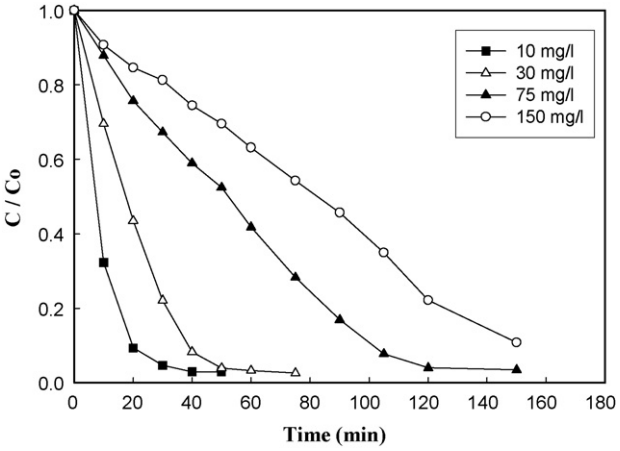

Fig. 6. Dimensionless phenol concentration as a function of time for different initial phenol concentrations. PVA volume = 300 ml; reactor temperature = 30 °C; air flow rate 3 l/min; liquid flow rate = 10 ml/min. PVA particle size = 10 mm.

taminants, including phenol, are known to have inhibitory effect on the activity of the biomass. Experiments were carried out at different phenol concentrations ranging from 10 to 150 mg/l. The reactor temperature was fixed at 30 °C and the volume of PVA pellets in the reactor was kept at 300 ml or 30 vol% of the total working volume. The air and liquid flow rates were also kept constant at 3 l/min and 10 ml/min, respectively.

A plot of the dimensionless phenol concentration (C/C_0) as a function of time for different initial phenol concentrations is shown in Fig. 6. The plot indicates that at high initial phenol concentrations the rate of reduction in phenol concentration remains constant for a longer time compared to that for low initial phenol concentration, which seems to decrease with time. This could be attributed to mass transfer limitations at low phenol concentrations, where the bacteria within the PVA particles may have a limited access to phenol. The estimated initial biodegradation rate of phenol as a function of the initial phenol concentrations is shown in Fig. 7. It is obvious that the biodegradation rate increased with increasing the initial phenol concentration. It is interesting to notice that substrate inhibition was not encountered in the range of substrate used, which is mainly due to the immobilization of the bacteria within the PVA matrix that prevents it from direct contact with the high phenol concentration and the continuous dilution effect.

3.4. Effect of PVA particle size

In the previous sections, the PVA particles used were cubical in shape with a particle size of about 10 mm. In this section, these PVA particles were cut into halves and then quarters. Experiments were then carried out to evaluate the effect of PVA particle size on the continuous biodegradation of phenol. One would expect that reducing the particle size would improve mixing inside the reactor, and at the same time enhance mass transfer through making the biomass more accessible to phenol.

A comparison of the reduction of phenol concentration for the three particle sizes, namely 10, 5 and 2.5 mm, for air flow of 1 l/min is shown in Fig. 8. A similar comparison, but at a higher air flow rate of 5 l/min, is shown in Fig. 9. It is obvious from these two figures that the PVA particle size has a considerable effect on the continuous biodegradation of phenol. As expected, the biodegradation rate was enhanced by reducing the particle size and this effect was more pronounced for high air flow rates. For low air flow rate (1 l/min), the difference in the reduction of phenol concentration for the 10 and 5 mm PVA particle sizes seemed to be negligible, as shown in Fig. 8. On the other hand, for a higher air flow rate (5 l/min), the difference in the reduction of phenol concentration for the three particle sizes was rather apparent as shown in Fig. 9. This could be

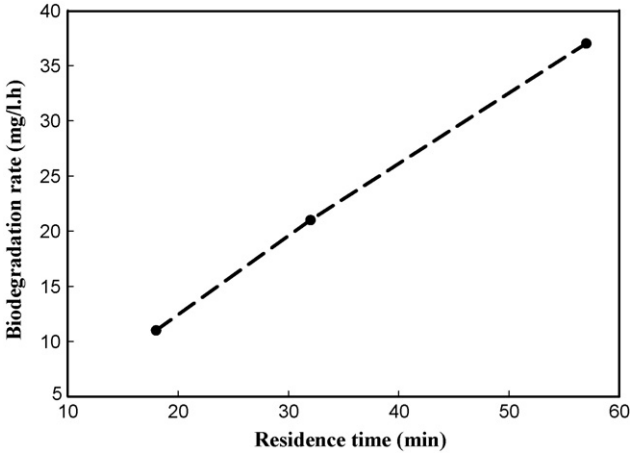

Fig. 5. Biodegradation rate of phenol as a function of residence time. Initial phenol concentration = 30 mg/l; PVA volume = 300 ml; reactor temperature = 30 °C; air flow rate 3 l/min.

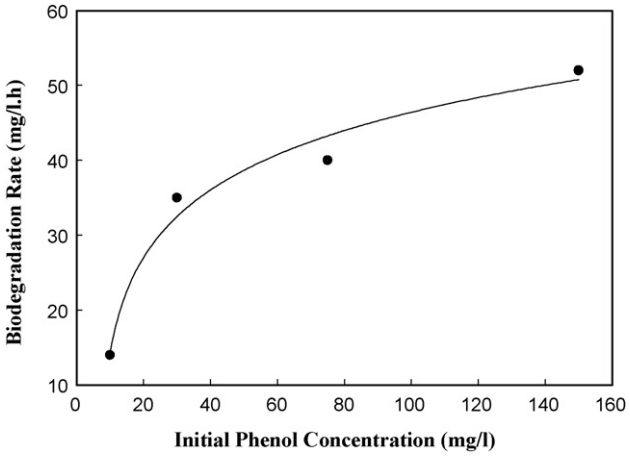

Fig. 7. Initial biodegradation rate of phenol as a function of initial phenol concentration. PVA volume = 300 ml; reactor temperature = 30 °C; air flow rate 3 l/min; liquid flow rate = 10 ml/min. PVA particle size = 10 mm.

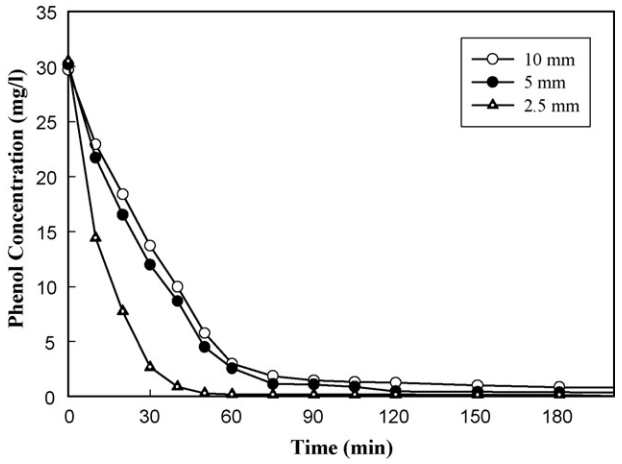

Fig. 8. Concentration of phenol as a function of time for different PVA particle sizes. PVA volume = 300 ml; reactor temperature = 30 °C; air flow rate 1 l/min; liquid flow rate = 10 ml/min.

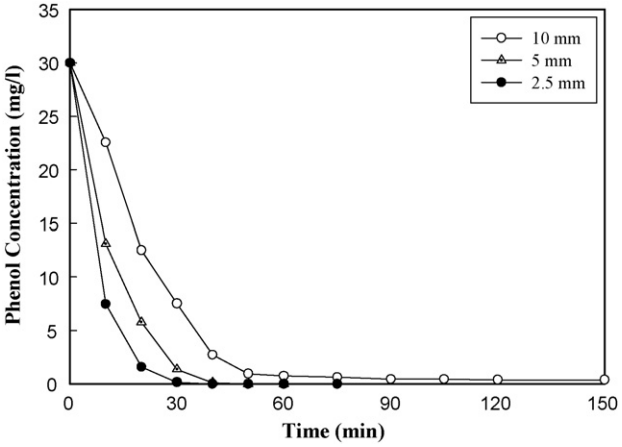

Fig. 9. Concentration of phenol as a function of time for different PVA particle sizes. PVA volume = 300 ml; reactor temperature = 30 °C; air flow rate 5 l/min; liquid flow rate = 10 ml/min.

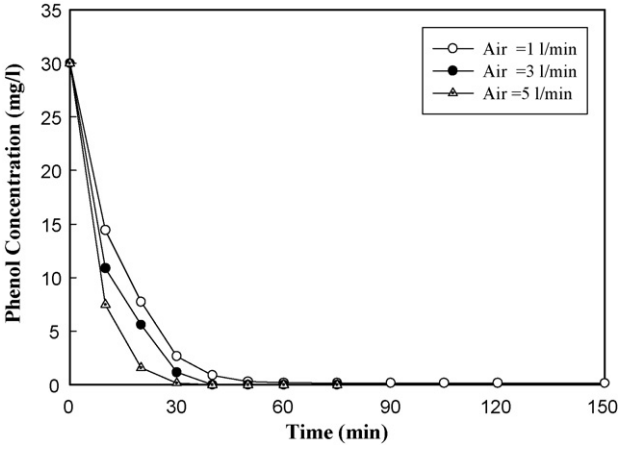

Fig. 10. Concentration of phenol as a function of time for different air flow rates. PVA volume = 300 ml; reactor temperature = 30 °C; liquid flow rate = 10 ml/min. PVA particle size =2.5 mm.

attributed to improvements in mass transfer inside the reactor due to the combined effect of good mixing and reduced particle size.

A plot of the phenol concentration as a function of time for the smallest PVA particle size (2.5 mm) at three different air flow rates is shown in Fig. 10. This plot reiterates the importance of mixing in enhancing mass transfer and consequently improving the biodegradation process, which is especially true for small PVA particles. For large particles, the biodegradation rate could not be improved by raising the air flow rate beyond 3 l/min, as explained in Section 3.1 and shown in Fig. 2.

4. Conclusions

The continuous biodegradation of phenol was evaluated using a special strain of the bacterium *P. putida*, immobilized in PVA gel matrices in a spouted bed bioreactor at different conditions. The effects of initial phenol concentration, aeration, air flow rate, liquid flow rate and PVA particle size were investigated. The experimental results indicated that the rate of continuous biodegradation of phenol increased with increasing the initial phenol concentration and increasing the air flow rate and decreased with increasing the liquid flow rate. It is believed that mass transfer limitations have considerable effect on the biodegradation rate. Mass transfer and hence the accessibility of the biomass to phenol was enhanced by decreasing the PVA particle size and increasing the air flow rate. The intense mixing associated with the SBBR makes it an effective reactor for the biodegradation of phenol and other biodegradation processes that may involve significant mass transfer limitations.

Acknowledgements

The authors would like to acknowledge the financial support provided by the Japan Cooperation Center, Petroleum (JCCP) and the technical support of the Nippon Oil Research Institute Co., Ltd. (NORI). They would also like to thank Abu Dhabi Oil Refining Company (TAKREER), e-FORS and the Research Affairs at the UAE University for their support.

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