Malays. Appl. Biol. (2007) 36(1): 73–78

THE KINETICS OF PHENOL DEGRADATION BY IMMOBILIZED *PSEUDOMONAS* SP IN A REPEATED-BATCH PROCESS

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ABSTRACT

The best operating conditions for phenol degradation by immobilized *Pseudomonas* sp in packed column were determined, and then evaluated in repeated batch cultures. The maximum degradation rate occurred in i) the support with 1.0 cm diameter or less, ii) loading rate of 2.5 ml/min, and iii) in culture supplemented with nutrient. At these conditions, the immobilized cells managed to remove 100% of 1000 ppm phenol within 24 hours, and repeated the same performance in the next six consecutive batches. This achievement was comparable to published data. The approach employed in this study provides a useful guideline in treating phenolic contaminants using packed reactor system.

Keadaan terbaik untuk menjalankan degradasi fenol oleh *Pseudomonas* sp yang tersekatgerak dalam turus padat telah ditentukan dan dinilai dalam kultur kelompok berulang. Kadar penguraian yang paling tinggi berlaku i) dalam penyokong yang bersaiz 1.0 cm atau kurang, ii) kadar alir 2.5 ml/min, dan iii) dalam kultur yang dibekal nutrien. Pada keadaan terbaik ini, sel tersekatgerak ini berupaya mengurai 100% fenol yang berkepekatan 1000 ppm dalam masa 24 jam, dan mengulangi prestasi yang sama dalam enam kelompok seterusnya berturut-turut. Pencapaian ini setanding dengan data yang pernah dilaporkan. Pendekatan yang dijalankan dalam penyelidikan ini memberi garis panduan yang berguna dalam rawatan fenol menggunakan sistem turus padat.

Key words: *Pseudomonas*, phenol degradation, bioceramic, repeated batch, packed column

INTRODUCTION

The revolution of industrial field has led to the extensively introduction of relatively high concentrations of foreign compounds into the environment. Phenol and its derivatives are the common organic pollutant that originated from many industrial wastewaters including steel industries, pharmaceutical, petrochemical, oil refineries, textiles and coal refining (Nuhoglu and Yalcin, 2004). The toxicity of phenol has been widely documented and their disastrous effect toward human and environment is a great concern (Hannaford and Kuek, 1999; Chen *et al*., 2002; Prieto *et al*., 2002).

Traditionally, phenol was removed by costly physicochemical methods, but recently biological treatment has been widely used as a low-cost alternative and offering the possibility of phenol complete mineralization (Collins and Daugulis, 1997). Moreover, the use of pure cultures, especially adapted to metabolize the contaminant, can be envisaged as an attractive alternative (Nurdan *et al*., 2004). The biodegradation is referred to the biological transformation of phenol to another form of non-toxic compounds (Grady, 1985). It is an enzymatic microbial metabolic process that utilizes hydrocarbon as carbon sources (Chen *et al.*, 2002). Many aerobic bacteria are capable of using aromatic compounds as their sole carbon source and energy. The microorganisms that normally used in phenol biodegradation are mainly from *Pseudomonas* sp (Wang and Loh, 1999; Kumar *et al*., 2004; Nurdan *et al*., 2004; Prieto *et al*., 2002;), *Candida tropicalis* (Chen *et al*., 2002), *Azotobacter* sp (Hughes *et al*., 1983), *Rhodococcus* sp (Prieto *et al*., 2002), *Alcaligenes* sp (Valenzuela *et al*., 1997) and *Acinetobacter* sp. (Hao *et al*., 2002).

Immobilization of the microorganisms in inert supports shows an increasing interest to remove phenol contaminants since this technology reveals many potential advantages over suspension cultures (Aksu and Bulbul, 1999; Hannaford and

Kuek, 1999; Chen *et al.*, 2002; Prieto *et al.*, 2002). Beside provides high cell concentration, immobilization system allows the reuse of cell so that eliminates the cost of cell recovery processes. The immobilization of microorganisms in packed reactor was further advantage in phenol removal since the active microorganisms are held in a stationary surface. They can be resistant to washout and phenol shocks load (Kim *et al.*, 2001).

In this study, phenol degradation was conducted in the packed reactor and operated in repeated batch culture. Variables operational parameters were investigated including loading rates, support's diameter, and nutrient limitation to study the kinetics of phenol degradation in immobilization system.

MATERIALS AND METHODS

Culture Preparation

A pure culture of *Pseudomonas* sp was isolated from a local residential wastewater treatment plant, periodically sub-cultured on nutrient agar (NA), and maintained at 4ºC. The bacteria was grown on Ramsay medium supplemented with NH_4NO_3, 2.0g/l; KH_2PO_4, 0.5 g/l; K_2HPO_4, 1.0 g/l; $MgSO_4.7H_2O$, 0.5 g/l; $CaCl_2.2H_2O$, 0.01 g/l; KCl, 0.1 g/l; and Yeast extract, 0.06 g/l (Ramsay *et al.*, 1983). The medium was autoclaved at 121ºC for 15 min. Phenol (0.3 g/l) was separately sterilized by membrane filter before aseptically added into the sterile medium. The culture was shook on a shaker (Infors AG, Germany) at room temperature and harvested at exponential phase which occurred between 15 to 18 hour cultivation.

Analytical Methods

Cell density was estimated by optical density measurement at 600 nm using a UV- spectrophotometer (Prieto *et al.*, 2002). Based on the dry weight calibration curve, 1 unit of an optical density is corresponds to approximately 1 g/L of cell. Phenol was calorimetrically assayed by using Folin-Ciocalteau reagent (Box, 1981). 0.1 ml of the reagent was added into the mixture of 2 ml samples and 0.3 ml of 200g/l sodium carbonate. The mixture was incubated for 60 minutes at room temperature to allow complete reaction and measured at 750 nm. A standard calibration curve was prepared with concentration range from 0 – 0.01 g/l.

Packed Reactor Set up

The full grown culture were first inoculated into a fresh Ramsay medium (10%, v/v) and circulated using a peristaltic pump through a seven cm internal diameter and 40 cm length of column packed with 500 g support. The support was a crushed bioceramic that was made of hwang-to and oyster shells (Haejoong, Korea). Due to its inert and highly porous nature, the original purpose of these materials was for making artificial coral reef in fishery industries. The batch degradation was performed by passing 600-ml 1000 ppm phenol at constant rate of 2.5 ml/min through the column. This gave a total of six passes in 24 hours. In batch degradation studies, the whole experiment was then repeated with fresh 600-ml 1000 ppm phenol solution. The experimental set up is illustrated in Figure 1.

RESULTS AND DISCUSSION

One of the merits employing immobilized culture is the reusability and retention of cells. However, in comparison to the suspended cell system, immobilized system is subjected to a number of variables that affect the reactor performance. In this work, factors such as support's adsorption capacity on phenol, support's diameters, loading rates, and nutrient limitation were investigated. The best outcomes from these investigations were then employed to evaluate the kinetic of phenol degradation in a repeated-batch by immobilized culture in a packed column.

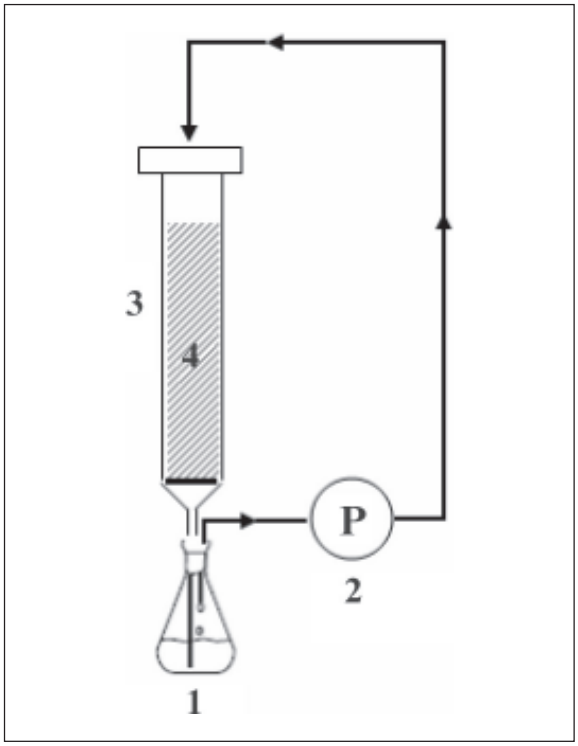

Fig. 1. Experimental set up (1) Feed reservoir, (2) Peristaltic pump, (3) Packed column, (4) Support

The percent of phenol degradation that measured in the liquid may be resulted from the combination of degradation by immobilized microbes, and phenol adsorbed by the support. The effect was investigated by comparing the phenol reduction rate in the liquid with and without culture. A 600-ml 1000 ppm phenol solution was circulated through the packed column using peristaltic pump at the rate of 2.5 ml/min for 24 hours. Result in Figure 2 reveals that complete removal of phenol was witnessed in the experiment with culture, while only 177 ppm phenol was reduced from the liquid without culture. The level of phenol level in the liquid without culture gradually and steadily declined until it reached at equilibrium state after 16 hours. The reduction was most likely due to the adsorption on the support surface (Nurdan and Azmi, 2004). This gave the bioceramic's maximum specific adsorption capacity of 0.354 ppm phenol per gram bioceramic. With this low adsorption capacity, for the sake of convenience, all phenol degradation results presented in this work were assumed solely contributed by the microbial degradation.

Larger particles increased their distances and significantly enhanced the problem of lower mass and gas transfer (Chung *et al*., 2003). In addition, the distances among supports reduced the ratio of surface area to volume resulted in poorer degradation rates due to relatively smaller cell populations per particle. The ability of carrier to trap and retain high amount of bacteria is also greatly influencing the degradation of phenol. Trend in Figure 3 shows that the support diameters beyond 1.0 cm would result in significant decline of degradation rate. Nevertheless, all support diameters experienced similar sharp decline of degradation rate after seven days. It seems that all tested particle diameters are suitable to be used in a real site. However, it was recommended to used 1.0 cm of particle or less to give constant phenol removal performance. It is also important to note that the experiments with different set of operating conditions (e.g. loading rate, column diameter etc) may result in different observations.

Optimum loading rate ensures that sufficient time is given for the transport of phenol to the immobilized cells, and finally degradation occurs. At high loading rate, phenol probably has insufficient time to diffuse within the carrier particles resulted in lower substrate bioconversion rate (Nurdan and Azmi, 2002). From the process point of view, high throughput is favorable; however this may lead to cell washout, and lack of solid-liquid equilibrium. Both would significantly reduce the phenol removal capacity. Figure 4 shows that the loading rate responsible for cell detachment, and consequently led to the decline of phenol degradation rate. It is clearly shown that whenever the loading rate was ramped up from 0.5 to 3.5 ml/min., the increasing phenol degradation rates were balanced with the cell detachment. Beyond the loading rate of 3.5 ml/min, a significant drop of degradation rate was seen due to the cell detachment. Thus, the feeding beyond this rate was inappropriate to be used in the next cycle of phenol degradation in repeated batch process. Meanwhile, the loading rate of 2.5 mg/min was the best to give both highest degradation rate, and reasonable cell detachment.

The addition of nutrient, especially a carbon or nitrogen source is common to give a balanced growth. In this study, phenol being used as the carbon source, while other nutrients (nitrogen etc) are limited. By comparing result of degradation

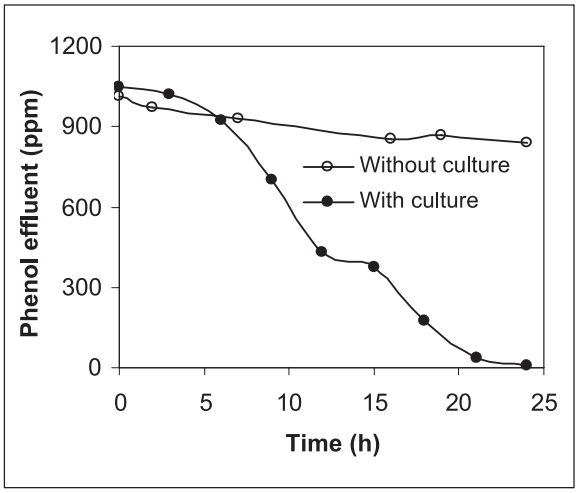

Fig. 2. Degradation of phenol with and without presence of culture.

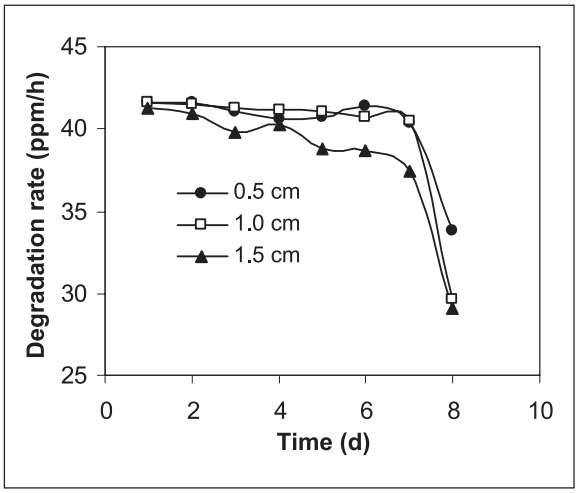

Fig. 3. Diameters of Support beyond 1.0 cm resulted in a significant decline of the degradation rate.

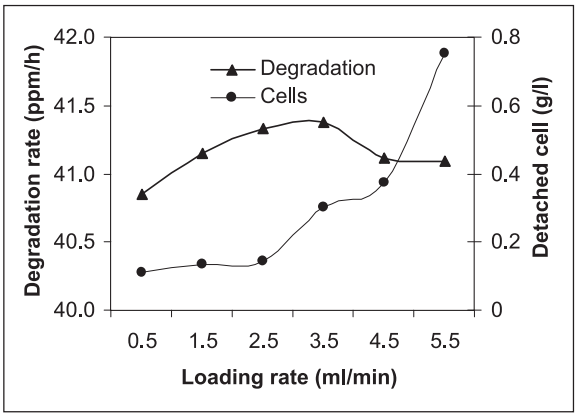

Fig. 4. The loading rate beyond 3.5 ml/min gives a significant drop of degradation rate due to the cell detachment.

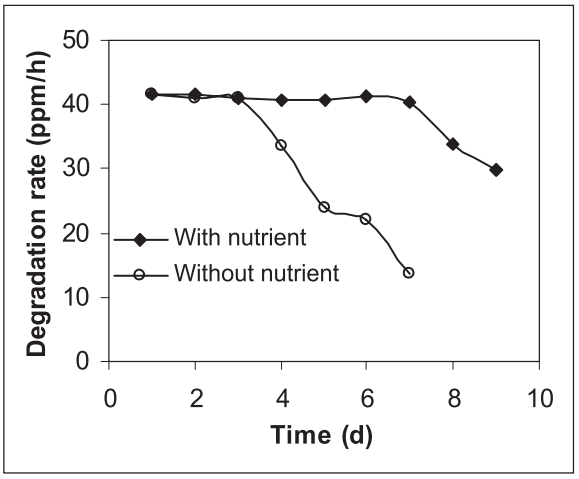

Fig. 5. Nutrient limitation significantly reduces the immobilized cells' capacity to degrade phenol.

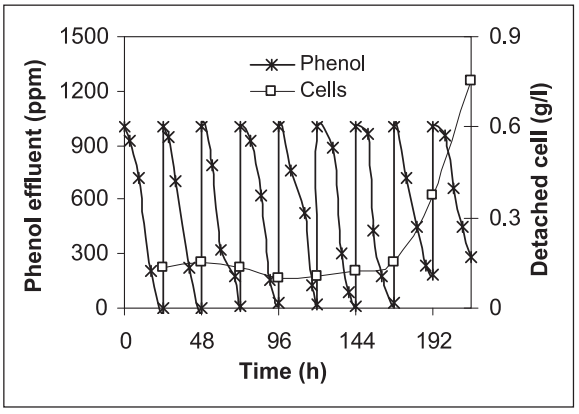

Fig. 6. Phenol concentration profile in repeated-batch mode

rate of cultures with and without medium, the level of limitation could be easily estimated. As demonstrated in Figure 5, the maximum degradation rate in culture without nutrient supplement only lasted for three days, and then followed by a drastic decline. Insufficient nutrient in the medium caused more cells to be killed and detached from supports (Chen *et al*., 2002). The supplement of nutrients however, has extended the degradation rate limit up to seven days. The suppplement helped the builing up of cell mass and attributed to the attenuation of phenol toxiciy (Lob and Tar, 2000).

The final experiments employed all best conditions that previously discussed. The study aimed at comparing the performance of our system to the reported literatures. The batch degradation was performed by passing a 600-ml 1000 ppm phenol solution at constant rate of 2.5 ml/min through the column. This gave a total of six passes in 24 hours. The whole experiment was then repeated with fresh 600-ml 1000 ppm phenol solution until the degradation rate started to show a decline (Nurdan and Azmi, 2004). Result in Figure 6 indicated that the immobilized culture managed to achieve 100% phenol degradation until seven batches before experiencing the first decline in the eighth batch. That means the culture endured 42 cycles of total 4.2 liter 1000 ppm phenol solution in seven days. This gives the specific removal of 42 ppm/hour. The decline of phenol degradation also nicely coincided with the cell leakage trend. The cell leakage was monitored by detecting the sample's optical density at 600 nm. In a solid support, microbes form a biofilm. The growing biofilm within support attributed to substrate diffusion limitations (Shuler and Kargi, 2002). Likewise, diffusional limitations may cause inadequate supply of substrate, oxygen, or nutrient to the inner portions of the biofilm, which may weaken the biofilm matrix and cause cell death. This circumstance was indicated by the increase of leakage cell concentration. Data in Table 1 summarizes published data on phenol degradation. It is evidently seen that in general, the immobilized cell systems produced better results over suspended or free cell systems. Most importantly, the outcome of this study has indicated that the performance of our isolated microbe exceeded some of the published work.

CONCLUSION

Key factors that determined the performance of phenol degradation rate by immobilized *Pseudomonas* sp in repeated-batch in packed column have been successfully investigated. A support's diameter of 1.0 cm or lower resulted in the highest degradation rate. The best result also being witnessed if the loading rate was set at 2.5 ml/min. This was mainly due to the minimal cell

Table 1. Outcome of this study as compared to other published data

Author	Microorganism	System	Mode	Degradation rate (ppm/h)
Prieto *et al*., 2002	*Rhodococcus erythropolis*	Imm. cell	Continuous	300
Mordocco *et al*., 1999	*Pseudomonas putida*	Imm. Cell	Continuous	108
Collins and Daugulis, 1997	*Pseudomonas putida*	Free Cell	Fed batch	69 (4 batches)
This study, 2006	*Pseudomonas* sp	Imm. cell	Repeated batch	42 (7 batches)
Nurdan *et al*., 2004	*Pseudomonas putida*	Imm. cell	Repeated batch	28 (5 batches)
Gonzalez *et al*.,2001	*Pseudomonas putida*	Imm. cell	Continuous	21
Hannaford and Kuek,1999	*Pseudomonas putida*	Imm. cell	Batch	250
El-Haleem *et al*., 2003	*Acinetobacter sp*	Imm. cell Free cell	Batch Batch	33 4
Kumar *et al*., 2004	*Pseudomonas putida*	Free cell	Batch	6

detachment. The addition of medium allowed a balanced growth, and hence prolonged the maximum phenol degradation rate from only three days to seven days. In a batch study that employed all the best operating conditions, the immobilized culture managed to degrade 100% fed phenol until seven batches. This concludes that our system's performance was comparable or even better in comparison to some published data. The approach in this study also provides a useful guideline in treating the phenolic contaminants in industrial wastewaters and environment.

ACKNOWLEDGEMENTS

This research was financially supported by UTM Short Term Grant (vote no: 75200) and UTM-PTP Scholarship. We wish to thank the technicians and postgraduates in Bioprocess Engineering Department for their contributions and technical supports in completing this study.

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