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Enhancement of phenol degradation using immobilized microorganisms and organic modified montmorillonite in a two-phase partitioning bioreactor

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ABSTRACT

A study was conducted to determine the potential of a two-phase partitioning bioreactor (TPPB) for enhancing the treatment of phenol at high initial concentrations. TPPBs are characterized by a cell-containing aqueous phase and an immiscible and biocompatible organic phase that partitions toxic substrates to the biocatalyst on the basis of their metabolic demand and the thermodynamic equilibrium of the system. In the present work, in order to enhance the degradation of phenol in TPPB, the polysulfone capsule containing organic modified montmorillonite (OMMT-PSF capsule) was used as organic phase, and polyurethane foam immobilized microorganism (PUF-immobilized microorganism) was used as biocatalyst. Experiments showed that OMMT-PSF capsules offered improved sorption capacity (30.2 mg phenol/g OMMT-PSF capsules at the fixed initial phenol concentration of 2030.2 mg/L) and a greater sorption rates (the equilibriums were reached at about 6 h). The characters of vast sorption capacity and rapid sorption rates are in accordance with the desire of delivery agent in TPPB, further testing demonstrated that OMMT-PSF capsules using as a reservoir in TPPB played a significant role. The phenol biodegradation rates of batch fermentation were examined, the maximum volumetric consumption rate of phenol decreased in the order: immobilized microorganisms with OMMT-PSF capsules in a TPPB (342.4 mg/(L h)) > immobilized microorganisms without OMMT-PSF capsules (300 mg/(L h)) > free microorganisms with OMMT-PSF capsules in a TPPB (208.4 mg/(L h)) > free microorganisms without OMMT-PSF capsules (125.8 mg/(L h)). This work demonstrates that the use of immobilized microorganisms and OMMT-PSF capsules in TPPB offers improved degradation of phenol.

1. Introduction

Aromatic compounds are a class of compounds regarded as ubiquitous pollutants. Many aromatic compounds exhibit carcinogenic, teratogenic or mutagenic properties [1–3]. Phenol is an aromatic compound that is frequently involved in contamination of the environment by transport, accidental discharge, the disposal of petroleum products or through industrial effluents [4,5]. Based on severe chronic toxicity, phenol has been classified as a high concern priority pollutant by the EPA [6]. Therefore, the treatment of phenol effluent is important. Phenol removal by biological methods is generally preferred to physicochemical methods because of lower costs and the possibility of complete mineralization. The biodegradation of phenol by free microorganisms has been extensively studied [7–9]. However, the use of free microorganisms for wastewater treatment involves many serious problems such as substrate inhibition and microorganism separation. Several strategies have been proposed to avoid these problems; microorganism immobilization is one of the most attractive alternatives [10,11]. In contrast with free microorganisms, microorganism immobilization may not only maintain high concentration of microorganisms in carriers even in continuous bioreactors without losing large number of microorganisms, but also increase the ease of inoculants storage and re-usage. Compared to free microorganisms, the immobilized microorganisms can biodegrade higher substrate concentrations. However, the problem of substrate inhibition could not be solved completely, the immobilized microorganisms could be inhibited if substrate concentrations were exceeding high [12–14].

However, TPPB allows biodegradation of higher pollutant concentrations, and presents a treatment option for contaminants that are difficult to remediate by conventional biological methods. The TPPB concept is based on the use of a water–immiscible and biocompatible organic solvent that is allowed to float on the surface of a cell-containing aqueous phase [15]. Traditionally, a TPPB uses

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an immiscible organic liquid that acts as a reservoir and delivery agent to effectively partition toxic substrates to/from the aqueous phase [16]. However, liquid second phases may have limitations that hinder TPPB performance such as bioavailability (most TPPB systems using immiscible organic solvents are limited to the use of single pure microbial species that are chosen for their inability to utilize the solvent as a carbon/energy source), cytotoxicity [17,18]. Moreover, TPPB requires large quantities of organic solvent to preconcentrate the phenol together with vigorous mixing or aeration in order to achieve a high interfacial surface area and mass transfer rate. This agitation or aeration frequently results in the formation of stable emulsions which are difficult to separate and the losses of solvent and cell [19]. These limitations could be overcome by using a polymer phase as the absorption/desorption component of the two-phase partitioning bioreactor [20]. At the same time, from the research results of Daugulis, we can easily find that the polymer using absorption/desorption component have a low capacity and rate of absorption and some polymers are very expensive [16,21–26].

In order to overcome these drawbacks, we use organic modified montmorillonite (OMMT) as delivery agents for phenol to microorganisms in a TPPB. OMMT has previously been demonstrated by some researcher to be effective as a sorbent of phenol [27–29]. At the same time, phenol which is adsorbed by OMMT could be completely released into water and removed by microorganism. Subsequently, OMMT could be recycled [30]. Compared with the organic solvents and polymer, OMMT has the following advantages: (i) OMMT exhibits a high sorption capacity and rates for phenolic compounds [31,32]; (ii) OMMT has no cytotoxicity, and could not be degraded by microorganisms; (iii) great aeration rates could not produce excessive foaming and lead to solvent and cell losses using OMMT as a reservoir in TPPB, on the contrary, if we use an immiscible organic liquid as a reservoir, aeration rates could not be greater than 0.5 vvm [19]; (iv) the abundance of montmorillonite (MMT) in most continents of the world and its low cost make it a strong candidate as an adsorbent for the removal of phenolic pollutants from wastewaters. However, powder OMMT has a small diameter and this causes a fatal problem in the application of TPPB: after the treatment of wastewater, there will be quantity of active silt of absorbent that is very hard to disposal and be separated from water. To resolve this problem, the powder of OMMT was wrapped within polysulfone (PSF) to form OMMT-PSF capsules which were used as a reservoir in TPPB.

In the present work, in order to enhance the degradation of phenol in TPPB, OMMT-PSF capsule and PUF-immobilized microorganism were firstly prepared. Subsequently, phenol was degraded by PUF-immobilized microorganism in a two-phase bioreactor in which the partitioning phase is OMMT-PSF capsule. The adsorption capability of OMMT-PSF capsules and the biodegradation rates of phenol in TPPB were investigated.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used for the culturing of microorganisms were purchased from Beijing Chemical Reagents Company (China). Phenol (99.5%, assay) was purchased from Tianjin Guangfu Fine Chemical Research Institute (China). Hexadecyl trimethyl ammonium bromide (CTAB), with 99% purity, used as cationic surfactant, was purchased from Beijing Chemical Reagents Company (China). Polysulfone (PSF) with intrinsic viscosity of 0.56 was purchased from DaLian Polysulfone Plastic Co. Ltd. (China). Montmorillonite with a cation exchange capacity (CEC) of 110 cmol/kg was obtained from ZheJiang Fenghong Clay Chemicals Co. Ltd. (China). All materials were used as received without any further purification.

2.2. Microorganisms immobilization and culture conditions

A mixed culture (B350) purchased from BIO-SYSTEMS Co. (USA) was used throughout the study [33]. A mineral salt medium (MSM) was used as the standard growth medium. MSM comprised (in grams per liter): KH_2PO_4, 1; K_2HPO_4, 1; $(NH_4)_2SO_4$, 1.5; NaCl, 0.1; $CaCl_2$, 0.1; $MgSO_4$, 0.1; and $FeCl_3$, 0.1.

2.2.1. Preparation of free microorganism

Microorganisms were grown on phenol as the sole carbon and energy source and the mineral medium was used. Phenol concentration is 800 mg/L. MSM comprised (in grams per liter): KH_2PO_4, 1; K_2HPO_4, 1; $(NH_4)_2SO_4$, 1.5; NaCl, 0.1; $CaCl_2$, 0.1; $MgSO_4$, 0.1; and $FeCl_3$, 0.1. Microorganisms were harvested after 12–24 h growth period (logarithmic growth phase) and stored at 4 °C for further research.

2.2.2. Preparation of immobilized microorganisms

Polyurethane foam (PUF) which was synthesized by our lab [34], density is about 1.0 g/cm^3 and their specific surface area is 28,000 m^2/m^3 with ratio of surface area to weight is between 60 and 100 m^2/g, was cut into cubes (0.5 cm × 0.5 cm × 0.5 cm). Prior to microorganism immobilization, PUF was washed twice with distilled water and dried. 1 g of B350 was added to a 2 L bioreactor containing PUF (14 g) and MSM at 30 °C, aerating at 1 vvm. Phenol concentration is 1000 mg/L. The component of MSM was the same as in Section 2.2.1. The fresh phenol and MSM were added again when they were consumed. This operation was repeated four times, and then, PUF containing the immobilized microorganisms were washed with saline and stored at 4 °C for further research.

2.3. Preparation of OMMT

OMMT was prepared as follows. The organic modification of natural montmorillonite was carried out in a batch reactor, which was put into a temperature controlled water bath. The temperature was controlled at 80 °C. 5 wt.% of natural montmorillonite solution was added to 1 wt.% of CTAB solution with a ratio of 1:2 (volume/volume) and stirred for 240 min. OMMT particles were then separated by gravity sedimentation followed by several cycles of washing with distilled water until no Br^- can be detected from the supernatant and dried at 70 °C for 24 h under the condition of vacuum. The sample was then desiccated and ground in an agate mortar and finally allowed to filter through a 200 mesh sieve (75 μm) before use.

2.4. Preparation of OMMT-PSF capsules

OMMT-PSF capsules were prepared as follows [35]: 8 g of PSF was dissolved in 100 mL of N-methyl-2-pyrrolidinone (NMP) to obtain the PSF solution. Then 24 g of powder OMMT was added into the solution of PSF and stirred for 60 min under the room temperature, and the dispersed phase of OMMT and PSF was obtained. The dispersed phase of OMMT and PSF was injected into the solidification solution (25 wt.% ethanol in aqueous solution) using a 0.4 mm diameter syringe needle, and stirred by magnetic stirrer to obtain OMMT-PSF capsules. Then, they were washed with deionized water several times and kept in deionzed water for the extraction process; finally, OMMT-PSF capsules were dried at 30 °C for 48 h under the condition of vacuum. The obtained capsules were characterized by means of scanning electron microscopy (SEM).

2.5. Batch adsorption test of OMMT-PSF capsules

The experiment was carried out in an Erlenmeyer flask, which was put into a constant temperature water bath shaker. 6 g of

OMMT-PSF capsules was added to each of four 125 mL Erlenmeyer flasks containing 100 mL of phenol/salts medium solution at phenol concentrations of 1000, 2000, 3000, and 4000 mg/L. The flasks were sealed with rubber stoppers, agitated at 180 rpm, and maintained at 30 °C for a period of 8 h. The uptake of the phenol on the absorbent was determined. The variation of the uptake of phenol with adsorption time was investigated in kinetic experiments.

2.6. Degraded phenol by free and immobilized microorganisms

Batch fermentations of free microorganism (7.6 g dry microorganism/L) and PUF-immobilized microorganism (14.2 g dry PUF-immobilized microorganism/L, 1.15 g dry microorganisms/g dry foam cubes) were performed in a 2 L (working volume) bioreactor at phenol concentration 1000 mg/L. The bioreactor was made from glass column and contained four sections: air inlet, air outlet, sample inlet and sample outlet. Oxygen was supplied by means of air gasification through the liquid phase using a diffuser to obtain small air bubbles. The bioreactor was put into the constant temperature incubator to maintain a constant temperature. The process of batch fermentation was carried out at 30 °C, aerating at 1 vvm. Samples were taken each hour for residual phenol analysis. Control bioreactors were incubated in parallel under the same conditions to ascertain the evaporation losses of phenol.

2.7. Batch fermentation in a TPPB using free and immobilized microorganisms

To compare the degradation efficiency of phenol by free and immobilized microorganisms used in TPPB, batch fermentation in TPPB using free microorganisms and PUF-immobilized microorganisms were performed, respectively. In these experiments, 5 g of phenol was dissolved into the aqueous phase to provide an initial concentration of 4793.1 mg/L; and then the addition of 100 g OMMT-PSF capsules decreased the aqueous phenol concentration to approximately 1500 mg/L in 7 h; finally, at the time of 7 h, freely suspended microorganisms (7.6 g dry microorganisms/L) and PUF-immobilized microorganisms (14.2 g dry PUF-immobilized microorganisms/L, 1.15 g dry microorganisms/g dry foam cubes) were added to two bioreactors, respectively. The system was aerated at 1 vvm, with the temperature and pH controlled at 30 °C and 7.0, respectively. Control bioreactors were incubated in parallel under the same conditions to ascertain the evaporation losses of phenol.

2.8. Selection of the feeding strategies

Two feeding strategies of TPPB fermentations were performed to demonstrate the ability of a solid–liquid TPPB to degrade phenol. In the feeding strategy A, 5 g of phenol was dissolved into the aqueous phase to provide an initial concentration of 4793.1 mg/L; and then the addition of 100 g OMMT-PSF capsules decreased the aqueous phenol concentration to approximately 1500 mg/L in 7 h; finally 28.4 g PUF-immobilized microorganisms (1.15 g dry microorganisms/g dry foam cubes) were added to the bioreactor. In the feeding strategy B, we added 100 g OMMT-PSF capsules and 28.4 g PUF-immobilized microorganisms (1.15 g dry microorganisms/g dry foam cubes) to a 2 L 4793.1 mg/L phenol solution simultaneously. The system was aerated at 1 vvm, with the temperature and pH controlled at 30 °C and 7.0, respectively.

2.9. Analytical methods

2.9.1. Dry cell weight determinations

Biomass was determined turbid metrically at 600 nm and converted to dry cell weight (DCW) with a standard conversion curve.

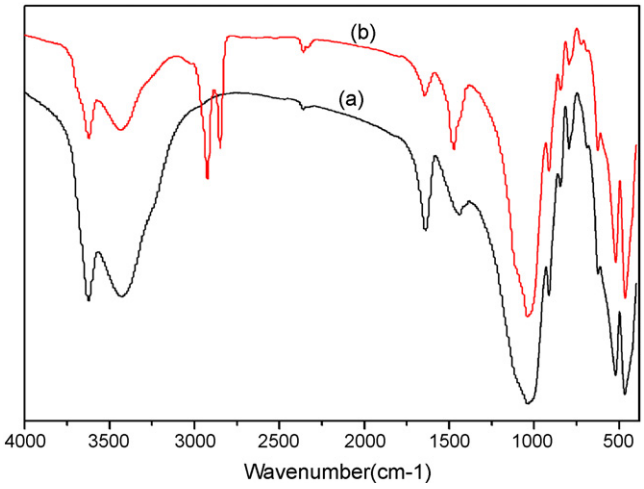

Fig. 1. FTIR spectra of the unmodified and the organically modified montmorillonite (a: MMT and b: OMMT).

The dry weight of immobilized microorganisms was determined as follows: (i) two pieces of PUF-immobilized microorganisms were washed by sonic waves in the solution of 50 mL 1 M sodium hydroxide for 30 min; (ii) they were put out and washed by distilled water (20 mL) for four times; (iii) the solution of microorganisms was collected and mixed, and then, biomass was determined with a standard conversion curve.

2.9.2. Residual phenol concentration

Phenol was estimated colorimetrically according to the method previously described by Yang and Humphrey [36] based on rapid condensation with 4-aminoantipyrene followed by oxidation with alkaline potassium ferricyanide and the absorbance read at 510 nm. Before phenol was determined, samples were filtered (0.22 μm) under vacuum to ensure all biomass was removed.

3. Results and discussion

3.1. Preparation of OMMT-PSF capsules

Fig. 1 shows the FTIR spectra of the unmodified and the organically modified montmorillonites in the wave number range 500–4000 cm^{-1}. FTIR spectra of two samples display typical absorption peaks of montmorillonite. The peak at about 3624.16 is the result of –OH stretching vibration of the structural OH groups in montmorillonite. The peaks at about 1639.35 cm^{-1} are characteristic of the stretching and bending vibrations of sorbed water. The strong absorption peak at about 1037.78 cm^{-1} is the result of Si–O–Si stretching vibrations. FTIR spectra of OMMT reveal the presence of characteristic absorptions of CTAB [37]: the absorption peaks at about 2923.64 and 2850.7 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of $-CH_2-$, respectively, the peak at 1471.98 cm^{-1} corresponds to C–N vibrations. The FTIR spectrum results showed that montmorillonite was modified by CTAB successfully.

A liquid–liquid phase separation technique was employed to fabricate the OMMT-PSF capsules [38]. When the OMMT-PSF solution drop contacts with the solidification solution (25 wt.% ethanol in aqueous solution), the rapid exchange of the solvent NMP and water occurred, at the same time, the influx of water was small, a skin layer formed due to the rapid phase separation. With the completion of the exchange between the solvent and the non-solvent, the porous OMMT-PSF capsules were prepared, and many pores existed in the spheres. Fig. 2 shows the SEM picture of the

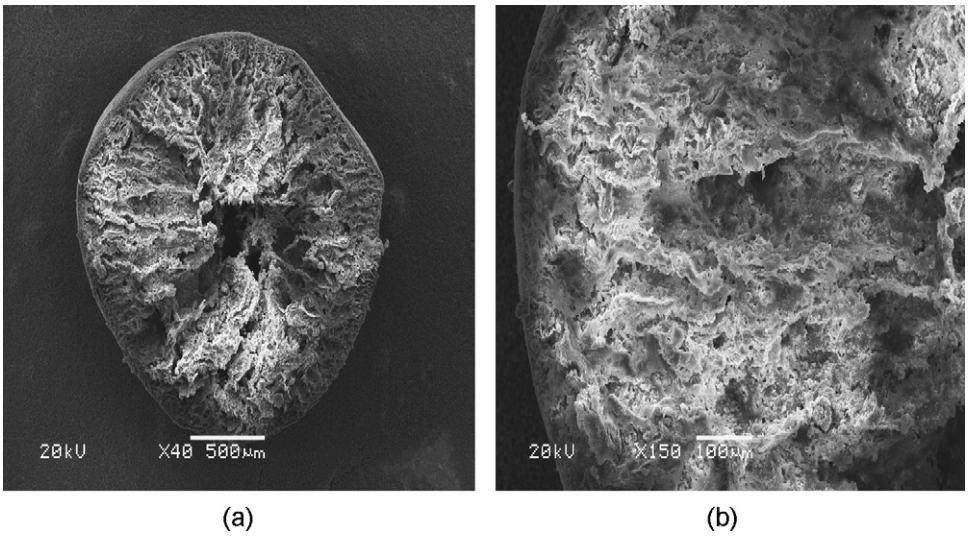

Fig. 2. Cross-section of the OMMT-PSF capsule.

cross-section of the OMMT-PSF capsules. In Fig. 2(a), a dense thin layer was found on the outer surface of the capsule. Very large pores are present in the center of the capsule, Smolders and coworkers [39] suggested the large pore in the center was formed by anomalous growth of nuclei. From Fig. 2(b), we find that there is a thick sponge layer, in which large numbers of OMMT particles were filled.

3.2. Sorption kinetics of phenol

The relationship between reaction time and sorption amounts at different initial phenol concentrations of phenol was presented in Fig. 3. The results showed that equilibrium time required for the adsorption of phenol on OMMT-PSF capsules is almost 6 h. These results also indicated that the adsorption process can be considered very fast because of large amount of phenol attached to the absorbent within the first 1 h of adsorption. After a rapid sorption, the phenol sorption rates declined slowly and the equilibriums were reached at about 6 h. This process is consistent with that of previous reports [40,41]. OMMT-PSF capsules offered improved sorption capacity (30.2 mg phenol/g OMMT-PSF capsules at a fixed initial phenol concentration of 2030.2 mg/L) and a greater sorption rates (the equilibriums were reached at about 6 h) in comparison with the sorption capacity and rates of previously used polymer (19 mg phenol/g polymer and 14 h, respectively) [16]. The characters of vast sorption capacity and rapid sorption rates also are in accordance with the desire of delivery agent in TPPB, therefore we suppose that OMMT-PSF capsules using as a reservoir in TPPB should play a significant role, this assumption was proved by the following research.

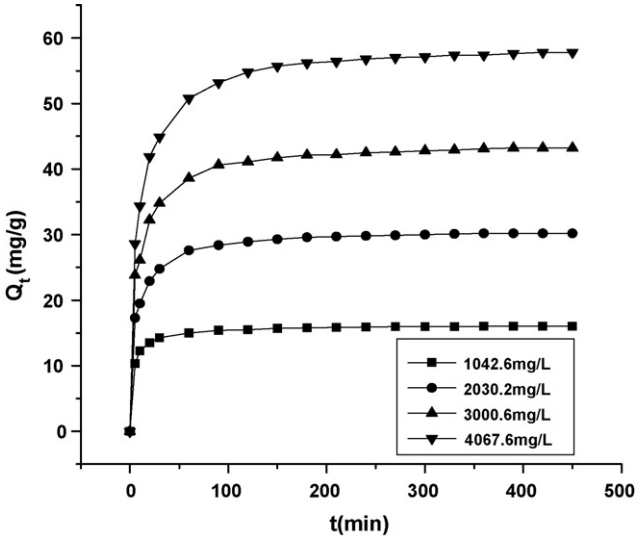

Fig. 3. The relationship between phenol uptake and reaction time.

In order to investigate the kinetics of adsorption of phenol on OMMT-PSF capsules, the pseudo-first- and pseudo-second-order models were used.

$$\log(Q_{e1} - Q_t) = \log Q_{e1} - \frac{k_1 t}{2.303} \quad \text{(pseudo-first-order)}$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_{e2}^2} + \frac{t}{Q_{e2}} \quad \text{(pseudo-second-order)}$$

where Q_t is the amount of phenol adsorbed (mg/g) on OMMT-PSF capsules at various time t, Q_{e1} the maximum adsorption capacity (mg/g) for the pseudo-first-order adsorption, k_1 the pseudo-first-order rate constant for the adsorption process (min^{-1}), Q_{e2} the maximum adsorption capacity (mg/g) for the pseudo-second-order adsorption, k_2 the rate constant of pseudo-second-order for the adsorption (g/mg min).

The kinetics data are plotted as the linear form of the models (Fig. 4, Fig. 5), and the resultant parameters are given in Table 1. As can be seen from Table 1, the coefficients ($R_1{}^2$) for pseudo-first-order kinetic model are between 0.9477 and 0.9687 and the correlation coefficients ($R_2{}^2$) for pseudo-second kinetic model are between 0.9998 and 0.9999. These results show that the adsorption system of OMMT-PSF capsule obeys the pseudo-second-order kinetic model.

3.3. Sorption isotherm of phenol

Adsorption isotherm data have been described by the Langmuir adsorption isotherm and Freundlich equations.

$$\text{Langmuir equation :} \quad \frac{C_e}{Q_e} = \frac{1}{K_L Q_{\max}} + \frac{C_e}{Q_{\max}}$$

$$\text{Freundlich equation :} \quad \ln Q_e = \ln K + \frac{1}{n} \ln C_e$$

Table 1
The adsorption kinetic model rate constants for phenol on OMMT-PSF capsules at different initial phenol concentrations.

C_0 (mg/L)	Pseudo-first-order			Pseudo-second-order		
	Q_{e1} (mg/g)	K_1 (min^{-1})	R_1^2	Q_{e2} (mg/g)	K_2 (g/mg min)	R_2^2
1042.6	3.8	0.0175	0.9477	15.9	0.0186	0.9999
2030.2	10.3	0.0168	0.9606	30.4	0.0057	0.9998
3000.6	15.8	0.0161	0.9535	43.5	0.0036	0.9998
4067.6	23.6	0.0163	0.9687	58.1	0.0023	0.9996

where C_e (mg/L) is the concentration of the phenol solution at equilibrium, Q_e (mg/g) is the amount of sorption at equilibrium. In Langmuir equation, Q_{max} is the maximum sorption capacity and K_L is Langmuir constant. In Freundlich equation, K and $1/n$ are empirical constants.

The Langmuir and Freundlich equations, values of parameters and correlation coefficients of the experimental data are shown in Table 2. From Table 2, it was observed that the adsorption isotherms of phenol on the OMMT-PSF capsules are well represented by both the Langmuir isotherm model and Freundlich isotherm model. However, comparing R^2 values, Freundlich isotherm model is better than Langmuir isotherm model.

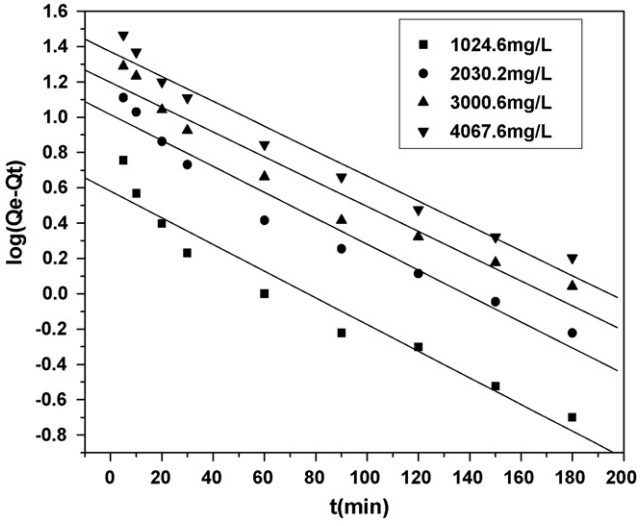

Fig. 4. The fitting of pseudo-first-order model for phenol on OMMT-PSF capsules for different initial concentrations.

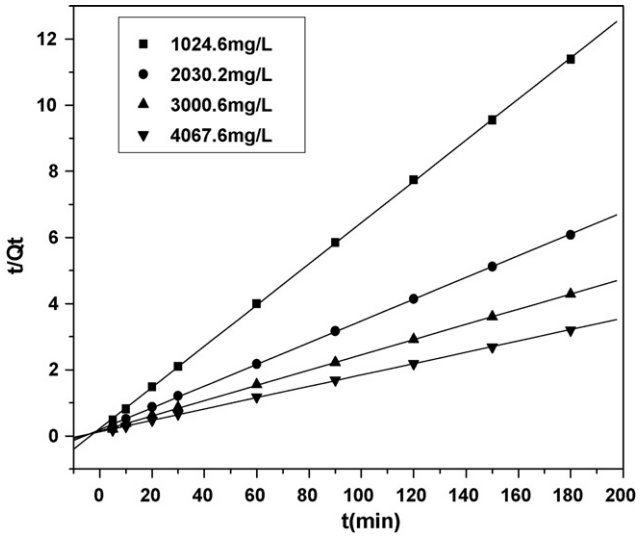

Fig. 5. The fitting of pseudo-second-order model for phenol on OMMT-PSF capsules for different initial concentrations.

3.4. Degraded phenol by free and immobilized microorganisms

In Fig. 6(a), the general structure of PUF is illustrated at a low magnification. The carrier structure consists of some micro-pores, each of diameters 0.5 mm. The pores provide space for gas and liquid to pass through the microorganisms. Fig. 6(b) shows an electronmicrograph of microorganisms immobilized in PUF. Comparison of the two electronmicrographs indicates that in Fig. 6(b) there are a much higher number of microorganisms in PUF than in Fig. 6(a). This means that the microorganisms were immobilized in PUF successfully. From Fig. 6(c and d), we can find the microorganisms which were immobilized in PUF are some filamentous and globular microorganisms.

In order to investigate the degradation capability of phenol by freely suspended microorganisms and PUF-immobilized microorganisms, batch fermentations were carried out. The results of these studies are given in Fig. 7; it was shown that phenol was completely degraded within 8 h by freely suspended microorganisms. However, for PUF-immobilized microorganisms, phenol was completely degraded within 3 h. The reasons which the degradation efficiency of phenol was increased by PUF-immobilized microorganisms are illustrated as follows: (i) phenol could be adsorbed by the PUF-carrier of immobilized microorganisms partially [42], therefore, the initial concentration of phenol could be reduced rapidly when PUF-immobilized microorganisms were added, microorganisms could not be restrained by the high phenol concentration and (ii) porous PUF may protect the enzymatic system of microorganisms against the detrimental effect of shear forces thereby improving the microorganisms activity.

From the above results, we can suppose that PUF-immobilized microorganisms used in TPPB should have higher phenol degradation efficiency than freely suspended microorganisms, this assumption was proved by the following research.

3.5. Batch fermentation in a TPPB using free and immobilized microorganisms

Fig. 8 provides data for a comparative experiment. In the comparative experiment, free and immobilized microorganisms were used to degrade phenol in TPPB, respectively. In batch fermentation using free microorganisms, the OMMT-PSF capsules were added into the TPPB containing high phenol concentration firstly, equilibration with the headspace after about 7 h resulted in an aqueous phenol concentration of approx. 1500 mg/L. Free

Table 2
The Langmuir and Freundlich equations, the values of parameters and correlation coefficients.

Langmuir			Freundlich		
Q_m (mg/g)	K_L (L/mg)	R^2	K (mg/g) (L/mg)$^{1/n}$	n	R^2
97.18	0.0022	0.9703	0.51	1.34	0.991

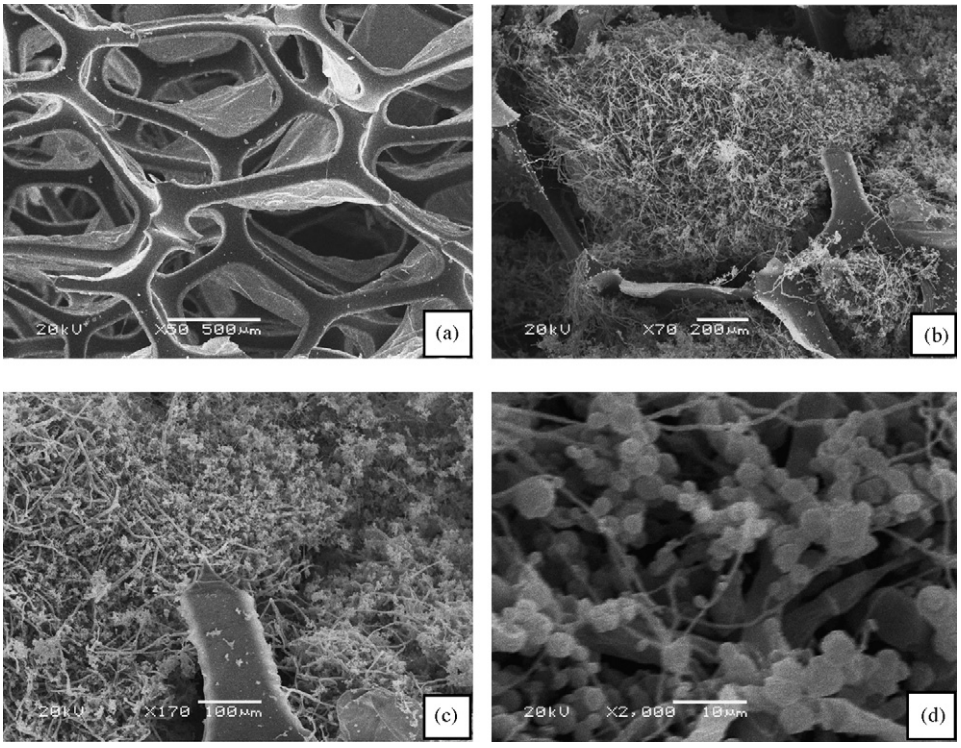

Fig. 6. SEM microphotographs of the immobilized microorganisms with polyurethane foam: (a) 50× general view of the polyurethane foam, (b) 70× microorganisms immobilized in polyurethane foam, (c) 170× microorganisms immobilized in polyurethane foam and (d) 2000× microorganisms immobilized in polyurethane foam.

microorganisms were added into the TPPB subsequently, the higher loading (approx. 1500 mg/L) resulted in a prolonged lag phase of about 8 h for free microorganisms as shown in Fig. 8. Phenol was degraded completely by free microorganisms after 24 h. However, in batch fermentation using immobilized microorganisms, PUF-immobilized microorganism was added into the TPPB after equilibration with the headspace. The phenol loading (approx. 1500 mg/L) in aqueous phase did not result in a prolonged lag phase, and phenol was degraded completely after 14 h. The batch system has been shown to have the volumetric phenol consumption rates of approximately 208.4 and 342.4 mg/(L h) for free and immobilized microorganisms, respectively, which are higher than the results of previous reports (Table 3). The high phenol degradation rate attributed to the finer absorption/desorption capability of OMMT-PSF capsules and the high catalytic activity of PUF-immobilized microorganism. These are also significantly higher than the maximum volumetric consumption rates reported in above research of approximately 125.8 and 300 mg/(L h) for free and immobilized microorganisms without OMMT-PSF capsules, respectively. Even more, the aqueous phenol concentration of 4793.1 mg/L was not degraded by free or immobilized microorganisms without OMMT-PSF capsules. Therefore, the TPPB described here provides an excellent option for the biodegradation of phenol, and the degradation efficiency of phenol was increased by PUF-immobilized microorganisms.

3.6. Batch fermentation in a TPPB with different feeding strategy

In order to discuss the relationship between phenol degradation rate and the time of adding the PUF-immobilized microorganisms into TPPB, we used two different feeding strategies. In the feeding strategy A, the OMMT-PSF capsules were firstly added into the TPPB containing high phenol concentration. Subsequently, PUF-immobilized microorganisms were added into the TPPB when equilibrium was reached. However, in the feeding strategy B, OMMT-PSF capsules and PUF-immobilized microorganisms were added into the TPPB containing high phenol concentration simultaneously. Fig. 9 shows the decline in phenol concentration in the bioreactor for both the feeding strategy A and B.

The feeding strategy A was the same as "batch fermentation in TPPB using immobilized microorganisms" (in Section 3.5). The volumetric phenol consumption rate of the feeding strategy A was determined to be 342.4 mg/(L h). In the feeding strategy B, during the lag phase of 0–7 h, the phenol concentration was reduced to approximately 1200 mg/L for the adsorption of OMMT-PSF capsules and biodegradation, the adsorption of OMMT-PSF capsules played a

Table 3
Recent examples of batch fermentation in TPPB for treating phenol.

Biocatalyst	Organic phase	Initial aqueous phenol concentration	Degradation rate	Refs
Pseudomonas putida ATCC 11172	2-Undecanone	168 mg/L	80 mg/(L h)	[15]
P. putida ATCC 11172	Hytrel	580 mg/L	150 mg/(L h)	[16]
P. putida ATCC 11172	IL109	400 mg/L	131.7 mg/(L h)	[18]
P. putida ATCC 11172	2-Undecanone	420 mg/L	175 mg/(L h)	[19]
P. putida ATCC 11172	Poly(ethylene-co-vinyl acetate)	718 mg/L	71.4 mg/(L h)	[20]
P. putida F1 (strain ATCC 700007)	2-Undecanone	200 mg/L	18 mg/(L h)	[43]
PUF-immobilized microorganisms	OMMT-PSF capsules	1500 mg/L	342.4 mg/(L h)	Current work
Free microorganisms	OMMT-PSF capsules	1500 mg/L	208.4 mg/(L h)	Current work

major role. Compared to the feeding strategy A, the feeding strategy B has a longer lag phase. Experienced 21 h, the phenol was consumed completely, and the volumetric phenol consumption rate was determined to be only 236.3 mg/L h. The reasons are as follows: in the feeding strategy B, OMMT-PSF capsules and PUF-immobilized microorganisms were added into the TPPB containing high phenol concentration (4793.1 mg/L) at the same time, microorganisms could be restrained by the high phenol concentration in aqueous phase. PUF-immobilized microorganisms started to degrade phenol with the decline of phenol concentration in aqueous phase, which was caused by the adsorption of OMMT-PSF capsules. Phenol was degraded rapidly when the phenol concentration was reduced to approximately 1500 mg/L for the adsorption of OMMT-PSF capsules. However, in the feeding strategy A, the phenol concentration in aqueous phase had been reduced to approximately 1500 when PUF-immobilized microorganisms were added into the TPPB, therefore microorganisms could not be restrained.

From above results, we can conclude as follows: (i) compared to the feeding strategy B, the feeding strategy A has a higher volumetric phenol consumption rate; it suggests that the batch fermentation in this TPPB should use the feeding strategy A and

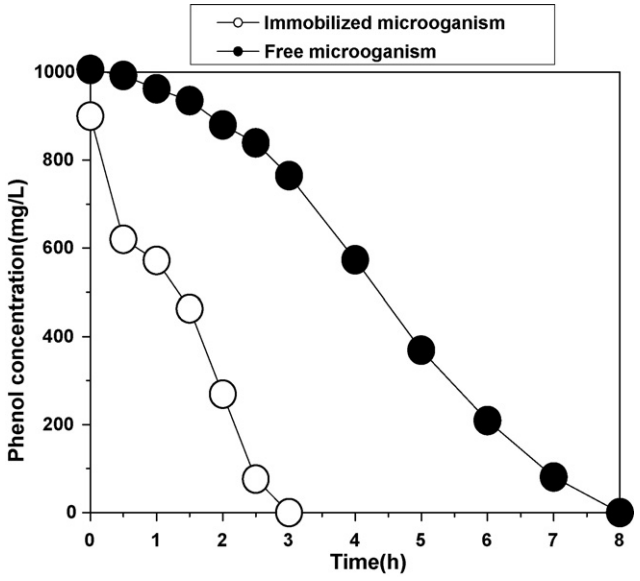

Fig. 7. Degradation of phenol by free and immobilized microorganisms.

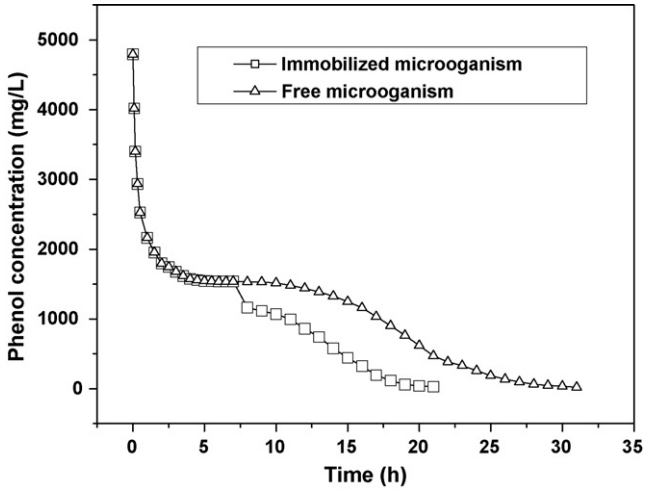

Fig. 8. Batch fermentation in a TPPB with free and immobilized microorganisms.

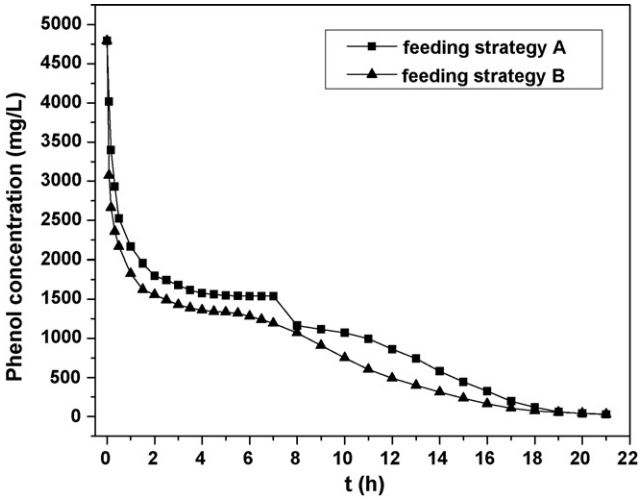

Fig. 9. Batch fermentation in a TPPB with different feeding strategy.

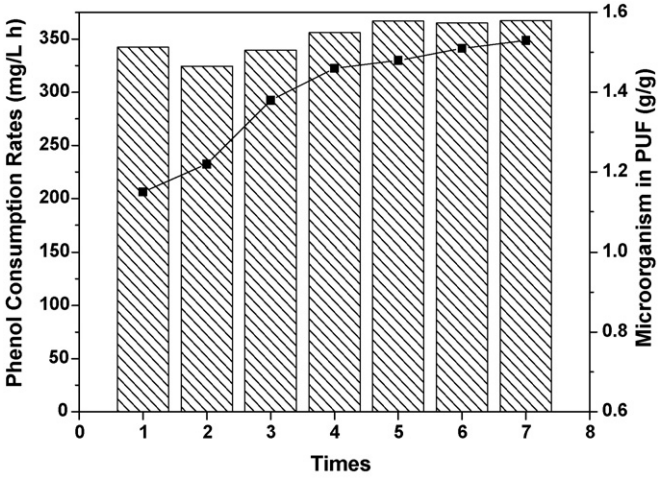

Fig. 10. Repeating batch fermentation in a TPPB with OMMT-PSF capsules and PUF-immobilized microorganisms. (The column represents the volumetric phenol consumption rate, and the square represents the amount of microorganisms in PUF.)

(ii) the continuous fermentation of this TPPB system will be researched in the next work. The aqueous phase containing high phenol concentration is continuously added into TPPB containing PUF-immobilized microorganisms and OMMT-PSF capsules, and PUF-immobilized microorganisms will suffer from high phenol concentration. The feeding strategy B is batch fermentation, and PUF-immobilized microorganisms also suffered from high phenol concentration in aqueous phase (4793.1 mg/L), but phenol was degraded completely. Therefore, we can suppose that the continuous operation of this TPPB system is feasible.

3.7. The repeating examination of the batch fermentation in a TPPB

To assess the reusability of OMMT-PSF capsules and PUF-immobilized microorganisms, the repeating examination of the feeding strategy A was performed in a TPPB that contained a known quantity of phenol (4793.1 mg/L): at the time of complete disappearance of phenol, the OMMT-PSF capsules and PUF-immobilized microorganisms were separated from the TPPB to do the next repeating examination, and the method of repeating examina-

tion was the same as feeding strategy A. As can be seen from Fig. 10, the volumetric phenol consumption rates were decreasing primarily and increasing afterwards. There are some reasons to explain this phenomenon: the adsorption capacity of the OMMT-PSF capsules had a little decrease at the second times since phenol was not released completely (the data was not shown), and the adsorption capacity did not keep decreasing compared to the second times in the subsequently repeating examination, subsequently, the decrease of adsorption capacity caused the increase of phenol concentration in TPPB, which made the lag phase prolonged and the consumption rate decreased; however, the amount of microorganisms in PUF was increasing as the times of repeating examination was increasing, and the increase of microorganisms made the consumption rate increased, but the space of PUF was limited, that was to say the amount of microorganisms in PUF would not be increasing when PUF was filled with microorganisms completely, at that time, the volumetric phenol consumption rate would not be increasing too. From these results, it is reasonable to suppose that TPPB will not suffer any loss of performance due to the use of reused OMMT-PSF capsules and PUF-immobilized microorganisms.

4. Conclusion

A low-cost solid delivery agent exhibiting superior capacity and delivery of phenol in TPPB, i.e. OMMT-PSF capsules, was successfully prepared, and microorganisms were immobilized in PUF successfully in this work. We used OMMT-PSF capsules and PUF-immobilized microorganisms in TPPB for the degradation of phenol, the experimental results show that this use is feasible because of the high volumetric degradation rate, and the reusability of the immobilized microorganisms and OMMT-PSF capsules. This indicates the suitability of such a system for application to a continuous operation, which we will research in the future.

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