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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