Biochemical Engineering Journal 79 (2013) 274–282

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Two-phase biodegradation of phenol in trioctylphosphine oxide impregnated hollow fiber membrane bioreactor

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ARTICLE INFO

Article history:
Received 12 April 2013
Received in revised form 14 August 2013
Accepted 31 August 2013
Available online 8 September 2013

Keywords:
Biodegradation
Liquid–liquid extraction
Membrane bioreactors
Phenol
Substrate inhibition
Two phase partitioning bioreactor

ABSTRACT

A hollow fiber membrane bioreactor using trioctylphosphine oxide (TOPO) impregnated in polypropylene hollow fiber membranes was developed for two-phase biodegradation of phenol using *Pseudomonas putida* ATCC 11172. Scanning electron microscopy revealed white deposits of TOPO impregnated non-uniformly within the cross sections and surfaces of the membranes. The extractant impregnated membranes exhibited high adsorption capacity and rates, whereas biodegradation of 800–2500 mg/L phenol at 200 mL volume in the extractant impregnated hollow fiber membrane bioreactor (EIHFMB) was characterized by high cell growth and biodegradation rates. For example, 1000 mg/L phenol was completely degraded within 12 h at a specific growth rate of 0.73 h^{-1} while the biomass yield and average biodegradation rate were 0.31 g/g and 86 mg/L h, respectively. The biodegradation capacity and rate in the EIHFMB were improved by increasing the effective length of the fibers by 50%, as demonstrated during the biodegradation of 3000 mg/L phenol. The adsorption/desorption rates were also enhanced with increasing aqueous phase flow rate. EIHFMB performance remained unchanged over 400 h of operation under various operating conditions suggesting the stability of TOPO impregnation within the membrane. These results indicate the use of EIHFMB as a promising technology in solvent-free two-phase biodegradation of phenolic compounds.

1. Introduction

Two phase partitioning bioreactors (TPPB) are based on the controlled delivery of a toxic substrate from a non-aqueous phase (NAP) having high affinity for the substrate to an aqueous phase containing biodegrading bacteria [1]. TPPBs can mitigate substrate inhibitions effectively and have been widely used in biodegradation of aromatic compounds using both liquid [2–4] and solid [5–7] NAPs. TPPBs achieve high cell growth and biodegradation rates, and identification of hydrophobic microorganisms which can live and degrade the substrate within the NAPs further boosts their efficiency [8,9].

The use of liquid NAPs in TPPBs has the advantages of high distribution coefficient and rapid equilibrium. But the limitations are biocompatibility and non-bioavailability of organic solvents, and phase dispersion which results in foaming, emulsification and downstream phase separation problems [10]. Solid NAPs are typically biocompatible, non-volatile and non-biodegradable [11]. However, these NAPs exhibit low sorption capacities and low diffusion coefficient for the substrates [10,12]. Furthermore, TPPBs operate at high agitation rates irrespective of the NAPs. Agitation rate as high as 600 RPM has been reported in biphenyls biodegradation using Hytrel as the partitioning phase [13].

One approach to combine the advantages of both solid and liquid NAPs is the encapsulation of the organic solvents into a semi-permeable polymeric support [14]. Such liquid-core polymeric microcapsules can prevent the challenges associated with TPPBs and have found application in extraction of metals and aromatics [15,16], and in two-phase biodegradation of pyrene and atrazine [17,18]. However, preparing these microcapsules involves controlled polymerization [15] and a significant amount of solvent is wasted during the process [19].

Recently, a novel technique has been developed to impregnate hydrophobic hollow fiber membranes with solid organic extractants [19]. The resulting extractant impregnated hollow fiber membranes (EIHFM) combine the advantages of high distribution coefficient of extraction, non-dispersive mass transfer of adsorption and the large interfacial area of the hollow fiber membranes. In the absence of any polymerization reactions, EIHFM preparation is simple, requires little quantities of the extractant and the carrier solvent, and results in a solventless configuration. The use of EIHFMs as NAP in two-phase biodegradation can prevent phase dispersion, while achieving large distribution coefficient and high substrate uptake rate. The resulting extractant impregnated hollow

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http://dx.doi.org/10.1016/j.bej.2013.08.012

fiber membrane bioreactor (EIHFMB) will extricate interphasic mass transfer of substrate from agitation speed, while the modular configuration will be easier to scale-up. Two-phase biodegradation in the EIHFMB will also be different from that in other membrane bioreactor reported in literature as the role of membranes in the EIHFMB will not only be limited to physical separation of the aqueous and the organic phases, but the membranes will also act as the partitioning phase.

In our previous research, the feasibility of using supported liquid membranes in TPPB has been evaluated [20], and the use of EIHFMs as the partitioning phase in conventional TPPB has been demonstrated [19]. In this research, the objective was to develop modular EIHFMB configuration for biodegradation of inhibitory phenol concentration. A shell and tube membrane module with polypropylene membrane has been synthesized and the membranes have been impregnated with high concentration of trioctylphosphine oxide (TOPO). The effects of operating parameters on phenol biodegradation and the long-term stability of the EIHFMB have been examined. Phenol was chosen as the model pollutant because it is a common contaminant in industrial wastewater. Phenol is toxic and recalcitrant, and exerts severe substrate inhibition on microorganisms [21]. TOPO was the preferred extractant due to its crystalline form at experimental conditions, low water solubility and high affinity for phenol [22]. Polypropylene membranes were used as the support due to their high chemical and mechanical stability.

2. Materials and methods

2.1. Microorganisms, culture conditions, and chemicals

Pseudomonas putida ATCC 11172 was used throughout this study. Stock cultures were maintained on nutrient agar (Oxoid, Hampshire, UK) slants at 4 °C. The microorganisms were grown in a chemically defined mineral medium supplemented with phenol in Erlenmeyer flasks on a shaking water bath (GFL 1092, Burgwedel, Germany) at 30 °C and 150 rpm. The composition of the mineral medium has been described elsewhere [23]. All media (except phenol), pipette tips, and Erlenmeyer flasks fitted with cotton plugs were autoclaved before use. Prior to inoculation, cells were induced into mineral medium containing 200 mg/L phenol as the sole carbon source. Activated cells in the late exponential growth phase were used as inoculum for all the experiments.

All the chemicals used in this research were of analytical grade. Phenol was dissolved in 0.02 M sodium hydroxide to prepare a stock solution of 10 g/L. TOPO was dissolved in dichloromethane to prepare a stock solution of 400 g/L.

2.2. Extractant impregnated hollow fiber membrane bioreactor (EIHFMB)

2.2.1. EIHFM preparation

The membrane contactors were fabricated by potting hollow fiber membranes into glass modules using epoxy resins (Araldite, England). Specifications for the membrane contactors are given in Table 1. The weight of the membranes used in the EIHFMB was 2.4 g. To impregnate TOPO into polypropylene fibers in the membrane contactor, 400 g/L of TOPO dissolved in dichloromethane was pumped into the shell side of the membrane contactor at 3 mL/min, while the lumen side was blocked at both the ends to trap TOPO in the lumen. After 1 h, the solution was drained out, and any remaining solution in the shell side was flushed out by washing the shell side with water for 10 min. Dry air was then blown into the shell side for 24 h to completely evaporate dichloromethane from the membranes and impregnate solid TOPO into the membrane pores. After drying, loosely attached TOPO on the outer membrane surface was removed by washing twice with water for 10 min each.

Table 1
Characteristics of the membrane contactor.

Characteristics	Values
Casing material	Glass
Casing inner diameter	0.7 cm
Membrane inner diameter	280 μm
Membrane thickness	50 μm
Pore size	0.2 μm
Porosity	0.5
Effective fiber length	60 cm
Number of fibers	150
Effective contactor volume	15.5 mL

The EIHFMs were characterized using a scanning electron microscope (SEM) (JEOL JSM-5600LV) after sputtering with platinum. The biocompatibility and biodegradability of the EIHFMs were investigated by following the protocols suggested by Collins and Daugulis [24] using glucose as the carbon source, with 0.5 g of EIHFMs as the NAP.

2.2.2. EIHFMB setup

Fig. 1 shows the schematic diagram of the experimental setup. Two identical EIHFM contactors were connected in series to get the effective fiber length of 60 cm. A peristaltic pump (L/S modular pump, Easy-Load II pump head, Masterflex, USA) was used to pump the aqueous solution from a 500 mL Erlenmeyer flask to the shell side of the EIHFMB. Purified air saturated with water was sparged into the bioreactor at 2 gas volume per reactor volume per minute (VVM).

2.2.3. EIHFMB operation

The distribution coefficient of phenol between water and EIHFM was estimated by carrying out the adsorption of phenol on the EIHFMs under abiotic conditions. Aqueous solution containing 500–3000 mg/L phenol was pumped into the EIHFMB at 8 mL/min until equilibrium was reached. The equilibrium concentration in the aqueous phase was measured and the corresponding concentration in the EIHFMs was calculated by mass balance. Phenol concentration in the EIHFMs (mg-phenol/g-EIHFM) was plotted against the corresponding aqueous phase phenol concentration (mg/L) to obtain the distribution coefficient (L/g) [25].

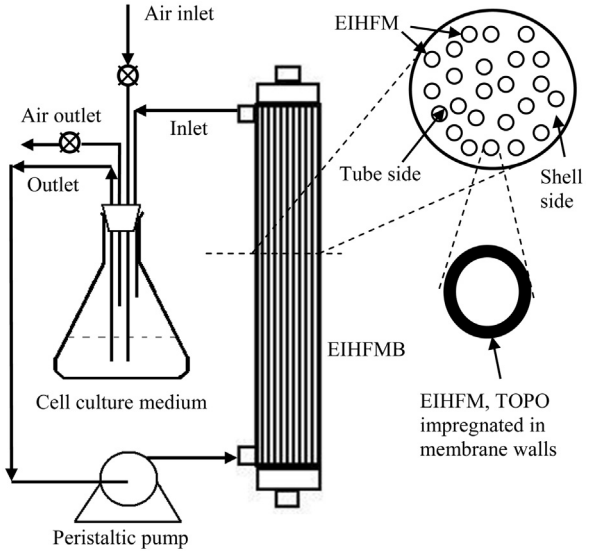

Fig. 1. Schematic diagram of the EIHFMB.