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## Kinetics modeling of two phase biodegradation in a hollow fiber membrane bioreactor



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#### ABSTRACT

A hollow fiber membrane bioreactor (HFMB) was used for two-phase biodegradation of phenol directly from wastewater. In a non-dispersive approach, phenol was extracted from the wastewater to 2-undecanone, and concomitantly back-extracted into the cell culture medium for biodegradation by suspended cells of *Pseudomonas putida* ATCC 11172. Cell growth in the HFMB was characterized by the absence of lag phase, high cell growth and biodegradation rates. For example, 1000 mg/L phenol was metabolized within 28 h, with specific growth rates and average biodegradation rates of 0.51 h<sup>-1</sup> and 59 mg/L-h, respectively. A kinetics model based on steady state mass transfer and Haldane growth kinetics was formulated to examine the mass transfer and biodegradation of phenol in the HFMB. The model described the experimental data with reasonable accuracy and the overall mass transfer coefficient on the shell and the tube sides were estimated using empirical correlations as  $3.83 \times 10^{-8}$  and  $1.26 \times 10^{-6}$  m/s, respectively. Phenol diffusion through the shell side boundary layer was the rate-limiting step. The model indicated that about 18% of the biomass in the HFMB was present on the surfaces as biofilms and the biodegradation kinetics of the biofilms was same as that of the suspended cells. Model simulations were carried out to examine the effects of various operating conditions on bioreactor performance, and HFMB performance improved significantly by increasing shell side flow rate.

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#### 1. Introduction

Two phase partitioning bioreactors (TPPBs) are based on the controlled delivery of a toxic substrate from an immiscible organic solvent to an aqueous cell culture medium, and subsequent biodegradation of the substrate in the aqueous phase [1]. By sequestering the bulk of the substrate into the organic phase, TPPBs enable suspended cells to withstand and metabolize inhibitory substrate concentrations. That these suspended cells could achieve high biodegradation rates has been demonstrated in the biodegradation of several aromatic compounds in TPPBs [2–5].

The drawbacks of aqueous/organic TPPBs arise mainly from phase dispersion, which results in foaming, emulsification and secondary effluent generation [6]. The physical proximity between the bacteria and the organic solvent requires biocompatible and non-bioavailable solvents, failing which cell growth and biodegradation may be jeopardized [7]. TPPBs also incur high operating costs on account of the high agitation speeds required to boost the mass transfer rates, and the large quantity of the solvent which is difficult to recycle. Furthermore, it is difficult to achieve simultaneous

extraction and biodegradation of the substrate directly from the wastewater in TPPBs.

One approach to mitigate these operating problems is the introduction of hollow fiber membranes between the aqueous cell culture medium and the organic solvent in TPPBs [8,9]. The resulting hollow fiber membrane bioreactor (HFMB) has the advantages of non-dispersive mass transfer, compact design, independent phase flow rates, high specific interfacial area and flexible configuration. Solvent-free aqueous phase in the HFMB also provides a better cell growth environment where microorganisms do not exhibit any lag phase [10]. However, the presence of membrane between the aqueous and the organic phases also results in additional mass transfer resistance during substrate diffusion from one phase to another [11]. Consequently, biodegradation rate in the HFMB may be limited by the substrate diffusion rate under a diminishing concentration gradient between the organic and the aqueous phases [9]. Moreover, bacteria in the HFMB readily attach to membrane to form biofilms. While biofilms lower the observed biomass yield in suspension, their presence can also influence biodegradation rate in the HFMB. In order to address these challenges, it is imperative that the substrate mass transfer mechanism and cell growth kinetics in the HFMB be elucidated, and contribution of biofilms in biodegradation be estimated through mathematical modeling.

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In this research, an HFMB was operated for simultaneous extraction and biodegradation of phenol from wastewater. A kinetics model has been formulated to gain insights into the mass transfer mechanism, cell growth and biodegradation in the HFMB. Phenol was chosen as the model pollutant as it is a highly toxic and recalcitrant aromatic compound commonly found in industrial effluents. The solvent 2-undecanone, which is biocompatible with *Pseudomonas putida* ATCC 11172 and has high affinity for phenol [2], was chosen as the organic phase.

#### 2. Materials and methods

#### 2.1. Microorganisms, culture conditions, and chemicals

*P. putida* ATCC 11172 was used throughout this study. 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 [12]. All media (except phenol), pipette tips, and Erlenmeyer flasks fitted with cotton plugs were autoclaved at 121 °C for 20 min 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

**Table 1**Characteristics of the membrane contactor.

Characteristics	Values
Casing material	Glass
Casing Inner diameter	0.7 cm
Membrane inner diameter, $d_{in}$	280 μm
Membrane thickness, $d_{out}$	50 μm
Effective fiber length, L	30 cm
Number of Fibers	150
Membrane packing density, $\phi$	0.44
Membrane porosity, $\varepsilon$	0.5
Membrane tortuosity, $ au$	3
Interfacial area, lumen, A <sub>in</sub>	0.0396 m <sup>2</sup>
Interfacial area, shell, A <sub>out</sub>	0.0537 m <sup>2</sup>
Hydraulic diameter, $d_h$	$4.8\times10^{-4}\text{m}$

inoculum. All the chemicals used in this research were of analytical grade.

#### 2.2. Hollow fiber membrane bioreactor (HFMB)

#### 2.2.1. Bioreactor setup

The membrane contactors were fabricated by potting commercial polypropylene hollow fiber membranes (Accurel PP 50/280, Membrana GmbH, Germany) into glass modules using epoxy resins (Araldite, England). Specifications for the HFMB are given in Table 1. Polypropylene membranes were chosen because they exhibited high stability during prolonged contact with 2-undecanone and the extraction performance remained stable for long periods of time.

Fig. 1 shows the schematic diagram of the experimental setup. Two peristaltic pumps (Masterflex, USA) were used to pump the aqueous feed and the cell culture medium from 500 mL Erlenmeyer flasks to the lumen of the respective membrane contactors, whereas 2-undecanone was recirculated into the shell side using a third peristaltic pump. The shell and lumen side flow rates were set at 4 and 6 mL/min, respectively. Purified air was sparged into the cell culture medium at 2 gas volume per reactor volume per minute (VVM). One of the membrane contactors was used for extraction of phenol from the feed solution to the organic solvent; phenol was then back-extracted from the solvent into the cell culture medium in the second membrane contactor.

#### 2.2.2. Bioreactor operation

Prior to HFMB operation, the membranes were wetted by pumping pure 2-undecanone in the shell side. The lumen side was then washed with sterilized ultrapure water to remove any solvent leaked into the lumen. The organic and aqueous phase volumes were 40 and 200 mL, respectively. Samples were collected from the three phases periodically to determine biomass and phenol concentrations. The pH of the cell culture was frequently measured and maintained in the range of 6.5–7.0. At the end of each run, the lumen was washed first with 1 M sodium hydroxide solution to remove loosely bound cells on the fibers and the tubings, and then twice with sterilized ultrapure water to wash away any remaining sodium hydroxide in the HFMB. Biodegradation in the

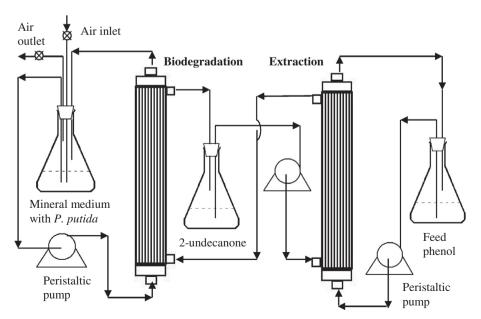


Fig. 1. Schematic diagram of the HFMB.

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