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External-loop fluidized bed airlift bioreactor (EFBAB) for the cometabolic biotransformation of 4-chlorophenol (4-cp) in the presence of phenol

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Abstract

The advantages from a 4-l external-loop inversed fluidized bed airlift bioreactor (EIFBAB) reported by Loh and Liu [2001. Chemical Engineering Science 56, 6171–6176] was synergized with preferential adsorption by granular activated carbon (GAC) for the enhanced cometabolic biotransformation of 4-chlorophenol (4-cp) in the presence of phenol as a growth substrate. This was achieved by incorporating a GAC fluidized bed in the lower part of the riser with the gas sparger relocated above this fluidized bed to avoid the presence of a 3-phase flow in the fluidized bed consequently providing larger gas holdup. Expanded polystyrene beads (EPS) were used as the supporting matrix for immobilizing *Pseudomonas putida* ATCC 49451, in the downcomer of the bioreactor. The hydrodynamics of the bioreactor system was characterized by studying the effect of the extent of valve opening, under cell-free condition, on gas holdup and liquid circulation velocity at different gas velocities and solids loading (EPS and GAC). The experimental data for gas holdup were modeled using power law correlations, while a Langmuir–Hinshelwood kinetics model was used for culation velocity. The bioreactor was tested for batch cometabolic biotransformation of 4-cp in the presence of phenol at various concentration ratios of phenol and 4-cp (ranging from 600 mg l^{-1} phenol: 200 mg l^{-1} 4-cp to 1600 mg l^{-1} phenol: 200 mg l^{-1} 4-cp) at 9% EPS loading and 2.8% (10 g) GAC loading. The 4-cp and phenol biotransformations were achieved successfully in the bioreactor system, which ascertained the feasibility of the bioreactor. Biotransformation of high 4-cp and phenol concentrations, which was oxygen limited, was also effectively achieved by increasing the gas holdup in the riser. This was possible in the current EFBAB system because of the synergistic effect of the GAC fluidized bed, the globe valve and cell immobilization by EPS.

Keywords: Airlift; Fluidized bed; Cell immobilization; Granular activated carbon adsorption; Gas holdup; Biodegradation

1. Introduction

Recalcitrant chlorinated organic solvents are often biodegraded through cometabolic pathways (Alvarez-Cohen and McCarty, 1991; Alexander, 1994; Wang and Loh, 1999). In cometabolism, the non-growth substrate is very often biotransformed in the presence of a specific growth substrate. In this case, the specific growth substrate is responsible for cell mass production but more importantly for the induction of the necessary enzymes that will catalyze the transformation of not only the growth substrate, but also the cometabolic transformation of the non-growth substrate.

Phenol and 4-chlorophenol (4-cp) constitute such a well studied cometabolic system (Saez and Rittmann, 1993; Wang and Loh, 1999). Here, phenol induces the NADH (or NADPH)-dependent monooxygenase and catechol dioxygenase, the first two enzymes required for its transformation. These same two enzymes can catalyze transformation of 4-cp to 4-chlorocatechol and 2-hydoxy-5-chloromuconic-semialdehyde, respectively. Given that phenol and 4-cp are structurally analogous, competitive inhibition exists when the cells are used to degrade these substrates. The deterioration in cell growth rate and degradation efficiency is further exacerbated by high

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concentrations of the growth substrate since phenol exerts substrate inhibition on cell growth and 4-cp is itself cytotoxic.

In a recent study, Wang (2003) recommended the use of granular activated carbon (GAC) for enhancing cometabolic biotransformation of 4-cp. It was found that GAC could preferentially adsorb 4-cp over phenol both thermodynamically and kinetically. As a result, competitive inhibition between phenol and 4-cp was minimized. Adsorption also caused a lowering of the phenol concentration in solution with a corresponding reduction in the substrate inhibition effect on cell growth.

Loh and Liu (2001) had developed a novel external-loop inversed fluidized bed airlift bioreactor (EIFBAB) for treating high strength phenolic wastewater. By installing a globe valve between the riser and the downcomer, gas holdup could be increased without significantly increasing the liquid circulation velocity. The feasibility of the EIFBAB for treating phenol up to 3000 mg l^{-1} was confirmed. In this setup, the bacteria were immobilized onto the fluidized expanded polystyrene (EPS) beads, which provided the much needed barrier between the cells and the toxic substrates/metabolites. We anticipated that the advantages reported for GAC could be incorporated into the EIFBAB for the enhanced cometabolic biotransformation of 4-cp and phenol at high substrate concentrations, hence resulting in this proof-of-concept investigation.

The specific objectives of this research were therefore to integrate a fluidized bed of GAC into the EIFBAB, to investigate and characterize the hydrodynamics of the bioreactor system so as to establish the relationship between gas holdup, gas flow rate, liquid circulation velocity, solids (EPS and GAC) loading and valve opening based on the experimental data and finally to examine the feasibility of using the modified external-loop fluidized bed airlift bioreactor (EFBAB) system for the cometabolic biotransformation of 4-cp in the presence of phenol.

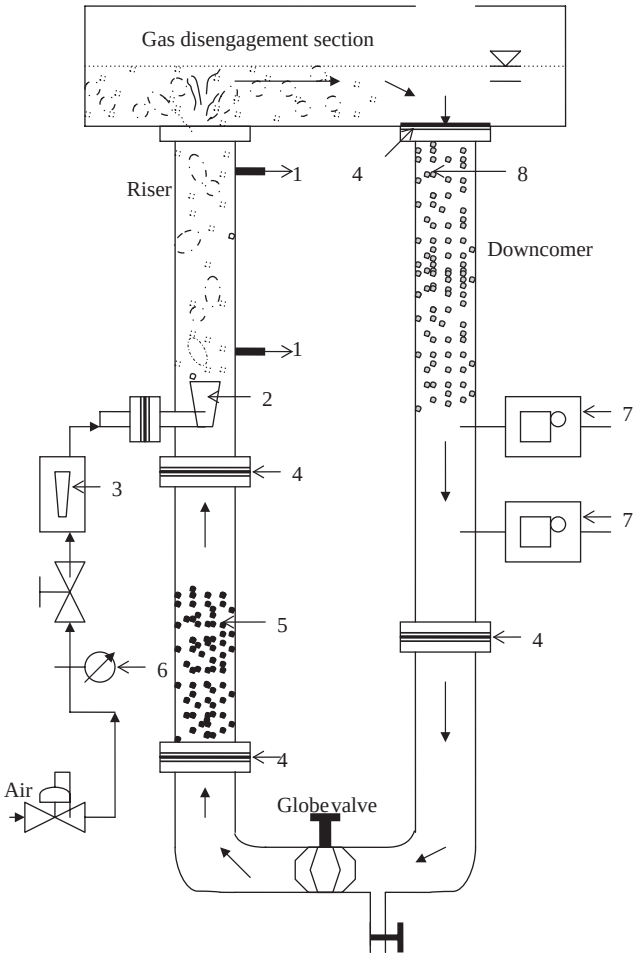

Fig. 1. Schematic diagram of the EFBAB system: (1) Manometer probes; (2) Air sparger; (3) Rotameter; (4) Stainless steel screens; (5) GAC; (6) Pressure gauge; (7) Conductivity probe and meter; (8) EPS beads.

2. Materials and methods

2.1. Bioreactor setup

Fig. 1 depicts the schematic representation of the EFBAB. The bioreactor had a working volume of 4 l and was constructed of Perspex material. Both the riser and the downcomer had inner diameters of 0.03 m. The downcomer was 0.786 m high and the riser was divided into two sections, one was a 0.45-m air sparged section and the other a 0.4-m GAC fluidization section. A stainless steel screen (mesh size #14) prevented GAC from overflowing from the fluidization section into the air sparged section. The effect of the stainless screen on gas holdup and liquid circulation velocity has been separately investigated. In both of these, the presence of the stainless steel screen exerted minimal effect. At the lowest gas velocity investigated (2.36 cm s^{-1}), the increase in gas holdup due to the screen was 2.5% and the decrease in liquid circulation velocity was 2%, while at the highest gas velocity studied (12 cm s^{-1}), gas holdup was increased only by 0.5% and liquid circulation velocity decreased by a mere 0.25%. Since, the bioreactor was operated well under the elutriation velocity of GAC, clogging was avoided to a considerable extent throughout the study. The rest of the details are similar to the EIFBAB system described by Loh and Liu (2001).

2.2. Microorganism and culture medium

Pseudomonas putida ATCC 49451, which grows on phenol and forms a biofilm on EPS beads, was used throughout this study. Nutrient agar slants were used to grow and maintain the stock cultures (Loh and Liu, 2001), which were stored at 4 °C.

A chemically defined medium (Loh and Liu, 2001) consisting of a mineral salt medium and a trace mineral solution