Journal of Environmental Chemical Engineering 4 (2016) 1523–1529

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

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

Biodegradation of phenol by calcium-alginate immobilized *Bacillus cereus* in a packed bed reactor and determination of the mass transfer correlation

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ARTICLE INFO

Article history:
Received 4 November 2015
Received in revised form 12 January 2016
Accepted 7 February 2016
Available online 10 February 2016

Keywords:
Phenol biodegradation
Packed bed reactor
Bacillus cereus
External film diffusion
Mass transfer coefficient

ABSTRACT

Biodegradation of phenol by calcium-alginate immobilized *Bacillus cereus* AKG1 MTCC9817 and *B. cereus* AKG2 MTCC9818 in a re-circulated up-flow packed bed reactor was performed. The observed biodegradation rate constants were calculated at various flow rates by assuming first-order biodegradation kinetics. The effect of external mass transfer combined with intrinsic biodegradation reaction on the observed biodegradation was investigated by the correlation between the Colburn factor (J_D) and Reynolds number (N_{Re}) as $J_D = K N_{\mathrm{Re}}^{-(1-n)}$ with values of K and n in the range of present experimentation as 1.34 and 0.35, respectively.

1. Introduction

Phenols, polycyclic aromatic hydrocarbons (PAH) and various heavy metals are the main toxic constituents of petroleum wastewater [1]. Phenol and other phenolic compounds, among these hazardous pollutants, pose serious environmental issues by contaminating nearby water courses easily due to their excellent water solubility and high toxicity [2]. Phenol can affect the aquatic micro-flora and fauna at a very low concentration of 5 mg/L and is lethal to fish at concentration of 5–25 ppm [3]. Human exposure to phenol causes critical health hazards and possible carcinogenesis [1–4]. Hence, the issue of reducing phenol level in industrial effluents to environmentally acceptable limits before releasing into the environment deserves critical attention [5]. The biodegradation of phenol, in this regard, appears to be the most environmentally benign method as compared to the conventional physico-chemical methods which are costly and also produce secondary hazardous pollutants [6,7]. However, substrate inhibition of microbial growth at higher concentrations of toxic pollutants such as phenol remains a critical challenge in biodegradation of industrial wastewater [8]. The immobilization of microorganisms on various supports has shown its potential for improving biodegradation efficiency in terms of sustainability as compared to free cells [9]. Whole cell entrapment in bio-polymeric beads [10] is one of the most well established methods of cell immobilization where immobilized cells show improved cell viability and can tolerate higher concentrations of toxicants for longer duration [11,12]. In this respect, Ca-alginate beads have been extensively studied for efficient entrapment of microbial cells owing to their low toxicity, ease of use and low cost [13–16].

The continuous mode of degrading toxic pollutants by the immobilized biomass, as compared to the batch mode, offers several advantages including the simplicity of automation and control resulting in a reduced operational cost and enhanced degradation [17,18]. The packed bed reactor (PBR) is the most convenient one among the wide range of reactor designs [19,20] available for continuous operation with immobilized cells. From the process-engineering perspective, a PBR has various advantages including high-yield operation, ease of scaling-up, possible automation of separation process leading to high degrees of purification, opportunity of treating large volume of wastewater continuously by a specified quantity of immobilized cells, and reuse of biomass [17,21]. Successful biodegradation of various toxic materials by immobilized cells in PBRs have been widely reported [22–24]. There are few reports on the continuous degradation of

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http://dx.doi.org/10.1016/j.jece.2016.02.012
2213-3437/

Nomenclature

Symbols

A	Column cross sectional area (cm^2)
A_m	Surface area per unit weight of immobilized bead (cm^2/g)
C	Outlet phenol concentration (mg/L)
C_0	Initial phenol concentration (mg/L)
C_S	Phenol concentration at cell surface (mg/L)
d_p	Immobilized bead diameter (cm)
D_f	Phenol diffusivity (cm/s)
G	Mass flux of phenol solution (g/cm^2 h)
h	Height of the reactor bed (cm)
J_D	Colburn factor (dimensionless)
k	Intrinsic first-order degradation rate constant (L/cm^2 h)
k_m	Mass transfer coefficient (L/cm^2 h)
k_p	Observed first-order biodegradation rate constant (L/g h)
K	Constant in Eq. (11)
N	Parameter in Eq. (13)
N_{Re}	Reynolds number ($=d_pG/\mu$)
Q	Volumetric flow rate (L/h)
r	Phenol removal rate (mg/g h)
r_m	Mass transfer rate (mg/g h)
W	Dry weight of biomass (g)
dz	Change in height of column (cm)
ε	Void fraction in packed bed
μ	Feed viscosity (g/cm s)
ρ	Feed density (g/cm^3)
ρ_p	Bead density (g/cm^3)

phenol in PBRs using cell-immobilized alginate beads as packing material. In one of the early reports, Aksu and Bülbül [21] investigated the combined effects of external mass transfer and biodegradation rates on phenol removal by immobilized *Pseudomonas putida* in a PBR. Later, Sheeja and Murugesan [18] systematically studied the biodegradation of phenol and phenolic effluents by alginate immobilized *Pseudomonas pictorum* in an up-flow PBR. Apart from the extensively studied *Pseudomonas* sp., recent attention has been drawn towards isolating and employing novel microorganisms for degrading phenol and phenolics more efficiently. To this end, the biodegradation of phenol by Ca-alginate immobilized *Ralstonia eutropha* in a PBR has been reported by Tepe and Dursun [17]. Furthermore, the parameters related to mass transfer are critical for the scale-up of PBR operation in recycle mode as the internal and external mass transfer resistances have been demonstrated to limit the mass transfer effects on immobilized particles in PBR [25].

In continuation to our research on the microbial treatment of petroleum wastewater, we have recently isolated and characterized two hyper phenol-tolerant *Bacillus cereus* strains (AKG1 MTCC9817 and AKG2 MTCC9818) from oil refinery and exploration sites, respectively [26]. Both the strains have shown promising potential for degrading high concentration of phenol in synthetic as well as real waste water sample [27,28]. To the best of our knowledge there is hardly any literature on the phenol degradation performances by the immobilized *B. cereus* strain in a PBR. Hence, in order to evaluate the feasibility in industrial application, it is of significant importance to further study the performance of these two strains, in the form of immobilized beads, in the degradation of phenol in a PBR. In the present report, the biodegradation of phenol by *B. cereus* immobilized Ca-alginate beads was investigated in a continuous packed bed reactor. Also, an attempt has been made to correlate the combined effect of external mass transfer and biochemical reaction rates on the biodegradation of phenol in order to estimate the reactor performance.

2. Theory

2.1. Biodegradation and observed rate constant

The material balance for phenol (substrate) in the PBR at steady state, considering spherical immobilized beads, no axial dispersion and plug flow, can be established as [17,29]:

$$\left(\frac{hQ}{W}\right)\frac{dC}{dz} = -r \tag{1}$$

where h is the height of the column (cm), Q is the volumetric flow rate (L/h), W is the total amount of dried cells in the immobilized particles (g), dC/dz is the concentration gradient along the column length (mg/L cm) and r is the biodegradation rate (mg/g h).

Assuming first-order biodegradation (a valid assumption at low phenol concentrations [30]), the relation between the observed biodegradation rate constant k_p (L/g h) and phenol concentration C (mg/L) in PBR can be expressed as:

$$r = k_pC \tag{2}$$

Substituting Eq. (1) with Eq. (2), the following equation can be obtained:

$$\left(\frac{hQ}{W}\right)\frac{dC}{dz} = -k_pC \tag{3}$$

Integrating Eq. (3) with boundary condition of $C = C_0$ at $z = 0$ and $C = C$ at $z = h$, Eq. (4) is obtained:

$$\ln\left(\frac{C_0}{C}\right) = \frac{W}{Q}k_p \tag{4}$$

where, C_0 and C are the initial and outlet phenol concentrations (mg/L), respectively. Different values of k_p can be calculated by using Eq. (4) at different initial flow rates with constant dried biomass amount in the immobilized beads [17,29,31].

2.2. Mass transfer and external film diffusion

As the fluid passes over the immobilized beads in a PBR, regions develop near the periphery of the beads where the velocity of the fluid is very low [31]. A near-stagnant film of fluid exists in such regions around the surface of the beads. Now, the substrate (such as phenol in the present study) needs to be transported through this fluid film, primarily by molecular diffusion. The observed reaction rate, hence, can be significantly affected by this external film diffusion [21,31].

The rate of film diffusion of the substrate (phenol) from the bulk fluid to the surface of the immobilized cells should be proportional to the area for mass transfer and the driving force for mass transfer which can be expressed by the concentration difference between the bulk and the external surface of the immobilized biomass. Hence, the following equation can be developed for the mass transfer:

$$r_m = k_mA_m(C - C_S) \tag{5}$$

where r_m is the mass transfer rate (mg/g h), k_m is the external mass transfer coefficient (L/cm^2 h), A_m is the surface area per unit weight of dried cells for mass transfer (cm^2/g), C is the phenol concentration in bulk liquid and C_S is the phenol concentration