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Combined effects of external mass transfer and biodegradation rates on removal of phenol by immobilized *Ralstonia eutropha* in a packed bed reactor

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Abstract

Biodegradation of phenol by calcium-alginate immobilized *Ralstonia eutropha* was carried out in a batch stirred and a packed bed reactor. In the batch system studies, the effect of initial phenol concentration on biodegradation was investigated at 30 °C and pH 7 while in the continuous system studies, the effects of flow rate and inlet phenol concentration on biodegradation were tested at the same temperature and pH. The observed biodegradation rate constant was calculated at different flow rates with the assumption of first-order biodegradation kinetics. Various external mass transfer correlations were evaluated and a new correlation of the type $J_D = K(N_{Re})^{-(n-1)}$ was developed with the values of $K = 1.34$ and $n = 0.65$. The intrinsic first-order biodegradation rate constants and the external mass transfer coefficients were calculated then the combined effects of these rates on the observed first-order biodegradation rate constants were also investigated.

Keywords: Phenol; Ca-alginate gel-immobilized *Ralstonia eutropha*; Biodegradation; Packed bed reactor; External mass transfer coefficient

1. Introduction

Phenols are considered as priority pollutants since they are harmful to organisms at low concentrations and many of them have been classified as hazardous pollutants because of their potential to harm human health. They exist in different concentrations in wastewaters originated from coking, synthetic rubber, plastics, paper, oil, gasoline, etc. Biological treatment, activated carbon adsorption and solvent extraction are the most widely used methods for removing phenol and phenolic compounds from wastewaters [1–4].

Biological treatment has been shown to be economical, practical and the most promising and versatile approach as it leads to complete mineralization of phenol. Many aerobic bacteria are capable of using phenol as the sole source of carbon and energy. Batch and continuous processes, employing either suspended or immobilized cultures are in use for the degradation of phenol [5–8].

Cell immobilization for removing of pollutants has been widely studied during the last decades due to a number of technical and economic advantages over free cell systems. Immobilization allows higher biomass concentration, minimization of inhibition, resistance to chemical environments and column operations and immobilized systems may be well suited for non-destructive recovery. Immobilization may also improve biomass performance, increase mechanical strength and facilitate separation of biomass from pollutant-bearing solution. Although numbers of advantages of using immobilized microorganisms, diffusion limitations are main disadvantages of the process. When biomass is immobilized the number of binding sites easily accessible to pollutant in solution is greatly reduced since the majority of sites will lie within the bead. So a good support material used for immobilization should be rigid, chemically inert and cheap, should bind cells firmly, should have high loading capacity and should have a loose structure for overcoming diffusion limitations. The entrapment of cells in Ca-alginate is a promising method for microbial degradation of toxic substances and has been used since 1975. Ca-alginate is not toxic to cells and the immobilization method is practical [9–13].

For continuous operation with immobilized biomass, the most convenient configuration is that of a packed column. Con-

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Nomenclature

a_s	surface area per unit weight of dried cells for mass transfer ($cm^2\ g^{-1}$)
A	column superficial cross-section area (cm^2)
C	outlet substrate (phenol) concentration ($mg\ L^{-1}$)
C_s	substrate (phenol) concentration ($mg\ L^{-1}$)
C_0	inlet substrate (phenol) concentration ($mg\ L^{-1}$)
d_p	particle diameter at the cell surface (cm)
D_S	substrate (phenol) diffusivity ($cm^2\ h^{-1}$)
G	mass flux of phenol solution ($G = Q\rho/A$) ($g\ cm^{-2}\ h^{-1}$)
h	height of the column (cm)
J_D	dimensionless group
k	intrinsic first-order biodegradation rate constant ($mL\ g^{-1}\ h^{-1}$)
k_L	mass transfer coefficient ($cm\ h^{-1}$)
k_p	observed first-order biodegradation rate constant ($mL\ g^{-1}\ h^{-1}$)
K	constant in Eq. (5)
N	the number of measurements
n	exponent in Eq. (5)
N	parameter given by Eq. (17)
N_{Re}	the Reynolds number in the packed bed ($N_{Re} = d_p G/\mu(1-\varepsilon)$)
N_{Sc}	the Schmidt number in the packed bed ($N_{Sc} = \mu/\rho D_S$)
Q	volumetric flow rate ($mL\ min^{-1}$)
r	biodegradation rate ($mg\ g^{-1}\ h^{-1}$)
r_m	mass transfer rate ($mg\ g^{-1}\ h^{-1}$)
X	the dried microorganism concentration ($g\ L^{-1}$)
w	dried microorganism quantity in packed bed (g)
z	the change of the height of the column (cm)

Greek letters

ε	void fraction in packed bed
μ	feed fluid viscosity ($g\ cm^{-1}\ h^{-1}$)
ρ	feed fluid density ($g\ mL^{-1}$)

tinuous packed bed process has a number of process engineering advantages including high yield operations and relatively easy scaling up from a laboratory-scale procedure. The stages in the separation protocol can also be automated and high degrees of purification can often be achieved in a single step process. A large volume of wastewater can be continuously treated using a defined quantity of immobilized cells in the column. Reuse of microorganism is also possible [11,14–16].

A large number of studies on the biodegradation of phenol by *Pseudomonas putida* have been made because of its high removal efficiency [14,17,18]. For this purpose, cells were entrapped in alginate beads [19] or in cellulose acetate [20] and hollow fibre polysulphone membranes [21]. Pazarlioğlu and Telefoncu [15] investigated the usage of the pumice for the immobilization of *P. putida* (DSMZ 50026) for biological phenol treatment systems. Also phenol biodegradation by suspended cells of *Rhodococcus* sp. P1 in continuous culture systems [22] and *Rhodococcus* sp. immobilized in calcium-alginate beads or on granular activated carbon [23] have been reported. Prieto et al. [24] evaluated the performance of a laboratory-scale reactor consisting of a column packed with Biolite® beads which support on its external surface an adsorbed network of *Rhodococcus erythropolis* UPV-1 cells in biodegradation of phenol. Murugesan and Sheeja [25,26] studied on degradation of phenolic effluents. For this purpose, a phenol degrading strain *Pseudomonas pictorum* (NCIM 2077) was immobilized on alginate and activated carbon-alginate mixtures, respectively and used as packing material in a packed bed reactor. Phenolic effluents were degraded in the packed bed reactor at different flow rates.

Although, *Pseudomonas* sp. have been widely used to treat phenol/phenolics, recently considerable attention has been directed towards new and more efficient microorganisms for this purpose. *Ralstonia eutropha* is one of these microorganisms and little is known about the biodegradation of phenol by Ca-alginate immobilized *R. eutropha* in a packed bed reactor. In the present study, the biodegradation of phenol was performed in a batch stirred and continuous packed bed column reactor using Ca-alginate immobilized *R. eutropha*. At steady state, the combined effect of external mass transfer with biochemical reaction on the observed reaction rate was evaluated and a new correlation was developed.

2. Theory

2.1. Biodegradation

Considering steady state, plug flow, no axial dispersion and spherical immobilized particles. The material balance for phenol in the packed bed reactor can be written as the following equation [14,27]:

$$\left(\frac{hQ}{w}\right)\frac{dC}{dz} \times 6 \times 10^{-2} = -r \quad (1)$$

where h is the height of the column (cm), Q the volumetric flow rate ($mL\ min^{-1}$), w the total amount of dried cells in the immobilized particles (g), dC/dz the concentration gradient along the column length ($mg\ L^{-1}\ cm^{-1}$) and r is the biodegradation rate ($mg\ g^{-1}\ h^{-1}$).

The relationship between the biodegradation rate and the substrate (phenol) concentration in the column is given as Eq. (2) assuming that first-order biodegradation (this is a correct assumption especially at low phenol concentrations [8]):

$$r = k_p C \quad (2)$$

where k_p is the observed first-order biodegradation rate constant ($mL\ g^{-1}\ h^{-1}$). After substituting Eq. (2) into Eq. (1) and integrating this equation with boundary conditions of at $z = 0$; $C = C_0$ and at $z = h$; $C = C$, Eq. (3) is obtained:

$$\ln\left(\frac{C_0}{C}\right) = \frac{w}{Q}k_p\left(\frac{10^3}{60}\right) \quad (3)$$

where C_0 and C are the inlet and outlet substrate concentrations (mg L^{-1}), respectively. k_p values can be calculated from Eq. (3) at different flow rates for a constant dried cell quantity in the immobilized particles [27,28].

2.2. Combined mass transfer and biodegradation reaction

Fluid passing over the surface of immobilized particles in a packed bed reactor develops a boundary layer in which the velocity varies rapidly over a very short distance. Very near the surface the fluid velocity is low there is a little mixing. Transport normal to the surface is by molecular diffusion and external mass transfer resistant may cause significant reduction in the observed reaction rate [29–32].

The mass transfer rate (r_m) of phenol from the fluid bulk to the surface of the immobilized beads is proportional to the area of mass transfer (a_s) and the concentration difference between the bulk (C) and the external surface of the immobilized beads (C_s):

$$r_m = k_L a_s (C - C_s) \times 10^{-3} \quad (4)$$

where k_L is the external mass transfer coefficient (cm h^{-1}). It is common practice to correlate the mass transfer coefficient with fluid properties in term of J_D-factor, which is defined as the following equation [14,27,32,35]:

$$J_D = \frac{k_L \rho}{G} N_{Sc}^{2/3} = K N_{Re}^{-(1-n)} \quad (5)$$

where ρ is the fluid density (g mL^{-1}), G the mass flux (g cm^{-2} h^{-1}) calculated from following equation, N_{Sc} and N_{Re} the Schmidt and Reynolds numbers, respectively [27,31].

Different external mass transfer correlations, which have various K and n values, have been developed in literature. The value of n varies from 0.1 to 1.0 in these correlations.

Nath and Chand [28] presented the following correlation for bioconversion of sugars to ethanol in the immobilized yeast cells on activated bagasse chips:

$$J_D = 5.7 N_{Re}^{-0.59} \quad (6)$$

Another correlation (Eq. (7)) was proposed by McCune and Wilhelm [33]. Rovito and Kittrell [34] showed the applicability of this correlation to glucose oxidase enzyme immobilized on porous glass beads system:

$$J_D = 1.625 N_{Re}^{-0.507} \quad (7)$$

This correlation was also applied successfully for biodegradation of phenol by immobilized *P. putida* [14] and biodegradation of ferrous(II) cyanide complex ions by immobilized *Pseudomonas fluorescens* [35].

Wilson and Geankoplis [36] showed the applicability of the following correlation for the external mass transfer from the liquids in the packed bed reactor:

$$J_D = 1.34 N_{Re}^{-0.28} \quad (8)$$

Assuming that nonporous immobilized particles and first-order biodegradation reaction, the biodegradation rate can be given as follows [13]:

$$r = k C_s \quad (9)$$

where k is the intrinsic first-order biodegradation rate constant (mL g^{-1} h^{-1}). At steady state, the rate of mass transfer equals to rate of the biodegradation. Thus equating Eq. (4) with Eq. (9) and solving for the unknown surface concentration Eq. (10) is obtained [27]:

$$C_s = \frac{k_L a_s C}{k + k_L a_s} \quad (10)$$

The effects of mass transfer and biodegradation rates on the observed biodegradation rate are given in the following equations:

$$k_p = \frac{k k_L a_s}{k + k_L a_s} \quad (11)$$

or

$$k_p = \frac{1}{(1/k) + (1/k_L a_s)} \quad (12)$$

Eq. (12) shows that the effects of reaction and mass transfer are additive. Rearranging Eq. (5) and solving for mass transfer coefficients, the equation becomes:

$$k_L = N G^n \quad (13)$$

where

$$N = \frac{K}{\rho} \left(\frac{\mu}{\rho D_S} \right)^{-2/3} \left(\frac{d_p}{\mu} \right)^{-(1-n)} \quad (14)$$

Substituting Eq. (13) into Eq. (11) and rearranging to yield:

$$\frac{1}{k_p} = \frac{1}{N a_s} \frac{1}{G^n} + \frac{1}{k} \quad (15)$$

The plots of $(1/k_p)$ versus $1/G^n$ for different K and n values in mass transfer correlations, yields straight lines of slope $(1/Na_s)$ and intercept $(1/k)$. The external mass transfer coefficients can be calculated from Eq. (13) for different flow rates. Then using a_s, k and k_L values, new k_p values can be calculated from Eq. (11). The J_D correlation predicts accurately our experimental data can be chosen for this system by comparing these k_p constants with the k_p constants found from Eq. (3). Then, comparing the k and $k_L a_s$ constants, it is also possible to decide which step has limited the biodegradation rate.

3. Material and methods

3.1. Microorganism and culture conditions

R. eutropha (NRRL B-14690), provided by American Type Culture Collection was used throughout the study. Growth was performed at 30 °C in a agitated (100 rpm) enrichment medium which contained (amounts given per L); glucose, 3 g; yeast extract, 2 g; peptone, 2 g; KH_2PO_4, 1 g; K_2HPO_4, 1 g; $(NH_4)_2SO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; 15 g L^{-1} agar was used for solid growth media. All media were sterilized by

autoclaving at 1.1 atm and 121 °C. After the culture was inoculated into 100 mL enrichment medium (in 1:10 ratio) in a 250 mL conical flask, it was incubated in an agitated shaker for 24 h.

3.2. Preparation of immobilized R. eutropha beads

In the stationary phase of growth, *R. eutropha* cells were centrifuged and resuspended in 2% sodium alginate. The ratio of Na-alginate to biomass was chosen as 3.0. Then this mixture was dropped into 20% calcium chloride solution by using a peristaltic pump. The drops of Na-alginate solution gelled into 0.2 cm diameter sphere upon contact with calcium chloride solution. Ca-alginate gel-immobilized *R. eutropha* particles have been stored in calcium chloride solution at 4 °C for at least 2 h to complete gel formation. Thus insoluble and stable immobilized beads were obtained.

3.3. Batch stirred reactor studies

In immobilized microorganism studies, the salts containing K^+, Mg^{2+}, PO_4^{3-} ions caused the dissolution of calcium-alginate beads. So the composition of the biodegradation medium was optimized to improve the stability of beads [12,13]. The biodegradation medium was prepared by diluting 1.0 g L^{-1} of stock phenol solution to desired concentration and adding sufficient quantities of nutrients as: yeast extract, 0.01 g L^{-1}; peptone, 0.04 g L^{-1}; $(NH_4)_2SO_4$, 0.015 g L^{-1}; KH_2PO_4, 0.007 g L^{-1} and $MgSO_4 \cdot 7H_2O$, 0.05 g L^{-1}. After the sterilization by autoclaving at 1.1 atm and 121 °C, the pH of the medium was adjusted to the desired value by using sterilized H_2SO_4 or NaOH solutions.

Biodegradation studies were carried out in 0.25 L Erlenmeyer flasks containing 0.1 L biodegradation medium on a rotary shaker at 100 rpm constant agitating rate. After the transference of a known weight of immobilized microorganism (containing 0.121 g of dried *R. eutropha* cells) into the biodegradation medium, samples were taken out at fixed time intervals and analyzed for phenol as described below. The experiments were continued for 48 h. Small differences (<0.5) were observed in pH during the biodegradation so pH changes during the experiments were neglected. Control experiments in a microorganism free medium (including phenol and other constituents) were also done in order to evaluate the possible degree of phenol removal with volatilization and it was seen that the phenol concentration remained unchanged. All the experiments were carried out in duplicates and average values were used for further calculations.

3.4. Packed bed reactor studies

A packed bed reactor with a 2.0 cm inside diameter was used in the studies. The bed depth was kept at 27.5 cm and the column contained 68.34 g of immobilized cell beads contain 48.52 g of wet cells which is equal to 10.45 g of dried microorganism. Average immobilized particle size 0.2 cm and immobilized particle density is 1.25 g mL^{-1}. The temperature of column during the experiments was kept constant at 30 °C. Feed solution was prepared as explained in Section 3.3. The schematic of the experimental set-up was shown in Fig. 1.

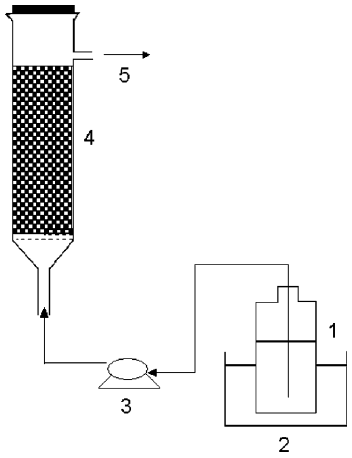

Fig. 1. Schematic of the experimental set-up: (1) Feed solution tank; (2) constant temperature water bath; (3) pump; (4) packed bed reactor; (5) outlet stream.

3.5. Analysis

The concentration of residual phenol in the biodegradation media was determined spectrophotometrically (Spectronic 20D). The absorbance of the colored complex of phenol and *p*-nitroaniline was read at 470 nm [37].

4. Results and discussion

4.1. Batch stirred reactor studies

The previous batch reactor studies on biodegradation of phenol by free and calcium-alginate gel immobilized *R. eutropha* showed that, immobilization increased the stability of the cells and the immobilized cells could tolerated the changes in pH and temperature of the biodegradation medium while free cells were more sensible against pH and temperature [8]. The optimum pH and temperature values were chosen as 7.0 and 30 °C, respectively for further experiments.

The biodegradation rate of phenol in a batch system is defined as

$$r = -\frac{1}{X}\frac{dC_s}{dt} \tag{16}$$

where X is the dried microorganism concentration (g L^{-1}). The biodegradation rate was determined from the slope of phenol consumption versus time plot. The fixed dried cell concentration in the immobilized beads was used as the cell concentration (X). The phenol removal yield was defined as removal $\% = 100 \times (C_0 - C)/C$, where C_0 and C are initial and phenol concentration at $t = 24$ h (mg L^{-1}).

In order to determine the effect of initial phenol concentration on phenol removal rate a series batch experiments were conducted. In these experiments, initial phenol concentration varied in the range 25–500 mg L^{-1}. Fig. 2 shows that immobilized *R. eutropha* could tolerate the toxicity of phenol

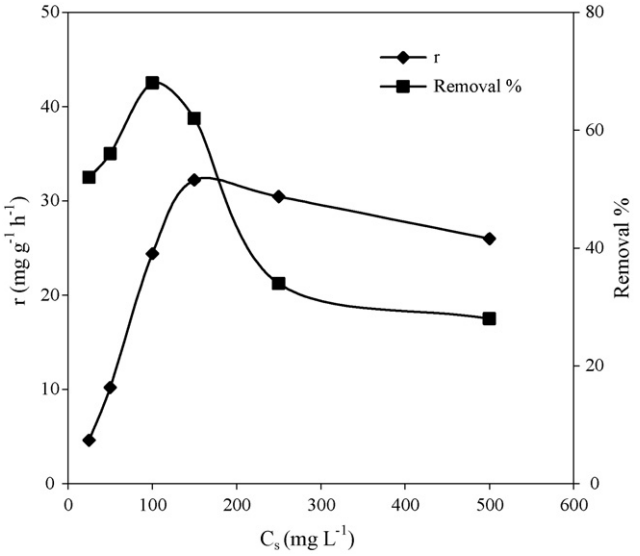

Fig. 2. Effect of initial phenol concentration on phenol biodegradation (pH 7, $T = 30\,^{\circ}C$, agitation rate = 100 rpm, $X = 0.121$ g).

up to high phenol concentration without loss of cell viability. So phenol removal rate increased from 4.6 mg g^{-1} h^{-1} to 32.2 mg g^{-1} h^{-1} with increasing initial phenol concentration from 25 mg L^{-1} to 150 mg L^{-1}, than remained nearly constant. Fig. 2 also shows that, phenol removal yields showed similar trend as the initial phenol concentration was increased. The maximum phenol removal % was determined as 68% at 100 mg L^{-1} initial phenol concentration. Then it started decreasing.

4.2. Packed bed studies

In a specific continuous packed bed reactor, with up-flow mode of operation, the mass transfer consideration indicates that, the biodegradation rate can be affected by operating parameters such as flow rate, substrate concentration, particle and column size and biomass quantity. As the flow rate was one of the most important parameters affecting the observed biodegradation rate, at the first stage of the column studies, the effect of flow rate was investigated. The flow rate was varied from 0.12 mL min^{-1} to 0.71 mL min^{-1} while initial phenol concentration kept constant at 100 mg L^{-1}. The observed biodegradation rates were evaluated from Eq. (1) and the variation of these values with flow rates was presented in Fig. 3. At low flow rates, low biodegradation rates were obtained ($r = 0.061$ mg g^{-1} h^{-1} for $Q = 0.12$ mL min^{-1}) because of mass transfer resistance at liquid film layer. An increasing in flow rate caused reduction in these mass transfer limitations so higher reaction rates were obtained at higher flow rate ($r = 0.153$ mg g^{-1} h^{-1} for $Q = 0.52$ mL min^{-1}). It was also observed that, a further increase in flow rate resulted in a decrease in reaction rate because of insufficient residence time of the reactants in the column. The experimental values of Q, C/C_0 and k_p evaluated from Eq. (3) at 100 mg L^{-1} of inlet phenol concentration are given in Table 1. It is reported that the observed first-order biodegradation rate constant increased from

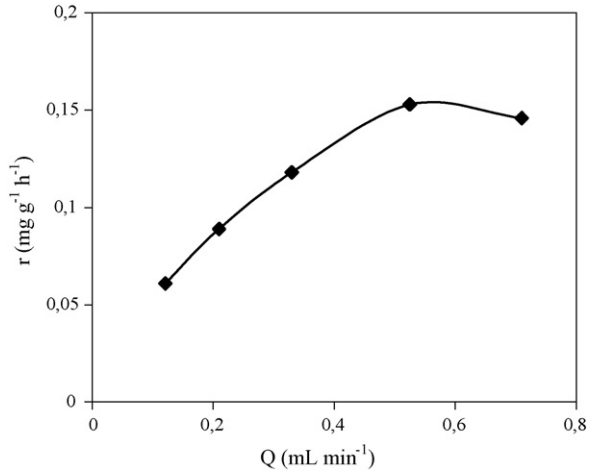

Fig. 3. Effect of flow rate on the observed reaction rate (pH 7, $T = 30\,^{\circ}C$, $C_0 = 100$ mg L^{-1}, $w = 10.45$ g).

1.47 mL g^{-1} h^{-1} to 2.14 mL g^{-1} h^{-1} with increasing flow rate from 0.12 mL min^{-1} to 0.52 mL min^{-1}.

The effect of inlet phenol concentration on the observed biodegradation rate was investigated at 0.33 mL min^{-1} flow rate and inlet phenol concentration was varied from 25 mg L^{-1} to 500 mg L^{-1}. The immobilized microorganism could be exposed to high phenol concentrations without loss of cell viability because of the protecting effect of immobilization. Thus observed biodegradation rates increased from 0.036 mg g^{-1} h^{-1} to 0.152 mg g^{-1} h^{-1} with increasing inlet phenol concentration from 25 mg L^{-1} to 150 mg L^{-1} and no significant changing was obtained with further increasing phenol concentration (Fig. 4). A high initial phenol concentration also provided an important driving force to overcome all mass transfer limitations. Hence a higher initial phenol concentration increased the observed reaction rate up to 150 mg L^{-1} inlet phenol concentration.

Pazarlioğlu and Telefoncu [15] found that, the maximum phenol degradation level of 99% was reached at a phenol loading rate of 0.001–0.002 g L^{-1} for *P. putida* immobilized on pumice. Prieto et al. [24] reported that, immobilized cells of *R. erythropolis* UPV-1 were able to degrade phenol at a maximum rate of 18.0 g L^{-1} day^{-1} at 200 mg L^{-1} initial phenol concentration and at 2.5 mL min^{-1} flow rate. Mordocco et al. [19] determined the phenol degradation rate as 1.4 g L^{-1} day^{-1} for *P. putida* immobilized in calcium-alginate beads and Pai et al. [23] found the phenol degradation rate as 2.1 g L^{-1} day^{-1} or 2.9 g L^{-1} day^{-1} for *Rhodococcus* sp. immobilized in calcium-alginate beads or on granular activated carbon, respectively. Although, the results obtained in this study are difficult to be

Table 1
The experimental values of Q, C/C_0 and k_p calculated from Eq. (3) (pH 7, $T = 30\,^{\circ}C$, $C_0 = 100$ mg L^{-1}, $w = 10.45$ g)

Q (mL min^{-1})	C/C_0	k_p (mL g^{-1} h^{-1})
0.12	0.120	1.47
0.21	0.261	1.62
0.33	0.375	1.86
0.52	0.491	2.14

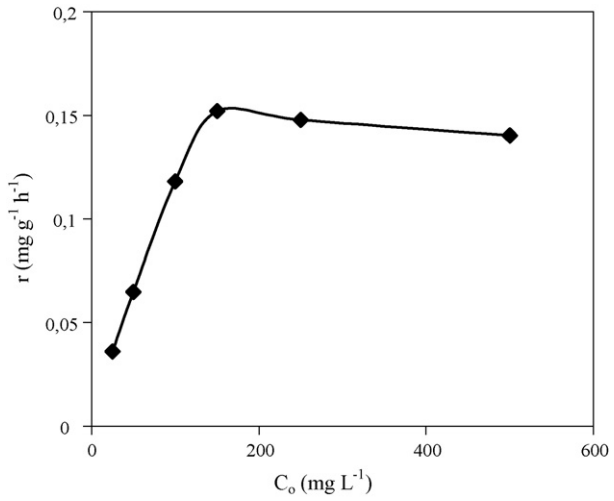

Fig. 4. Effect of inlet phenol concentration on the observed reaction rate (pH 7, $T = 30\,^{\circ}C$, $Q = 0.33\,mL\,min^{-1}$, $w = 10.45\,g$).

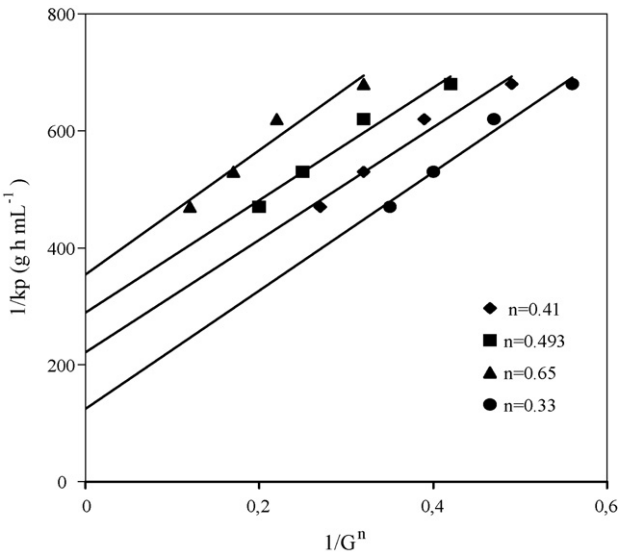

Fig. 5. Plots of $1/k_p$ vs. $1/G^n$ for various values of n.

compared with those obtained in the literature because they are usually expressed in different units and proceed from experiments performed under different experimental conditions using distinct microbial strains, the comparison of the results obtained in this study with those obtained in the literature shows that, the phenol biodegradation rate ($0.153\,mg\,g^{-1}\,h^{-1}$ at $100\,mg\,L^{-1}$ initial phenol concentration and at $0.52\,mL\,min^{-1}$ flow rate) found in this work is comparable to these values found in the literature.

For investigating the external film diffusion effects on the observed biodegradation rate, mass fluxes and Reynolds numbers at studied flow rates were also calculated using $d_p = 0.2\,cm$, $\mu = 0.845\,cp$, $\rho = 0.99814\,g\,mL^{-1}$, $\varepsilon = 0.33$ and $A = 3.14\,cm^2$ values which were determined from experimental data. Table 2 shows, the calculated values of G, N_{Re}, $1/k_p$ and $1/G^n$ at all studied flow rates for different n values ($n = 0.33$, 0.41 and 0.493). In the previous studies it was found that the biodegradation of phenol by *R. eutropha* followed first-order reaction kinetics at low phenol concentrations with high correlation coefficients ($R^2 = 0.97$ at $100\,mg\,L^{-1}$ initial phenol concentration) [8]. So in the present study, the first-order reaction kinetics was used. The plots of $1/k_p$ versus $1/G^n$ for all n values were presented in Fig. 5. The values of intrinsic first-order biodegradation rate constant and a_s were calculated from the intercepts and slopes of the plots and were given in Table 3.

Using phenol diffusivity found from the literature at $T = 30\,^{\circ}C$ [38], mass transfer coefficients were obtained from Eq. (13) for all mass transfer models. The $k_L a_s$ values were also calculated using a_s values found at the same n values (Table 4). Since the k and $k_L a_s$ values were known, k_p values were determined from Eq. (11) again and in order to demonstrate the validity of the mass transfer models, these values were compared with the k_p constants determined from Eq. (3) in Table 5. As seen from the table, although mass transfer models could give satisfactory straight lines, there were some differences between k_p values found from Eqs. (11) and (3). So it was decided that, none of the mass transfer correlations examined were suitable for our system. In order to obtain a new correlation that represent the experimental data more accurately, a range of assumption was made for different k and n values ($0 < n < 1.0$). New k_p values were obtained and these values were tested as explained before. After several assumption it was reported that for the estimated value of $K = 1.34$ and $n = 0.65$, the k_p values were in good agreement for all flow rates studied. The results of the calculations for the estimated values of K and n were also presented in Fig. 5 and Tables 2–5. In order to compare the validity of the new mass transfer correlation more definitely, a normalized deviation

Table 3
The values of k and a_s for various values of n

K	n	k (mL g^{-1} h^{-1})	a_s (cm^2 g^{-1})
3.333	0.330	8.02	0.98
5.700	0.410	4.51	0.89
1.625	0.493	3.46	4.72
1.340	0.650	2.82	11.47

Table 2
Calculated values of G, N_{Re}, $1/k_p$ and $1/G^n$ found at various flow rates (pH 7, $T = 30\,^{\circ}C$, $C_0 = 100\,mg\,L^{-1}$, $w = 10.45\,g$)

G (g cm^{-2} h^{-1})	N_{Re}	$k_{p(3)}$ (mL g^{-1} h^{-1}	$1/k_p$ (g h mL^{-1})	$1/G^{0.33}$	$1/G^{0.41}$	$1/G^{0.493}$	$1/G^{0.65}$
5.78	0.037	1.47	0.68	0.56	0.49	0.42	0.32
10.00	0.065	1.62	0.62	0.47	0.39	0.32	0.22
15.76	0.103	1.86	0.53	0.40	0.32	0.26	0.17
25.07	0.164	2.14	0.47	0.35	0.27	0.20	0.12

Table 4
The variation of k_L and k_La_s values with flow rates for various values of n

Q (mL min^{-1})	$n=0.33$		$n=0.41$		$n=0.493$		$n=0.65$	
	k_L (cm h^{-1})	k_La_s (mL g^{-1} h^{-1})	k_L (cm h^{-1})	k_La_s (mL g^{-1} h^{-1})	k_L (cm h^{-1})	k_La_s (mL g^{-1} h^{-1})	k_L (cm h^{-1})	k_La_s (mL g^{-1} h^{-1})
0.12	1.49	1.46	1.72	1.53	0.32	1.51	0.26	2.98
0.21	1.67	1.64	1.94	1.73	0.36	1.70	0.37	4.24
0.33	1.85	1.81	2.14	1.90	0.40	1.89	0.49	5.62
0.52	2.05	2.00	2.37	2.10	0.44	2.08	0.67	7.68

Table 5
The comparison of the observed first-order biodegradation rate constants calculated from Eq. (3) with the ones calculated from Eq. (14) found at all flow rates for various values of n

Q (mL min^{-1})	$k_{p(3)}$ (mL g^{-1} h^{-1})	$n=0.33$		$n=0.41$		$n=0.493$		$n=0.65$	
		$k_{p(11)}$ (mL g^{-1} h^{-1})	$\Delta\%$	$k_{p(11)}$ (mL g^{-1} h^{-1})	$\Delta\%$	$k_{p(11)}$ (mL g^{-1} h^{-1})	$\Delta\%$	$k_{p(11)}$ (mL g^{-1} h^{-1})	$\Delta\%$
0.12	1.47	1.24		1.14		1.05		1.45	
0.21	1.62	1.36	19.3	1.25	26.6	1.14	32.9	1.69	2.5
0.33	1.86	1.48		1.34		1.22		1.87	
0.52	2.14	1.60		1.43		1.30		2.06	

Table 6
The comparison of the combined effects of intrinsic of first-order biodegradation rate constant and mass transfer coefficients on the observed of first-order biodegradation rate constant

k_P (mL g^{-1} h^{-1})	k (mL g^{-1} h^{-1})	$1/k$ (g h mL^{-1})	% Contribution of k	k_La_s ($\times10^3$ mL g^{-1} h^{-1})	$1/k_La_s$ (g h^{-1} mL^{-1})	% Contribution of k_La_s
1.45			51	2.98	0.335	49
1.69	2.82	0.354	60	4.24	0.235	40
1.87			66	5.62	0.178	34
2.06			73	7.68	0.130	27

($\Delta\%$) was calculated as follows:

$$\Delta\% = \frac{\sum_{i=1}^{N} \left| \frac{k_{P(3)} - k_{P(11)}}{k_{P(3)}} \right|}{N} \times 100 \quad (17)$$

where the subscripts 'p(3)' and 'p(11)' show the k_p values calculated from Eqs. (3) and (11) at various n values, respectively and M is the number of measurements. The normalized deviation values were given in Table 5. It was reported that ($\Delta\%$) values obtained for $n=0.65$ is lower than that of other n values. In the view of these results, it can be said that, with the estimated values of $K=1.34$ and $n=0.65$, the mass transfer correlation:

$$J_D = 1.34 N_{Re}^{-0.35} \quad (18)$$

accurately predicts our experimental data for the biodegradation of phenol in the packed bed reactor.

The value of the constant K was determined by Sheeja and Murugesan as 1.56 and 2.26 ($n=0.72$) for *P. pictorum*-alginate beads and activated carbon-*P. pictorum*-alginate beads, respectively [25,26]. In another study, the values of K and n for biodegradation of phenol by Ca-alginate immobilized *P. putida* were reported as 1.625 and 0.49 by Aksu and Bülbül, respectively [14].

Table 6 shows, the comparison of combined effects of intrinsic first-order biodegradation rate constant and external mass transfer coefficients on the observed first-order biodegradation rate constants for $n=0.65$. It was reported that both the external film diffusion and the biochemical reaction limited the biodegradation of phenol and an increase in flow rate caused reduction in external mass transfer limitations.

5. Conclusion

Biodegradation of pollutants by immobilized cells in packed bed reactor is a technically efficient and economically feasible technology for removal of organic pollutants. In the present research, the biodegradation of phenol from aqueous solution by immobilized *R. eutropha* was performed in a batch stirred and a packed bed reactor. Maximum phenol removal efficiency was determined as 68% in the batch studies. Continuous system results showed that, the biodegradation rate of phenol is dependent both the flow rate and the inlet phenol concentration. The combined effect of external mass transfer with biochemical reaction on phenol removal is analyzed and a mass transfer correlation (Eq. (18)) was developed which represents the present experimental data accurately. This proposed correlation would

be useful for the design and development of up-flow packed bed reactors for the continuous degradation of phenol. Furthermore the methodology used in this work permits us to make realistic engineering estimates of the effects of external mass transfer on the observed biodegradation rates in immobilized packed bed reactors. It was also reported that the effect of external mass transfer limitations on observed reaction rate was significant and should not be ignored in any engineering analysis but it decreased with increasing flow rate.

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