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# Internal mass transfer effect on biodegradation of phenol by Ca-alginate immobilized *Ralstonia eutropha*

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**Abstract**

Phenol biodegradation by free and Ca-alginate immobilized *Ralstonia eutropha* was performed in batch system. Optimum initial pH and temperature were determined as 7 and 30 °C, respectively for free cells, while a wide pH and temperature range were obtained for immobilized cells. Phenol had a strong inhibitory effect on the microbial growth and Haldane model was used to describe the substrate inhibition. Model parameters were determined as $\mu_{max} = 0.89\,h^{-1}$, $K_S = 55.11\,mg\,dm^{-3}$ and $K_I = 257.94\,mg\,dm^{-3}$ by non-linear regression analysis. The effective diffusion coefficient of phenol in immobilized particles was calculated. For this purpose, using biodegradation rates experimental effectiveness factors were determined for different sized immobilized particles. The Thiele modulus was evaluated from experimental effectiveness factors. Then the average effective diffusion coefficient was calculated as $1.21 \times 10^{-7}\,cm^2\,s^{-1}$. These results showed that intraparticle diffusion resistance was important for this system and could not be ignored.


*Keywords:* Phenol; Biodegradation; Immobilized *Ralstonia eutropha*; Mass transfer; Diffusion limitation; Effective diffusion coefficients

## 1. Introduction

Many industries such as petroleum and petrochemical, coal conversion, pesticide, paint, paper and pulp industries release phenolic compounds to the environment. Phenols are toxic to microorganisms, animals and human being at low concentrations and they have been classified as hazardous pollutants. The maximum concentration of total phenols in drinking water is given as $0.5\,\mu g\,dm^{-3}$ by the European Union [1–3]. Different methods designed to remove phenols have been proposed. Adsorption by activated carbons is the most frequently used method [4,5]. But activated carbon is expensive and the higher the quality, the greater the cost. Both chemical and thermal regeneration of the poisoned carbon are expensive impractical on a large scale, produce additional effluent and results in considerable loss of the adsorbent. Other methods include, aerobic and anaerobic biodegradation, oxidation by ozone and ion exchange, etc. [1,6,7]. Among these methods, biological methods have been shown to be practical, economical as it leads to complete mineralization of phenol. Several aerobic microorganisms are capable of utilizing phenols as the sole carbon and energy sources in either pure or mixed cultures [1,7–9].

A large number of studies on the biodegradation of phenol by *Pseudomonas putida* have been made because of its high removal efficiency [9,10]. Recently, considerable attention has been directed towards new, more efficient microorganisms for this purpose. *Ralstonia eutropha* is one of these microorganisms and little is known about the biodegradation of phenol by free and Ca-alginate immobilized *R. eutropha* and effect of internal diffusion limitations to the biodegradation.

Immobilization of microorganisms on inert supports shows an increasing interest since this strategy allows obtaining much more profit from the process. Immobilization may improve microbial performance, and provide good operational stability. The main advantages in the use of immobilized cells in comparison with suspended ones include the

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**Nomenclature**

| | |
|---|---|
| $C$ | phenol concentration within Ca-alginate bead (mg dm$^{-3}$) |
| $C_0$ | initial phenol concentration (mg dm$^{-3}$) |
| $C_S$ | substrate concentration at the Ca-alginate immobilized *R. eutropha* bead surface (mg dm$^{-3}$) |
| $d_p$ | particle diameter (cm) |
| $D_e$ | effective diffusivity of phenol within Ca-alginate immobilized *R. eutropha* bead (cm$^2$ s$^{-1}$) |
| $k$ | first-order biodegradation rate constant (dm$^3$ g$^{-1}$ h$^{-1}$) |
| $k'$ | first-order biodegradation rate constant given in Eq. (6) (dm$^3$ dm$^{-3}$ s$^{-1}$) |
| $K_I$ | substrate inhibition constant (mg dm$^{-3}$) |
| $K_S$ | substrate affinity constant (mg dm$^{-3}$) |
| $r$ | radial position within the bead (cm) |
| $R$ | radius of particle (cm) |
| $t$ | time (h) |
| $T$ | temperature (°C) |
| $v$ | actual biodegradation (phenol removal) rate (mg g$^{-1}$ h$^{-1}$) |
| $v_s$ | biodegradation (phenol removal) rate at the outer surface of Ca-alginate immobilized *R. eutropha* beads (mg g$^{-1}$ h$^{-1}$) |
| $w$ | the amount *R. eutropha* of dried cells in the immobilized beads (g) |
| $X$ | dried microorganism concentration (g dm$^{-3}$) |

*Greek letters*

| | |
|---|---|
| $\eta$ | effectiveness factor |
| $\mu$ | microorganism's specific growth rate (h$^{-1}$) |
| $\mu_{max}$ | microorganism's maximum specific growth rate (h$^{-1}$) |
| $\rho_p$ | density of dried microorganisms (g dm$^{-3}$) |
| $\varphi$ | Thiele modulus |

retention in the reactor of higher concentrations of microorganisms, protection of cells against toxic substances and eliminates the costly processes of cell recovery and cell recycle. Besides these advantages, the use of immobilized microorganism has some disadvantages. One of the major problems of immobilization is diffusion limitation. In such case, the control of micro-environmental conditions is difficult because of the resulting heterogeneity in the system. With viable cells, growth and gas evolution can lead to significant mechanical disruption of the immobilizing matrix [10–14].

In the present study, biodegradation of phenol by free and Ca-alginate immobilized *R. eutropha* was performed in a batch system. The Haldane equation was used to describe the phenol inhibition. The effect of internal mass transfer limitations on the degradation in the immobilized cells was studied and the effectiveness factor and effective diffusion coefficient were calculated.

### 1.1. Model description

#### 1.1.1. Cell growth and substrate degradation kinetics

The specific degradation rate of phenol $v$ (mg g$^{-1}$ h$^{-1}$), in a batch system is given in the following equation:

$$v = -\frac{1}{X}\frac{dC_S}{dt} \quad (1)$$

where $X$ is dried microorganism concentration (g dm$^{-3}$), $C_S$ the phenol concentration in the biodegradation medium (mg dm$^{-3}$). When the free cells were used, the biodegradation rate was determined from the slope of phenol consumption versus time plot at the exponential growth phase in which the dried microorganism concentration is obtained from the exponential growth phase. For the studies with immobilized microorganisms, the biodegradation rate was calculated as the same as the free cells for a fixed dried cell concentration in Ca-alginate beads.

Although many microorganisms have been shown to grow on phenol as the sole source of carbon, most of these microorganisms show sign of substrate inhibition. The Haldane equation has frequently used to describe this inhibition (Eq. (2)) [15].

$$\mu = \frac{\mu_{max} C}{K_S + C + (C^2/K_I)} \quad (2)$$

where $\mu_{max}$ is the maximum specific growth rate (h$^{-1}$), $K_S$ the substrate affinity constant (mg dm$^{-3}$) and $K_I$ the substrate inhibition constant (mg dm$^{-3}$).

#### 1.1.2. Kinetic model in immobilized microorganism

When microorganism is attached to a porous carrier matrix the internal mass-transfer limitations have a great influence on the intrinsic kinetics. It is necessary to develop comprehensive models that quantitatively account for the internal diffusion effects in addition to biochemical reaction as the substrates and product diffuse into and out of the immobilized particles.

Assuming that the porous structure is isothermal and spherical in shape, microorganisms are uniformly distributed in the particle and there is no partitioning of the substrate between the exterior and interior of the support, the following equation can be written stating that diffusion rate is equal to reaction rate at steady state.

$$\frac{d^2C}{dr^2} + \frac{2}{r}\frac{dC}{dr} = \frac{\rho_p}{D_e} v \quad (3)$$

where $C$ is the phenol concentration within the immobilized particles (mg dm$^{-3}$), $r$ the radial position within the bead, $\rho_p$ and $D_e$ the density of dried microorganism (g dm$^{-3}$) and effective diffusion coefficient of phenol within the bead respectively. $v$ is the actual biodegradation rate. Assuming

that the biodegradation of phenol by immobilized *R. eutropha* follows first order kinetics (it is a correct assumption, especially at low phenol concentrations), biodegradation rate can be defined as Eq. (4):

$$v = kC \tag{4}$$

where, $k$ is the first-order biodegradation rate constant ($dm^{-3}$ solution $g^{-1}$ dried microorganism $h^{-1}$).

Solving Eq. (3) with boundary conditions $C = C_S$ at $r = R$ and $dC/dr = 0$ at $r = 0$, Eq. (5) can be obtained.

$$\frac{C}{C_S} = \frac{\sin h(3\varphi r/R)}{(r/R)\sin h3\varphi} \tag{5}$$

where $R$ is the radius of the bead (cm), $C_S$ the phenol concentration at the bead surface and $\varphi$ the Thiele modulus for a spherical particle and given as the following equation:

$$\varphi = \frac{R}{3}\sqrt{\frac{k'}{D_e}} \tag{6}$$

where $k'$ is the rate constant ($k' = k\rho_p(1/3600)$).

The effectiveness factor, here symbolized by $\eta$, is defined as the ratio of the actual reaction rate to the rate evaluated at outer surface conditions (without diffusion limitation) ($v_S$).

$$v = \eta v_S \tag{7}$$

Assuming that the actual biodegradation rate is equal to the diffusion rate of substrate into the immobilized particle at $R$, Eq. (8) can be written as:

$$v = 4\pi R^2 D_e \left(-\frac{dC}{dr}\right)_{r=R} \tag{8}$$

The biodegradation rate evaluated at the outer surface conditions can be written as:

$$v_S = \tfrac{4}{3}\pi R^3 k' C_S \tag{9}$$

After the necessary regulations, the effectiveness factor is given by Eq. (10) as a function of $\varphi$ [18].

$$\eta = \frac{1}{\varphi}\left[\frac{1}{\tan h3\varphi} - \frac{1}{3\varphi}\right] \tag{10}$$

For small values of $\varphi$, $\eta \to 1$ and intraparticle mass transport has no effect on the rate. The rate at the centre is the same as the rate at the outer surface, all the volume is fully effective. The biodegradation step controls the rate. For large $\varphi$, $\eta \ll 1$, intraparticle diffusion has a large effect on the rate. Practically, these conditions mean that diffusion into the particle is relatively slow, so that biodegradation occurs before the substrate has diffused far into the particle and only the surface near the outer periphery of the particle is effective [10,12,13,15–18]. For $\varphi > 5$ a good approximation for Eq. (10) is:

$$\eta = \frac{1}{\varphi} \tag{11}$$

## 2. Materials and methods

### 2.1. Microorganism and growth conditions

*R. eutropha* (NRRL B-14690) obtained from American Type Culture Collection was used throughout the present study. The nutrient medium contained the following ingredients (in g $dm^{-3}$) glucose, 3; yeast extract, 2; peptone, 2; $KH_2PO_4$, 1; $K_2HPO_4$, 1; $(NH_4)_2SO_4$, 1; $MgSO_4{\cdot}7H_2O$ 0.05. The pH of the medium was adjusted to 7 and it was sterilized by autoclaving at 121 °C for 20 min. The microorganism was incubated at 30 °C in an agitated shaker (100 rpm) for 24 h. The culture was acclimatized in phenol (100 mg $dm^{-3}$) containing nutrient medium, then it was transferred (in 1:10 ratio) into the biodegradation medium containing phenol as sole source of carbon and energy.

### 2.2. Biodegradation studies

Initial experiments on biodegradation of phenol by *R. eutropha* showed that the presence of another carbon source such as glucose in the biodegradation medium affected the biodegradation of phenol and the microorganism could degrade phenol after consuming of glucose. So phenol was used as the sole sources of carbon and energy in the study.

In suspended cell studies, the biodegradation medium was prepared by diluting 1.0 g $dm^{-3}$ of stock phenol solution to desired concentration and adding sufficient quantities of salts as $KH_2PO_4$, 0.5 g $dm^{-3}$; $K_2HPO_4$, 0.5 g $dm^{-3}$; $(NH_4)_2SO_4$, 0.5 g $dm^{-3}$ and $MgSO_4{\cdot}7H_2O$, 0.05 g $dm^{-3}$. After the sterilization, the pH of the medium was adjusted to the desired value by using sterilized 1.0 M $H_2SO_4$ or NaOH solutions.

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]. It contained $KH_2PO_4$, 0.035 g $dm^{-3}$; $(NH_4)_2SO_4$, 0.3 g $dm^{-3}$; $MgSO_4{\cdot}7H_2O$, 0.05 g $dm^{-3}$ and phenol at desired concentration. After the solution was sterilized, the pH of the medium was adjusted to the required value.

At the first stage of the studies, free cells experiments were performed and the effects of initial pH, temperature and initial phenol concentration on the biodegradation was investigated. Then immobilized microorganisms were used. Biodegradation studies were carried out in 0.25 $dm^3$ Erlenmeyer flasks containing 0.1 $dm^3$ biodegradation medium on a rotary shaker at 100 rpm constant agitating rate. After the transference of free cells or 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 biomass and phenol as described below. The experiments were continued for 48 h. Small differences ($\leq$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.

### 2.3. Analytical methods

The residual phenol concentration in the biodegradation medium was determined spectrophotometrically. The absorbance of the colored complex of phenol and *p*-nitroaniline was read at 470 nm [19]. For free cell studies the biomass concentration was determined by measuring the absorbance at 400 nm using a standard curve of absorbance against dry cell weight.

## 3. Results and discussion

### 3.1. Effect of initial pH

pH effects the activity of enzymes and therefore the microbial growth rate. Fig. 1 shows the effects of pH on phenol biodegradation by free and immobilized cells. For free cells, specific growth and phenol removal rates increased from 0.138 to 0.470 $h^{-1}$ and 74 to 216 mg $g^{-1}$ $h^{-1}$ with increasing pH from 4 to 7, respectively and then decreased sharply. Variations in the pH of medium result in changes in the ionic form of the active site and changes in the activity of the enzyme and hence the biodegradation rate. Changes in pH may also alter the three-dimensional shape of the enzymes in microorganism. For these reasons, enzymes in microorganisms are only active over a certain pH range and different microorganisms have different pH optima.

Fig. 1 also shows that, lower phenol removal rates were obtained for Ca-alginate immobilized *R. eutropha* beads

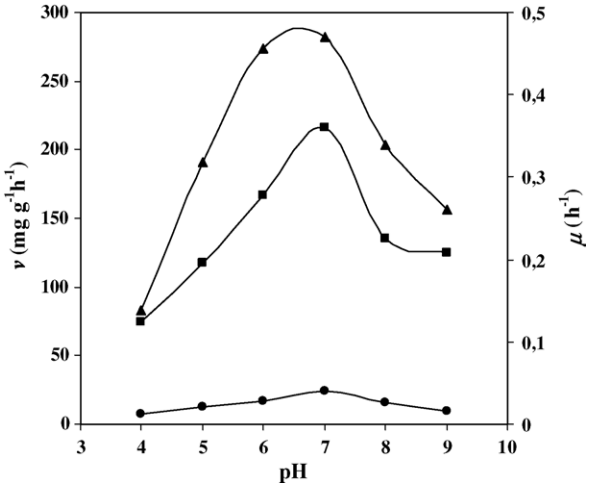


Fig. 1. Effect of initial pH on the phenol degradation for free and immobilized cells ($C_{So}$ = 100 mg $dm^{-3}$, $T$ = 30 °C, agitation rate = 100 rpm) (▲: $\mu$, ■: $v_{free\ cells}$ and ●:$v_{imm.\ cells}$).

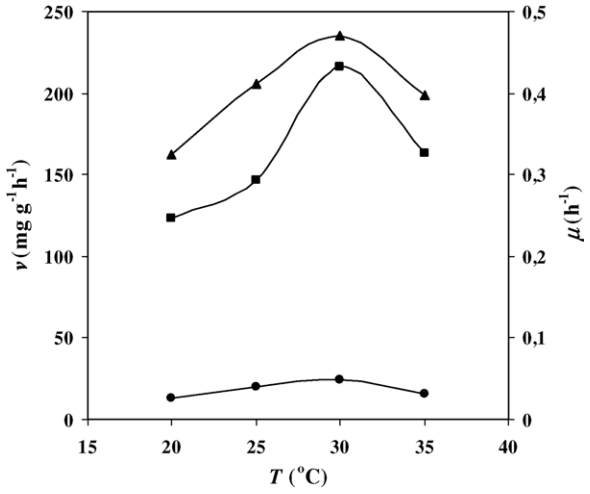


Fig. 2. Effect of temperature on the phenol degradation for free and immobilized cells ($C_{So}$ = 100 mg $dm^{-3}$, pH 7, agitation rate = 100 rpm) (▲: $\mu$, ■:: $v_{free\ cells}$ and ●:$v_{imm.\ cells}$).

because of internal mass transfer limitations. It was also reported that the immobilized cells could tolerated the changes in pH of the medium and less affected from the pH changes than free cells. The maximum substrate removal rate was determined as 24.4 mg $g^{-1}$ $h^{-1}$ at pH 7.

### 3.2. Effect of temperature

The effect of temperature on phenol biodegradation for free and immobilized microorganism was given in Fig. 2. The optimal temperature was determined as 30 °C for both cases. Temperature is an important factor affecting the performance of cells. *R. eutropha* is an mesophilic bacteria. As the temperature is increased toward optimal growth temperature (30 °C), the specific growth rate increased. Thus higher phenol removal rates were obtained. Above the optimal temperature, thermal death occurred so the specific growth and phenol removal rates decreased. It was also observed that, free cells were more sensible against temperature than immobilized microorganism and immobilization increased the thermal stability of the cells.

### 3.3. Microbial growth and phenol degradation kinetics

Typical phenol biodegradation results for free cells at pH 7, 30 °C and 100 mg $dm^{-3}$ initial phenol concentration was presented in Fig. 3. Phenol degradation was higher during the exponential growth phase of the microorganism. Most of the phenol degradation was achieved within 10 h. No significant change was observed in microbial growth and phenol consumption later.

### 3.4. Effects of initial phenol concentration

In order to determine the effect of initial phenol concentration on microbial growth and phenol removal rates a

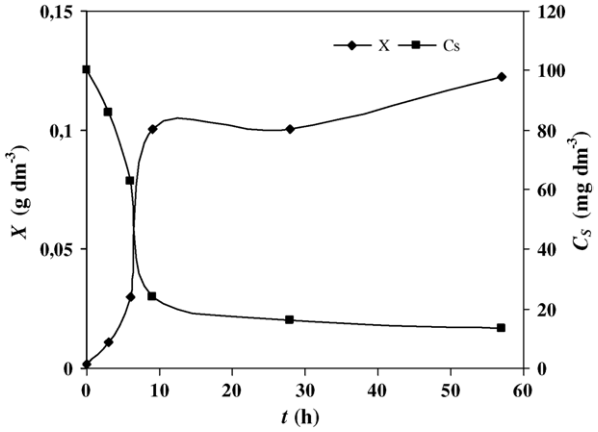


Fig. 3. The variation of growth and phenol consumption with time ($C_{So}$ = 100 mg dm$^{-3}$, pH 7, $T$ = 30 °C, agitation rate = 100 rpm).

series batch experiments were conducted. In these experiments, initial phenol concentration varied in the range 25–500 mg dm$^{-3}$. Figs. 4 and 5 show that, free *R. eutropha* could degrade low phenol concentrations easily and maximum specific growth rate was determined as 0.47 h$^{-1}$ at 100 mg dm$^{-3}$ initial phenol concentration while maximum phenol removal rate was obtained as 270 mg g$^{-1}$ h$^{-1}$ at 150 mg dm$^{-3}$ initial phenol concentration. Then rates started decreasing due to substrate inhibition since *R. eutropha* could not tolerate the toxicity of phenol at higher levels. The Haldane model gave an adequate fit to plot of $\mu$ versus $C_S$. The values of three biokinetic constants of the Haldane equation were determined as $\mu_{max}$ = 0.89 h$^{-1}$, $K_S$ = 55.11 mg dm$^{-3}$ and, $K_I$ = 257.94 mg dm$^{-3}$ by non-linear regression analysis. The dependences of the microbial specific growth rate on phenol concentration and fitted curve were given in Fig. 5. As seen from the figure, the predicted and experimental values were in good agreement ($R^2$ = 0.99).

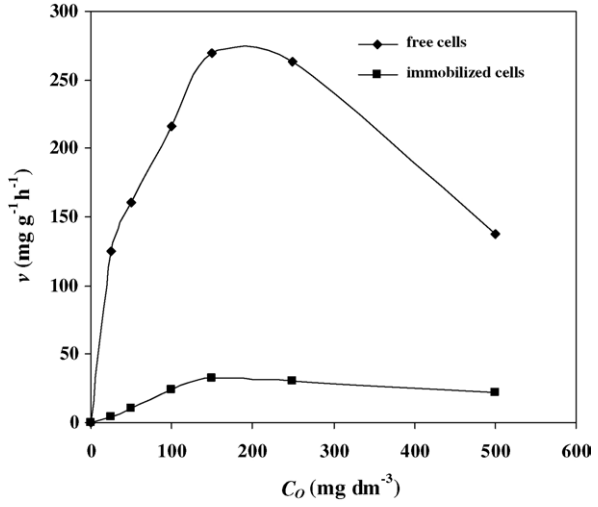


Fig. 4. Effect of initial phenol concentration on phenol biodegradation for free and immobilized cells (pH 7, $T$ = 30 °C, agitation rate = 100 rpm).

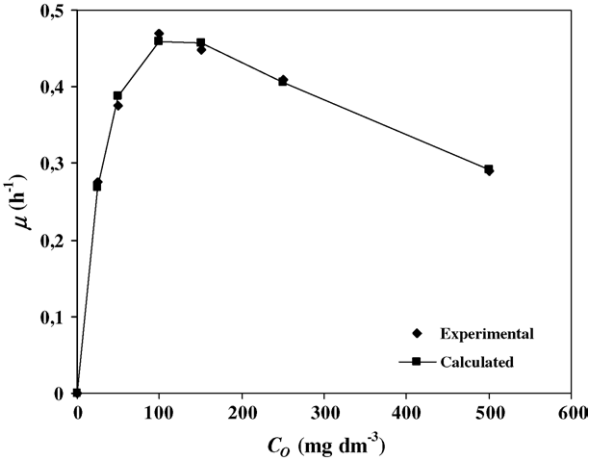


Fig. 5. The dependence of specific growth rate on initial phenol concentration (pH 7, $T$ = 30 °C, agitation rate = 100 rpm).

The comparison of specific growth and phenol removal rates of *R. eutropha* obtained in this study with those obtained in the literatures shows that *R. eutropha* is efficient microorganism for biodegradation of phenol. Hill and Robinson reported the maximum specific growth rate as 0.48 h$^{-1}$ for *P. putida* [20]. Monerio et al. [21] determined the maximum specific growth rate of *P. putida* as 0.288 h$^{-1}$ at 25 mg dm$^{-3}$ initial phenol concentration. Aksu and Bülbül [13] reported the maximum phenol removal rate of *P. putida* as 41.9 mg g$^{-1}$ h$^{-1}$ at 100 mg dm$^{-3}$ initial phenol concentration. The obtained results show that *R. eutropha* has high phenol biodegradation rate.

In the case of the immobilized microorganisms, lower substrate removal rate values were obtained than free cell because of internal mass transfer limitations. It was also clear that, immobilized microorganism could expose to higher phenol concentration without loss of cell viability (Fig. 4).

### 3.5. Effect of particle size

At 100 mg dm$^{-3}$ initial phenol concentration, three different particle sizes (0.2; 0.3 and 0.4 cm) were used to investigate the effect of particle size on the substrate removal rate. As seen from Fig. 6, the highest phenol removal rate was obtained as 24.4 mg g$^{-1}$ h$^{-1}$ for smallest particles ($d_p$ = 0.2 cm). An increase in particle size caused an increase in diffusion limitations in the particles thus, lower substrate removal rates were found for bigger particles.

In immobilized microorganism systems, diffusion resistances should be eliminated by using small particles, a high degree of turbulence around the particles and high substrate concentrations. For maximum conversion rates, the particle size should be as small as possible within the constraints of particle integrity, resistance to compression and the nature of the particle recovery systems.

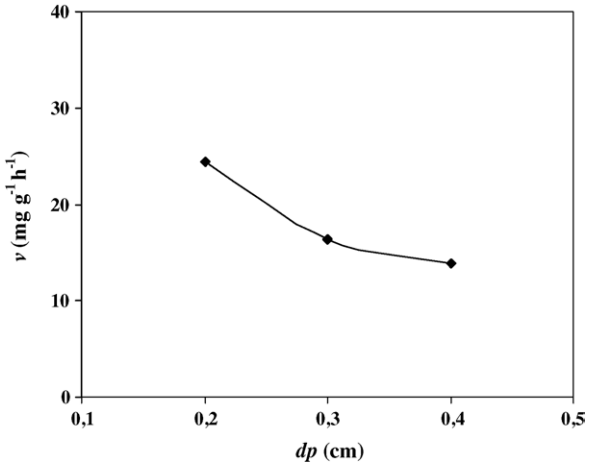


Fig. 6. The effect of particle size on phenol biodegradation (pH 7, $T = 30\,^{\circ}C$, agitation rate = 100 rpm, $w = 0.12$ g).

### 3.6. Mass transfer effect in immobilized cells

Using biodegradations rates obtained with immobilized particles (biodegradation rate with diffusion limitation) and free cells (biodegradation rate without diffusion limitation), experimental effectiveness factors were calculated from Eq. (7) at 100 mg dm$^{-3}$ initial phenol concentration (it was assumed that the external mass transfer resistance is negligible and the surface concentration $C_S$ is equal to bulk value). As seen from Table 1, the effectiveness factors determined for three different sized immobilized particles were much smaller than 1.0. It was clear that, the effect of diffusion resistance on the biodegradation rate was very significant and the biodegradation of phenol by immobilized *R. eutropha* was diffusion limited. It was also observed that the effectiveness factor decreased from 0.113 to 0.064 with increasing the particle size from 0.2 to 0.4 cm.

Table 2 shows the Thiele modulus values calculated from Eq. (11) for each particle size. As seen from table, the Thiele modulus were found to be 8.85–15.50, showing the importance of internal diffusion effects on the overall biodegradation rate. As the particle diameter becomes very small, $\varphi$ decreases, so that the effectiveness factor approaches 1, and the reaction is surface-reaction-limited. On the other hand, when $\varphi$ is large, the effectiveness factor is small and the reaction is diffusion-limited within the pellet. Assuming that the biodegradation of phenol by *R. eutropha* follows first order reaction kinetics at low phenol concentrations, first-order biodegradation rate constants were obtained from $\ln(C_0/C_S)$ versus $t$ plots at 100 mg dm$^{-3}$ initial phenol concentration (it is a correct assumption, especially for low phenol concentrations) with high correlation coefficients (for free cell $R^2 = 0.91$, for immobilized cell $R^2 = 0.97$ at 100 mg dm$^{-3}$ initial phenol concentration).

Table 1
The effect of particle size on the biodegradation rate and experimental effectiveness factor

| $d_p$ (cm) | $v$ (mg g$^{-1}$ h$^{-1}$) | $\eta$ |
|---|---|---|
| Free cells | 216.0 | 1.000 |
| 0.2 | 24.4 | 0.113 |
| 0.3 | 16.4 | 0.076 |
| 0.4 | 13.9 | 0.064 |

pH 7, $T = 30\,^{\circ}C$, $C_0 = 100$ mg dm$^{-3}$ and $w = 0.121$ g.

Table 2
Calculated values of Thiele modulus, first-order biodegradation rate constants and effective diffusion coefficients

| $d_p$ (cm) | $\varphi$ | $k'$ (dm$^3$ dm$^{-3}$ h$^{-1}$) | $D_e$ ($\times 10^{-7}$ cm$^2$ s$^{-1}$) |
|---|---|---|---|
| 0.2 | 8.85 | 50.62 | 1.99 |
| 0.3 | 13.16 | 64.00 | 1.14 |
| 0.4 | 15.50 | 39.20 | 0.51 |

The effective diffusion coefficients given in Table 2 were calculated from Eq. (6). The pores in the porous particles are not straight and cylindrical; rather, they are a series of tortuous, interconnecting paths of varying cross-sectional areas. The effective diffusion coefficient is determined by the molecular diffusivity and the pore structure of the immobilized particles. It was supposed to be independent from the particle size [18]. As seen from Table 2, small differences were obtained in the values of the effective diffusion coefficient for three different immobilized particles. These differences can be explained by the differences in pore structure of immobilized particles occurred during the immobilization. Average effective diffusion coefficient was calculated as $1.21 \times 10^{-7}$ cm$^2$ s$^{-1}$. This values smaller than phenol's diffusion coefficient in water at 30 °C ($0.92 \times 10^{-5}$ cm$^2$ s$^{-1}$) [22]. This can be explained by the exclusion effect (the biopolymer reduces the volume available for the phenol to move in) and the obstruction effect (the impenetrable part of biopolymer increases the path length for movement of phenol). The predictive value of these concepts is limited but is useful in visualizing the process.

Similar diffusion limitation effect was reported for phenol degradation by different microorganisms. For instance Aksu and Bülbül investigated the biodegradation of phenol by Ca-alginate immobilized *P. putida* and they reported that the biodegradation process was intraparticle diffusion limited [13].

## 4. Conclusions

In the present study, free and Ca-alginate immobilized *R. eutropha* were used for phenol degradation. Phenol can be utilized as sole source of carbon and energy by *R. eutropha*. Free microorganism studies showed that the specific growth rate was highly affected by pH, temperature and initial phenol concentration. Maximum phenol removal rate was obtained as 270 mg g$^{-1}$ h$^{-1}$ at pH 7, 30 °C and 150 mg dm$^{-3}$ initial phenol concentration. Phenol had a strong inhibitory effect on the growth of free cells and it was well described by the Haldane model. It was also indicated that immobilized cells could tolerate a higher level of phenol concentration,

although the biodegradation rate was slower. Kinetic analysis of biodegradation of phenol by immobilized microorganism showed that there was a serious internal mass transfer limitation. The maximum experimental effectiveness factor was determined as 0.113 for the smallest immobilized beads ($d_p$ = 0.2 cm), while the average effective diffusion coefficient was obtained as $1.21 \times 10^{-7}$ cm$^2$ s$^{-1}$. These results indicated that the biodegradation process (by immobilized cells) was diffusion limited.

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