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Full Length Research Paper

Mass transfer analysis for terephthalic acid biodegradation by immobilized *Pseudomonas* sp. in a packed-bed reactor

Zhi-Jiang Wang*, Jie Zhang, Li-hua Teng and Zhao-Xi Ge

College of Biological and Environmental Science, Zhejiang Wanli University, Ningbo 315100, China.

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Biodegradation of terephthalic acid (TA) by polyvinyl alcohol (PVA)-alginate immobilized *Pseudomonas* sp. was carried out in a packed-bed reactor. The effect of inlet TA concentration on biodegradation was investigated at 30°C, pH 7 and flow rate of 20 ml/min. The effects of flow rate on mass transfer and biodegradation rate of TA were analyzed with the assumption of first-order reaction kinetics. Based on the experimental data, various external mass transfer correlations were evaluated and the correlations $J_D=5.7Re^{-0.3}$ was found to be adequate to predict the biodegradation of TA. The intrinsic biodegradation rate constants and external mass transfer constants were calculated and the combined effects on the whole biodegradation of TA were also investigated.

Key words: Terephthalic acid, mass transfer analysis, PVA-alginate immobilization, packed-bed reactor.

INTRODUCTION

Terephthalic acid (TA) is an important industrial chemical and has been widely used for the syntheses of plastics, dyes, pesticides and chemical fibers (Savostianoff and Didier, 1993). Generally, 3 to 10 ton of wastewater containing high concentration of TA would be created for the production of 1 ton of TA (Karthik et al., 2008; Kleerebezem et al., 1997; Zhang et al., 2005). Thus, wastewater from these industries creates a great pollution problem. TA can inhibit microbial growth, cause bladder stones and blander cancer, and impairment of renal functions and testicular functions (Dai et al., 2005; Scholz, 2003; Wolkowski et al., 1982; Zhang et al., 2010).

Recently, researches on degradation of TA had been done, such as by physical and chemical methods (Thiruvenkatachari et al., 2007; Wen et al., 2006) and microbial degradation methods (Karthik et al., 2008). In our previous study, we carried out the research on degradation of TA by free cells (Wang et al., 2011). Cell-immobilization techniques have many advantages such as higher cell concentration in the reactor, easier solid–liquid separation for reaction solution, and more stable than free cells (Lee et al., 1994; Lu et al., 1996). Moreover, immobilization techniques need a good support material. Polyvinyl alcohol (PVA) is the good material that has been extensively used for cell immobilization because of its nontoxic to microorganisms (Hsia et al., 2008; Hsieh et al., 2002). Immobilized cells usually are packed in a column when they work. However, the removal of substrate in the packed column system is affected strongly by solute diffusion into the pore of beans and internal pore diffusion effects (Halim et al., 2009; Mudliar et al., 2008; Murty et al., 2004). Therefore, to establish a reaction and mass transfer control model for studying the degradation of TA by immobilized cells is very meaningful.

In this study, *Pseudomonas* sp. was immobilized by PVA and packed in a column. A quantitative analysis of mass transfer was performed in combination with biochemical reaction rates for degradation of TA. Experimental data on the flow rates and the first-order rate constants for the removal of TA were analyzed.

*Corresponding author. E-mail: wangzj1978@126.com. Tel: +860574-88222679.

Abbreviation: TA, Terephthalic acid; **PVA,** polyvinyl alcohol.

Theory

Biodegradation rate constant

The transport processes in this study include transfer of TA from bulk liquid to immobilized bioactive surface and simultaneous diffusion and bioreaction of TA within the bioactive layer (Kathiravan et al., 2010; Nath and Chand, 1996). Assuming the packing beads are spherical, without axial diffusion, and plug flow is in steady state, the material balance for TA in packed-bed reactor can be written as the following equation (Aksu and Bŭlbŭl, 1998);

$$(\frac{HQ}{W})\times\frac{\mathrm{d}C}{\mathrm{d}Z}\times\frac{60}{10^3}=-r \quad (1)$$

Where, H is the height of the column (cm); Q is the volumetric flow rate (ml/min); W is the total amount of dried cells in the immobilized beads (g); $\mathrm{d}C/\mathrm{d}Z$ is the concentration gradient along the column height (mg/L/cm) and r is the biodegradation rate (mg/g/h). The relationship between the biodegradation rate and the TA concentration in the reactor is given as Equation (2), assuming the reaction rate can be written in terms of bulk TA concentration (Dursun and Tepe, 2005);

$$r=k_{\mathrm{P}}C \quad (2)$$

Where, k_P is the observed first-order biodegradation rate constant (L/g/h) and C is the substrate concentration in the bulk liquid (mg/L). Substituting Equation (2) into Equation (1), and integrating this equation for the following boundary conditions; at $Z=0$ of $C=C_0$ and at $Z=\mathrm{H}$ of $C=C$, Equation (3) is obtained as;

$$\ln(\frac{C_0}{C})=\frac{W}{Q}\times k_{\mathrm{P}}\times\frac{10^3}{60} \quad (3)$$

Combined mass transfer and biochemical reaction

When fluid flows though, the surface of immobilized beads in packed bed reactor, a boundary layer near the surface of immobilized beads where the fluid velocity is very low, is developed. Observed reaction rate can be significantly reduced by mass transfer of diffusion (Radovich, 1985). The mass transfer rate of the TA from the bulk fluid to the surface of the immobilized beads is considered to be proportional to the area for mass transfer and the difference of TA concentrations between the bulk and the external surface of the immobilized beads (Nath and Chand, 1996). The external mass transfer rate of TA can be expressed as:

$$r_{\mathrm{m}}=k_{\mathrm{L}}\times a_{\mathrm{S}}\times(C-C_{\mathrm{S}})\times10^{-3} \quad (4)$$

Where, r_m is the external mass transfer rate of TA (mg/g/h); k_L is the external mass transfer constant (cm/h); a_s is the external surface area for mass transfer (cm^2/g) and C_s is the TA concentration at the surface of the immobilized cell (mg/L).

The first-order biodegradation rate of the immobilized cells can be written as:

$$\mathrm{r}=\mathrm{k_S}\cdot\mathrm{C_S} \quad (5)$$

Where, k_s is the intrinsic first-order biodegradation rate constant (L/g/h). At steady state, the mass transfer rate is equal to the biodegradation rate. Thus, Equation (6) is obtained for solving the unknown surface concentration, from Equations (4) and (5).

$$C_{\mathrm{S}}=\frac{k_{\mathrm{L}}\cdot a_{\mathrm{S}}\times10^{-3}\cdot C}{k_{\mathrm{S}}+k_{\mathrm{L}}\cdot a_{\mathrm{S}}\times10^{-3}} \quad (6)$$

Substituting Equation (6) into Equation (5);

$$r=\frac{k_{\mathrm{S}}\cdot k_{\mathrm{L}}\cdot a_{\mathrm{S}}\cdot C\times10^{-3}}{k_{\mathrm{S}}+k_{\mathrm{L}}\cdot a_{\mathrm{S}}\times10^{-3}} \quad (7)$$

From Equations (2) and (7);

$$k_{\mathrm{P}}=\frac{k_{\mathrm{S}}\cdot k_{\mathrm{L}}\cdot a_{\mathrm{S}}\times10^{-3}}{k_{\mathrm{S}}+k_{\mathrm{L}}\cdot a_{\mathrm{S}}\times10^{-3}} \quad (8)$$

Rearranging for external mass transfer constant, Equation (8) is derived as:

$$k_{\mathrm{L}}=\frac{k_{\mathrm{S}}\cdot k_{\mathrm{P}}}{(k_{\mathrm{S}}-k_{\mathrm{P}})\cdot a_{\mathrm{S}}\times10^{-3}} \quad (9)$$

Empirical model

It is common practice to correlate the mass transfer constant with fluid properties in term of J_D-factor, which is defined as the following equation (Dursun and Aksu, 2000; Kathiravan et al., 2010);

$$J_{\mathrm{D}}=\frac{k_{\mathrm{L}}\cdot\rho}{G}(\frac{\mu}{\rho\cdot D_f})^{2/3}=K\cdot R_e^{n-1} \quad (10)$$

Where, ρ is the fluid density (g/ml); μ is the feed fluid viscosity (g/cm/h); K is the constant; J_D is the colburn factor; D_f is the substrate diffusivity (m^2/s) and G is the mass flux of TA solution (g/cm^2/h) calculated from the

following equation:

$$G=\frac{Q\cdot\rho}{\Omega}\times 60 \quad (11)$$

Where, Ω is the column superficial cross section area (cm^2). In addition, *Re* is the Reynolds number in the packed-bed, calculated from the following equation:

$$R_e=\frac{d_P\cdot G}{\mu(1-\varepsilon)} \quad (12)$$

Where, ε is the void fraction and d_P is the partial diameter (cm). From Equation (10), the mass transfer constant can be solved as;

$$k_{\mathrm{L}}=\frac{K}{\rho}\left(\frac{\rho\cdot D_f}{\mu}\right)^{2/3}\left[\frac{d_{\mathrm{P}}}{\mu(1-\varepsilon)}\right]^{n-1}G^n \quad (13)$$

Or:

$$k_{\mathrm{L}}=AG^n \quad (14)$$

$$A=\frac{K}{\rho}\left(\frac{\rho\cdot D_f}{\mu}\right)^{2/3}\left[\frac{d_{\mathrm{P}}}{\mu(1-\varepsilon)}\right]^{n-1} \quad (15)$$

Substituting Equation (14) into Equation (9);

$$\frac{1}{k_{\mathrm{P}}}=\frac{1}{A\cdot a_{\mathrm{S}}\times 10^{-3}}\frac{1}{G^n}+\frac{1}{k_{\mathrm{S}}} \quad (16)$$

MATERIALS AND METHODS

Reagents

TA with greater than 99% purity, and polyvinyl alcohol with an average degree of polymerization of 1750±50 were both purchased from Shanghai Chemical Reagent Factory, China.

Microorganisms and culture condition

Pseudomonas sp. previously isolated from polyester wastewater was used. Seed medium consisted of TA 1 g/L, $MgSO_4$ 0.25 g/L, KH_2PO_4 3 g/L, NH_4Cl 1 g/L, NaCl 0.5 g/L and Na_2HPO_4 7 g/L. Batch medium contained KH_2PO_4 3 g/L, NaCl 0.5 g/L, TA 1 g/L, $MgSO_4$ 0.25 g/L, Na_2HPO_4 7 g/L and urea 1.4 g/L, with pH of 7.0. *Pseudomonas* sp. was cultured on the seed medium for 12 h at 30℃ and then inoculated into a 500 ml conical flask containing 100 ml of sterilized batch medium by inoculating 4% seed culture with its concentration of 1.7×10^5 CFU/ml and cultured at 30℃ for 48 h on an orbital shaker of 140 rpm.

Immobilization of *Pseudomonas* sp.

The cells were collected by centrifugation at 8000 rpm for 10 min, and then washed with physiological saline twice. Cell immobilization by PVA method was carried out as previously described (Hsia et al., 2008; Wang et al., 2007). In the experiment, the solution containing 8% w/v PVA and 0.3% w/v Na-alginate was prepared by mixing and heating until dissolved. After cooling down to 34~37℃, the concentrated cells were added with adequate mixing and the final cell concentration was 50 g wet cells per liter. The mixture was dropped into a solidifying solution (saturation H_2BO_3 containing 0.10 mol/L $CaCl_2$) with agitation, and immersed in the solidifying solution for 1 h to enforce the spherical gel beads. The porous spherical beads were formed with this method, and the diameter of these beads was about 0.3 cm. These beads were washed several times with large amount of physiological saline to remove extra alginate or PVA.

Experimental setup

The schematic of the packed bed reactor is shown in Figure 1. The diameter of the reactor used in this study was 1.0 cm. The bed depth was kept at 15 cm. The reactor contained 8.58 g of immobilized beads with 0.84 g wet cells which is equal to 0.043 g dried microorganism. The beads were placed loosed inside the reactor, and the temperature of the reaction was kept constant at 30℃. The TA solution (pH 7.0) was located at the lower end of the reactor and the top end was used for effluent. The solution of 50 ml was re-circulated though the reactor with a variable speed peristaltic pump for 10 h.

Analysis

The measurement of TA concentration was carried out as described by our previous report (Wang et al., 2011).

RESULTS AND DISCUSSION

Effect of inlet TA concentration on TA biodegradation

The effect of inlet TA concentration was researched at 20 ml/min flow rate and inlet TA concentration was varied from 100 to 500 mg/L, with pH 7.0 and temperature of 30℃. The *Pseudomonas* sp. could be exposed to these TA concentrations (Wang et al., 2011). Figure 2 shows the biodegradation rates increased from 13.85 to 27.99 mg/g/h with the increase of inlet TA concentration from 100 to 200 mg/L. With further increase of concentration, the biodegradation rate shows a slight downward trend. It could be said that an increase in the inlet TA concentration to raise the biodegradation rate in packed-bed is limited.

External mass transfer

The experiment values of Q (20, 30 and 40 ml/min), C_0/C, and k_P were presented in Table 1. k_P studied at three different flow rates was evaluated from Equation

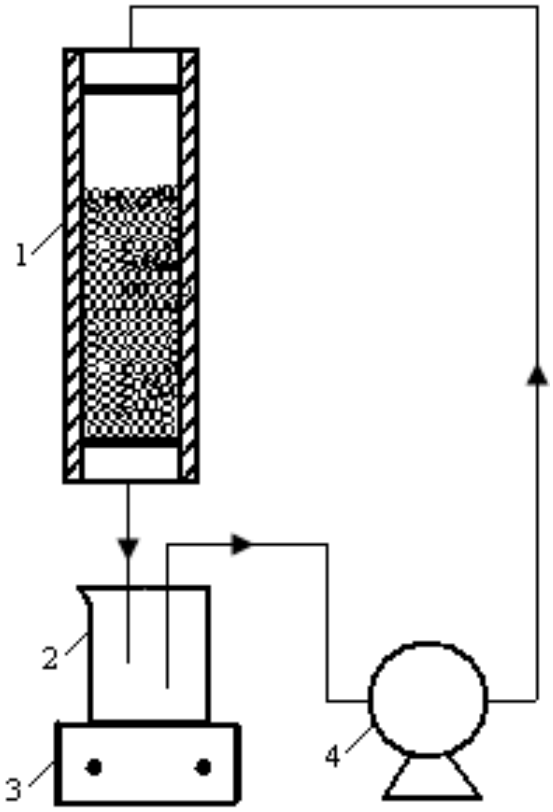

Figure 1. Schematic representation of the packed-bed reactor. 1, Thermostatic jacket; 2, substrate container; 3, magnetic stirrer; 4, feed pump.

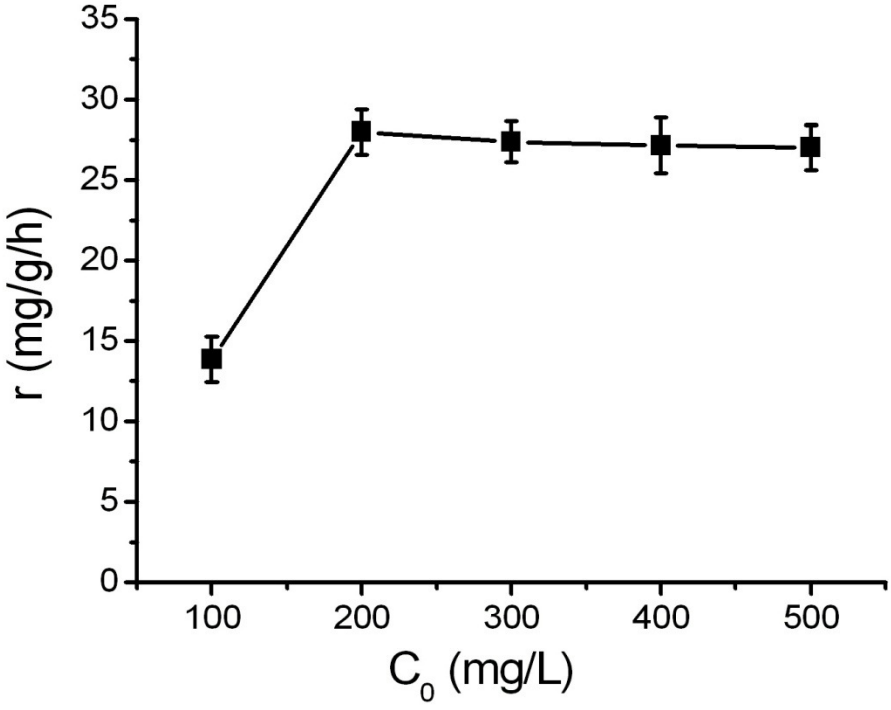

Figure 2. Effect of inlet TA concentration on the biodegradation rate.

Table 1. The experimental value of Q, C_0/C, and k_P.

Q (mL/min)	C_0/C	k_P (L/g/h).
20	24.01	88.70
30	11.00	100.38
40	10.29	130.11

Table 2. Slope and intercept values from the plots of 1/ k_P vs 1/G^n for different n values.

n	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Slope	0.1084	0.1163	0.1663	0.2673	0.4581	0.8172	1.4988	2.8047	5.3288
Intercept	<0	<0	<0	<0	<0	0.0015	0.0026	0.0035	0.0042

Table 3. Calculated values of 1/k_p and G^n with different flow rates.

Q (ml/min)	1/k_p(3)(L/g/h)	G	1/$G^{0.6}$	1/$G^{0.7}$	1/$G^{0.8}$	1/$G^{0.9}$
20	0.01128	1525.61	0.012301	0.00591	0.00284	0.001364
30	0.00996	2288.41	0.009645	0.00445	0.002053	0.000947
40	0.00769	3051.21	0.008116	0.003638	0.001631	0.000731

Table 4. The estimated values of k_s, A and a_s in relation to K=5.7 and different n values.

n	k_s (L/g/h)	K=5.7	
		A	$a_s \times 10^{-3}$
0.6	666.67	0.258	4.737
0.7	384.62	0.162	4.129
0.8	285.71	0.101	3. 528
0.9	238.09	0.063	2.969

(3). Results show that the observed first-order biodegradation rate constant increased from 88.70 to 130.11 L/g/h with flow rate increasing from 20 to 40 ml/min. For researching the external diffusion effects on the observed biodegradation rate, mass fluxes and dimensionless numbers were calculated for $\mu = 1.3\times10^{-2}$ g/cm/s, $\varepsilon = 0.3$, $\rho = 0.998$ g/ml, and $D_f = 0.75\times10^{-5}$ cm^2/s. Using Equation (16), 1/kp vs 1/G^n (0<n<1) was plotted for different values of n. Negative intercepts were obtained for n<0.6 as shown in Table 2, therefore, these n values were not considered for further analysis. The slope and intercept increased for the values of n increased from 0.6 to 0.9. Calculated values of 1/k_p and G^n found at different flow rates were presented in Table 3. The further analysis was carried out with K value of 5.7 to determine A (Nath and Chand, 1996). Using Equation (15) and Equation (16), the value of A, a_s and k_s were obtained for different n values, the results are presented in Table 4.

Using Equation (13), the k_L, $k_L a_s$ were calculated and presented in Table 5. As k_L, $k_L a_s$ values were calculated, k_P values were determined from Equation (8) again. In order to demonstrate the validity of mass transfer models, these k_P (8) values were compared with the k_P (3) values calculated from Equation (3). A normalized deviation (Δ%) used to compare the validity of the mass transfer correlation was calculated was follows:

$$\Delta\% = \frac{\sum_{i=1}^{N}\left|\frac{k_P(3)-k_P(8)}{k_P(3)}\right|}{N}\times 100\% \quad (17)$$

Where, k_P (3) and k_P (8) mean the k_P values calculated from Equations (3) and (8) at different n values, and N is the number of measurements. The normalized deviation values were given in Table 6. Results show that the value of normalized deviation for n = 0.7 is the lowest, so it could be said that with the estimated value of K=5.7, the mass transfer correlation was:

$$J_D = 5.7\,\mathrm{Re}^{-0.3} \quad (18)$$

Table 5. The variation of k_L and $k_L a_s$ values for various n values at different flow rates.

Q (ml/min)	G (g/cm^2/h)	0.6		0.7		0.8		0.9	
		k_L (cm/h)	$k_L a_s$(ml/g/h)	k_L(cm/h)	$k_L a_s$(ml/g/h)	k_L(cm/h)	$k_L a_s$(ml/g/h)	k_L(cm/h)	$k_L a_s$(ml/g/h)
20	1525.61	20.97	99.35×10^3	27.41	113.17×10^3	35.56	125.49×10^3	46.17	137.10×10^3
30	2288.41	26.75	126.71×10^3	36.41	150.32×10^3	49.20	173.57×10^3	66.51	197.48×10^3
40	3051.21	31.79	150.59×10^3	44.52	183.86×10^3	61.93	218.49×10^3	86.17	255.84×10^3

Table 6. Comparison of the experimental k_p calculated from Equation (3) with the ones calculated from Equation (8).

Q (ml/min)	k_p (3)(L/g/h)	0.6		0.7		0.8		0.9	
		k_p (8)	Δ%	k_p (8)	Δ%	k_p (8)	Δ%	k_p (8)	Δ%
20	88.70	86.46		87.44		87.20		87.00	
30	100.38	106.48	4.73	108.08	4.49	107.97	4.70	107.94	4.88
40	130.11	122.84		124.40		123.81		123.32	

Table 7. Comparison of intrinsic and external mass transfer resisting forces.

k_p (L/g/h)	1/k_p (g h/L)	k_s (L/g/h)	1/ k_s (g h/L)	% contribution of k_s	$k_L a_s$($\times10^3$mL/g/h)	1/($k_L a_s$) ($\times10^{-3}$g h/ml)	%contribution of $k_L a_s$
87.44	0.011436			23	113.17	0.008836	77
108.08	0.009252	384.62	0.0026	28	150.32	0.006652	72
124.40	0.008039			32	183.86	0.005439	68

The model accurately predicted the experimental date for biodegradation of TA in the packed-bed reactor. From Table 7, it could be said that both the intrinsic biodegradation and external diffusion are the resisting force. For n = 0.7, the external mass transfer resisting force is the main force on limiting the biodegradation of TA. So, increase in flow rate would reduce the external mass transfer limitation, and raise the whole biodegradation rate of TA in packed-bed.

Conclusion

In this study, biodegradation of TA was conducted with immobilized cells by PVA media in packed bed bioreactor. The combined effect of external mass transfer with biochemical reaction on TA was analyzed. The mass transfer correlation $J_D = 5.7\times Re^{-0.3}$ was established for the removal of TA in the bioreactor. This proposed correlation would be useful for the design and development of down-flow packed bed reactor for the continuous degradation of TA. This work was adequate to predict the effects external mass transfer on the observed biodegradation rates in immobilized packed bed reactors.

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NOMENCLATURE

a_s The external surface area for mass transfer (cm^2/g)
C The substrate concentration in the bulk liquid (mg/L)
C_s The TA concentration at the surface of the immobilized cell (mg/L)
C_0 The inlet TA concentration (mg/L)
D_f The substrate diffusivity (m^2/s)
d_P Partical diameter (cm)
G The mass flux of TA solution ($g/cm^2/h$)
H The height of the column (cm)
J_D The colburn factor
K Constant
k_L The external mass transfer constant (cm/h)
k_P The observed first-order biodegradation rate constant (L/g/h)
k_s The intrinsic first-order biodegradation rate constant (L/g/h)
N The number of measurements
Q The volumetric flow rate (ml/min)
Re The Reynolds number in the packed-bed
r The biodegradation rate (mg/g/h)
r_m The external mass transfer rate of TA (mg/g/h)
W The total amount of dried cells in the immobilized beads (g)
ε The void fraction
ρ The fluid density (g/ml)
μ The feed fluid viscosity (g/cm/h)

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