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External mass transfer analysis for simultaneous removal of carbohydrate and protein by immobilized activated sludge culture in a packed bed batch bioreactor

Nadir Dizge[a,b], Berrin Tansel[a,*]

[a] Florida International University, Civil and Environmental Engineering Department, Miami, FL, United States
[b] Gebze Institute of Technology, Department of Environmental Engineering, Gebze, Kocaeli, Turkey

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ABSTRACT

External mass transfer effects were analyzed for removal of carbohydrate and protein by immobilized activated sludge culture in a packed bed bioreactor. The bioreactor was made from 52 cm glass tubing with 5.0 cm inner diameter (with a total volume of 1020 cm^3). The microbial culture was immobilized on microporus polyurethane cut into cubic pieces of approximately 1.5 cm in length. The effect of flow rate on mass transfer and removal of carbohydrate and protein were analyzed theoretically and compared with experimental data. The rate constants were estimated using external film diffusion models at different flow rates (900, 1200, 1800 $cm^3\ h^{-1}$). Based on the experimental data, correlations between the Colburn factor (J_D) and Reynolds number (Re) as $J_D = 5.7 \times Re^{-0.90}$ and $J_D = 5.7 \times Re^{-0.18}$ were found to be adequate to predict the removal of carbohydrate and protein, respectively.

1. Introduction

Packed bed reactors provide high specific surface area, allowing development of highly active biofilms which are tolerant to changes in feed quality and environmental conditions. In recent years, packed bed reactors have gained acceptance for wastewater treatment applications [1–7]. Operation of packed beds in recycle mode allows control of effluent quality with small foot print while maintaining high metabolic rates [8–12].

Presence of a static media with its own microporous structure as well as porosity of the packing arrangement (i.e., particle size and shape of media inside the bioreactor) creates a complex system from both mass transport and reaction kinetics perspectives. Experimental studies with biofilms indicate that reaction/diffusion phenomena within the biofilm control the thickness of the biofilm, hence, oxygen or substrate (or both) can be limiting the metabolic rate within the biofilm. Mudliar et al. [13] developed a steady state model to estimate the external liquid film diffusion and internal pore diffusion effects in an immobilized biofilm system under continuous mode. Murty et al. [14] reported that the mass transfer effects on immobilized beads in packed bed reactor systems were limited by internal and external mass transfer resistances. Halim et al. [15] studied reaction and mass transfer control models for biodiesel production with immobilized cells in packed bed reactor. In packed bed bioreactors, biocatalysts are usually attached to the supporting surfaces to allow high volumetric loadings and to provide a long retention time without the need to separate or recycle the bed [16,17]. Although there are a number of advantages for using immobilized microorganisms, diffusion limitations are the main disadvantages of these processes [12].

In this study, a quantitative analysis of mass transfer was performed in combination with biochemical reaction rates for utilization of carbohydrate and protein. Experimental data on the flow rates and the pseudo first-order rate constants (k_s) for the removal of carbohydrate and protein were analyzed and compared with external film diffusion theory. The mass transfer coefficients (k_m) were estimated as a function of the mass velocity (G) and the Reynolds number (Re) for carbohydrate and protein.

2. Model theory

2.1. Biochemical reaction

A four-step mechanism has been proposed for biofilm growth as (1) formation of a thin layer by microorganisms, (2) increase of the biofilm thickness, (3) breakage of biofilm clusters and release of particles (biomass) due to excess growth, and (4) formation of a small pellet by detached particles [18]. The mass transport through the biofilm takes place by several mechanisms such as diffusion of the substrate from solution to the biofilm, diffusion–reaction through the biofilm, and adsorption–diffusion through the immobilization surface. The micro scale reaction and diffusion phenomena

* Corresponding author. Tel.: +1 305 348 2928; fax: +1 305 348 2802.
E-mail address: tanselb@fiu.edu (B. Tansel).

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Nomenclature

a_m	surface area per unit weight of cells for mass transfer ($cm^2\ g^{-1}$)
A	parameter given by Eq. (14)
C	substrate concentration ($mg\ L^{-1}$)
C_o	substrate concentration in the bulk ($mg\ L^{-1}$)
C_s	substrate concentration on the surface of immobilized cell layer ($mg\ L^{-1}$)
d_p	sponge diameter ($cm^2\ s^{-1}$)
D_f	substrate diffusivity ($m^2\ s^{-1}$)
H	height of the column (cm)
G	mass flux based on the superficial velocity ($g\ cm^{-2}\ h^{-1}$)
J_D	Colburn factor (dimensionless)
k_m	external mass transfer coefficient ($cm\ h^{-1}$)
k_p	observed first-order substrate removal rate constant ($L\ g^{-1}\ h^{-1}$)
k_s	intrinsic first-order reduction rate constant ($cm\ h^{-1}$)
K	constant (dimensionless)
Q	volumetric flow rate ($cm^3\ h^{-1}$)
r	reaction rate ($mg\ g^{-1}\ h^{-1}$)
r_m	mass transfer rate ($mg\ g^{-1}\ h^{-1}$)
Re	Reynolds number (dimensionless)
u	flow velocity ($cm\ h^{-1}$)
V	reactor volume (m^3)
W	microorganism quantity in packed bed reactor (g)
z	height of the column (cm)
ε	void fraction in packed bed (dimensionless)
μ	feed fluid viscosity ($g\ cm^{-1}\ h^{-1}$)
ρ	liquid density ($g\ cm^{-3}$)

are especially important in biofilms as these processes control growth of the microorganism by affecting the availability of nutrients and oxygen within the bioactive layer; how different layers within the biofilm result in differentiation of organisms; and availability of oxygen and substrate which in turn affect the biofilm characteristics (i.e., structure, thickness, sub-layers).

2.2. Mass transfer: external film diffusion

The transport processes that occur in packed bed reactors with immobilized cultures include (1) transfer of substrate from bulk liquid to immobilized bioactive surface; and (2) simultaneous diffusion and reaction of substrate within the bioactive layer (i.e., biocatalyst) [19,20]. According to the film-theory, there exists a laminar film next to the surface which is in contact with a moving fluid. The transport of substrate to biofilm occurs primarily by molecular diffusion and is called external mass transfer. In this study, the mass transfer model was developed according to the approach described by Aksu and Bülbül [21].

2.3. Removal rate constant

The material balance for carbohydrate and protein in the packed bed column was developed by assuming cube shaped packing media, plug flow, no axial dispersion and steady state conditions. The corresponding material balance equation can be written as [19,20]:

$$\left(\frac{HQ}{W}\right)\frac{dC}{dZ}\times\left(\frac{60}{10^3}\right)=-r \tag{1}$$

where r is the reaction rate ($mg\ g^{-1}\ h^{-1}$), Q is the volumetric flow rate ($mL\ min^{-1}$), H is the height of the bioreactor (cm), W is the amount of immobilized organisms (g), dC/dZ is the concentration gradient along the column height ($mg\ L^{-1}\ cm^{-1}$). Eq. (2) relates the apparent removal rate and bulk substrate concentration in the column. Assuming first-order kinetics, the reaction rate can be written in terms of bulk substrate concentration (i.e., carbohydrate and protein):

$$\left(\frac{HQ}{W}\right)\frac{dC}{dZ}\times\left(\frac{60}{10^3}\right)=-k_pC \tag{2}$$

where k_p is the observed first-order reaction rate constant ($L\ g^{-1}\ h^{-1}$) and C is the bulk substrate concentration ($mg\ L^{-1}$). For the boundary conditions 1 and 2, below:

Boundary condition 1: at $Z=0$ of $C=(\text{Substrate})_{in}$ and

Boundary condition 2: at $Z=H$ of $C=(\text{Substrate})_{out}$

Eq. (2) can be solved as follows:

$$\frac{\text{Ln}(\text{Substrate})_{in}}{(\text{Substrate})_{out}}=\frac{W}{Q}\times k_p\times\left(\frac{60}{10^3}\right) \tag{3}$$

where $(\text{Substrate})_{in}$ concentration at the inlet ($mg\ L^{-1}$) and $(\text{Substrate})_{out}$ is concentration at the outlet ($mg\ L^{-1}$). The outlet concentration of the re-circulated packed bed reactor can be written as [20]:

$$(\text{Substrate})_{out}=(\text{Substrate})_{in}\times e^{-N} \tag{4}$$

where,

$$N=\frac{W}{Q}\times k_p\times\left(\frac{10^3}{60}\right) \tag{5}$$

2.4. Combined mass transfer and biochemical reaction

When fluid flows through a packed bed, there are regions near the surface of the packing media where the fluid velocity is very low. In such regions around the exterior of packing media, the substrate transport takes place primarily by molecular diffusion. Since this rate may be very slow, the observed reaction rate can be significantly decreased by the external film diffusion. The local rate of film diffusion of the substrate from the bulk fluid to the surface of the immobilized cells may be considered to be proportional to the area for mass transfer and the driving force for mass transfer (i.e., concentration difference between the bulk and the external surface of the immobilized cell) [19]. For simplicity purposes, assuming biofilm acts as a catalyst for conversion of nutrients to waste products, mass transfer rate of substrate (carbohydrate and protein) from the bulk liquid to the surface of the immobilized culture can be expressed as:

$$r_m=k_m a_m(C-C_s) \tag{6}$$

where r_m is the external mass transfer rate of substrate ($mg\ g^{-1}\ h^{-1}$), k_m is the external mass transfer coefficient ($cm\ h^{-1}$), a_m is the external surface area for mass transfer ($cm^2\ g^{-1}$), C is the substrate concentration in the bulk liquid ($mg\ L^{-1}$), and C_s is the substrate concentration at the surface of the immobilized cell ($mg\ L^{-1}$).

For the packing media, the value of a_m can be determined as [19]:

$$a_m=\frac{6(1-\varepsilon)}{d_p} \tag{7}$$

where d_p is the particle diameter (cm) and ε is the porosity.

The first-order biochemical reaction rate of the immobilized culture can be written as:

$$r=k_s a_m C_s \tag{8}$$

where k_s is the intrinsic first-order rate constant ($cm\ h^{-1}$).

At steady state, the rate of mass transfer is equal to the rate of reaction ($r = r_m$). From Eqs. (6) and (8):

$$C_s = \frac{k_m C}{k_s + k_m} \tag{9}$$

Substituting Eq. (9) to Eq. (8):

$$r = \frac{k_s k_m a_m C}{k_s + k_m} \tag{10}$$

For first-order kinetics, reaction rate can be written as:

$$r = k_p C \tag{11}$$

From Eqs. (10) and (11):

$$k_p = \frac{k_s k_m a_m}{k_s + k_m} \tag{12}$$

After rearranging for external mass transfer coefficient (k_m) as:

$$k_m = \frac{k_p k_s}{k_s a_m - k_p} \tag{13}$$

2.5. Empirical model

The external mass transfer coefficient (k_m) can be expressed in terms of operational parameters (e.g., flow rate) and the properties on the fluid by the dimensionless group [19,20]:

$$J_D = \frac{k_m \rho}{G}\left(\frac{\mu}{\rho D_f}\right)^{2/3} = K(Re)^{n-1} \tag{14}$$

where J_D is the Colburn factor and Re is the Reynolds number. The value of n depends on the mass transfer conditions and varies from 0.1 to 1.0 depending on the flow characteristics. From Eq. (14), the mass transfer coefficient can be expressed as:

$$k_m = \left(\frac{K}{\rho}\right)\left(\frac{\mu}{\rho D_f}\right)^{-2/3}\left(\frac{d_p}{\mu}\right)^{n-1} G^n \tag{15}$$

or:

$$k_m = AG^n \tag{16}$$

where,

$$A = \left(\frac{K}{\rho}\right)\left(\frac{\mu}{\rho D_f}\right)^{-2/3}\left(\frac{d_p}{\mu}\right)^{n-1} \tag{17}$$

By substituting Eq. (16) into Eq. (13):

$$\frac{1}{k_p} = \left(\frac{1}{A a_m}\right)\left(\frac{1}{G^n}\right) + \left(\frac{1}{k_s a_m}\right) \tag{18}$$

The plot $1/k_p$ vs $1/G^n$ for different values of n yields straight lines with slope $(1/Aa_m)$ and intercept $(1/k_s a_m)$. Assuming K and n values, the value of a_m can be determined. The a_m values estimated were compared with a_m values to determine the adequate set of K and n values for carbohydrate and protein in the packed bed.

3. Materials and methods

3.1. Experimental setup

Fig. 1 presents a schematic of the packed bed bioreactor. The system consisted of a tubular reactor, feed pump, feed solution container, flow meter, air pump, thermometer, and Tygon tubing with fitted valves for sampling ports. The reactor was made from 52 cm glass tubing with 5.0 cm inner diameter (with a total volume of 1020 cm^3), which was mounted in vertical position. The substrate feed (synthetic wastewater) and air lines were located at the lower end of the reactor and port 3 was used for the effluent. Fifty grams of packing material consisting of polyurethane foam was cut into about 1.5 cm × 1.5 cm × 1.5 cm cube shaped pieces and placed

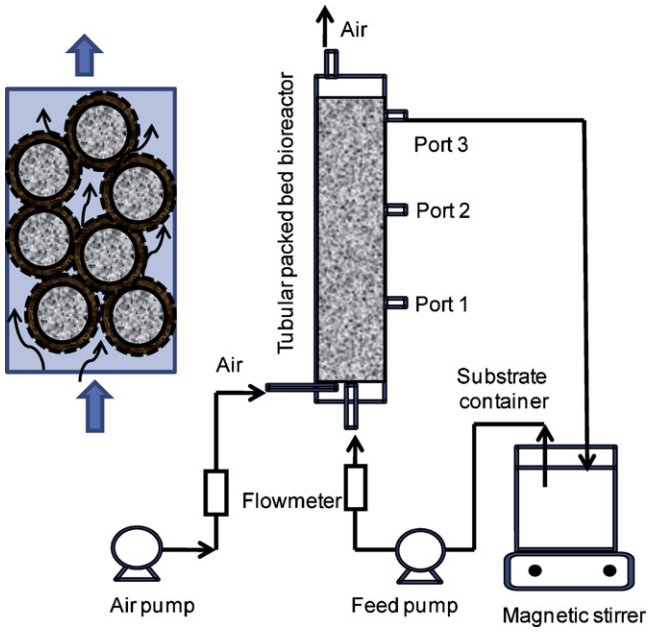

Fig. 1. Schematic representation of the packed bed bioreactor.

loosely inside the reactor. Inside the reactor, the polyurethane foam filled up to 38 cm of the column (i.e., loosely filling 745 cm^3 of reactor volume). Air and wastewater flow rates were monitored by flow meters on their respective lines. The outlets for air and effluent were located at the top of the column. Tygon tubing was used for air supply, feed line, and sampling ports. The system was aerated through a coarse bubble diffuser placed at the base of the column.

3.2. Immobilization of microbial culture and operation of bioreactor

Activated sludge culture was obtained from the aeration tank at South Dade Wastewater Treatment Plant in Miami, Florida. The culture was acclimated to the synthetic wastewater using a completely aerated batch stirred tank reactor for 20 days. The synthetic wastewater was prepared to contain 650 mg L^{-1} glucose, 50 mg L^{-1} peptone, 100 mg L^{-1} urea, 50 mg L^{-1} $(NH_4)_2SO_4$, 50 mg L^{-1} KH_2PO_4, and 5 mg L^{-1} K_2HPO_4. $NaHCO_3$ was used as buffer to adjust the influent pH to about 7.3 ± 0.2. Required trace metals derived from $CaCl_2$ (10 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (50 mg L^{-1}), NaCl (50 mg L^{-1}), KCl (10 mg L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.1 mg L^{-1}), $FeCl_3{\cdot}6H_2O$ (10 mg L^{-1}), $ZnCl_2$ (0.25 mg L^{-1}), $CoCl_2{\cdot}6H_2O$ (0.45 mg L^{-1}), and $MnSO_4{\cdot}7H_2O$ (7 mg L^{-1}) were provided to the system. All chemicals were analytical grade.

The activated sludge culture was re-circulated (15 mL min^{-1}) through the reactor using a variable speed Master Flex peristaltic pump (Cole Parmer, Illinois) for 20 days, replacing fresh culture medium containing 2000 ± 100 mg L^{-1} MLSS every 2 days. During the start up of the tubular bioreactor, the system was filled with the synthetic wastewater and operated in batch mode for 20 days until the biofilm was established on the packing material. The pH level was monitored and adjusted to 7.3 ± 0.2 on daily basis. The decrease in turbidity over the first 10 h of recirculation indicated a net flux of biomass from the culture container and immobilization of cells on the support matrix in the reactor. Attachment and proliferation of the biomass on the porous packing material was visually observed until the entire packing material was coated with biofilm. The system was operated for another 10 days before daily monitoring of carbohydrate and protein. During this period, the bioreactor was fed daily with 1.5 L of synthetic wastewater which was re-circulated through packed bed. The feed quality was maintained

Table 1
Bioreactor test data.

Q (cm^3 h^{-1})	Carbohydrate		Protein	
	Inlet (mg L^{-1})	Outlet (mg L^{-1})	Inlet (mg L^{-1})	Outlet (mg L^{-1})
900	560 ± 15	9.37 ± 2.33	40 ± 5	6.90 ± 1.12
1200	560 ± 15	25.47 ± 3.45	40 ± 5	10.40 ± 1.94
1800	560 ± 15	67.67 ± 5.87	40 ± 5	15.65 ± 2.09

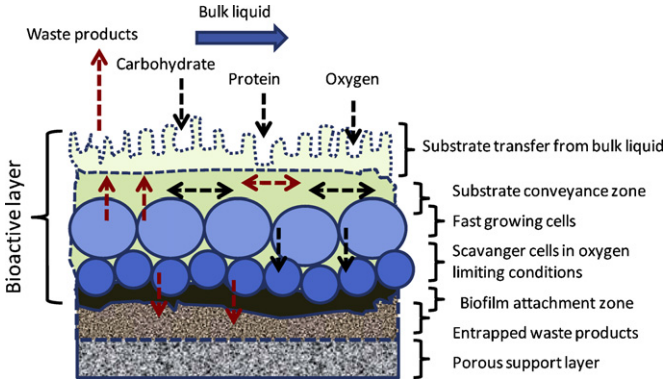

Fig. 2. Schematic representation of immobilized culture.

at 560 ± 15 mg L^{-1} for carbohydrate and 40 ± 5 mg L^{-1} for protein levels and outlet concentrations for three different flow rates (900, 1200, 1800 cm^3 h^{-1}) were represented in Table 1.

3.3. Sampling and monitoring methods

Samples collected from the reactor inlet and outlet was analyzed for carbohydrate and protein. The pH measurements were conducted with a glass electrode (WTW multi 340i model pH meter, Multi Parameter Instrument). Temperature and dissolved oxygen (DO) were monitored by a DO multimeter (HACH HQ 40d multi).

3.4. Carbohydrate and protein analysis

Samples (5 mL) were centrifuged (13,200 × g, 20 min, 4 °C) (Sigma 318-K) and analyzed for carbohydrate and protein. The protein content was analyzed by the method described by Lowry et al. [22]. BSA was used as the standard and the results expressed in mg equivalent of BSA per gram of liter. Carbohydrate was determined by the phenol–sulfuric acid method as described by Dubois et al. [23]. Glucose was used as the standard and results were expressed in mg equivalent of glucose per gram of liter. The samples were analyzed using a UV–vis spectrophotometer (Turner Spectrophotometer SP 830) at the wavelength of 490 nm for carbohydrate or at the wavelength of 660 nm for protein. Calibration curves were prepared for the range between 1 and 200 mg L^{-1}. Precision of the parallel measurements was ±3% SD. All the analyses were conducted in duplicates and their average values were within ±0.2 mg L^{-1} for carbohydrate and ±0.5 mg L^{-1} for protein.

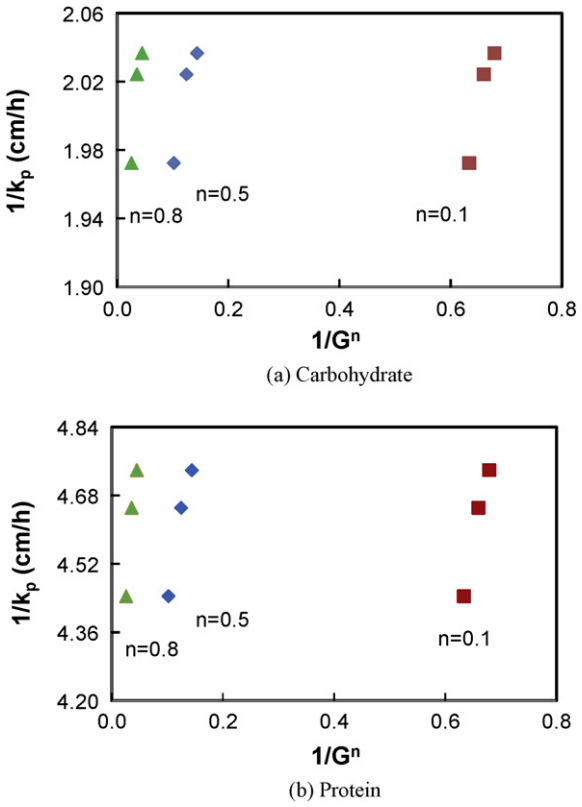

Fig. 3. Variation of $1/k_p$ in relation to $1/G^n$ for different n values (n = 0.1, 0.5, and 0.8) for (a) carbohydrate, and (b) protein.

4. Results and discussion

4.1. External mass transfer

Fig. 2 provides a schematic of the immobilized culture as the microorganism establish on the media. The influence of flow rate on the removal rate constant (k_p) was studied at three different flow rates (900, 1200, 1800 cm^3 h^{-1}) as presented in Table 2. The experimental values of Q and k_p and the calculated values of $1/k_p$,

Table 3
Slope and intercept values from the plots of $1/k_p$ vs $1/G^n$ for various values of n.

n	Carbohydrate		Protein	
	Slope (g^n cm^{-2n} h$^{(1-n)}$)	Intercept	Slope (g^n cm^{-2n} h$^{(1-n)}$)	Intercept
0.09	NA	<0	NA	<0
0.10	1.458	1.052	6.701	0.203
0.12	1.321	1.212	6.076	0.935
0.15	1.199	1.371	5.516	1.668
0.20	1.110	1.531	5.106	2.400
0.25	1.095	1.626	5.042	2.839
0.30	1.125	1.690	5.185	3.132
0.40	1.283	1.770	5.919	3.498
0.50	1.560	1.817	7.203	3.717
0.60	1.974	1.849	9.126	3.863
0.80	3.407	1.889	15.794	4.045
1.00	6.260	1.913	29.084	4.154

Table 2
Characteristics of the bioreactor operational parameters and experimentally determined k_p values for carbohydrate and protein.

Q (cm^3 h^{-1})	Carbohydrate		Protein		Re	G(g cm^{-2} h^{-1})	$1/G^n$				
	k_p (L g^{-1} h^{-1})	$1/k_p$ (g h L^{-1})	k_p (L g^{-1} h^{-1})	$1/k_p$ (g h L^{-1})			n = 0.25	n = 0.3	n = 0.4	n = 0.5	n = 0.8
900	0.491	2.037	0.211	1.543	1.543	48.153	0.379	0.313	0.212	0.144	0.045
1200	0.494	2.022	0.215	2.058	2.058	64.294	0.353	0.287	0.189	0.125	0.036
1800	0.507	1.972	0.255	3.087	3.087	96.306	0.319	0.254	0.161	0.102	0.026

Table 4
Estimated values of surface area for mass transfer (a_m), A and rate constant (k_s) in relation to n (K = 5.7).

n	$A \times 10^{-4}$ ($g^{-n}\,cm^{(2n+1)}\,s^{-(1-n)}$)	Carbohydrate		Protein	
		k_s ($cm\,h^{-1}$)	a_m ($cm^2\,cm^{-3}$)	k_s ($cm\,h^{-1}$)	a_m ($cm^2\,cm^{-3}$)
0.10	5.30	1.164	0.817	27.745	0.177
0.12	5.82	0.856	0.964	5.099	0.209
0.15	6.71	0.619	1.178	2.340	0.256
0.20	8.51	0.432	1.512	1.268	0.329
0.25	10.79	0.338	1.820	0.891	0.395
0.30	13.69	0.281	2.104	0.699	0.457
0.40	22.00	0.217	2.603	0.507	0.564
0.50	35.38	0.182	3.019	0.411	0.654
0.60	56.88	0.161	3.367	0.355	0.728
0.80	147.01	0.136	3.881	0.295	0.837
1.00	380.00	0.124	4.204	0.266	0.905

Re, u, G, and $1/G^n$ ($0 < n < 1$) for the immobilized culture packed bed reactor at steady state conditions are presented in Table 2. The k_p values increased with increasing flow rate due to higher turbulence which reduces boundary layer effects [20]. Although the first-order rate constant also increased with flow rate; the overall carbohydrate and protein removal decreased. This may be due to the lower residence times at higher flow rates, which may have increase the short circuiting inside the reactor.

The relevant dimensionless numbers and mass fluxes were calculated for $\mu = 1.3 \times 10^{-2}\,g\,cm^{-1}\,s^{-1}$, $\rho = 1.05\,g\,cm^{-3}$, $D_f = 0.75 \times 10^{-5}\ cm^2\,s^{-1}$, and $\varepsilon = 0.8$. Using Eq. (18), $1/k_p$ vs $1/G^n$ was plotted for different values of n. Negative intercepts were obtained for $n < 0.05$ for carbohydrate and $n < 0.10$ for protein; hence, these n values were not considered for further analysis. The slopes and intercepts in relation to n are provided in Table 3. The slope decreased for the values of n between 0.10 and 0.25 and increase for values higher than 0.3. The intercept values increased with increasing n values. All n values gave a satisfactory straight line fit; however, not all n values resulted in a good estimate for the values of a_m calculated by experimental data.

The analysis was carried out with two different K values 1.625 and 5.7 to determine A [19]. The values of A obtained with Eq. (17) were used to calculate a_m from the slope of $1/k_p$ vs $1/G^n$ plots. Example plots of $1/k_p$ vs $1/G^n$ for $n = 0.1$, 0.5, and 0.8 are depicted in Fig. 3. The values of K, A, a_m, and k_s obtained for different n values are presented in Table 4. The value a_m obtained for $K = 5.7$ and $n = 0.1$ for carbohydrate and $K = 5.7$ and $n = 0.8$ for protein were similar to the experimental values of a_m ($0.800\,cm^2\,cm^{-3}$). The K value of 1.625 did not give adequate estimated for a_m that were similar to the experimental values. Hence, it was not considered for further evaluation. Table 4 provides the estimated a_m values in relation to n which were used to calculate the rate constant (k_s) from the intercept ($1/k_s a_m$).

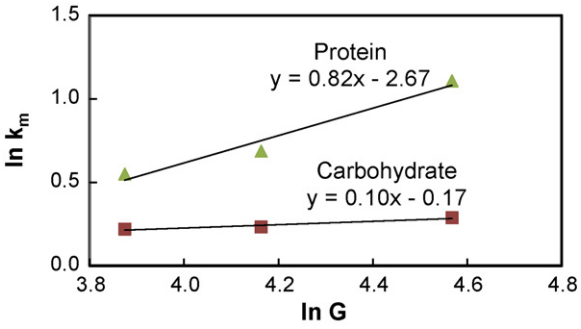

Fig. 4. Variation of external mass transfer coefficient (k_m) in relation to superficial mass velocity (G) for carbohydrate and protein.

The mass transfer coefficient (k_m) was estimated by Eq. (15) as presented in Table 5. From Eq. (16), it can be seen that the plot $\ln k_m$ vs $\ln G$ yields intercept as $\ln A$ and slope as n. The slope and intercept values obtained for the present study are 0.10 and −0.17 for carbohydrate and 0.82 and −2.67 for protein, respectively (Fig. 4). The value of A obtained was $5.30 \times 10^{-4}\,g^{-0.1}\,cm^{1.2}\,s^{-0.9}$ for carbohydrate and $135.13 \times 10^{-4}\,g^{-0.8}\,cm^{2.6}\,s^{-0.2}$ for protein. These values are similar to the calculated values of A ($5.30 \times 10^{-4}\ g^{-0.1}\,cm^{1.2}\,s^{-0.9}$ for

Table 5
Estimated values of external mass transfer coefficient (k_m) at different mass velocities (G) for K = 5.7, n = 0.10 for carbohydrate and K = 5.7, n = 0.82 for protein).

Q ($cm^3\,h^{-1}$)	G ($g\,cm^{-2}\,h^{-1}$)	Carbohydrate k_m ($cm\,h^{-1}$)	Protein k_m ($cm\,h^{-1}$)
900	48.153	1.246	1.733
1200	64.294	1.261	1.987
1800	96.306	1.333	3.029

Table 6
Comparison of experimental k_p values obtained from Eq. (3) and calculated k_p values obtained from Eq. (12) at various flow rates (900, 1200, 1800 $cm^3\,h^{-1}$) in relation to n for (a) carbohydrate, and (b) protein.

Q ($cm^3\,h^{-1}$)	Exp. k_p ($L\,g^{-1}\,h^{-1}$)	Calculated k_p ($L\,g^{-1}\,h^{-1}$)							
		n = 0.10	n = 0.12	n = 0.20	n = 0.30	n = 0.40	n = 0.60	n = 0.80	n = 1.00
Carbohydrate									
900	0.491	0.491	0.489	0.485	0.483	0.481	0.479	0.477	0.475
1200	0.494	0.494	0.492	0.486	0.484	0.482	0.480	0.478	0.476
1800	0.507	0.507	0.503	0.493	0.489	0.486	0.483	0.480	0.478
Protein									
900	0.211	0.290	0.271	0.241	0.227	0.221	0.215	0.211	0.209
1200	0.215	0.329	0.299	0.254	0.236	0.228	0.219	0.215	0.212
1800	0.225	0.485	0.398	0.294	0.259	0.245	0.232	0.225	0.221

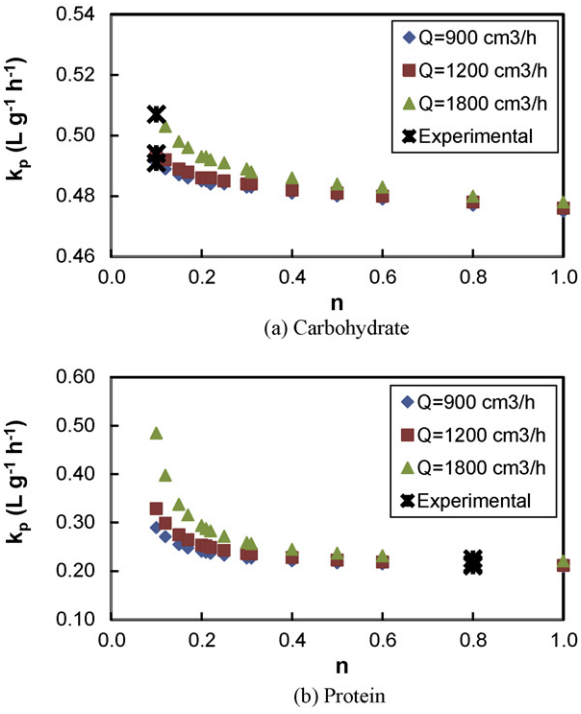

Fig. 5. Comparison of experimental and calculated k_p values at different flow rates (Q = 900, 1200, 1800 cm^3 h^{-1} min^{-1}) in relation to n for (a) carbohydrate, and (b) protein.

carbohydrate and 147.01×10^{-4} g$^{-0.8}$ cm$^{2.6}$ s$^{-0.2}$ for protein) from Eq. (15) at K and n values of 5.7, 0.1 for carbohydrate and 5.7, 0.8 for protein.

The model adequately predicted the external mass transfer influence during carbohydrate and protein removal by the immobilized activated sludge culture. The estimated k_p values at different n values are presented in Table 6 and Fig. 5. The estimated k_p values were similar to that of experimental k_p at $K = 5.7$ and $n = 0.10$ for carbohydrate and $K = 5.7$ and $n = 0.82$ for protein.

The mass transfer correlations which adequately predict the carbohydrate and protein removal in the re-circulated packed bed bioreactor were developed as follows:

$$\text{For carbohydrate:} \quad J_D = 5.7 \times Re^{-0.90} \tag{19}$$

$$\text{For protein:} \quad J_D = 5.7 \times Re^{-0.18} \tag{20}$$

As presented in Fig. 5, removal of carbohydrate is more sensitive to changes in flow rate; hence, more sensitive to *Re*. On the other hand, removal of protein is not sensitive to changes in flow rate. The SEM images of polyurethane packing medium showed significant accumulation of bioactive layer as presented in Fig. 6.

Table 7 compares the correlations developed for Colburn factors for bioreactors with packed media and immobilized cultures. The absolute value of the exponent of *Re* (i.e., 0.90) is larger than the exponents reported for other cultures and substrates. This may be due to high metabolization rate for the carbohydrate used (i.e., glucose). For protein, the exponent of *Re* has smaller absolute value (i.e., 0.18) may be due a number of factors such as: (1) some protein may be available within the biofilm due to decaying cells, (2) demand for protein is less than the demand for carbohydrate, and (3) protein used (BSA) has significantly higher molecular weight in comparison to that for the carbohydrate used (i.e., glucose); hence lower diffusion ability into the biofilm.

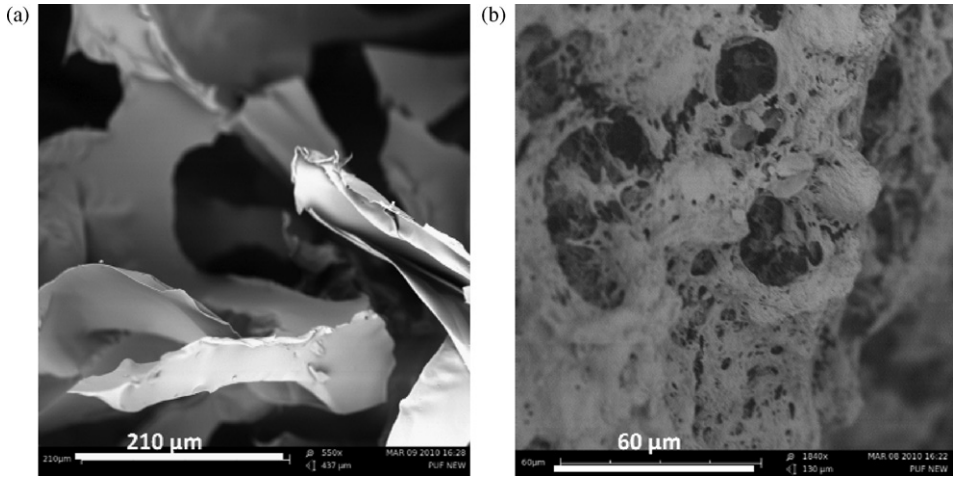

Fig. 6. SEM micrographs of (a) clean packing media and (b) sample removed from the bioreactor.

Table 7
Comparison of Colburn factor correlations by different studies using packed media and immobilized cultures.

Packed bed bioreactor and culture media	Bed void fraction	Packing diameter (cm)	Model	Reference
Immobilized lipase for hydrolysis of palm olein	0.04	0.065	$J_D = 0.056$	[24]
Immobilized glucose oxidase enzyme on porous glass beads			$J_D = 1.625 \times Re^{-0.507}$	[25]
Immobilized collagen enzyme chips			$J_D = 5.7 \times Re^{-0.78}$	[26]
Immobilized *Saccharomyces cerevisiae* on activated bagasse chips	0.45	0.986	$J_D = 5.7 \times Re^{-0.59}$	[19]
Immobilized *Bacillus* sp. on calcium alginate to reduce hexavalent chromium	0.5	0.21	$J_D = 5.7 \times Re^{-0.70}$	[20]
Immobilized activated sludge culture on polyurethane foam to remove carbohydrate	0.8	1.5	$J_D = 5.7 \times Re^{-0.90}$	This study
Immobilized activated sludge culture on polyurethane foam to remove protein	0.8	1.5	$J_D = 5.7 \times Re^{-0.18}$	This study

5. Conclusions

Experimental results indicate that reaction/diffusion phenomena within the biofilm control the thickness of the biofilm, hence; oxygen and/or substrate availability can within the biofilm can limit the metabolic rate of the culture. In this study, microbial removal of carbohydrate and protein was conducted using packed bed bioreactor with immobilized activated sludge culture on polyurethane media. Based on the analyses, $J_D = 5.7 \times Re^{-0.90}$ for carbohydrate and $J_D = 5.7 \times Re^{-0.18}$ for protein were found to be adequate to predict the removal of carbohydrate and protein in the bioreactor.

References

[1] D.C. Sau, S. Mohanty, K.C. Biswal, Minimum fluidization velocities and maximum bed pressure drops for gas–solid tapered fluidized beds, Chem. Eng. J. 132 (1–3) (2007) 151–157.

[2] C. Juneson, O.P. Ward, A. Singh, Biodegradation of dimethyl phthalate with high removal rates in a packed-bed reactor, World J. Microbiol. Biotechnol. 18 (1) (2002) 7–10.

[3] E.O. Obanijesu, O.O. Bello, F.A.O. Osinowo, S.R.A. Macaulay, Development of a packed-bed reactor for the recovery of metals from industrial wastewaters, Int. J. Environ. Pollut. 22 (6) (2004) 701–709.

[4] K.L. Johnson, P.L. Younger, Rapid manganese removal from mine waters using an aerated packed-bed bioreactor, J. Environ. Qual. 34 (2005) 987–993.

[5] W. Parawira, M. Mutto, R. Zvauya, B. Mattasson, Comparative performance of a UASB reactor and an anaerobic packed-bed reactor when treating potato waste leachate, Renew. Energy 31 (6) (2005) 893–903.

[6] H. Zilouei, B. Guieysse, B. Mattiasson, Biological degradation of chlorophenols in packed-bed bioreactors using mixed bacterial consortia, Process Biochem. 41 (5) (2006) 1083–1089.

[7] A. Razaee, H. Godini, N. Naimi, H. Mosombaigi, A. Yazanbaksh, H. Gholamreza, R. Kazemnejad, High nitrate removal in a packed bed reactor using microbial cellulose, Res. J. Environ. Sci. 2 (6) (2008) 424–432.

[8] B.E. Logan, D. LaPoint, Treatment of perchlorate- and nitrate-contaminated groundwater in an autotrophic, gas phase, packed-bed bioreactor, Water Res. 36 (2002) 3647–3653.

[9] N Fiol, C. Escudero, J. Poch, I. Villaescusa, Preliminary studies on Cr(VI) removal from aqueous solution using grape stalk wastes encapsulated in calcium alginate beadsb in a packed bed up-flow column, React. Funct. Polym. 66 (2006) 795–807.

[10] A. Cordoba, P. Vargas, J. Dussan, Chromate reduction by Arthrobacter CR47 in biofilm packed bed reactors, J. Hazard. Mater. 151 (2008) 274–279.

[11] K.S. Ashish, T. Conneely, K.R. Nosslein, S.J. Ergas, Biological perchlorate reduction in packed bed reactors using elemental sulfur, Environ. Sci. Technol. 43 (2009) 4466–4471.

[12] O. Tepe, A.Y. Dursun, Combined effects of external mass transfer and biodegradation rates on removal of phenol by immobilized *Ralstonia eutropha* in a packed bed reactor, J. Hazard. Mater. 151 (2008) 9–16.

[13] S. Mudliar, S. Banerjee, A. Vaidya, S. Devotta, Steady state model for evaluation of external and internal mass transfer effects in an immobilized biofilm, Bioresour. Technol. 99 (2008) 3468–3474.

[14] V.R.C. Murty, J. Bhat, P.K.A. Muniswaran, External mass transfer effects during the hydrolysis of rice bran oil in immobilized lipase packed bed reactor, Chem. Biochem. Eng. 19 (2005) 57–61.

[15] S.F.A. Halim, A.H. Kamaruddin, W.J.N. Fernando, Continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: optimization using response surface methodology (RSM) and mass transfer studies, Bioresour. Technol. 100 (2009) 710–716.

[16] S.A. Beg, M.M. Hassan, M. Arshad, S. Chaudhry, Effect of sinusoidal variation of feed concentration and temperature on the performance of a packed-bed biological reactor – a theoretical study, Chem. Eng. Technol. 19 (1996) 43–49.

[17] M. Deront, M. Falilou, S.N. Adler, P. Peringer, Volumetric oxygen mass transfer coefficient in upflow cocurrent packed-bed bioreactor, Chem. Eng. Sci. 53 (1998) 1321–1330.

[18] C. Di Iaconi, R. Ramadori, A. Lopez, R. Passino, Influence of hydrodynamic shear forces on properties of granular biomass in a sequencing batch biofilter reactor, Biochem. Eng. J. 30 (2) (2006) 152–157.

[19] S. Nath, S. Chand, Mass transfer and biochemical reaction in immobilized cell packed-bed reactors: correlation of experiment with theory, J. Chem. Technol. Biotechnol. 66 (1996) 286–292.

[20] M.N. Kathiravan, R.K. Rani, R. Karthick, K. Muthukumar, Mass transfer studies on the reduction of Cr(VI) using calcium alginate immobilized Bacillus sp. in packed bed reactor, Bioresour. Technol. 101 (2010) 853–858.

[21] Z. Aksu, G. Bülbül, Investigation of the combined effects of external mass transfer and biodegradation rates on phenol removal using immobilized *P. putida* in a packed-bed column reactor, Enzyme Microb. Technol. 22 (1998) 397–403.

[22] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[23] M.J. Dubois, K.A. Gills, J.K. Hamilton, P.A. Reber, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356.

[24] C.Y. Hoon, L.C. Tin, External mass transfer model for a recirculated packed-bed batch reactor on the hydrolysis of palm olein using immobilized lipase, in: Proceedings of the 1st International Conference on Natural Resources Eng. & Tech., July 24–25, Putrajaya, Malaysia, 2006, pp. 500–509.

[25] Rovito B.J., Kittrell J.R. J.R., Film and pore difuusion studies with immobilized glucose oxidase, Biotechnol. Bioeng. 15 (1973) 143–161.

[26] W.R. Vieth, K. Venkatasubramanian, A. Constantinides, B. Davidson, Design and analysis of immobilized flow reactors, in: L.B. Wingard, E.K. Katzir, L. Goldtein (Eds.), Immobilized Enzyme Principles, Academic Press, New York, Appl. Biochem. Bioeng. 1 (1976) 221–327.