5 Investigation of External Mass Transfer during Biodegradation of Congo Red Dye in a Recirculating Packed Bed Bioreactor

Large amounts of color containing industrial effluents are released into the environment due to the widespread use of azo dyes in food, textiles, cosmetics, leather, plastics, paper printing, pharmaceutical, photography, and pigment production. Azo dyes are the most common (60–70% of all dyes) of synthetic commercial dyes. It makes them one of the significant environmental contaminants (Srinivasan, Kathiravan, and Gopinath 2011). Azo dyes are aromatic compounds containing one or more (R–N=N–R) groups. Such hazardous colored compounds in the industrial effluent raise severe health and environmental issues (Pinheiro, Touraud, and Thomas 2004; Sessa and Palmquist 2008). The traditional methods for decolorizing textile dyes include membrane filtration, chemical precipitation, coagulation, electrochemical degradation, and adsorption (Phugare et al. 2011). The methods mentioned above successfully remove color, but they use a lot of energy and chemicals. Therefore, they are expensive and generate a lot of secondary pollutants. An efficient and cost-effective treatment technology is required for environment-neutral manufacturing in the color-related industry (Chaturvedi et al. 2021c). Various microorganisms, including bacteria, fungi, and yeasts, have been discovered to decolorize azo dyes. Among them, a thorough investigation of the bacterial breakdown of azo dyes in free cell cultures has already been published (Gopinath et al. 2009; Ip, Barford, and McKay 2010; Jadhav et al. 2011; Junghanns, Neumann, and Schlosser 2012; Manu and Chaudhari 2002; Niebisch et al. 2010). When the free cells are employed at industrial scale, it witnesses several operational issues, such as shear forces, cell toxicity, cell stability under agitated environment, and biomass-effluent separation.

In recent years, researchers have focused on developing immobilized bacterial cells for the degradation of azo dyes. It offers many advantages such as high stability, regeneration, reuse, simpler separation, accelerated response rates, enhanced cell metabolism, limited cell wash-out, and efficient control. Many immobilized cell systems have been developed and

widely used for the complete degradation of textile dyes, including Basic Blue 41 (Elizalde-González, Fuentes-Ramírez, and Guevara-Villa 2009), Reactive Blue 4 (Binupriya et al. 2010), Remazol Brilliant Blue R (Osma, Toca-Herrera, and Rodríguez-Couto 2010), Reactive red 45 (Asgher and Bhatti 2010), Malachite Green (Yang et al. 2011), Anthraquinone (Champagne and Ramsay 2010), Direct Blue 1 (Karagoz et al. 2011) and Congo red (Chaturvedi et al. 2021a). Several researchers have utilized continuous immobilized bioreactors such as air-lift, packed bed, trickling bed, fluidized bed, membrane-based, and rotating biological contactor to decolorize textile dyes. The packed-bed reactors are generally preferred among the other bioreactors for continuous and scalable wastewater treatment applications (Parawira et al. 2006; Zilouei, Guieysse, and Mattiasson 2006). The dyeing effluents have also been treated using a packed bed bioreactor (PBBR) with a single or multi-column configuration (Fiol et al. 2006; Córdoba, Vargas, and Dussan 2008; Sahu et al. 2009).

The internal and external mass transfer resistances play a critical role in the overall reaction kinetics of the immobilized bioreactors, and hence, are essential in the design and scale-up of bioreactors. Many researchers have investigated the effects of mass transfer on the overall reaction rate in the immobilized packed bed reactor systems and concluded that the reaction kinetics were restricted by internal and external mass transfer resistances (Banerjee and Ghoshal 2016; Dizge and Tansel 2010; Geed, Kureel, et al. 2018). Mudliar and coworkers (Mudliar et al. 2008) constructed a steady-state model for evaluating internal pore diffusion and exterior liquid film diffusion effects in an immobilized biofilm system in a continuous mode. However, there are few published studies on the external mass transfer aspects during the biodegradation of azo dyes in bioreactors operating in the 'recirculation' mode. The current study investigated the effect of external mass transfer on the biodegradation rate of Congo red dye in a recirculating packed bed bioreactor (RPBB). The RPBB employed isolated *Bacillus subtilis MN372379* species immobilized on the packing material of low-

density polyethylene (LDPE) foam cubes. The effect of recirculation was accounted for in the calculations of the 'observed' reaction rate constant in the RPBB. The input flow rate was varied to determine the empirical correlation for external mass transfer coefficient and the surface reaction rate constant.

5.1 Bioreactor configuration

For the biodegradation of SDW, a cylindrical bioreactor made of borosilicate glass with a total volume of about 1500 mL and a working volume of 1200 mL (outer diameter = 6 cm, height = 53 cm) was built. Since biodegradation is a slow process, it was impossible to completely degrade dye in a single pass through a packed bed bioreactor. Therefore, the biodegradation process was run in the recirculating mode. A total of 2000 mL of SDW with 200 mg/L dye concentrations was used at varied flow rates in every biodegradation experiment in the RPBB. The sample collecting ports were supplied at the top, and the air supply, influent feeding, and drainage ports were provided at the bottom of the RPBB. The bioreactor was filled with the washed and dried LDPE foam cubes (packing medium) up to a height of 43 cm. Stainless steel sieves were used to prepare the support for the fixed bed of packing material at the top and bottom of the bioreactor. A schematic of the RPBB used in this study is shown in Fig. 5.1.

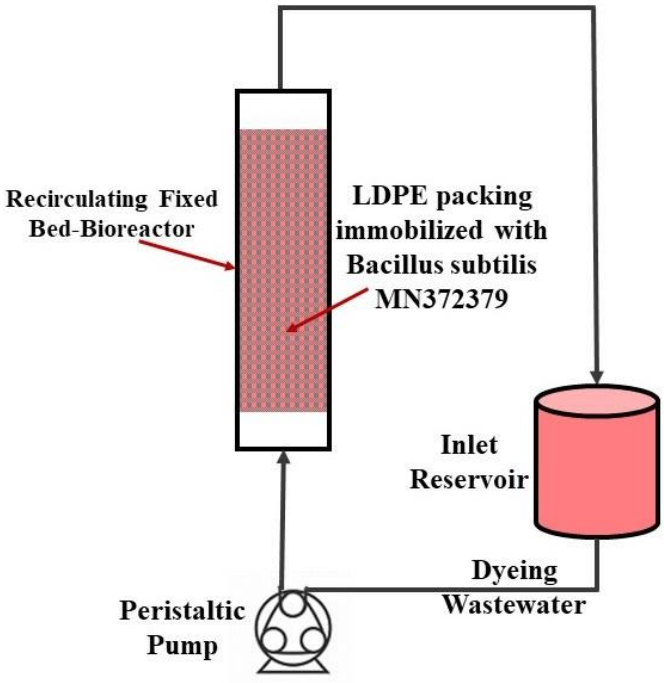

Figure 5.1 Schematic of the recirculating packed bed bioreactor (RPBB). The bioreactor was immobilized with B. subtilis on the LDPE foam cubes

5.2 Bacterial immobilization and acclimatization in the Congo Red dye environment in the RPBB

In the RPBB, the isolated *Bacillus subtilis MN372379* species (Chaturvedi et al. 2021a) were immobilized on the LDPE packing medium. The bacterial immobilization procedure took a total of 20 days to complete. Subsequently, the bacterial mass was acclimatized to the SDW (made of Congo Red dye) environment in the RPBB. The goal of the acclimatization procedure was to get the bacterial population adapted to the anaerobic environment of SDW. The dye concentration in the RPBB inlet stream was gradually raised from 50 to 500 mg/L. At each concentration, the RPBB was operated until the full decolorization of SDW was achieved, followed by a successive cycle of decolorization of SDW at the same dye loading. The second cycle of decolorization at every inlet dye concentration exhibited a relatively higher dye-breakdown rate than the prior cycle implying that the bio-mass had been adapted to the SDW environment in the RPBB.

5.3 Mass Transfer Study: Theoretical Method

In the RPBB, the bacterial species are immobilized onto porous LDPE foam cubes. According to the film hypothesis of bio-transport, the surface of the packing medium (in contact with a moving fluid) develops a fictional laminar boundary layer engulfing the attached biomass (Banerjee and Ghoshal 2016; Kathiravan et al. 2010). Therefore, the substrate (Congo Red dye) must be transported from bulk liquid to the biofilm through the surrounding laminar layer *via* molecular diffusion. After the substrate is diffused to the surface of biomass attached to the LDPE foam, the biochemical reactions occur at the biofilm. While the presence of a laminar layer offers a mass transfer barrier to the transport of a substrate, it enhances the toxicity tolerance of the microorganism present in the biofilm.

5.3.1 Concentration profile and observed biodegradation rate in the RPBB

For a steady-state plug flow with no axial dispersion through immobilized LDPE foam in a packed bed bioreactor (PBB), the following material balance equation is used to calculate the overall rate of biodegradation of dye:

$$\left(\frac{Q}{W/h}\right)\frac{dC}{dz} = -k_p C \qquad (5.1)$$

where Q is the feed flow rate (L/h), W represents the dry cell mass (biocatalyst) in the immobilized foam cubes (g), h represents the bioreactor column height (cm), *W/h* is the dry cell mass in the unit length of the bioreactor, dC/dz represents the Congo Red dye concentration gradient along the length of the bioreactor (mg/L/cm), k_p (L/g/h) represents is the 'observed' (overall) biodegradation rate constant, and C (mg/L) is the substrate concentration. In reality, *W* will change with time because the growth and decay of the active biomass is a continuous process. However, considering the complexity of the biochemical systems and experimental limitations, the value of *W* was determined at the steady-state and assumed to be constant throughout the process.

The following equation is derived by integrating equation 1 from inlet (at z = 0) to outlet (at z = h):

$$ln\left(\frac{C_{in}}{C_{out}}\right) = \frac{W}{Q}k_p \qquad (5.2)$$

where C_{in} and C_{out} stands for the inlet and outlet dye concentrations (mg/L) from the column, respectively. Finally, the concentration of dye at the outlet of the PBB is given by the following expression:

$$C_{out} = C_{in}e^{-\frac{Wk_p}{Q}} \qquad (5.3)$$

5.3.2 Modification in the material balance equation to account for recirculation in an RPBB

Equations 5.1, 5.2, and 5.3 will be valid only for a single pass PBB. Few research papers published in the literature applied Eq. 5.2 for an RPBB that is incorrect (Swain et al. 2021; Banerjee and Ghoshal 2016). In an RPBB, the outlet stream is recycled back to the inlet, the C_{in} keeps changing with time. Therefore, the Eq. 5.1 & 5.2 will hold good only for a single residence time (τ) from any time t to ($t + \tau$). When a mass balance of dye is applied on the inlet reservoir, which was assumed to be a perfectly mixed tank in this study, it yields the following equation:

$$\frac{dC_{in}}{dt} = \frac{C_{in}(t+\tau) - C_{in}(t)}{\tau} \qquad (5.4)$$

Further substituting the Eq. 5.3 into Eq. 5.4, the following equation is obtained:

$$\frac{dC_{in}}{dt} \cong \frac{(C_{in}e^{-\frac{Wk_p}{Q}} - C_{in})}{\tau} \qquad (5.5)$$

Finally, the variations of dye concentration in the reservoir with time are obtained by integrating Eq. 5.5 along the height of the RPBB as:

$$\ln\frac{C}{C_o} = -\left(1 - e^{-W.\frac{k_p}{Q}}\right).\frac{t}{\tau} \qquad (5.6)$$

where C_0 is the initial concentration of dye for the RPBB. The observed dye degradation rate constant, k_p can be determined from the slope of the straight line plotted for *ln* (C/C_O) against time according to Eq. 5.6. At varied circulating flow rates, different values of k_p Can be obtained for the same RPBB.

5.3.3 Evaluation of external mass transfer in the RPBB

In the RPBB, the molecular diffusion of dye through the thin laminar film formed on the biomass immobilized on the LDPE foam might be significantly slow (Sonwani et al. 2020). This extrinsic film diffusion can considerably reduce the observed reaction rate. The rate of substrate diffusion from bulk fluid to the external surface of biomass layer can be described as follows (Dizge and Tansel 2010; Tepe and Dursun 2008b):

$$r_m = k_m A_m (C - C_s) \qquad (5.7)$$

where r_m is the mass transfer rate (mg/g/h), A_m is the surface area per unit mass of LDPE foam available for mass transfer (cm^2/g), k_m is the external mass transfer rate constant ($L/cm^2/h$), C and C_s are the dye concentration in bulk liquid and at the surface of the LDPE foam (mg/L). A_m of the porous foam can be determined from Eq. 5.8 (Kafshgari et al. 2013).

$$A_m = \frac{6(1-\varepsilon)}{\rho_p d_p} \qquad (5.8)$$

where d_p represents the equivalent spherical diameter of the foam cube (cm), ρ_p represents the density of the LDPE (g/cm^3) and ε is the bed porosity. The porosity (i.e., pore volume fraction) of the LDPE foam was determined from the values of ρ_p and the masses of the wet and dried foam cubes (Kafshgari et al. 2013).

5.3.3.1 Relation between the mass transfer coefficient, biochemical surface reaction, and overall dye removal rate constants

Suppose a first-order reaction rate is assumed for the biodegradation of dyes at the surface of the bacterial layer immobilized at the LDPE foam cubes. In that case, the biochemical reaction rate can be described as follows:

$$r = kA_m C_s \tag{5.9}$$

where r is the substrate (i.e., dye) removal rate (mg/g/h), and k is the biochemical surface reaction rate constant (L/cm^{2}h). The substrate removal rate becomes equal to the rate of external mass transfer at the steady-state. Hence, the surface concentration of the substrate can be determined using Eq. (5.7) and (5.9) as:

$$C_s = \frac{k_m C}{k + k_m} \tag{5.10}$$

The observed dye removal rate constant (k_p) can be calculated from equations (5.1), (5.9), and (5.10) as follows:

$$k_p = \frac{k k_m A_m}{k + k_m} \tag{5.11}$$

The rearrangement of Eq. (5.11) results in the following equation that relates the observed dye removal rate in the RPBB to the mass transfer and biochemical reaction rates:

$$\frac{1}{k_p} = \frac{1}{k_m A_m} + \frac{1}{k A_m} \tag{5.12}$$

5.3.3.2 Determination of the correlation for external mass transfer coefficient in the RPBB

The dimensionless Colburn factor, J_D Is used to link the external mass transfer coefficient to operational factors, e. g. reactor diameter, fluid flow rate, and fluid characteristics.

$$J_D = \frac{k_m \rho}{G} \left(\frac{\mu}{\rho D_f} \right)^{2/3} = K Re^{n-1} \tag{5.13}$$

where Re is Reynolds number, ρ is the feed density, K and n are empirical parameters, D_f is the diffusivity, μ is the feed viscosity, and G (g/cm^2.h) is the superficial mass velocity defined as $G = \frac{Q\rho}{A}$. The value of n is found in the range from 0.1 to 1.0 in the literature (Kathiravan et al. 2010). Eq. (5.13) is rearranged to express the external mass transfer rate constant as follows:

$$k_m = NG^n \tag{5.14}$$

where $N = \frac{K}{\rho}\left(\frac{\mu}{\rho D_f}\right)^{-2/3}\left(\frac{d_p}{\mu}\right)^{n-1}$ (5.14a)

Finally, the following equation for k_p is obtained after substituting equation (5.14) in Eq. (5.12):

$$\frac{1}{k_p} = \left(\frac{1}{NA_m}\right)\left(\frac{1}{G^n}\right) + \frac{1}{kA_m} \tag{5.15}$$

In this study, the experimentally observed values of $1/k_p$ is plotted against $1/G^n$ for different values of K and n. Next, the values of A_m are determined from the plot and compared with the calculated A_m (Eq. 5.8) to identify the correct values of K and n for the bioreactor. It enables the determination of a dimensionless correlation for the external mass transfer coefficient and the magnitude of biochemical surface reaction rate constant in the given RPBB.

5.4 Results and Discussion

Although the bacterial population uses dyes as the carbon source, the literature shows that the high concentration of dyes inhibits their metabolic activities (Kathiravan et al. 2014). The microbial strains are immobilized in this study's LDPE foam cube matrix to reduce the substrate inhibitory effect. This is because the microorganisms immobilized on a porous support medium are exposed to a relatively lower concentration of dyes due to the mass transfer barrier than the freely suspended biomass. It improves their tolerance against any

shock of dye loading in the inlet stream and enhances their dye-degrading efficiency. The biodegradation kinetics of immobilized bacterial cells in such cases are affected by various factors driving the mass transfer diffusion rate from bulk to the biofilm.

5.4.1 Effect of inlet mass flow rate on the overall rate of dye removal in the RPBB

The influence of variation in the recirculation flow rate on the overall 'observed' rate of dye-degradation in the RPBB was investigated by changing the feed flow rate between 25 and 100 ml/h while maintaining the dye loading constant 200 mg/L. Fig. 5.2 shows the overall observed dye removal rate constant (k_p) against the volumetric flow rates of the recirculating stream. The k_p was determined from Eq. 5.6 according to the method discussed in Section 5.2.2. The low values of k_p at a low flow rate indicated high mass transfer resistance, which is attributed to the formation of a thick laminar layer around the surface of the LDPE foam under a low Reynolds flow condition (Tepe and Dursun 2008a). A study by Kathiravan et al. (Kathiravan et al. 2010) has shown that the low residence time at high flow rates affects the diffusion of solutes into the pores of the LDPE foam. As the feed flow rate was increased, the thickness of the laminar layer around the LDPE foam was reduced. It led to the high concentration gradient of Congo Red dye within the boundary layer and, therefore, reduced external mass transfer resistance. It eventually increased the value of the overall observed dye removal rate constant in the RPBB.

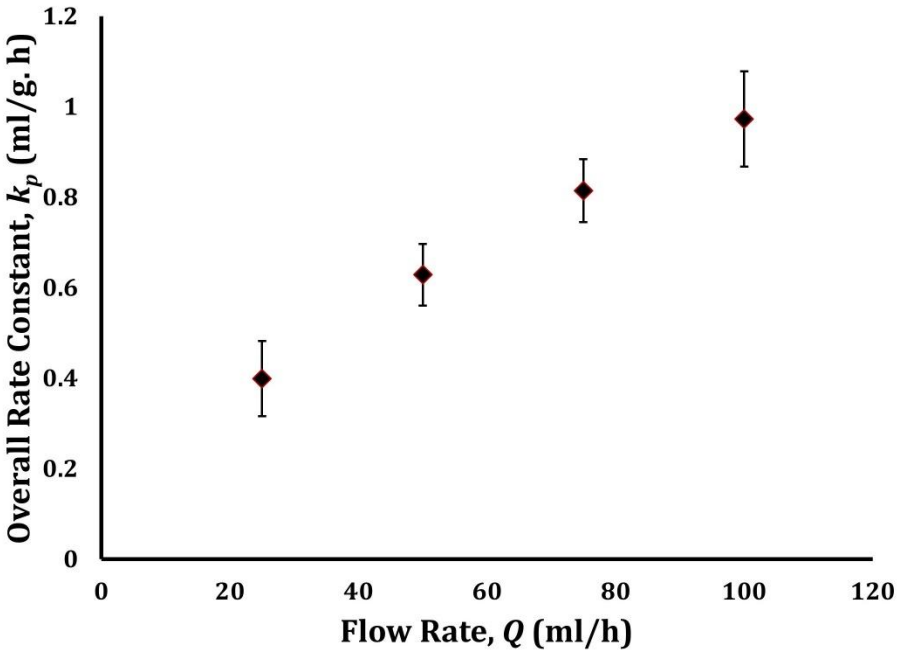

Figure 5.2 Effect of recirculating flow rate on the observed dye removal rate constant at 200 mg/l of inlet dye concentration

5.4.2 Determination of the correlation for external mass transfer coefficient

The mass transfer rate of the substrate into a porous immobilizing matrix plays a critical role in influencing the efficacy of a packed bed bioreactor system (Kureel et al. 2018). The external mass transfer and intra-particle diffusion are two types of diffusional limitations. The external diffusion resistance is caused by forming a laminar boundary layer between the immobilizing matrix and the surrounding fluid. In contrast, the intraparticle diffusion resistance is caused by the concentration difference of the substrate within the matrix. Out of these two, the external mass transfer restriction is significant in governing the biodegradation kinetics in a packed bed bioreactor system. Further, the external mass transfer resistance depends on the feed flow rate, substance concentration, diffusivity, nature of the support material, and other hydrodynamic conditions in a bioreactor.

This study determined the external mass transfer coefficient correlation according to Eq. 5.14, which involved two empirical parameters, *n* and *N*. The parameter *N* depends on another empirical constant, *K* (Eq. 5.14a). First, a range of random values for *n* and *K* are chosen

based on the literature data. k_p was determined (from Eq. 5.6) by running the biodegradation experiments at varied volumetric flow rates of dyeing water in the RPBB. Next, the experimentally determined $1/k_p$ were plotted against $1/G^n$ for various values of n in Fig. 5.3, and the corresponding data are summarized in Table 5.1. The straight-line nature of the plots in Fig. 5.3 was consistent with Eq. 5.15.

Table 5.1 Experimentally determined values of k_p and $1/k_p$, and calculated values of G, and $1/G^n$ at various volumetric flow rate (Q)

Q	k_p	G	$1/k_p$	$1/G^n$				
(ml/h)	(ml/g.hr)	(g/cm^2. h)	(g.hr/l)	n = 0.4	n = 0.6	n = 0.8	n = 0.9	n = 1.0
25	0.399	1.065	2.506	0.975	0.963	0.951	0.945	0.939
50	0.629	2.131	1.589	0.739	0.635	0.546	0.506	0.469
75	0.815	3.196	1.227	0.628	0.498	0.395	0.351	0.313
100	0.973	4.261	1.028	0.561	0.419	0.314	0.272	0.235

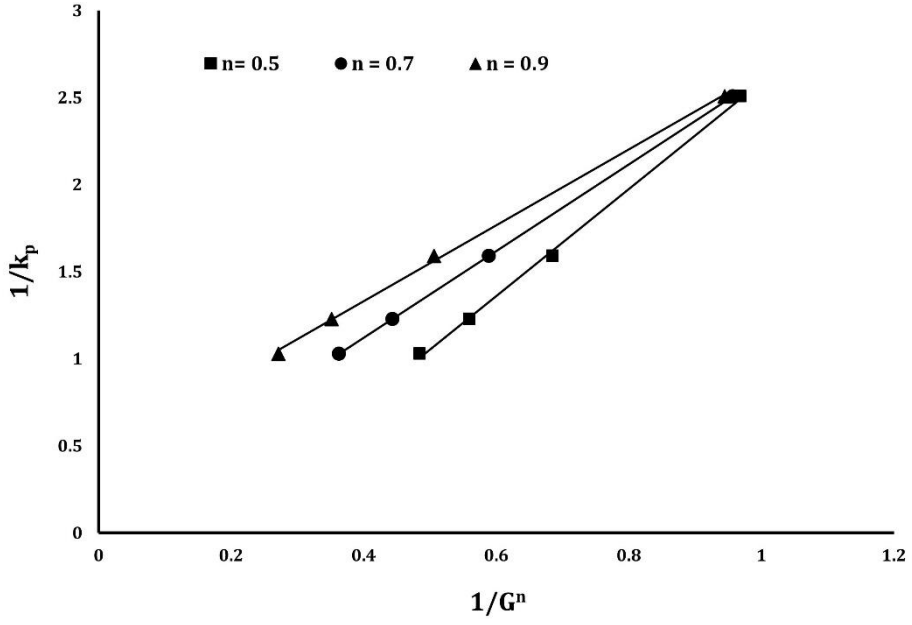

Figure 5.3 Plot of $1/k_p$ against $1/G^n$ at various values of n (= 0.5, 0.7, 0.9)

For the random values of *n* and *K*, the value of *N* was determined from Eq. 5.14a, as has been summarized in Table 5.2 for a few cases. The necessary physical parameters used in the computation were as follows: ρ = 1.205 g/cm^3, d_p = 1.817 cm, ε = 0.32, A_c = 28.286 cm^2, and μ = 0.0085 g/cm. s, D_f = 7.88 ×10^{-5} cm^2/s. It enables the determination of A_m from Fig. 5.3 and Eq. 5.15 for the given value of *N*. The same process was repeated for a series of random values of *n* and *K*, and the corresponding values of A_m were obtained. The calculated values of A_m have been summarized in Table 5.2 for some cases of *n* and *K*. It is apparent from Table 5.2 that the calculated value (3.22 cm^2/g) of A_m for n = 0.7 and K = 1.34 was very close to experimentally determined value (3.48 cm^2/g) of A_m. Therefore, the true values for n and K were selected as 0.7 and 1.34, respectively. The corresponding surface reaction rate constant (*k*) for the biodegradation in the biofilm is 2.5 mL/cm^2. h**.** Finally, the correlation for external mass transfer coefficient (k_m) was obtained (from Eq. 5.14) using the true values of the empirical parameters *n* and *N* as:

$$k_m = 0.125G^{0.7} \qquad (5.16)$$

Table 5.2 Calculated values of N, A_m, and k for various random values of n and K

n	**K=1.34**			**K=1.625**			**K=3.406**		
	N×10^2	**A_m (cm^2/g)**	**k (mL/cm^2.h)**	**N×10^2**	**A_m (cm^2/g)**	**k (ml/cm^2.h)**	**N×10^2**	**A_m (cm^2/g)**	**k (ml/cm^2.h)**
0.7	12.46	3.22	2.50	15.11	2.66	3.03	31.67	1.27	2.12
0.8	20.51	2.12	1.52	24.87	1.74	1.84	52.13	0.83	1.36
0.9	33.76	1.36	1.60	40.94	1.12	1.95	85.81	0.53	0.87
1	55.57	0.87	2.01	67.39	0.72	2.44	114.2	0.34	5.11

5.4.3 Determination of the Colburn factor for external mass transfer coefficient

The Colburn factor provides a dimensionless correlation for the external mass transfer coefficient. After obtaining the true values of the empirical parameters *n* and *k*, the following

Colburn factor was obtained for the biodegradation of Congo red dye using immobilized *Bacillus subtilis MN372379* in the RPBB used in this study (using Eq. 5.13).

$$J_D = 1.34\, Re^{-0.3} \qquad (5.17)$$

The above dimensionless correlation for the Colburn factor can be used in designing the scaled-up RPBB with optimized external mass transfer resistance.

5.4.4 Effect of recirculation flow rate on the external mass transfer

It is apparent from Eq. 5.16 that the mass transfer coefficient, k_m increases with increasing the recirculation flow rate. The values of k_m for various volumetric flow rates in the range of 25-100 ml/h were calculated and summarized in Table 5.3. The mass transfer coefficients were found to be 0.13 to 0.35 ml/cm^2.h for this flow rate range.

Table 5.3 Calculated values of mass transfer coefficient (k_m) at different values of mass flux (G) for n = 0.7 and K = 1.34

Q (ml/cm^{2}h)	G (g/cm^{2}h)	k_p (ml/g.h)	k_m (ml/cm^2.h)
25	1.065	0.399	0.131
50	2.131	0.629	0.212
75	3.195	0.815	0.282
100	4.261	0.973	0.345

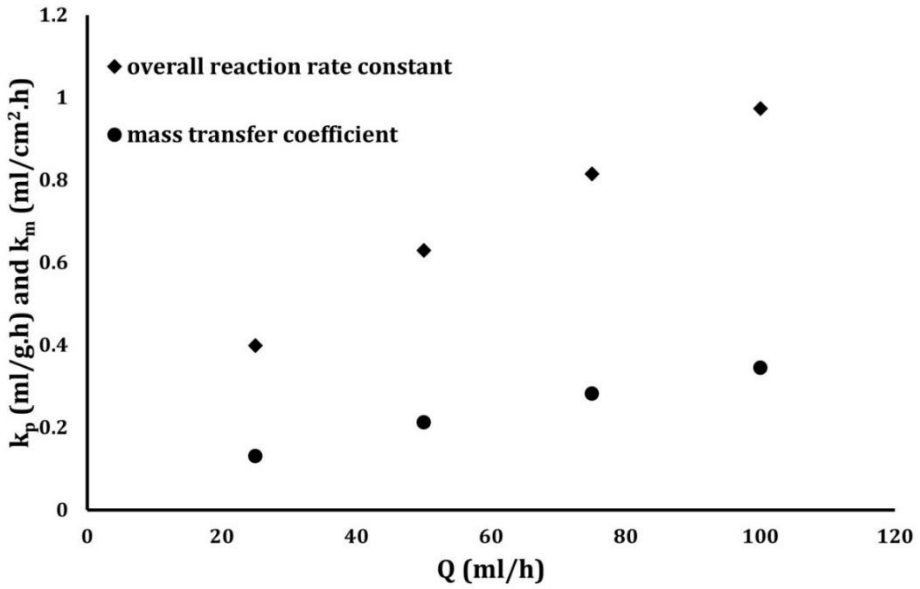

Figure 5.4 Variation in external mass transfer coefficient and the overall reaction rate constant with the recirculation flow rate

Figure 5.4 displays the external mass transfer coefficient (along with the overall dye degradation rate constant, k_p) as the recirculation flow rate function. The increase in k_p with the recirculation flow rate is attributed to the increased mass transfer coefficient at a high recirculation rate. When the recirculation flow rate was doubled, the overall biodegradation rate constant was increased by ~36 %. Generally, at a high feed flow rate through a packed bed reactor, the overall kinetic becomes reaction limited and independent of the mass transfer resistance. Therefore, a high recirculation flow rate would lower the external mass transfer barrier. However, an extremely high recirculation flow may destabilize the biofilm adhered to the LDPE packing; hence, the recirculation flow rate needs optimization to achieve maximum overall dye degradation while ensuring a stable biochemical operation in the RPBB.

5.5 Conclusion

The objective of the presented study was to investigate the effect of external mass transfer resistance on the overall dye removal rate obtained in an RPBB having LDPE packing immobilized with isolated microorganisms. The measured dye removal rates at varied feed flow conditions and the diffusion and reaction kinetics were used to develop an empirical

correlation for the external mass transfer coefficient in the given RPBB. The Chilton–Colburn analogy related the flow characteristics with the mass transfer parameters. The order of the biochemical reaction at the bacterial surface was taken as one. The observed rate of removal of Congo red dye in the RPBB was obtained and shown to be increasing with the operational parameters such as feed flow rate or recycle rate. A correlation to predict the non-dimensional Colburn factor to determine the mass transfer coefficient of Congo red dye in the RPBB was obtained as $J_D = 1.34\, Re^{-0.3}$. The surface reaction rate constant for the biodegradation of Congo Red dye was also determined as 2.5 ml/cm^2. h. Determining a dimensionless correlation for mass transfer coefficient will help assess and minimize the impact of external mass transfer barrier on the biodegradation of Congo red dye in an RPBB. Further, it would also enable the design and scale-up of an RPBB for the continuous treatment of dyeing wastewater.