implication of optimization technique, kinetic study, and pilot-scale IATP demonstrate that this work can also be used for the treatment of industrial wastewater.

CHAPTER 4

Performance evaluation of a packed bed bioreactor: Bio-kinetics and external mass transfer study

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4.1. Introduction

The biological processes are broadly classified as the suspended-growth and attached-growth. In the suspended-growth process, the microorganisms in the form of free cells are used for the biodegradation of pollutants. Whereas the attached-growth process is based on the microbial biofilm, which is developed and embedded onto the surface and pores of support or packing media (e.g., ceramics, polyvinyl alcohol (PVA), activated carbons, etc.) (Bharti et al., 2019; Geed et al., 2017; Shen et al., 2015;). In the suspended-growth process, the growth of microorganisms is substantially reduced at high pollutant (or substrate) concentration due to substrate inhibition, and it results in decrease in the overall performance of the system (Banerjee and Ghoshal, 2017; Kureel et al., 2017). However, the attached-growth system is more effective for the removal of pollutants due to the availability of high biomass concentration, large surface area, low hydraulic retention time (HRT), and high tolerance for high pollutant concentrations (Asri et al., 2018; Singh et al., 2010). In recent years, the packed bed bioreactor (PBBR), a type of attached-growth system is extensively used for the biodegradation of various kinds of pollutants (Asri et al., 2018; Swain et al., 2020). The successful biodegradation of pollutants such as pesticides, mono and polycyclic aromatic hydrocarbons, dyes, and pharmaceuticals in PBBRs is available in open literature (Kureel et al., 2017; Yadav et al., 2014; Zhang et al., 2020). The PBBRs have several merits like high-yield operation, ease of fabrication, treat a large amount of wastewater continuously, and reuse of biomass (Banerjee and Ghoshal, 2017). However, as the liquid flow through the attached biofilm, a stagnant liquid film is formed near the boundary of the attached biofilm, and this stagnant liquid film offers external mass transfer (EMT) resistance to the substrate diffusion through biofilm (Dizge and Tansel, 2010; Mudliar et al., 2008). The mass transfer (MT) of pollutants through biofilm is the key concern in operating PBBRs (Banerji and Ghosal, 2016). Therefore, it is a necessity to study the PBBR performance and mass transfer correlation at a different scale to predict the bioreactor behavior and scale-up of the process. To the best of our knowledge, there is no work available in the open literature on external mass transfer aspects of naphthalene biodegradation in PBBR.

Keeping the above fact in mind, the present work is to examine the performance of PBBR for naphthalene biodegradation by low-density polyethylene (LDPE) immobilized *Exiguobacterium* sp. RKS3 (MG696729). An effort has also been made to examine the correlation between the EMT coefficient and mass flux at various inlet flow rates (IFRs). The process variables like pH, temperature, and salinity were optimized to enhance the performance of PBBR. A comparative study between suspended and attached growth bioreactor was also carried out at optimized conditions. In addition, Andrew–Haldane kinetic model was used to study the substrate inhibition in the PBBR.

4.2. Mass transfer Model

4.2.1. Biodegradation of naphthalene and observed rate constant

In PBBR, the mass balance of naphthalene biodegradation was studied by assuming steady-state, plug flow, no axial diffusion, and cubical shape particle. The mass balance in a bioreactor is given as:

$$\left(\frac{HQ}{W}\right)\frac{dC}{dZ} = -(r) \tag{4.1}$$

where *H is the* height of PBBR (cm), *W* is the total amount of immobilized dried cells (g), *Q* represents the volumetric flow rate of feed solution (mL/h), and *r* is the biodegradation rate of naphthalene (mg/g.h). *dC/dZ* represents the concentration gradient along length of PBBR (mg/L.cm). Assuming first-order degradation (a valid assumption at low substrate concentration (Banerjee and Ghoshal, 2016), the rate of reaction can be denoted as:

$$r = k_p C \tag{4.2}$$

where k_p is the biodegradation rate constant (L/g.h).

Combining Eq. (4.1) and Eq. (4.2), the following Eq. (4.3) can get:

$$\left(\frac{HQ}{W}\right)\frac{dC}{dZ} = (-k_p\ C) \tag{4.3}$$

Integrating Eq. (4.3) using boundary conditions as $C = C_{in}$ at Z = 0 and $C = C_{out}$ at $Z = H$, Eq. (4.4) is obtained:

$$k_p = \left(\frac{Q}{W}\right) \times ln\left(\frac{C_{in}}{C_{out}}\right) \tag{4.4}$$

where C_{in} and C_{out} represent the inlet and outlet concentrations of naphthalene (mg/L), respectively.

4.2.2. Combined mass transfer and biochemical reaction

The performance of PBBR is adversely affected by fluid properties (e.g., viscosity, diffusivity, and density), operating process variables (e.g., pH, temperature, substrate, and concentration), and inlet loading rate (ILR) (Mudliar et al., 2008). In PBBR, the fluid passed through immobilized (attached biofilm) packing bed, and velocity near the periphery of the packing bed is very low (Dizge and Tansel, 2010; Tepe and Dursun, 2008). Due to the low velocity, a stagnant liquid film developed around the periphery of the packed bed, and the substrate transport primarily due to the molecular diffusion (Geed et al., 2018). Since the mass transfer rate may be slow, the corresponding observed biodegradation rate might be low due to limited external film diffusion (Banerjee and Ghoshal, 2016). The diffusion of naphthalene from the bulk liquid to immobilized biofilm can be given by the following equation (Dizge and Tansel, 2010):

$$r_m = k_m a_m (C - C_s) \tag{4.5}$$

where r_m, is the EMT rate of naphthalene (mg/g.h), a_m is the external surface area for mass transfer (cm^2/gm), and k_m is the EMT coefficient (mL/cm^2.h). C and Cs are the naphthalene concentration in the bulk liquid and immobilized support (mg/L), respectively.

The value of a_m can be calculated using the following equation (Geed et al., 2018):

$$a_m = \frac{6\,(1-\epsilon)}{\rho_p d_p} \tag{4.6}$$

where d_p, ρ_p, and ε represent the equivalent diameter of particle (cm), particle density (g/cm^3), and bed voidage (unitless), respectively.

The biodegradation rate (r) of naphthalene can be calculated by Eq. (4.7):

$$r = k_s a_m C_s \tag{4.7}$$

where k_s is the intrinsic first-order reaction rate constant (L/cm^2.h).

The EMT rate and naphthalene biodegradation will be equal at steady-state. Hence, Eq. (4.5) and (4.7) can be used to find out unknown surface concentration (C_s).

$$C_s = \frac{k_m C}{k_s + k_m} \tag{4.8}$$

Solving Eq. (4.7) and Eq. (4.8) and using with $r = k_p C$, the value of k_p can be calculated as:

$$k_p = \frac{k_s k_m a_m}{k_s + k_m} \tag{4.9}$$

Rearranging equation Eq. (4.9);

$$\frac{1}{k_p} = \frac{1}{k_m\, a_m} + \frac{1}{k_s\, a_m} \tag{4.10}$$

The term of k_m in Eq. (4.10) can be shown as:

$$k_m = \frac{k_p\, k_s}{(k_s\, a_m - k_p)} \tag{4.11}$$

4.2.3. An empirical model for naphthalene degradation

The value of EMT coefficient (k_m) is the function of variables, namely; IFR of fluid, the dimension of the bioreactor, and fluid properties and generally evaluated by following Eq. (4.12) (Dizge and Tansel, 2010; Kathiravan et al., 2010).

$$J_D = \frac{k_m \rho}{G}\left(\frac{\mu}{\rho D_f}\right)^{-2/3} = K(N_{R_e})^{(n-1)} \tag{4.12}$$

where J_D is the Colburn factor, G, D_f, μ, and ρ represent the mass velocity (g/cm^2.h), diffusivity (cm^2/s), viscosity (g/cm.s), and density (g/cm^3) of the fluid, respectively. The literature survey reveals that the value of n commonly varies from 0.1 to 1.0, which is generally depends on the flow characteristic of fluid (Mudliar et al., 2008). Using Eq (4.12), k_m can be expressed as:

$$k_m = NG^n \tag{4.13}$$

where

$$\mathrm{N} = \frac{K}{\rho}\left(\frac{\mu}{\rho \times D_f}\right)^{\left(-\frac{2}{3}\right)}\left(\frac{d_p}{\mu}\right)^{n-1} \tag{4.14}$$

Combining Eq. (4.10) with Eq. (4.13):

$$\frac{1}{k_p} = \left(\frac{1}{Na_m}\right)\left(\frac{1}{G^n}\right) + \frac{1}{k_s\, a_m} \tag{4.15}$$

The value a_m was estimated at various values of n and K and correlated with obtained data.

4.3. Materials and methods

4.3.1. Chemicals and materials

The modified mineral salt medium (MSM) composed of the following chemicals (g/L): KH_2PO_4 1.0, $K_2HPO_4 \cdot 2H_2O$ 1.0, $MgSO_4 \cdot 7H_2O$ 0.3, NaCl 1.0, $(NH_4)_2SO_4$ 0.3, $CaCl_2$ 0.2, and glucose 1.0, was used in the present study (Lin et al., 2010). MSM also contained microelements as (mg/L): $FeCl_3$ 2.3, $ZnSO_4$ 5.0, $(NH_4)_6Mo_7O_{24}$ 1.0, and $MnSO_4$ 5.0. The details of chemicals, materials, and synthetic wastewater (SW) preparation have been described in section 3.2.1.

4.3.2. Potential bacterial culture

The details of the soil sample, bacterial source, isolation, and enrichment procedure have been discussed in section 3.2.2 and 3.2.3. In this chapter, the second most potential bacterial species, i.e. RKS3 was used in the biodegradation study. The isolated bacterial strain was characterized by 16S rRNA technique and details of characterization have been given in section 3.2.4.

4.3.3. Packing media in packed bed bioreactor

The low-density polyethylene (LDPE) is generally used as the covering and packing material for electronic instruments (like, computers, spectrophotometer, etc.) during transportation, and after that, it is generally thrown in garbage as waste, which leads to solid waste generation. Here, an attempt has been given to utilize this plastic waste as packing (or support) media to immobilization of microorganisms in PBBR. LDPE was collected from the industrial pollution control lab, chemical engineering department, IIT (BHU) Varanasi, India (**Figure 4.1**). It is mainly composed of carbon and hydrogen. It is a low-cost, highly porous, durable, chemical and water-resistant, and reusable material. LDPE sheets were cut into cubical shapes having a size of approximately 0.5 cm. The sterilized packing media neither interfered with the biological process nor contaminated the treated water (Cheikh et al., 2013). Therefore, the packing media were washed with 70% ethanol and followed by five times washing with sterilized double-distilled water and vacuum drying at 40 °C for 24 hours. Before immobilization, the packing media were sterilized to avoid environmental contamination.

Figure 4.1. Low density polyethylene used as packing media in packed bed bioreactor.

4.3.4. Biodegradation of naphthalene in packed bed bioreactor

The PBBR was fabricated using a cylindrical borosilicate column of 60 cm length and 5.5 cm of internal diameter (**Figure 4.2**). The total capacity of PBBR was 1424.7 mL and the working capacity was about 1000 mL. The bioreactor bed (42.0 cm height) was packed with LDPE cubes followed by inoculation with potential isolated bacterial culture (i.e., *Exiguobacterium sp.* RKS3). The wastewater prepared in the lab was supplied in the bioreactor at different IFRs (20-100 mL/h) using a peristaltic pump. The air was supplied from a compressor (XP-AC-24L, Xtra Power, India) to maintain aerobic conditions in the PBBR. The air was filtered through a 0.22µm filter (GSWP, Merck Millipore Ltd) to prevent the possibility of contamination in PBBR. The flow rate of air was controlled by a rotameter (SRS-MG5, Eureka, India). The required sampling ports were provided in a bioreactor to facilitate the inlet and outlet of samples. At the top of the bioreactor, an outlet valve was provided for venting out the gases.

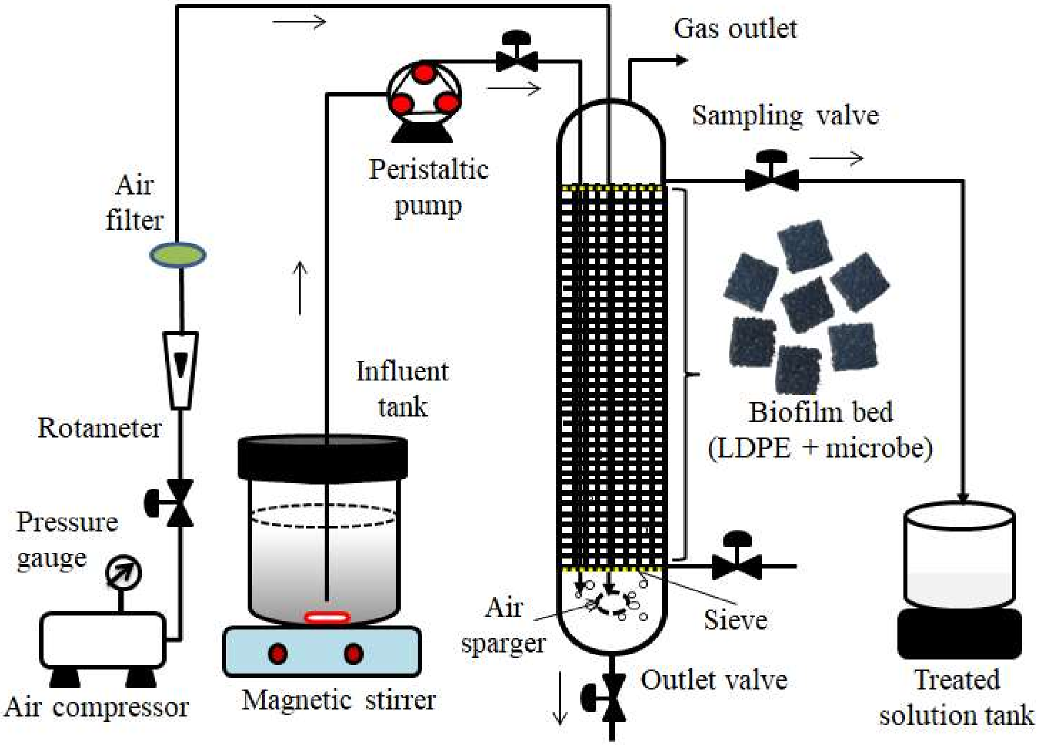

Figure 4.2. Schematic diagram of the experimental set-up for the biodegradation of naphthalene.

4.3.5. Bioreactor study

The batch experiments were carried out to optimize the process parameters such as pH (5.0, 6.0, 7.0, 8.0, and 9.0), temperature (25, 30, 35, and 40 °C), and salinity (0, 2, 4, 6, and 8.0 g/L). During the optimization study, the initial concentration of naphthalene was fixed at 20 mg/L. A comparative study between suspended and attached growth bioreactor was also carried out in 250 mL Erlenmeyer flask. Prior to continuous study, the bioreactor was operated for 15 days for the successful acclimatization and growth of biofilm onto the packing media. The biodegradation of naphthalene in PBBR under continuous mode was studied at different IFRs (20-100 mL/h). At every IFR, adequate time was given to reach equilibrium. The performance equations used in PBBR have been discussed in section 3.2.7 (Eq. 3.2, 3.3, and 3.4).

4.3.6. Analytical methods

The details of analytical instrument used in this chapter have been described in section 3.2.8. The scanning electron microscope (SEM) characterization of packing media (LDPE) were taken before and after the immobilization of bacterial culture. The packing media was vacuum dried at 40 °C for 24 hours in a vacuum oven (NSW 251, India) to remove the moisture. As the packing material (LDPE) was non-conducting in nature, it was coated with fine gold particles to obtain the effective resolution and analyzed using SEM (QUANTA 450 FEI, America) under a low vacuum to avoid any harm to developed biofilms (Kureel et al., 2017).

4.3.7. Growth kinetic study

In this work, the bio-kinetic parameters of the bacterial growth under substrate inhibition have been estimated by Andrews-Haldane model (Knight and Peters, 2006; Yadav et al., 2014).

$$\mu = \frac{\mu_{max} S}{K_s + S + \frac{S^2}{K_i}} \tag{4.16}$$

where μ, μ_{max}, K_s, and K_i represent the specific growth rate of the microorganisms (per day), the maximum specific growth rate of the microorganisms (per day), half-saturation rate constant (mg/L), and substrate inhibition constant (mg/L), respectively. S is the substrate (i.e., naphthalene) concentration (mg/L).

4.4. Results and discussions

4.4.1. Identification of potential species

The 16S rRNA sequence of potential bacterial species was compared with the sequences available in Gen Bank database, and the most homologous isolated species was found to be *Exiguobacterium sp*. Further, this nucleotide sequence was deposited in GenBank of NCBI and got the accession number (MG696729.1). The phylogenetic tree of *Exiguobacterium sp*. RKS3 was constructed by MEGA 7 software and shown in **Figure 4.3**.

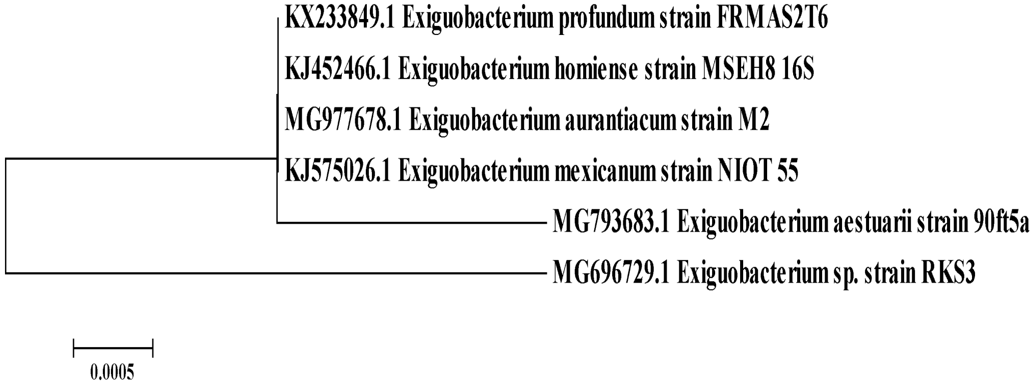

Figure 4.3. Phylogenetic tree of *Exiguobacterium sp.* RKS3 isolated from petroleum-contaminated site.

4.4.2. Morphological analysis of packing media

The SEM image of the packing media (LDPE) before immobilization shows the presence of the large number of micro-pores on the surface of LDPE (**Figure 4.4**). These micropores provide large surface area for biofilm growth. Also, the micropores are irregular in shape, which improves the bacterial binding ability during immobilization. Before the start-up of PBBR, no biofilm was observed on the surface of packing media or support, whereas dense biofilm was developed after 15 days of the experiment. This developed biofilm is responsible for the biodegradation of naphthalene in PBBR. The substrate passes through the developed biofilm by molecular diffusion and resulting biodegradation of substrate in PBBR observed (Geed et al., 2018). The parameters like pH, adaptive capability of bacterial species, temperature, and substrate concentration affect the biofilm formation on the packing support (Kureel et al., 2017). The obtained results indicate the successful immobilization of *Exiguobacterium sp.* RKS3 into LDPE.

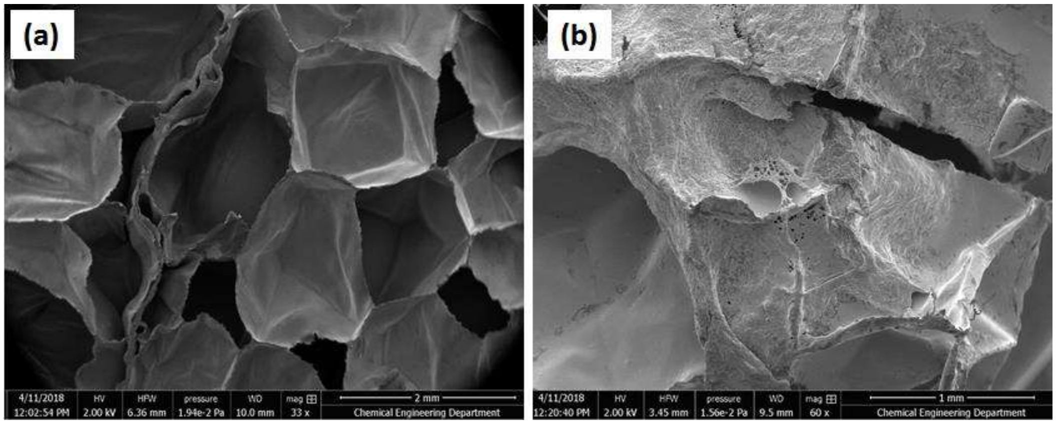

Figure 4.4. SEM image packing media: (a) LDPE before immobilization; and (b) LDPE immobilized with biofilm of *Exiguobacterium sp.* RKS3.

4.4.3. Process optimization

The biodegradation efficiency of substrates is adversely affected by the pH of the solution (Wong et al., 2002). The effect of pH (5.0-9.0) on naphthalene removal efficiency (RE %) is shown in **Figure 4.5**. It was found that the RE of naphthalene was increased with increasing the pH from 5.0 to 7.0, and beyond this pH, the naphthalene RE was decreased. The naphthalene RE of 53.2, 59.3, 95.8, 81.7, and 44.2 were obtained at pH of 5.0, 6.0, 7.0, 8.0, and 9.0, respectively. The microorganisms produce enzymes which act as biocatalyst and facilitate the substrate degradation (Yadav et al., 2014). These enzymes contain ionic groups on their active site, which must be in a suitable state (acidic or alkaline) to work effectively. Due to the variation of pH of the solution, the activity of the ionic site of enzymes was adversely affected and led to the slow rate of biodegradation, which is clearly indicated by low RE (**Figure 4.5**) under highly acidic and alkaline condition. Lin et al. (2010) have examined the naphthalene biodegradation using *Bacillus fusiformis*, and observed the low RE under acidic conditions because of lower enzymatic activity. Most of the researchers observed neutral pH as the suitable condition for the biodegradation of organic pollutants (Kureel et al., 2017; Swain et al., 2020).

The solubility and bioavailability of pollutants are increased with the temperature of the solution (Pugazhendi et al., 2017). In contrast, the dissolved oxygen (DO) level in solution is decreased with increasing temperature. Therefore, to obtain the optimum efficacy of the biodegradation process, it is vital to maintain the specific temperature range during the biodegradation. **Figure 4.5** shows the effect of temperature (25 to 40 °C) on the naphthalene RE. Initially, at 25 °C of temperature, the naphthalene RE was low, which further increased with increasing temperature up to 30 °C. Beyond this temperature, the naphthalene RE was started decreasing. The maximum naphthalene RE of 92.5% was found at 30 °C, whereas the RE was decreased to 79.2 and 66.7% at 25 and 40 °C, respectively. The enzymatic activity of microorganisms significantly reduced at a high temperature, which subsequently impedes the rate of biodegradation (Geed et al., 2017).

The effect of salinity (NaCl concentration) on naphthalene RE is shown in **Figure 4.5**. Initially, the RE was decreased slowly with increasing salinity up to 4.0 g/L. However, above 4.0 g/L of salinity, the RE was sharply decreased and reached to 39.9% at 8.0 g/L of salinity. These results showed that the high salinity adversely affects the growth and metabolic activity of the microorganism; hence the RE was decreased. The effect of salinity (0-5% NaCl) on pyrene biodegradation was examined by *Achromobactor xylosoxidans,* and found that 2.5% salinity was optimum for the effective removal of pyrene (Nzila et al., 2018). In another work, Lin et al. (2010) studied the effect of salinity (0-15 g/L) on naphthalene biodegradation and reported that the optimum salinity range for effective degradation was from 0 to 5.0 g/L and above this, the biodegradation efficiency was sharply decreased.

A comparative study between suspended and attached growth system was performed under optimum conditions. The attached-growth system was subjected to 94.1% of RE, whereas the suspended-growth system reveals 72% of RE within 5.0 days of the experiment. This clearly indicated that the attached-growth bioreactor had shown better performance than the suspended-growth bioreactor. Shen et al. (2015) studied the biodegradation of crude oil by free and semi-coke immobilized microbial consortium, and they also found that the attached-growth bioreactor had shown higher RE (1.78 times) of crude oil than suspended-growth bioreactor. Previous researchers have observed similar phenomena (Kureel et al., 2017; Yadav et al., 2014).

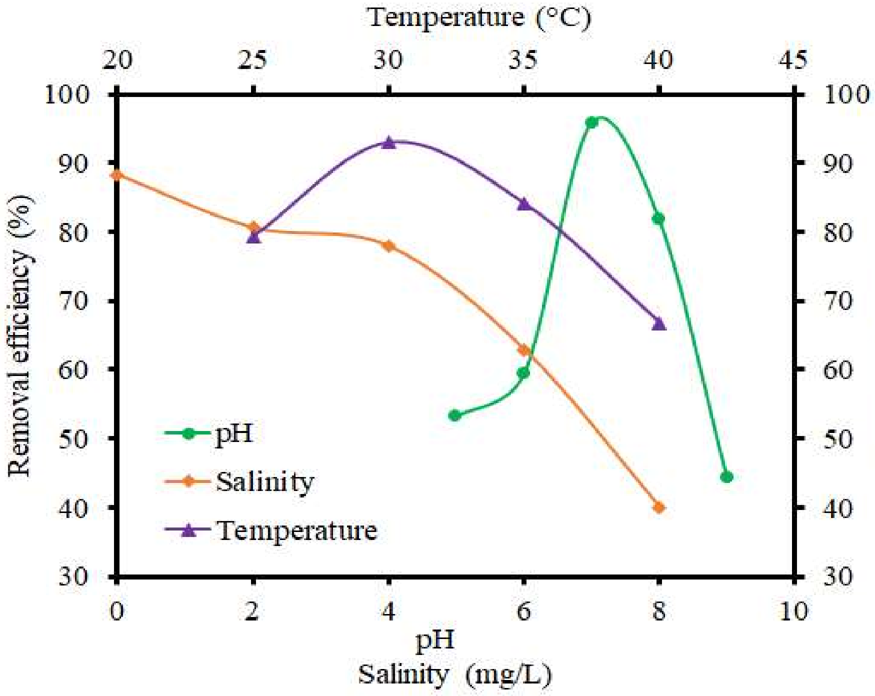

Figure 4.5. Effect of pH (5-9), temperature (25-40 °C), and salinity (0-8 g/L) on naphthalene removal efficiency.

4.4.4. Performance of a continuous packed bed bioreactor

In order to evaluate the performance of PBBR at different loading rates, it was operated for 64 days under ambient conditions (30 ± 3.0 °C). **Figure 4.6** shows the effect of IFRs on naphthalene RE and EC with respect to duration. Initially, the PBBR was operated at 20 mL/h with an ILR of 9.6 mg/L.day, while initial naphthalene concentration was kept constant at 20 mg/L. The % RE was increased with time and reached equilibrium on 15th day of experiments with 94.7% of RE, corresponding to which 9.1 mg/L.day of EC was observed. The summary of PBBR performance in terms of ILR, RE, and EC is given in **Table 4.1**. On 16th day, the IFR was increased from 20 to 40 mL/h. Initially, a sharp dip in the RE (71.2%) was observed, which further recovered and reached to almost steady-state on 28th day with 85.6% of RE and 16.4 mg/L.day of EC. On 29th and 40th day, the flow rates were again increased to 60 and 80 mL/h, respectively. The RE and EC were obtained to be 83.1% and 23.9 mg/L.day, respectively on 40th day at 60 mL/h, whereas RE was decreased to 77.9%, and EC was increased to 29.1 mg/L.day on 54th day at 80 mL/h. Again, as we increased the flow rate (100 mL/h), the RE was further reduced to 71.4%, while the EC was slightly improved to 34.3 mg/L.day. It can be noticed here that the EC is increased with ILRs, whereas RE is decreased with ILRs under similar conditions. Yadav et al. (2014) reported that more than 91% removal of Chlorpyrifos was obtained at 300 mg/L.day by *Pseudomonas sp.* in continuous PBBR. In another work, Kureel et al. (2017) studied the biodegradation of aromatic hydrocarbon (benzene) by *Bacillus sp.* in a continuous PBBR and reported the maximum RE of 93% was obtained on 38th days of operation.

Table 4.1 Profile of packed bed bioreactor performance at various inlet flow rates.

Flow rate (mL/h)	Time (day)	EC[a] (mg/L.day)	ILR[b] (mg/L.day)	RE[c] (%)
20	1-15	1.4-9.1	9.6	0-94.7
40	16-28	9.1-16.4	19.2	71.2-85.6
60	29-40	16.4-23.9	28.8	72.2-83.1
80	41-54	23.1-29.1	38.4	80.0-77.9
100	55-64	29.1-34.3	48.0	80.2-71.5

[a]Elimination capacity; [b]Inlet loading rate; [c]Removal efficiency

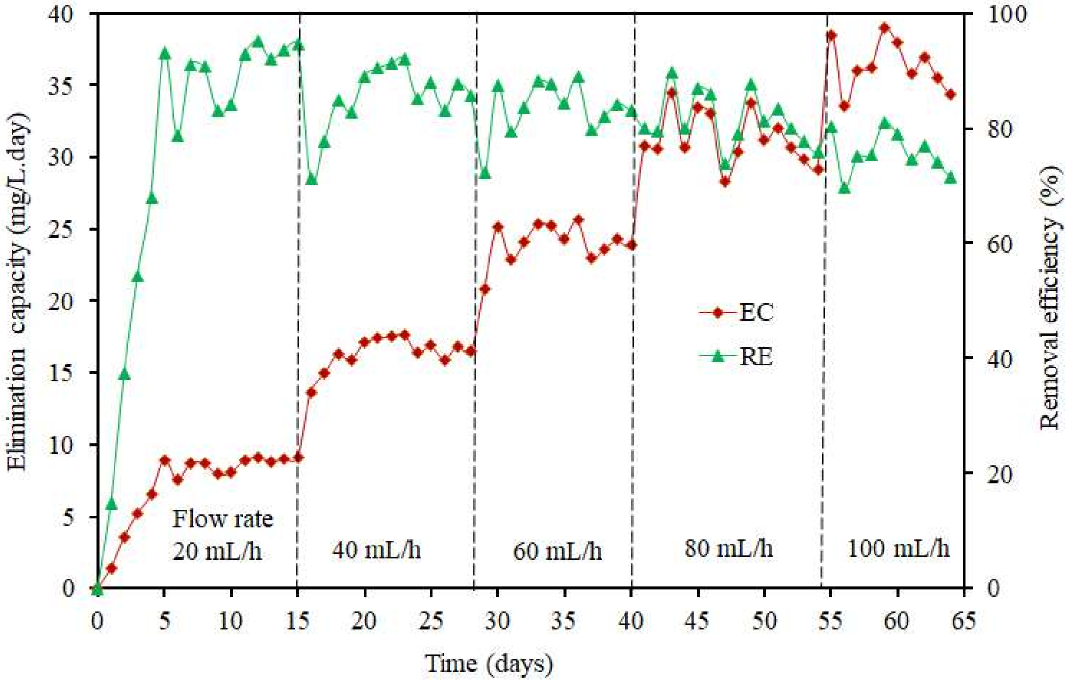

Figure 4.6. The performance of packed bed bioreactor at various flow rates.

4.4.5. Mass transfer studies

The effect of IFRs (20, 40, 60, 80, and 100 mL/h) on the biodegradation rate constant (k_p) is presented in **Figure 4.7a.** Initially, at a low IFR (20 mL/h), the value of k_p (2.99 mL/g.h) was low (**Table 4.2**). The lower value of k_p at low IFR was because of MTR offered by the stagnant liquid film. However, the value of k_p was increased with increasing IFR because at higher flow rate more turbulence was created in PBBR, which facilitates better mass transfer. The overall naphthalene RE was decreased slowly with an increase in IFR, and this may be due to insufficient residence time of pollutants in the bioreactor at higher IFRs (Dizge and Tansel, 2010). Banerjee and Ghosal. (2016) studied the biodegradation of phenol using calcium-alginate immobilized *Bacillus cereus* at various IFRs, and they reported that the overall RE was decreased because of the insufficient residence times in PBBR at high IFRs.

In order to analyze the external MTR effect on naphthalene biodegradation, the required dimensionless number and mass flux (G) were evaluated using following parameters; ρ = 1.13 g/cm^3, dp = 0.5 cm, ε = 0.342, a_m =23.75 cm^2, and μ = 0.0085 g/cm.s, D_f = 7.8× 10^{-6} cm^2/sec. The experimental values of k_p and calculated values of $1/k_p$ at various IFRs are summarized in **Table 4.2**. The negative value of the intercept was obtained for $n < 0.5$ and these values were not used in further study. It was observed that the value of slope decreased for n between 0.1 and 0.6, whereas the slope was increased for n higher than 0.6 (**Table 4.3**). The plot of $1/kp$ vs $1/G^n$ for n = 0.2, 0.5, 0.7, and 0.9 is shown in **Figure 4.7b**. To obtain the suitable value of N, a_m, and k, further calculation was carried out for different values of K (Banrjee and Ghosal, 2016). The estimated N, a_m, and k at various values of n and K are given in **Table 4.4**. From Eq. (4.15), the values of a_m and k_s were calculated. After several assumptions, the value of a_m (10.6 cm^2/g) obtained at K=5.7 and n=0.9 was very close to observed data. In order to verify the calculated value of K and n with observed data, the values of k_m were calculated at various flow rates and using values a_m and k found at K=5.7 and n=0.9 (**Table 4.5**). The graph of $ln\ k_m$ against $ln\ G$ is shown in **Figure 4.8**. The value of n and N was found to be 0.93 and 0.151, which was closest to the estimated value at K=5.7 and n=0.9. Further, the values of k_p were calculated and it was found that the calculated values of k_p were similar to the experimental k_p (**Table 4.6**). From obtained results, the EMT correlation for naphthalene biodegradation can be proposed as:

$$J_D = 5.7{N_{Re}}^{-0.1}$$

This empirical correlation can satisfactorily predict the EMT aspect of naphthalene biodegradation in PBBR. A summary of the mass transfer correlation reported in previous studies in PBBR reactor is represented in **Table 4.7**. These are empirical correlations and are extensively varied with pollutant types, operating conditions, and microorganisms used.

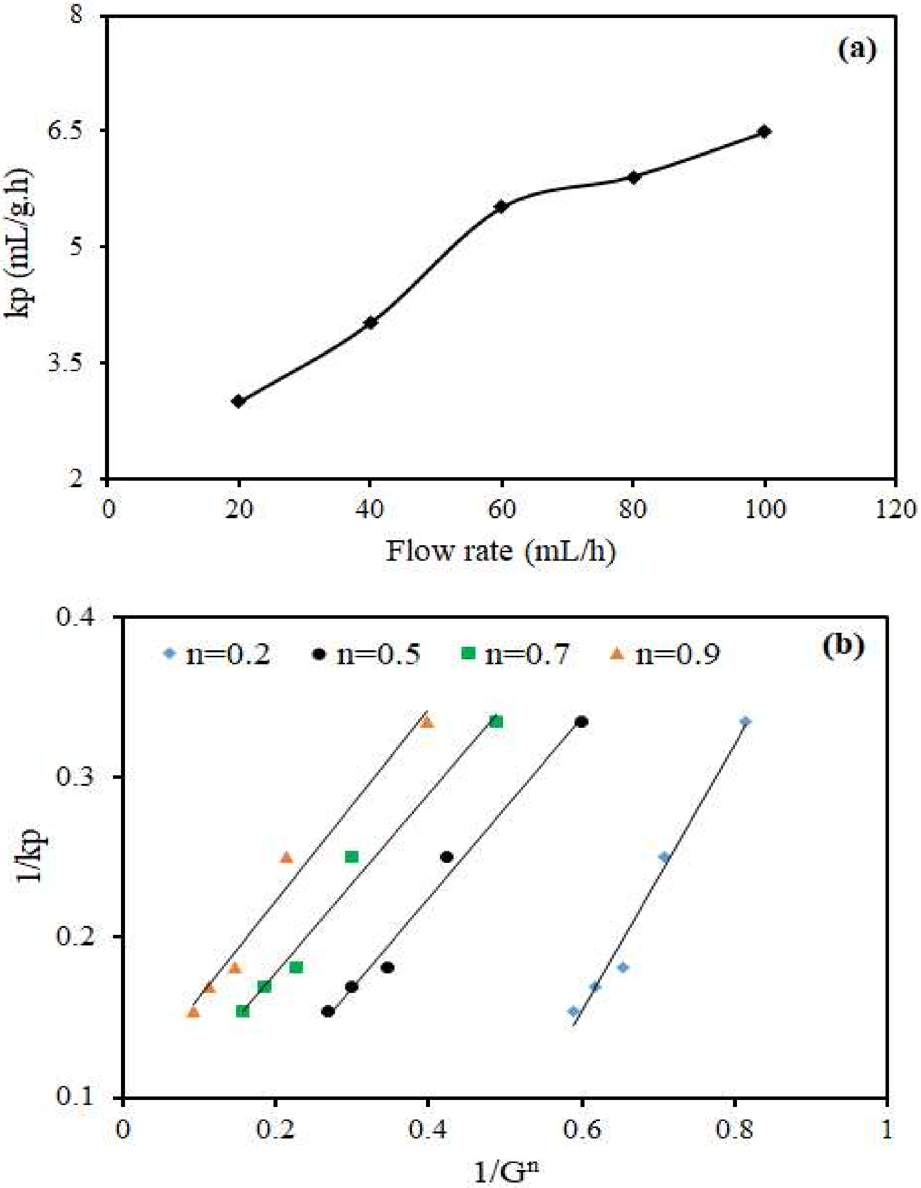

Figure 4.7. (a) Effect of flow rate on the biodegradation rate constant; (b) The observed profile of 1/kp vs. $1/G^n$ at different n values (n= 0.2, 0.5, 0.7, and 0.9).

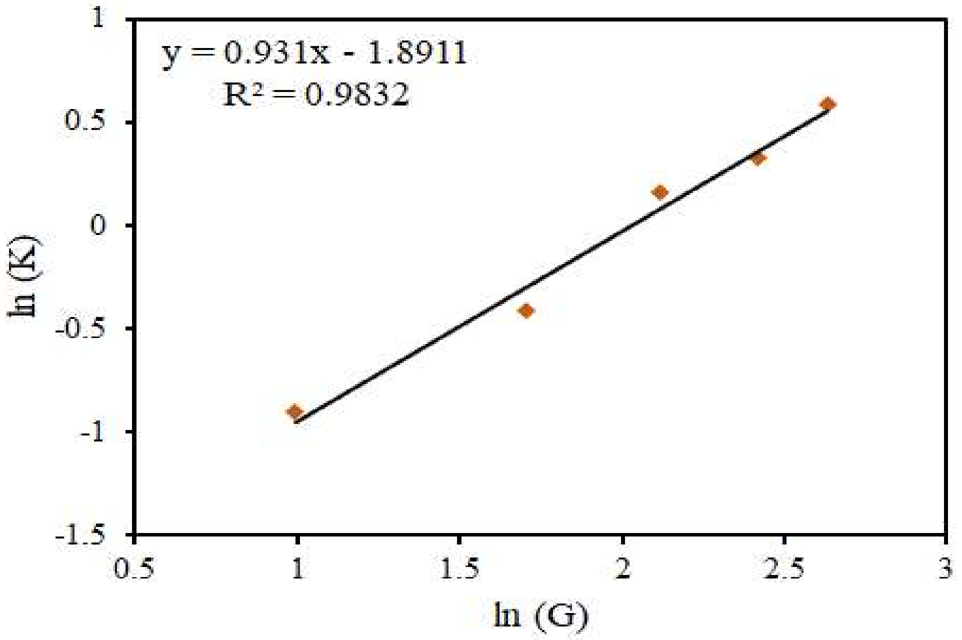

Figure 4.8. The variation of external mass transfer coefficient with mass velocity.

Table 4.2 Profile of the operational parameters of bioreactor: experimentally determined values of k_p and calculated data at various values of n.

Q[a] (mL/h)	k_p[b] (mL/g.h)	$1/kp$	G[c] (gm/cm^2.h)	$1/G^n$									
				$1/G^{0.1}$	$1/G^{0.2}$	$1/G^{0.3}$	$1/G^{0.4}$	$1/G^{0.5}$	$1/G^{0.6}$	$1/G^{0.7}$	$1/G^{0.8}$	$1/G^{0.9}$	$1/G^{1.0}$
20	2.99	0.334	2.78	0.902	0.814	0.735	0.664	0.599	0.541	0.488	0.441	0.398	0.359
40	4	0.25	5.56	0.842	0.709	0.597	0.503	0.423	0.357	0.301	0.253	0.213	0.179
60	5.51	0.181	8.34	0.808	0.654	0.529	0.427	0.346	0.279	0.226	0.183	0.183	0.119
80	5.9	0.169	11.1	0.785	0.617	0.485	0.381	0.299	0.235	0.185	0.145	0.114	0.089
100	6.49	0.153	13.9	0.768	0.590	0.853	0.348	0.268	0.206	0.158	0.121	0.093	0.071

[a]Inlet flow rate; [b]Biodegradation rate constant; [c]Mass velocity

Table 4.3 Profile of slope and intercept estimated from the plot of $1/k_p$ vs. $1/G^n$ for different values of n.

n	0.1	0.4	0.5	0.52	0.54	0.56	0.58	0.6	0.7	0.8	0.9	1
Slope	–	–	0.561	0.558	0.556	0.554	0.553	0.553	0.559	0.575	0.60	0.632
Intercept	Negative	Negative	0.001	0.009	0.017	0.024	0.032	0.038	0.065	0.086	0.101	0.114

Table 4.4 Calculated values of external mass transfer area (a_m), external mass transfer coefficient (k), and N at different values of n and K.

n	K=1.09			K=1.34			K=1.625			K=5.7		
	$N^a \times 10^{-3}$	$a_m{}^b$ (cm^2/g)	$k_s{}^c$ (L/cm$^2 \cdot$h)	$N^a \times 10^{-3}$	$a_m{}^b$ (cm^2/g)	$k_s{}^c$ (L/cm^2.h)	$N^a \times 10^{-3}$	$a_m{}^b$ (cm^2/g)	$k_s{}^c$ (L/cm^2.h)	$N^a \times 10^{-3}$	$a_m{}^b$ (cm^2/g)	$k_s{}^c$ (L/cm^2.h)
0.5	5.8	307.9	16.23	7.2	250.4	19.9	8.7	206.5	24.20	30.7	58.8	84.9
0.52	6.3	285.5	0.38	7.8	232.3	0.47	9.5	191.5	0.57	33.4	54.6	2.01
0.54	6.9	264.4	0.21	8.5	215.1	0.26	10.3	177.4	0.32	036.2	50.7	1.14
0.56	7.5	244.6	0.16	9.2	199.0	0.20	11.2	164.1	0.24	39.3	46.7	0.85
0.58	8.1	226.0	0.13	10.0	183.9	0.16	12.1	151.6	0.20	42.6	43.2	0.72
0.6	8.8	208.6	0.12	10.8	169.7	0.15	13.1	139.9	0.18	46.2	39.9	0.64
0.7	13.3	134.5	0.11	16.3	109.4	0.14	19.8	90.2	0.17	69.5	25.7	0.59
0.8	19.9	87.0	0.13	24.5	70.7	0.16	29.8	58.3	0.19	104.5	16.6	0.69
0.9	30.0	55.4	0.17	36.9	45.1	0.21	44.7	37.2	0.26	157.1	10.6	0.93
1	45.1	35.0	0.25	55.5	28.5	0.30	67.3	23.5	0.37	236.1	6.7	1.30

[a] Parameter defined in Eq. (4.14); [b] External mass transfer area; [c] Intrinsic first-order reaction rate constant

Table 4.5 The profile of k_m at different mass velocities for n= 0.9 and K= 5.7.

Q^a (mL/h)	G^b ($gm/cm^{2}\cdot h$)	km^c ($mL/cm^{2}.h$)
20	2.7	0.404
40	5.5	0.661
60	8.3	1.173
80	11.2	1.379
100	13.9	1.789

[a]Inlet flow rate; [c]Mass velocity; [c]External mass transfer coefficient

Table 4.6 Comparison of experimental k_p values and calculated k_p values obtained at various flow rates in relation to n.

Q^a (mL/h)	${k_p}^b$ (Experimental) (mL/g.h)	${k_p}^b$ Calculated) (mL/g.h)					
		n=0.5	n=0.6	n=0.7	n=0.8	n=0.9	n=1.0
20	2.99	2.986	2.986	2.984	2.982	2.988	2.988
40	4.02	4.077	4.078	4.099	4.098	4.097	4.088
60	5.51	5.488	5.502	5.501	5.505	5.502	5.502
80	5.90	5.873	5.897	5.898	5.899	5.883	5.983
100	6.49	6.474	6.492	6.494	6.497	6.499	6.498

[a]Inlet flow rate; [b]Biodegradation rate constant

Table 4.7 Different mass transfer correlation reported in previous studies in packed bed biofilm reactor.

Pollutant	Microorganism	Packing media	Mass transfer correlation	Reference
Phenol	*Ralstonia eutropha*	Calcium-alginate	$J_D = 1.34{N_{Re}}^{-0.35}$	Tepe and Dursun (2008)
Carbohydrate	Activated sludge	Polyurethane foam	$J_D = 5.7{N_{Re}}^{-0.9}$	Dizge and Tansel (2010)
Chromium (VI)	*Bacillus* sp.	Calcium-alginate	$J_D = 5.7{N_{Re}}^{-0.7}$	Kathiravan et al. (2010)
Phenol	*Bacillus cereus*	Calcium-alginate	$J_D = 1.34{N_{Re}}^{-0.65}$	Banerjee and Ghoshal (2016)
Fluorene	*Pseudomonas pseudoalcaligenes*	Low-density polyethylene	$J_D = 5.71{N_{Re}}^{-0.2}$	Sonwani et al. (2019a)
Phenol	*Bacillus flexus*	Low-density polyethylene	$J_D = 1.62{N_{Re}}^{-0.3}$	Swain et al. (2020)
Naphthalene	*Exiguobacterium sp.*	Low-density polyethylene	$J_D = 5.71{N_{Re}}^{-0.1}$	Present study

4.4.6. Biodegradation kinetic

The experimental and predicted specific growth rate versus initial naphthalene concentrations plot is shown in **Figure 4.9**. Initially, the specific growth rate (μ) was increased with naphthalene concentration up to 20 mg/L, whereas above 20 mg/L, the value of μ was decreased gradually. The bio-kinetic parameters; μ_{max}, K_s, and K_i were estimated to be 0.386 per day, 13.6 mg/L, and 20.54 mg/L, respectively. The coefficient of determinant (R^2) and root mean square error (RMSE) were obtained to be 0.97 and 0.02, respectively, and indicate that the obtained data were well fitted with Andrews-Haldane model. The high value of μ_{max} supports the fast rate of substrate biodegradation, whereas the low value of K_S indicates the affinity of microorganisms towards substrate degradation (Geed et al., 2017). The value of μ_{max}/K_s is reported as a useful index to signify the biodegradation potential of microorganisms (Tomei et al., 2004; Yadav et al., 2014).

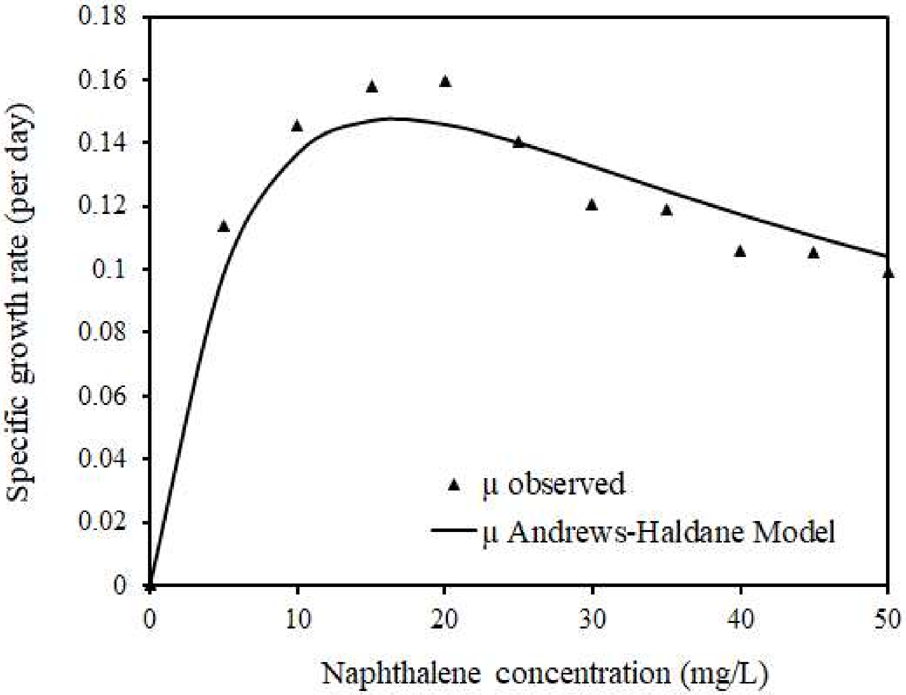

Figure 4.9. Profile of the kinetics and experimental data fitted with Andrews-Haldane model.

4.5. Conclusions

In this work, the biodegradation of naphthalene was studied in a PBBR. The most affecting process variable, namely temperature, salinity, and pH were optimized under the batch mode and found to be 30 °C, 4 g/L, and 7.0, respectively. The performance of a continuous PBBR was examined at different IFRs for the naphthalene biodegradation using LDPE immobilized *Exiguobacterium sp.* RKS3. The RE of naphthalene was decreased slowly with increase in IFRs. The combined effect of EMT and naphthalene biodegradation rate was studied, and a new mass transfer correlation was developed ($J_D = 5.71 {N_{Re}}^{-0.1}$) to satisfactorily predict the EMT aspect. The SEM analysis confirmed the successful formation of biofilm on LDPE. Also, the attached-growth