bioreactor has shown higher performance than the suspended-growth bioreactor. The growth-inhibition kinetics clearly indicated that the substrate inhibition had taken place at higher concentration of naphthalene.

CHAPTER 5

A comparative study of modified carriers in moving bed biofilm reactor for the treatment of wastewater: Process optimization and kinetic study

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5.1. Introduction

Moving bed biofilm reactor (MBBR) is an attached-growth type bioreactor and is presently employed in more than 50 countries. Fundamentally, It is a combination of the both activated sludge process (ASP) and fluidized bed bioreactor (FBBR) process (Nur et al., 2018). The merits of MBBR over a conventional activated sludge process and other biofilm-based techniques, include less clogging, effective mass transfer, elimination of sludge recirculation, and low hydraulic retention time (HRT) (Leyva-Díaz et al., 2020). The carriers play a significant role in the growth of biofilm and subsequently affect the performance of MBBR. The details of the MBBR has been discussed in section 2.8. It is found in the open literature that very limited attention has been given to the development of the specific configuration of the novel biofilm carrier. Therefore, special attention is required to develop new carriers to enhance the performance of MBBR.

In this chapter, the modified carriers, namely polypropylene (PP), low-density polyethylene-polypropylene (LDPE-PP), and polyurethane foam-polypropylene (PUF-PP) were developed and used in MBBR for the treatment of naphthalene. To the best of our knowledge, this type of specific configuration of carriers that integrate PP carriers with LDPE and PUF for biofilm growth is the novel advancement of this type configuration. This study also investigates the comparative performance of three MBBRs containing different carriers using central composite design (CCD) of response surface methodology (RSM). Further, the biodegradation kinetics of naphthalene removal in MBBR is evaluated by Modified Stover–Kincannon model.

5.2. Materials and Methods

5.2.1. Chemicals and 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. The enriched bacterial consortium isolated from petroleum-contaminated soil was used as an inoculant in MBBR.

5.2.2. Carriers used in bioreactor

Three modified plastic carriers, namely PP, LDPE-PP, and PUF-PP were used in this chapter. The PP was purchased from Netrox Aqua Solution, New Delhi, India and PUF sheet was purchased from Gyan Scientific Agencies, Varanasi, India. The details of LDPE has been given in section 4.3.3. PP plastic carriers have length and diameter of 12 and 25 mm, respectively, with a specific density of 0.93 g/cm^3 and specific surface area of 550 m^2/m^3. The LDPE and PUF were cut into the uniform size of 0.25×2.0 cm and fixed into alternate holes of the PP carriers to prepare the modified carriers; LDPE-PP, and PUF-PP, respectively. The average weight of these carriers, PP, LDPE-PP, and PUF-PP were 1.03±0.02, 1.0745±0.04, 1.19±0.03 g, respectively per unit carrier. These three types of carriers were used separately in three different MBBR.

5.2.3. Experimental set-up

The schematic diagram of the MBBR set-up has been shown in **Figure 5.1**. The set-up was made up of three MBBR reactors, wastewater feed tank, air pump (KNF, Labport, Germany), peristaltic pump, and (Miclins PP 30 EX), rotameter (SRS-MG5, Eureka, Pune, India). Each MBBR (MBBR-1, MBBR-2, and MBBR-3) having a total volume of 2.0 L (1.8 L of working volume) was fabricated using borosilicate glass. The

MBBR-1, MBBR-2, and MBBR-3 were filled with novel modified plastic carriers (40% of working capacity), namely PP, LDPE-PP, and PUF-PP, respectively. Air was supplied through air diffusers to maintain the aeration and circulation of carriers inside the bioreactors. The peristaltic pump was used to transfer the wastewater from the storage tank to the bioreactors. Prior to the start of the bioreactors, acclimatized bacterial consortia were inoculated into each of the MBBR for 15 days to ensure the biofilm formation onto the surface and pores of plastic carriers (Deng et al., 2016). The biofilm formed on the surface of carriers was visually observed and confirmed by SEM analysis. After the biofilm development, the wastewater was supplied to the MBBRs from the top of the reactor a using peristaltic pump. During the experiment, the DO was maintained to 4.0±1.0 mg/L. All the experiments were operated at the temperature of 30±3.0 °C.

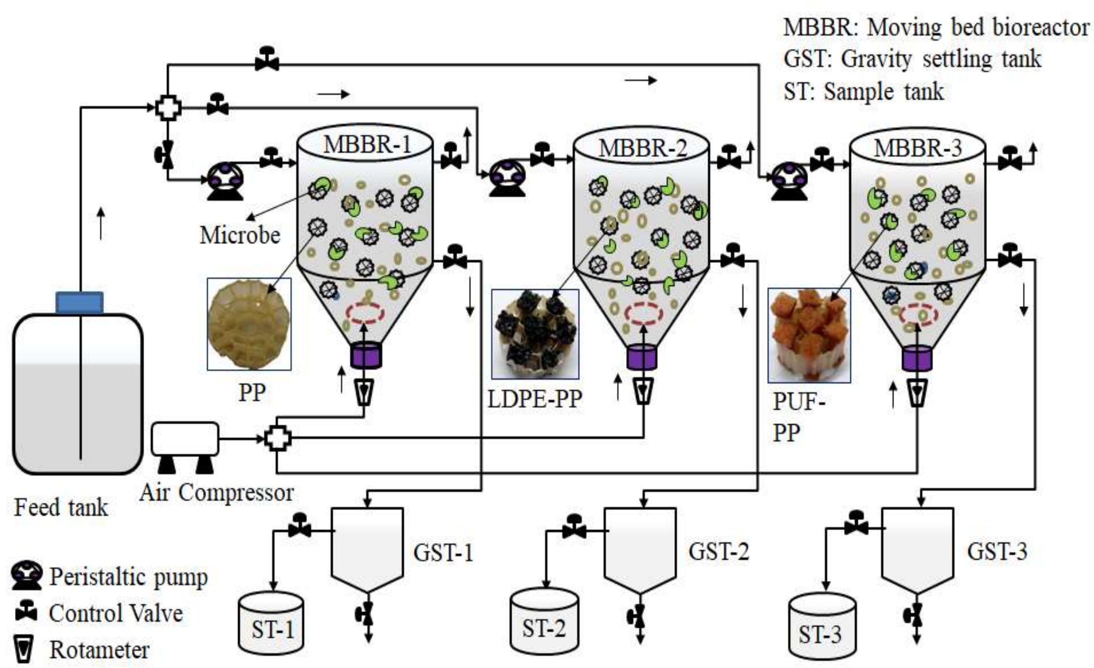

Figure 5.1. Schematic diagram of the moving bed biofilm reactor.

5.2.4. Design of experiments using response surface methodology

The design of the MBBR experiments and their statistical examination of the results were performed by CCD of RSM. The process variables, namely pH (5-9) and HRT (1-5 days) were selected as two independent variables and the type of carriers as a categorical factor. On the basis of the factorial design, 13 runs for each type of carriers were obtained and summarized in **Table 5.1**. To fit the obtained results of designed experiments and find out the appropriate model, the coefficients were evaluated by a second order polynomial equation (Eq. 5.1).

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ij} X_{ij} + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \ldots \quad (5.1)$$

where Y represents the response variable (% COD removal), β represents the correlation coefficient, and i and j represent the coefficients of linear and multi-degree.

Table 5.1 Summary of process variables and their range in optimization process.

Factor	Name	Level of variables		
A	pH	5.0	7.0	9.0
B	HRT[a] (day)	1.00	3.0	5.0
C	Type of carrier	PP[b]	LDPE-PP[c]	PUF-PP[d]

[a]Hydraulic retention time; [b]Polypropylene; [c]Low-density polyethylene-polypropylene; [d]Polyurethane foam-polypropylene

5.2.5. Analytical methods

The overall degradation of naphthalene was evaluated in terms of COD using standard protocols (APHA, AWWA, WEF 2005). The residual naphthalene was analyzed by a HPLC (ELICO HL 460, India). The details of analytical instruments (such as SEM, HPLC, spectrophotometer, etc.) used in this chapter have been discussed in section 3.2.8 and section 4.3.6.

5.2.6. Kinetic model

The mathematical models are helpful in predicting the performance of bioreactor, effect of process variables (such as volume, HRT, influent and effluent concentration), design and scale-up of the biological process (Brink et al., 2017; Derakhshan et al., 2018a). Generally, Stover– Kincannon mathematical model is most widely used to evaluate the kinetic parameters of biofilm reactors (Ahmadi et al., 2017; Jafarzadeh et al., 2009). The initial equation of the Stover-Kincannon model is given by Eq. (5.2) (Babaei et al., 2013; Derakhshan et al., 2018b).

$$\frac{dS}{dt} = \frac{Q(S_o\text{-}S_e)}{V} = \frac{U_{max}(\frac{QS_o}{A})}{K_B+(\frac{QS_o}{A})} \tag{5.2}$$

where S_0 and S_e represent the inlet and outlet concentrations of substrate (mg/L), V is the working volume of the bioreactor, Q is the inlet flow rate of influent (L/h). K_B, U_{max}, and A are the saturation constant (mg/L.day), maximum substrate removal rate (mg/L.day), and film surface area (m^2), respectively. If the surface area (A) is replaced by the working volume of the reactor (V), the modified Stover-Kincannon model can be represented as (Eq. 5.3) (Ahmadi et al., 2017; Derakhshan et al., 2018a).

$$\frac{dS}{dt} = \frac{Q(S_o\text{-}S_e)}{V} = \frac{U_{max}(\frac{QS_o}{V})}{K_B+(\frac{QS_o}{V})} \tag{5.3}$$

This equation can be expressed in linear form as given below (Eq. 5.4)

$$\frac{V}{Q(S_o\text{-}S_e)} = \frac{K_B V}{U_{max} Q S_o} + \frac{1}{U_{max}} \tag{5.4}$$

The values of kinetic parameters like K_B and U_{max} were evaluated by the slope and intercept of the above equation, respectively. Furthermore, Eq. (5.4) can be solved to predict the outlet concentration (Eq. 5.5) and volume of MBBR (Eq. 5.6).

$$S_e = S_o - \frac{U_{max} S_o}{K_B+(\frac{QS_o}{V})} \tag{5.5}$$

$$V = \frac{QS_o}{\left(\frac{U_{max}S_o}{S_o\text{-}S_e}\right)\text{-}K_B} \tag{5.6}$$

5.3. Results and discussion

5.3.1. Characterization of carriers

The morphology of MBBR carriers, namely PP, LDPE-PP, and PUF-PP before (no biofilm) and after biofilm formation, are represented in **Figure 5.2**. The carriers; LDPE-PP and PUF-PP show several micro-pores that provide an effective surface area for the biofilm formation. Initially (start-up of MBBR), no biofilm was observed on MBBR carriers (**Figure 5.2a, 5.2c,** and **5.2e**), whereas the dense biofilm appeared on the MBBR carriers after 15 days of operation (**Figure 5.2b, 5.2d,** and **5.2f**). The thickness of the biofilm on the support media depends on process variables such as pH, temperature, nutrient availability, substrate concentration, etc. (Geed et al., 2017). According to Wang et al. (2018), the formation of biofilm on the surface of the carrier depends on the metabolic activity of microorganisms and their extracellular polymers. Derakhshan et al. (2018a) reported that the microorganism secretes and releases extracellular polymers, which play an important role in the formation of stable biofilm. The observed morphology of carriers demonstrates that the biofilm was developed on the respective MBBR carriers.

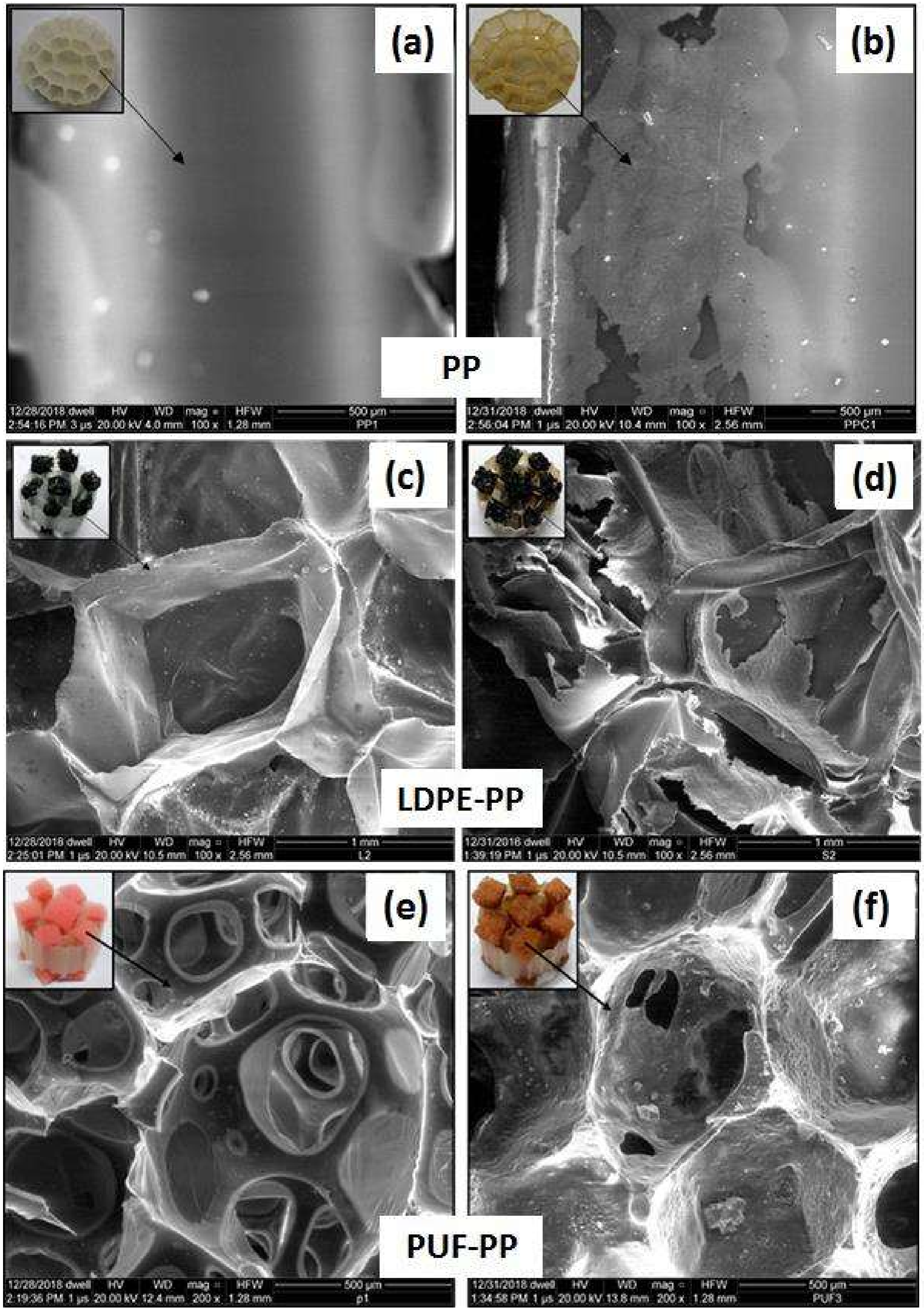

Figure 5.2. SEM image of MBBR carriers: (a) before and (b) after biofilm developed on PP; (c) before and (d) after biofilm developed on LDPE-PP; (e) before and (f) after biofilm developed on PUF-PP.

5.3.2. Optimization of process parameters

Total thirty-nine experimental runs (13 for each type of carrier) were performed in accordance with **Table 5.2** and their outcomes in terms of COD removal (%) are also reported in the **Table 5.2**. To study the model statistic, the experimental data were applied to linear and higher degree polynomial equations and results are shown in **Table 5.3**. To validate the fitting of the model, the values of coefficient of determination: R^2, adjusted R^2, and predicted R^2 were determined and found to be 0.966, 0.952, and 0.903, respectively for the quadratic model. The obtained values of the coefficient of determination: R^2, adjusted R^2, and predicted R^2 were larger than 0.8 which signifies that the quadratic model was valid for the optimization study.

ANOVA for naphthalene degradation was applied to study the significance of each variable and determine the consistency of the model. The correlation between the response (COD removal %) and independent variables has been presented in the form of equation. Eq. (5.7) represents the response of reduced models in terms of coded factors with significant terms.

$$\text{COD removal}(\%) = 69.19 - 5.67A + 17.49B + 0.764C - 4.16AB - 33.07A^2 - 13.16B^2 \tag{5.7}$$

The linear and square terms of the model were more significant ($p < 0.05$) (**Table 5.4**). The interaction among the variable A and B was significant ($p < 0.05$), while interactive terms between A and C and B and C were insignificant. Overall, the regression model was significant ($p<0.0001$) and represented that the quadratic model was adequate for the study of correlation between response and variables. According to Ghevariya et al. (2011) and Silva et al. (2018), the larger value of Fisher's F and the smaller value of p represent that the model has significant coefficient.

Table 5.2 Experimental conditions and results obtained by the central composite design of response surface methodology.

Run	Factor 1 A:pH	Factor 2 B:HRT[a] (day)	Factor C: Type of carriers	Response COD[b] removal (%)
1	9	1	PP	6.5
1	7	3	LDPE-PP	71.2
1	7	3	PUF-PP	79
2	7	3	PP	60
2	5	1	LDPE-PP	15.6
2	9	5	PUF-PP	37.2
3	9	5	PP	21
3	7	3	LDPE-PP	70.8
3	9	1	PUF-PP	12.5
4	5	5	PP	41
4	5	3	LDPE-PP	38.5
4	7	3	PUF-PP	78.9
5	7	3	PP	59.2
5	9	3	LDPE-PP	28
5	5	1	PUF-PP	14
6	7	3	PP	59.5
6	7	3	LDPE-PP	73
6	5	3	PUF-PP	44.2
7	7	3	PP	61
7	7	1	LDPE-PP	30.5
7	7	1	PUF-PP	33
8	7	3	PP	62.2
8	5	5	LDPE-PP	48
8	7	3	PUF-PP	80.2
9	9	3	PP	18.5
9	9	5	LDPE-PP	28
9	7	3	PUF-PP	80.4
10	7	5	PP	70.1
10	7	3	LDPE-PP	72.5
10	9	3	PUF-PP	34.5
11	7	1	PP	19.5
11	7	3	LDPE-PP	71.3
11	5	5	PUF-PP	56.2
12	5	3	PP	32.2
12	9	1	LDPE-PP	9.3
12	7	5	PUF-PP	86.5
13	5	1	PP	7.8
13	7	5	LDPE-PP	75.6
13	7	3	PUF-PP	79.8

[a]Hydraulic retention time; [b]Chemical oxygen demand; PP: Polypropylene; LDPE: Low-density polyethylene; PUF: Polyurethane foam

Table 5.3 Model Summary by ANOVA analysis.

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	
Linear	22.06	0.314	0.234	0.085	-
2FI	23.69	0.325	0.116	0.582	-
Quadratic	5.50	0.966	0.952	0.903	Suggested
Cubic	3.58	0.989	0.979	0.880	Aliased

Table 5.4 ANOVA for quadratic model by central composite design of response surface methodology.

Source	Sum of Squares	Mean Square	F-value	p-value
Model	23334	2121.3	70.2	< 0.0001
A-pH	578.00	578.00	19.1	0.0002
B-HRT[a]	5505.5	5505.5	182	< 0.0001
C-Type of carrier	1516.1	758.09	25.0	< 0.0001
AB	207.50	207.50	6.87	0.0142
AC	3.88	1.94	0.06	0.9380
BC	59.40	29.70	0.98	0.3872
A^2	9039.4	9039.4	299	< 0.0001
B^2	1435.6	1435.6	47.5	< 0.0001
Residual	815.93	30.22	-	-
Pure Error	11.41	0.9510	-	-

[a]Hydraulic retention time

5.3.3. Optimization study in moving bed biofilm reactor

5.3.3.1. Effect of pH

Figure 5.3 (a) shows the effect of pH on the COD removal efficiency (%). It was observed that with the increase in the pH of the solution from 5.0 to 7.0, the removal efficiency was increased, whereas further increase in the pH up to 9.0; the removal efficiency was reduced. At pH 7.0, the maximum COD removal of 61.0%, 72.5%, and 79.8% were observed in MBBRs filled with PP, LDPE-PP, and PUF-PP, respectively. The acidic or alkaline conditions of the solution affect the metabolic activity of the enzyme, and hence inhibit the substrate degradation. The optimum degradation of different pollutants was observed between 6.0 to 8.0 pH (Chen et al., 2006; Geed et al., 2017).

5.3.3.2. Effect of retention time

Figure 5.3 (b) shows the effect of retention time on COD removal (%) in MBBRs filled with carriers, namely PP, LDPE-PP, and PUF-PP. Initially, the removal efficiency was low, which further increased with increasing the retention time. The maximum COD removal of 70.1%, 75.6%, and 86.2% were observed for PP, LDPE-PP, and PUF-PP, respectively, in 5 days. The increase in the removal efficiency with increase in retention time is due to the fact that microorganisms get sufficient time to produce the enzyme in an adapted environment (Di Bella et al., 2015).

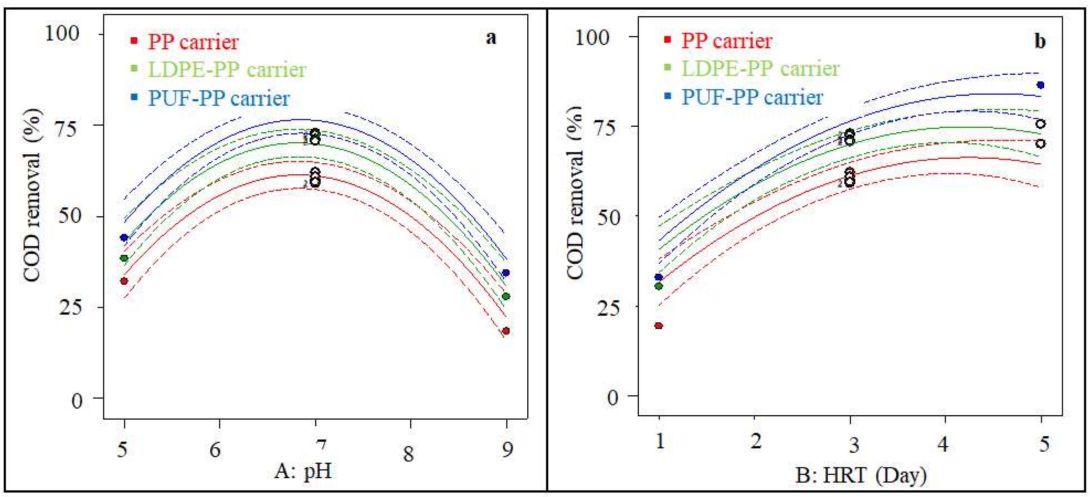

Figure 5.3. (a) Effect of pH (5-9) on COD removal (%); (b) Effect hydraulic retention time (HRT) (1-5 day) on COD removal (%) with various carriers: PP, LDPE-PP, PUF-PP.

5.3.3.3. Effect of type of carriers

Figure 5.3(a) and **5.3(b)** demonstrate the effect of type of carriers on COD removal (%) in the MBBR. It can be observed from **Figure 5.3**, the MBBR filled with PUF-PP carriers shows more removal efficiency than MBBR filled with LDPE-PP and PP. In the case of PP carriers, the biofilm mainly formed on the outer surface, while carriers;

LDPE-PP and PUF-PP provide large pores (**Figure 5.2**) in which the microorganisms were entrapped into pores and biofilm was formed on both inner and outer surface of the carriers. The higher efficiency may also be due to more biofilm growth into the pores of PUF-PP modified carriers than other carriers. Similar phenomena of biofilm development on sponge and plastic carriers were observed by the previous researchers (Deng et al., 2016; Zinatizadeh and Ghaytooli, 2015). Deng et al. (2016) reported that the biofilm on the plastic carriers was mainly developed on the outer surface while for the sponge, biofilm was developed in the inner and outer surface of the pores.

5.3.3.4. Simultaneous effect of pH and retention time

Surface and contour plots in **Figure 5.4 (a-f)** represent the simultaneous effects of pH and retention time on COD removal (%) for PP, LDPE-PP, and PUF-PP carriers in MBBRs. It can be observed from surface and contour plots (**Figure 5.4)** that by increasing the retention time from 1.0 to 5.0 day and pH from 5.0 to 7.0, the COD removal was increased in all three MBBR systems with the similar trend. The maximum COD removal was obtained to be 86.4, 75.6, and 70.1% for the MBBR filled with PUF-PP, LDPE-PP, and PP, respectively at the retention time of 5.0 days and pH of 7.0. The minimum response was found to be 15.6, 14.0, and 7.17% for the MBBR filled with PUF-PP, LDPE-PP, and PP carriers, respectively at the retention time of 1.0 day and pH of 5.0. The results indicate that the simultaneous increase or decrease in the pH and retention time has direct impact on the efficacy of MBBR. Ghevariya et al. (2011) reported that the biodegradation of PAH (chrysene) using CCD of RSM and they reported that under optimum condition, 85.96% of chrysene removal was found on 5^{th} day of the experiments. Similar kind of work was accomplished by Zinatizadeh and Ghaytooli (2015) in MBBR for the treatment of municipal wastewater with different carriers namely; Ring form and Kaldnes-3 at HRT of 4.0 hour. They reported that the optimum COD removal of 85% and 88% were observed for Ring form and Kaldnes-3 carriers, respectively in the MBBR.

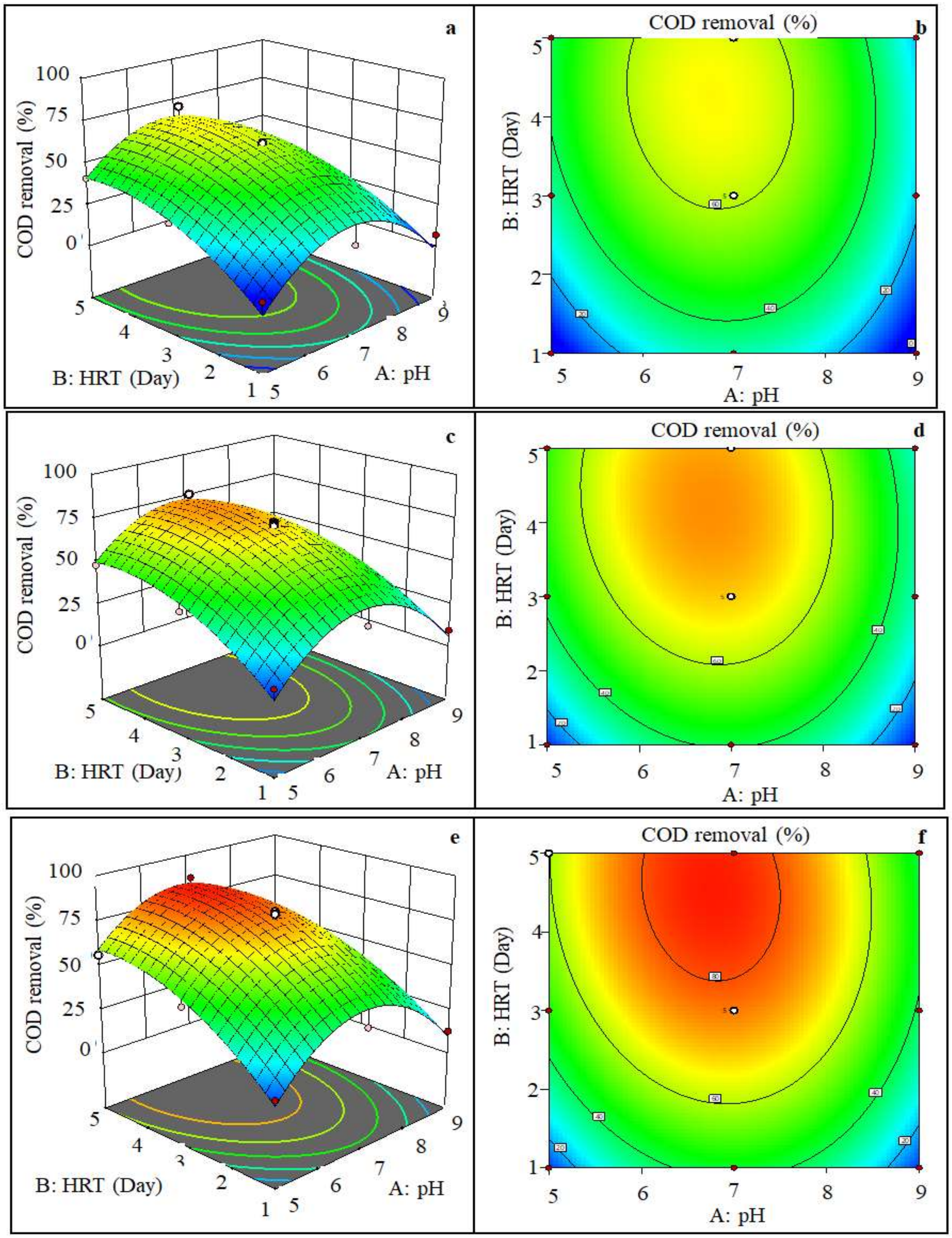

Figure 5.4. Simultaneous effect of retention time (1-5 day) and pH (5-9) on COD removal efficiency: (a, b) with polypropylene (PP); (c, d) with low-density polyethylene-polypropylene (LDPE-PP); (e, f) with polyurethane foam-polypropylene (PUF-PP) as carriers in MBBR.

5.3.3.5. Optimization and validation of the model

According to the CCD, the optimum conditions were predicted and found at 6.74 pH and 4.56 days of retention time with PUF-PP carriers for the maximum COD removal (84.6%). In the confirmation experiment, pH and retention time were rounded off to 7.0, and 5.0 day, respectively. Further, the experiments were performed and results were compared with the predicted model, which indicated an error of less than 5.0% (**Table 5.5**). At optimum condition, the maximum naphthalene removal efficiencies were found to be 72.4%, 84.4%, and 90.2% for PP, LDPE-PP, and PUF-PP carriers, respectively.

Table 5.5 Optimum conditions for the COD removal in moving bed biofilm reactor.

SN	Type of carriers	pH	HRT	Experimental values (%)	Predicted values (%)
1	PP[a]	7.0	5.0	64.5	67.8
2	LDPE-PP[b]	7.0	5.0	76.3	75.41
3	PUF-PP[c]	7.0	5.0	87.2	84.56

[a]Polypropylene; [b]Low-density polyethylene-polypropylene; [c]Polyurethane foam polypropylene

5.3.4. Kinetic evaluation by modified Stover-Kincannon model

Figure 5.5 represents the linearized plots of responses for different carriers in MBBR. The value of correlation coefficients R^2 (0.99) validates the efficacy of the model (**Table 5.6**). The values of U_{max} were found to be 0.476, 0.666, and 0.769 g/L.day for PP, LDPE-PP, and PUF-PP carriers, respectively. Similarly, the values of K_B were found to be 0.565, 0.755, and 0.874 g/L.day for PP, LDPE-PP, and PUF-PP carriers, respectively. The results demonstrate that value of kinetic coefficients, U_{max} and K_B of PUF-PP were larger than LDPE-PP and PP carriers. This could be because PUF-PP carrier provided a large number of pores in which the microorganisms were entrapped and the biofilm was formed on both inner and outer surface of the carriers which caused more substrate consumption (Deng et al., 2016; Oberoi and Philip, 2017).

Further, by putting the values of kinetic constants in Eq. (5.5) and Eq. (5.6), the effluent concentration and required MBBR volume can be predicted.

Oberoi and Philip (2017) studied the kinetic of polycyclic aromatic hydrocarbons and phenolic by modified Stover–Kincannon model. In their work, the value of U_{max} and K_B were obtained as 0.755 and 0.62 (g/L.day) for pyridine and 1.22 and 1.21 (g/L.day) for naphthalene, respectively. Similarly, Derakhshan et al. (2018b) studied the kinetics of atrazine removal by the modified Stover-Kincannon model in the MBBR. In their finding, the values of U_{max} and K_B were observed to be 7.42×10^{-3} and 1.24×10^{-2} (g/L.day), respectively and reported that this model was helpful for predicting the atrazine removal efficacy. The deviation of the kinetic coefficient in this study as compared with previous results may be due to the variation in the composition of influent wastewater, type of activated culture/consortia applied in the reactor, process conditions (pH, DO, HRT etc.), and the type of carriers in the reactor. The obtained results indicated that the model shows good prediction with experimental data and it can be used for the design of MBBRs.

Table 5.6 Summary of kinetics parameter obtained by modified Stover–Kincannon model during biodegradation of naphthalene.

Type of carriers	U_{max} (g/L.day)	K_B (g/L.day)	R^2	RMSE
PP	0.476	0.565	0.995	0.001
LDPE-PP	0.666	0.775	0.998	0.002
PUF-PP	0.769	0.874	0.998	0.001

PP: Polypropylene; LDPE: Low-density polyethylene; PUF: Polyurethane foam, RMSE: Root mean square error