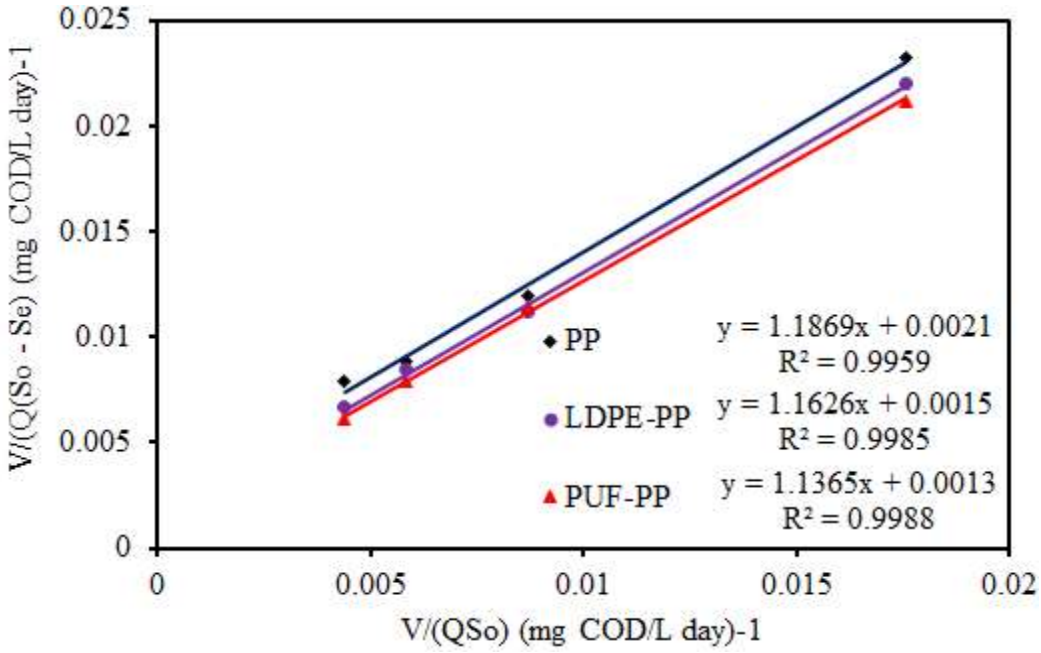
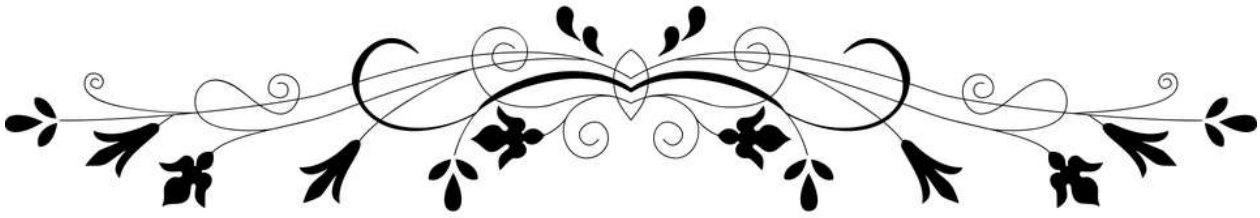


Figure 5.5. Modelling of COD removal in MBBR by modified Stover–Kincannon model for different carriers (PP: polypropylene; LDPE-PP: low-density polyethylene-polypropylene; PUF-PP: polyurethane foam-polypropylene carriers).

5.4. Conclusions

This study demonstrates the feasibility and performance of three MBBRs (MBBR1, MBBR2, and MBBR3) filled with three different types of modified carriers, namely PP, LDPE-PP, and PUF-PP. The SEM analysis demonstrated the successful growth of biofilm onto carriers. At optimum pH (7.0) and HRT (5 days), the maximum COD removal efficiencies were obtained as 72.4%, 84.4%, and 90.2% for MBBR filled with PP, LDPE-PP, and PUF-PP carriers, respectively. MBBR filled with PUF-PP carrier has shown the highest performance than other carriers. It can be a substitute to the conventional carriers to enhance the performance of MBBR for the treatment of a wide range of pollutants. The kinetic constants evaluated by the modified Stover–Kincannon model are helpful to predict the outlet concentration and MBBR volume.



CHAPTER 6

Biodegradation of Congo red dye in a moving bed biofilm reactor: Performance evaluation and kinetic modeling

CHAPTER 6

Biodegradation of Congo red dye in a moving bed biofilm reactor: Performance evaluation and kinetic modeling

6.1. Introduction

The wastewater containing the azo dye is a global environmental challenge among researchers (Bharti et al., 2019). The details of dyes, their source and adverse impact, and treatment techniques have been well discussed in chapter 1 and 2. Congo red dye is a type of azo dye and is widely used in textile industries and laboratories. It is anionic, stable, and highly water-soluble (1 g/30 mL in water) (Mittal et al., 2009). The presence of dyes in wastewater could lead to serious environmental and health concerns due to their toxic, carcinogenic, and mutagenic effects on humans and aquatic animals (Hameed and Ismail, 2018; Vikrant et al., 2018). Therefore, extensive efforts are needed to develop an economical, ecofriendly, and advanced technique to overcome the concern of wastewater containing azo dyes.

In this work, an effort has been given on the application of carrier, i.e., polyurethane foam-polypropylene (PUF-PP), which had shown the highest performance in MBBR to treat the wastewater, as discussed in chapter 5. Congo red [1-naphthalene sulfonic acid, 3,3′-(4,4′-biphenylenebis (azo)) bis(4-amino-) disodium salt] was selected as the model pollutants in the present study. The process variables, namely pH, Congo red concentration, and media filling ratio, were optimized by central composite design (CCD) of response surface methodology (RSM). The performance of a continuous MBBR was evaluated at different flow rates under optimized conditions. Further, Congo red degradation kinetics was estimated by a Modified Stover–Kincannon model.

The phytotoxicity effects of untreated and treated wastewater were performed using *Vigna radiata* seeds.

6.2. Materials and methods

6.2.1. Dye and chemicals

Congo red (CAS number 573-58-0) was purchased from Sigma-Aldrich, India. The modified mineral salt media (MSM) contains the following composition (g/L); KH_2PO_4 (1.8), NaH_2PO_4 (2.5), $FeCl_3$ (0.01), $MnSO_4.H_2O$ (0.2), $MgSO_4$ (0.2), beef extract (0.2), peptone (0.1), and supplemented with 0.2% (w/v) of glucose (Chen et al., 2018). The SW was prepared by adding MSM and different concentrations of Congo red dye in distilled water.

6.2.2. Bacterial species and carriers

It is well known that the dye degrading bacterial species should be present/survive in dye-contaminated soil and wastewater. Therefore, the dye-contaminated soil samples were collected from the near site of the textile industrial area of Bhadohi, UP, India, for the isolation of dye degrading bacterial species. The soil samples were collected in sterile polyethylene bags, and until further use, the samples were kept at 4 °C. The isolation and enrichment procedure of bacterial species from soil samples have been discussed in section 3.2.3. In brief, initially, the soil sample was enriched with MSM (100 mL) and Congo red dye (20 mg/L) in Erlenmeyer flasks (250 mL) and incubated at 150 rpm and 37 °C for five days. After completion of the incubation period, 2 mL of inoculums was transferred to another Erlenmeyer flasks containing fresh MSM (100 mL) and Congo red (50 mg/L) and further acclimatized until complete dye degradation observed under the similar conditions. This process was repeated three times with gradually increasing the Congo red dye. A serial dilution technique was applied to isolate the bacterial species. Based on batch experiments, most potential bacterial

species were selected and used in this study. In brief, the set of batch experiments were performed in 250 mL flasks. The flasks contained MSM (100 mL) and Congo red dye (50 mg/L) were inoculated with 2.0 mL of respective isolated bacterial species (D1, D2, D3, D4, and D5). All flasks were incubated at 35±2 °C in the rotary incubator at 150 rpm for 4.0 days. The most potential bacterial species were identified by 16S rRNA, and details of the molecular characterization procedure have been discussed in section 3.2.4. Similarly, the details of carriers used in MBBR has been well described in section 5.2.2.

6.2.3. Optimization by response surface methodology

The process parameters, namely pH (5.0-9.0), dye concentration (10-100 mg/L), and carrier filling ratio (10-60%) were selected to optimize the biodegradation of Congo red dye in MBBR (**Table 6.1**). The total runs were designed according to the following term $2^n + 2n + n_0$ using CCD of RSM. Where n and n_o represent the number of independent variables and repetitions of experiments at the center point, respectively. Therefore, 20 runs were performed in triplicate to attain the optimized conditions (**Table 6.2**).

Table 6.1 The range of the operating parameters in the biodegradation of Congo red dye.

Factor	Name	Unit	Minimum	Maximum	Mean
A	pH	-	5	9	7
B	Dye concentration	mg/L	10	100	55
C	Carrier filling ratio (%)	-	10	60	35

6.2.4. Biodegradation study

The biodegradation of Congo red dye was performed in the MBBR bioreactor, and details of the experimental set-up of MBBR have been discussed in section 5.2.3 (**Figure 6.1**). The bioreactor was filled with PUF-PP as moving carriers and isolated

potential bacterial sp. as an inoculant for Congo red degradation. The sterilized air (passed through 0.22 μm filter) was continuously provided in MBBR to keep the circulation of carriers and prevent contamination. The performance of a continuous MBBR at different flow rates was examined under optimum conditions obtained in the batch study. The wastewater was stored in 50 L plastic tank. The provisions were made to the continuous supply of dye solution and filtered air in MBBR. The dye solution (50 mg/L) was continuously supplied into MBBR at varying flow rates of 25-100 mL/h using a peristaltic pump. At every flow rate, adequate time was given to achieve the steady-state conditions. All the analyses were carried in triplicate and their average values were used for the data interpretation. The MBBR was continuously operated for 564 h and its performance can be estimated by equations discussed in section 3.2.7.

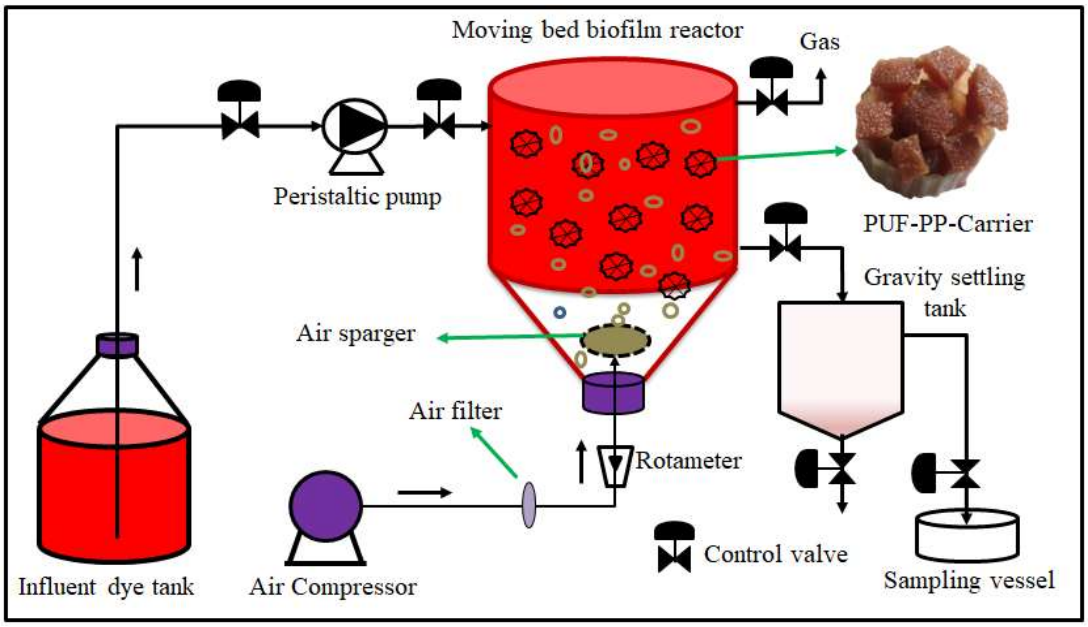


Figure 6.1. Schematic representation of experimental set-up for the biodegradation of Congo red dye.

6.2.5. Analytical techniques

The biodegraded samples were taken from MBBR at a regular time intervals. The Congo red concentration was measured using a UV-vis spectrophotometer (ELICO SL-2010, India). The samples were subjected to centrifugation at 5000×g for 10 min. After

that, the supernatant was used to analyze the Congo red concentration, whereas pellets were used for biomass estimation. The morphology of biofilm developed onto MBBR carriers was examined by a SEM (EVO-18, ZESIS, Germany). The PUF-PP carriers without bacterial cells were used as the control (0^{th} day). The pH was measured using a pH meter (HD 2305.0; Delta OHM; Italy). The functional group of Congo red dye and its biodegraded samples were analyzed by FR-IR analysis (NICOLET 5700, Japan).

6.2.6. Kinetics model and phytotoxicity analysis

Stover–Kincannon model was applied to evaluate the kinetics of dye degradation, and the mathematical equations have been described in section 5.2.6. In this study, the phytotoxicity test was performed using *Vigna radiata* seeds, and the experimental set-up has been described in section 3.2.10 and **Figure 3.3**. The details of the experimental set-up and procedure have been discussed in section 3.2.10. In brief, 20 seeds of *Vigna radiata* were kept between two filter paper and soaked in 15 mL of distilled water (control), untreated and treated water in three separate Petri plates. These plates were kept at 30±2.0 °C for 15 days. The seed germination was recorded after 2 days, whereas root (cm) and shoot (cm) length were measured after 15 days.

6.3. Results and discussion

6.3.1. Selection of potential bacterial species

The removal efficiency (RE %) of Congo red dye against the isolated bacterial species, namely D1, D2, D3, D4, and D5, is shown in **Figure 6.2.** It can be observed from the figure that the bacterial species D2 was found to be more effective for Congo red removal than the other bacterial species (D1, D3, D4, and D5). The removal efficiencies of 67%, 87.1%, 71.1%, 41%, and 34.4% were obtained by D1, D2, D3, D4, and D5 bacterial species, respectively. Therefore, D2 species was used in the present study.

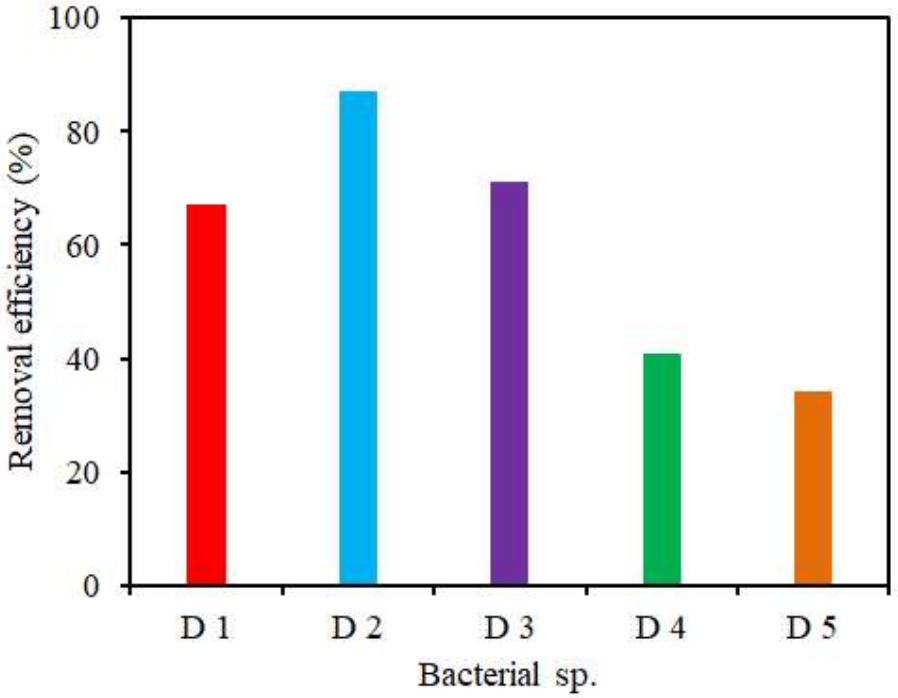


Figure 6.2. Removal efficiency of Congo red dye by isolated bacterial species

6.3.2. Identification of dye degrading bacterial species

The obtained 16S-rRNA sequence of dye degrading bacterial species was deposited in the GenBank database of NCBI and get the accession number (MH587030). The sequence was organized with maximum similarity, and five sequences were selected and exported in FASTA format. The phylogenetic tree of *Bacillus species* (MH587030.1) is given in **Figure 6.3**.

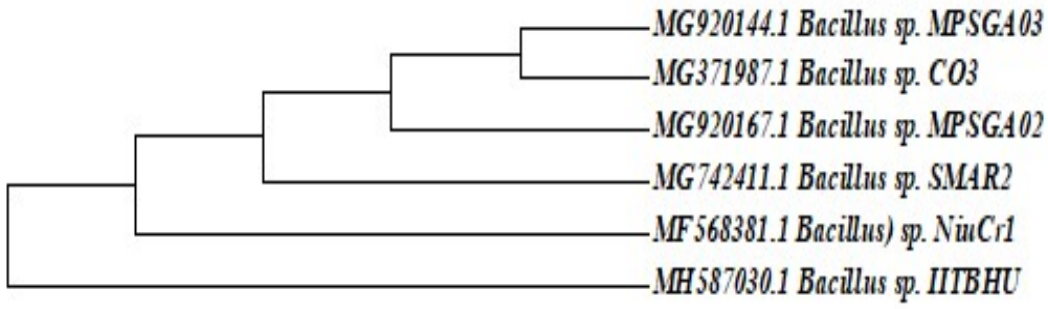


Figure 6.3. A phylogenetic tree of *Bacillus species* (MH587030.1) isolated from dye contaminated sites.

6.3.3. Morphological analysis of packing media

The morphology of PUF-PP before (0^{th} day) and after (15^{th} day) biofilm formation is given in **Figure 6.4**. The large number of micropores connected to each other was perceived on the PUF-PP surface before the biofilm formation. These micropores offer an enormous surface area for biofilm formation (Geed et al., 2018). Hence, dense bacterial biofilm is formed onto the surface and pores of PUF-PP and reveals the successful colonization of bacterial cells on carriers. The biofilm formation on moving bed carriers took place due to the accumulation of biomass on carrier/support matrix or dense growth of microbial populations. The extracellular polymers released by microorganisms support stable biofilm on carriers against the high hydraulic load (Derakhshan et al., 2018b). The *Bacillus cohnii* immobilized into PUF was used for the biodegradation of Reactive Red 120 dye in wastewater (Padmanaban et al., 2016). They reported that PUF provides a highly porous surface for the formation of stable biofilm. Similarly, Kureel et al. (2017) used PUF as the packing support for biofilm formation and reported that immobilized PUF-*Bacillus* sp. successfully degrade the benzene at the high concentration.

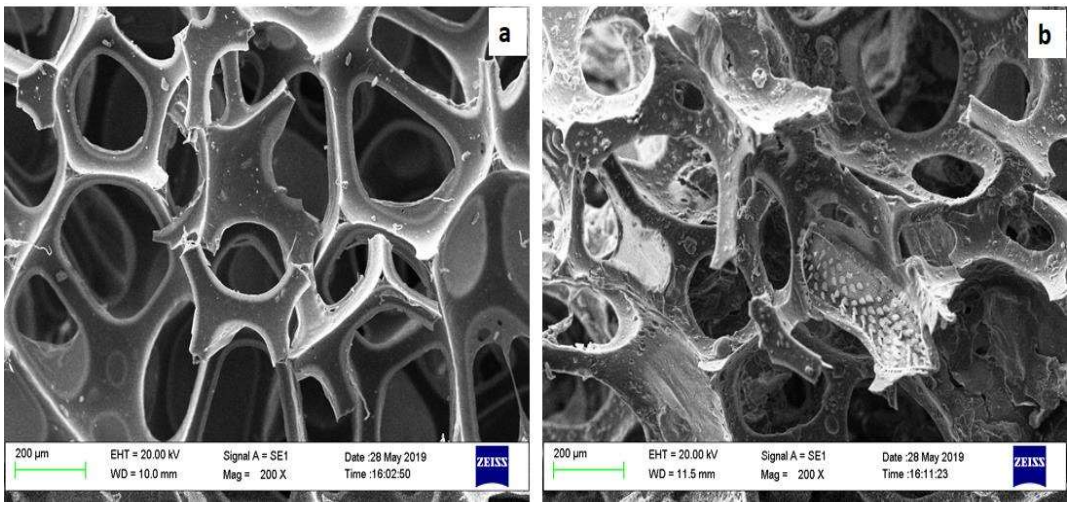


Figure 6.4. Scanning electron microscope image of PUF-PP carrier: (a) before immobilization (control); and (b) after biofilm development.

6.3.4. Statistical analysis

A total of 20 experiments were performed and their outcomes in terms of dye RE (%) are given in **Table 6.2**. To analyze the suitable model, the data were examined with linear, interaction, and quadratic models, and the obtained table is given in **Table 6.3**. The values of R^2 and adjusted R^2 were obtained as 0.98 and 0.96, respectively for the quadratic model. Furthermore, the predicted R^2 (0.89) value is in a reasonable covenant with adjusted R^2 (0.96) and seems to be well fitted with results. The obtained values of R^2, adjusted R^2, and predicted R^2 are reasonable with previous studies and suggest that the quadratic model is suitable for optimization study (Gusain et al., 2016; Singh et al., 2010). The experimental data fitted with the quadratic model is given in the following Eq. (6.2):

$$\text{Dye removal}(\%) = 91.9 - 1.0A - 13.4B + 8.7C - 3.4AB + 2.87AC + 0.63BC - 28.1A^2 - 6.1B^2 - 10.5C^2 \quad (6.1)$$

ANOVA analysis was used to examine the significance of each variable for dye degradation and the summary of the result is reported in **Table 6.4**. The *p*-value less than 0.05 designates that the model terms are significant, while the *p*-value greater than 0.1 shows insignificant terms (Kashefi et al., 2019; Silva et al., 2018). In this work, *B, C, AB,* A^2, B^2, and C^2 are significant model terms ($p < 0.05$). The obtained model *F*-value of 69.1 denotes that the overall model is suitable for dye degradation study. There are only a 0.01% error chance could occur due to noise. Therefore, the quadratic regression model was significant to correlate the response and independent variables for the optimization of the biodegradation system.

Table 6.2 Central composite design of experimental runs and their outcomes for the removal of Congo red dye.

Run	pH	Dye conc. (mg/L)	Carrier filling ration (%)	Dye removal (%)
1	7	55	35	90
2	7	55	35	92
3	5	100	10	33
4	5	10	10	56
5	7	55	10	69
6	5	10	60	63
7	9	10	60	72
8	9	100	60	38
9	7	55	35	92
10	5	55	35	62
11	9	55	35	69
12	7	55	35	89
13	7	55	35	93
14	5	100	60	44
15	9	10	10	52
16	7	10	35	99
17	9	100	10	17
18	7	55	35	89
19	7	55	60	97
20	7	100	35	76

Table 6.3 Summary of model statistics.

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	22.61	0.2386	0.0959	-0.3791	14810.35	
2FI	24.83	0.2536	-0.0910	-4.5478	59576.64	
Quadratic	4.12	0.9842	0.9699	0.8925	1154.87	Suggested
Cubic	2.55	0.9964	0.9885	-1.7791	29844.09	Aliased

Table 6.4 ANOVA summary for the quadratic model.

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	10568.69	1174.30	69.03	< 0.0001	significant
A-pH	10.00	10.00	0.5879	0.4610	
B-Conc.	1795.60	1795.60	105.56	< 0.0001	
C-Carrier filling	756.90	756.90	44.50	< 0.0001	

ratio					
AB	91.13	91.13	5.36	0.0432	
AC	66.13	66.13	3.89	0.0769	
BC	3.13	3.13	0.1837	0.6773	
A^2	2163.01	2163.01	127.16	< 0.0001	
B^2	100.51	100.51	5.91	0.0354	
C^2	305.82	305.82	17.98	0.0017	
Residual	170.11	17.01			
Lack of Fit	155.27	31.05	10.47	0.0111	significant
Pure Error	14.83	2.97			
Cor Total	10738.80				

6.3.5. Optimization study using central composite design

6.3.5.1. Interactive effect of pH and dye concentration

Figure 6.5 shows the three-dimensional surface response and counter plots of the interactive variables, namely pH, initial dye concentration, and carrier filling ratio against the removal of Congo red dye in MBBR. The **Figure 6.5a** and **6.5b** show the simultaneous effect of initial Congo red dye concentration and pH against the dye RE. The elliptical profile of a contour plot reveals a very significant interaction between dye concentration and pH. As we change the pH either acidic or alkaline, the dye RE in MBBR was decreased. At fixed carrier filling ratio (35%) and pH 7.0, the dye RE of 99% was achieved at 10 mg/L, whereas the RE decreased with increasing dye concentration and reached up to 76% at 100 mg/L. Furthermore, at similar carrier filling ratio and initial dye concentration of 55 mg/L, the RE of 92% was observed at pH 7.0, while RE decreased to 62 and 69 % at pH 5.0 and 9.0, respectively. The enzymatic activity of microorganisms is adversely affected in acidic and alkaline conditions, and corresponding dye RE was significantly reduced (Gopinath et al., 2009; Nath et al., 2019; Sutar et al., 2019). The decrease in the RE of dye with increasing dye concentration may lead to substrate (dye) inhibition (Talha et al., 2018). Padmanaban et al. (2016) have studied the degradation of reactive red dye in a PBBR by *Bacillus*

cohnii and reported that the efficacy of bioreactor becomes low due to the substrate inhibition at higher loading of dye.

6.3.5.2. Interactive effect of pH and carrier filling ratio

The pH of the solution is responsible for the transport of dye molecules through the cell membrane of microorganisms and considered an essential variable in biodegradation of dye (Chen et al., 2018). The maximum RE of dye was obtained at 7.0 pH (**Figure 6.5c** and **6.5d**). As we change the pH either acidic or alkaline at fixed carrier ratio, the RE of the system adversely decreased. At neutral pH, the dye RE was significantly enhanced by increasing the carrier filling ratio (%). However, above 45% of carrier filling ratio, very less enhancement in the dye RE was observed. The increase in the dye RE with carrier filling ratio may be due to the significant amount of biofilm developed at higher carrier filling (%) in the MBBR. More biomass can be retained in the MBBR with increasing the carrier filling ratio (Lopez-Lopez et al., 2012).

6.3.5.3. Interactive effect of initial dye concentration and carrier filling ratio

Figure 6.5e and **6.5f** demonstrate the simultaneous effect of initial dye concentration and carrier filling ratio on the dye removal. The dye RE was enhanced with carrier filling ratio, whereas an inverse relationship was observed against the dye concentration. For example, the dye RE of 69% was observed with 10% carrier filling ratio, while RE increased with carrier filling ratio and reached to 97% with 60 % of carrier filling at 55 mg/L of the dye. These results intimated that an increase in carrier filling in the MBBR caused more available surface area for the biofilm formation; hence, more active and stable biomass formation took place into MBBR carriers. High loading of pollutants unfavorably inhibited the metabolic activity of the bacterial species and corresponding reduced the biodegradation efficacy of the system (Bankole

et al., 2018; Yadav et al., 2014). A comparative study of Congo red dye removal at various concentrations with the previous literature is summarized in **Table 6.5**.

Table 6.5. Comparison of Congo red dye removal at various concentrations with the previous literature.

Dye	Microorganism	Reactor	Concentration (mg/L)	Removal efficiency (%)	Reference
Congo red	*Providencia stuartii*	Packed bed bioreactor	100	85.3	Goswami et al. (2020)
Congo red	*Bacillus sp.*	Moving bed biofilm reactor	50	93.6	Sonwani et al. (2020a)
Congo red	*Bacillus subtilis*	Fluidized bed bioreactor	100	92	Shalini and Setty (2019)
Congo red	*Brevibacillus parabrevis*	Packed bed bioreactor	300	63.3	Talha et al. (2018)
Congo red	Microbial consortium	Up-flow column reactor	100	100	Lade et al. (2015)
Congo red	*Acinetobacter Baumannii*	Flask	100	98.6	Li et al. (2015)
Congo red	*Acinetobacter baumannii*	Flask	100	90.3	Ning et al. (2014)
Congo red	*Bacillus* sp.	Moving bed biofilm reactor	50	95.7	Present study

6.3.6. Model validation

In order to obtain an optimal condition for the maximum removal of dye, CCD of RSM was used. The optimal values of pH, dye concentration, and carrier filling ratio were predicted and found as 7.1, 50 mg/L, 45.2%, respectively. In the validation process, pH, dye concentration, and carrier filling ratio were rounded off to 7.0, 50 mg/L, and 45%, respectively. The obtained experimental response was in good agreement with the predicted value and revealed only 1.57% of error with predicted result. The error of less than 5% indicates the validity of the model (Dubey et al., 2016).

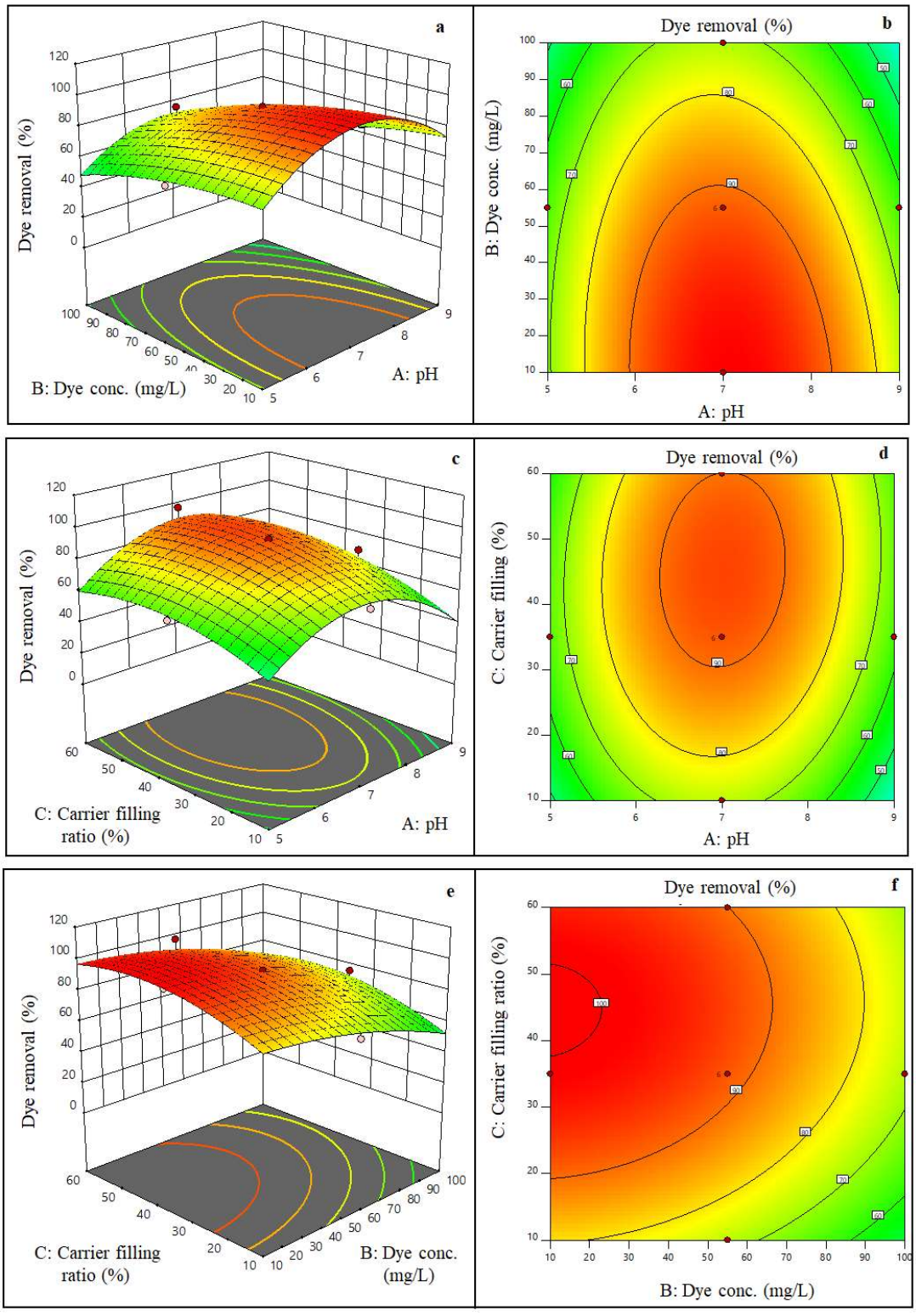


Figure 6.5. Surface and contour plots for the removal (%) of Congo red dye: (a, b) effect of pH (5-9) and dye concentration (10-100 mg/L) (at 35% carrier filling); (c, d) effect of carrier filling (10-60%) and pH (5-9) (at 55 mg/L Congo red concentration); (e, f) effect of carrier filling (10-60 %) and dye concentration (10-100 mg/L) (at 7.0 pH).

6.3.7. Biodegradation of Congo red dye in continuous bioreactor

The effect of flow rates on the dye RE was studied to achieve the suitable flow rate at which the MBBR could offer high performance. The performance of continuous MBBR was evaluated at various flow rates (25-100 mL/h) under optimum conations (pH=7.0, Congo red dye concentration=50 mg/L, and carrier filling ratio= 45%). Initially, the MBBR was run at 25 mL/h with an inlet loading rate (ILR) of 20 mg/L.day. The dye RE and elimination capacity (EC) were exponentially increased with time and reached equilibrium in 136 h with 95.7% of RE and 19.1 mg/L/day of EC (**Figure 6.6a**). The overall performance of MBBR at different flow rates has been given in **Table 6.6**. The inlet flow rate (IFR) was increased from 25 to 50 mL/h on 144th h and it was found that a sharp dip in RE observed. The RE was further recovered (91.82%) with time and became almost constant in 240 h. At the same time, the EC was increased from 19.1 to 36.1 mg/L.day, as the flow rate was increased from 25 to 50 mL/h. On 175th h, the flow rate was increased to 75 mL/h and it was found that after a sharp initial dip on RE in 264 h, the RE was reached to 88.9 % on 396th h. Further, on 408th h, as we increased the flow rate from 75 to 100 mL/h, a sharp drop of RE was observed, which again stabilized in 564th h with 72.29% of RE. The maximum EC of 57.6 mg/L.day was found at 100 mL/h of IFR.

The dye REs were obtained to be 95.7, 91.8, 88.9, and 72.9 % as the flow rates were increased to 25, 50, 75, and 100 mL/h, respectively. The high flow rate of dye solution could lead to short hydraulic retention time (HRT) inside MBBR, and corresponding RE was significantly reduced. It was reported that a sufficient HRT is required for the effective degradation of pollutants (Banerjee and Ghoshal, 2017; Leyva-Díaz et al., 2013; Sonwani et al., 2019b). The high IFRs inhibit the effective growth of bacteria into the surface of carriers and reduce the interaction of dye molecules; thereby less RE was

observed. The previous researcher performed similar work and informed that the RE of pollutants was reduced due to the short HRT at the high inlet flow rate (Geed et al., 2018; Kurade et al., 2017). Kurade et al. (2017) have studied the degradation of textile effluent at various flow rates by the bacterial-yeast consortium and found that as the IFR increased, the RE was enormously reduced. Similarly, Nath et al. (2016) studied the biodegradation of malachite green from synthetic dye solution at different flow rates by *Bacillus cereus* immobilized in calcium alginate and reported that the RE malachite green was decreased at the high inlet flow rate.

6.3.8. Rate control mechanism in dye degradation

The effect of ILR of dye solution on the RE and EC is shown in **Figure 6.6b**. As the inlet loading rate of dye solution was increased, the RE was slightly decreased up to 60 mg/L.day ILR and beyond this, the RE declined sharply. A linear relationship was found between the ILR and EC up to 60 mg/L.day of ILR, and after that the EC was increased very slowly with a non-linear pattern. The maximum EC of 57.67 mg/L.day was obtained at 80 mg/L.day of ILR. At a high flow rate, the performance of the bioreactor was significantly dropped due to the rate-controlling mechanism (Chung et al., 2003; Yadav et al., 2014). In the present work, two distinct zones, i.e. mass transfer and bio-reaction control were overserved during biodegradation of Congo red dye at different ILR. At low ILR, the diffusion of dye molecules through biofilm was slow, and it was mass transfer control. Due to the mass transport limitation, the internal layer of biofilm could be substrate deficient and the microorganisms did not utilize the substrate completely (Dizge and Tansel, 2010). The biodegradation of substrate was become bio-reaction control from mass transfer control due to high diffusional flux at high ILR (Yadav et al., 2014).

Geed et al. (2018) studied the biodegradation of Malathion at different ILR using *Bacillus* sp. immobilized into PUF and reported that the mass transfer control was observed at low ILR, whereas bio-reaction control limitation was found at high inlet loading rate. It is uneconomical to operate the PBBR in the mass transfer limitation zone (Yadav et al., 2014). From the practical point of view, the desirable lower limit of ILR in the bioreactor at which the controlling mechanism changes from mass transfer control to bio-reaction control, whereas the point of intersection of RE and EC may be considered as extreme limit (Geed et al., 2017; Yadav et al., 2014). In this work, the optimum operating range of the inlet loading rate has been obtained between 60 and 72.3 mg/L.day for the effective degradation of Congo red dye.

Table 6.6 Performance of a moving bed bioreactor for the biodegradation of CR dye.

Flow rate (mL/h)	Process time (h)	ILR (mg/L. day)	EC (mg/L.day)	RE (%)
25	1-132	20	19.1	95.7
50	144-240	40	36.1	91.8
75	252-396	60	52.0	88.9
100	408-564	80	57.6	72.2

ILR= Inlet loading rate; EC= Elimination capacity; RE= Removal Efficiency

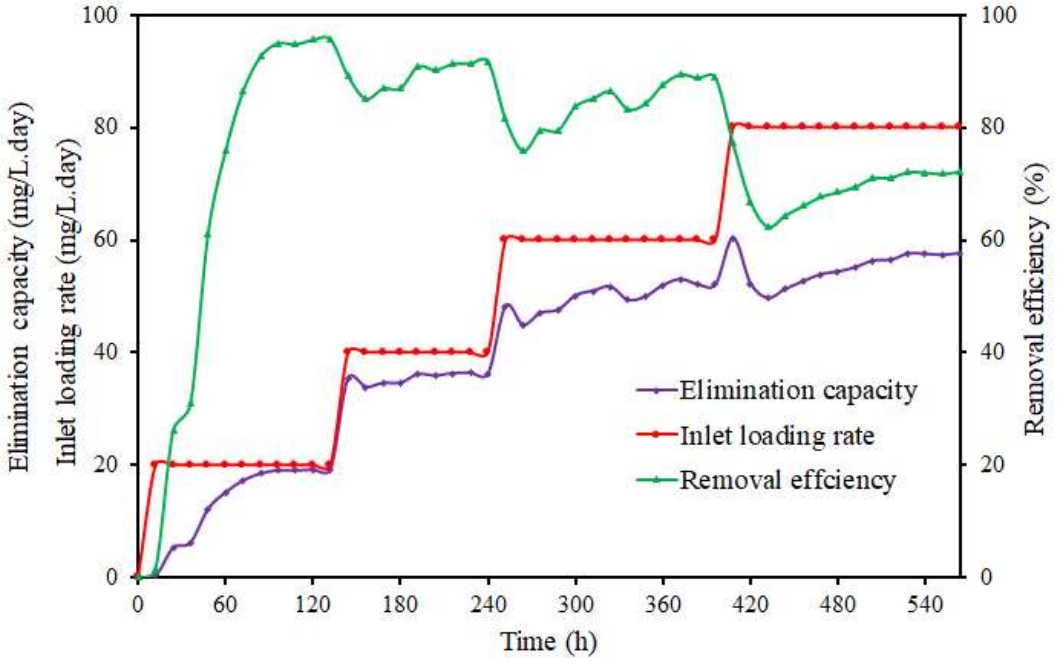


Figure 6.6a. Performance of a moving bed bioreactor at different inlet loading rate.

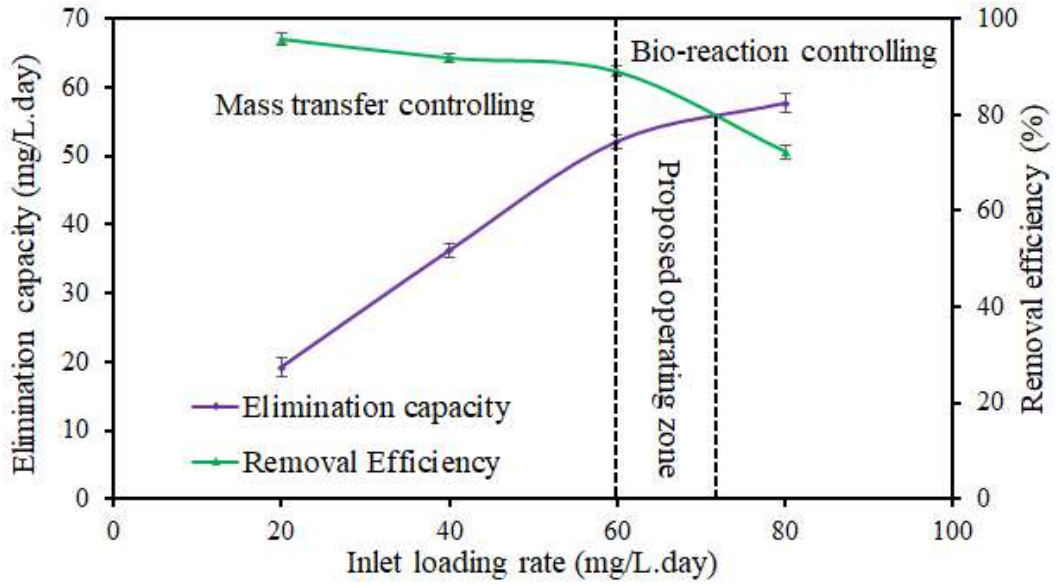


Figure 6.6b. Relation between mass transfer and bio-reaction controlling zone at various inlet loading rates.

6.3.9. Kinetic study by modified Stover-Kincannon model

In this work, the kinetics of Congo red dye degradation in a MBBR was studied using a modified Stover-Kincannon model. The experimental data fitted with model has been presented in **Figure 6.7**. The value of R^2 (0.99) and RMSE (0.002) indicate that the model is well fitted with experimental data. The value of kinetic parameters K_B and U_{max} were obtained to be 0.253 and 0.263 g/L.day, respectively (**Table 6.7**). Furthermore, by putting the value of kinetic constants in Eq. (5.5) and Eq. (5.6), the dye effluent concentration and the required volume of MBBR can be predicted by the following equations.

$$S_e = S_o - \frac{0.263 S_o}{0.253 + (\frac{QS_o}{V})} \quad (6.3)$$

$$V = \frac{QS_o}{\left(\frac{0.263\, S_o}{S_o - S_e}\right) - 0.253} \quad (6.4)$$

Kapdan (2005) studied the biodegradation kinetics of dyestuff (Reactive red 195) using a modified Stover-Kincannon model and kinetic constants: K_B and U_{max} were reported as 0.43 g/L.day and 0.47 g/L.day, respectively. The naphthalene

biodegradation kinetics was studied by a modified Stover-Kincannon model, and kinetic constants were observed as K_B: 0.769 g/L.day and U_{max}: 0.87 g/L.day (Sonwani et al., 2019b). The variation of the kinetic parameter compared with previous work may be due to the distinct in the composition and inlet loading rate of target pollutants, types of bacterial species used, operating process parameter, and bioreactor design.

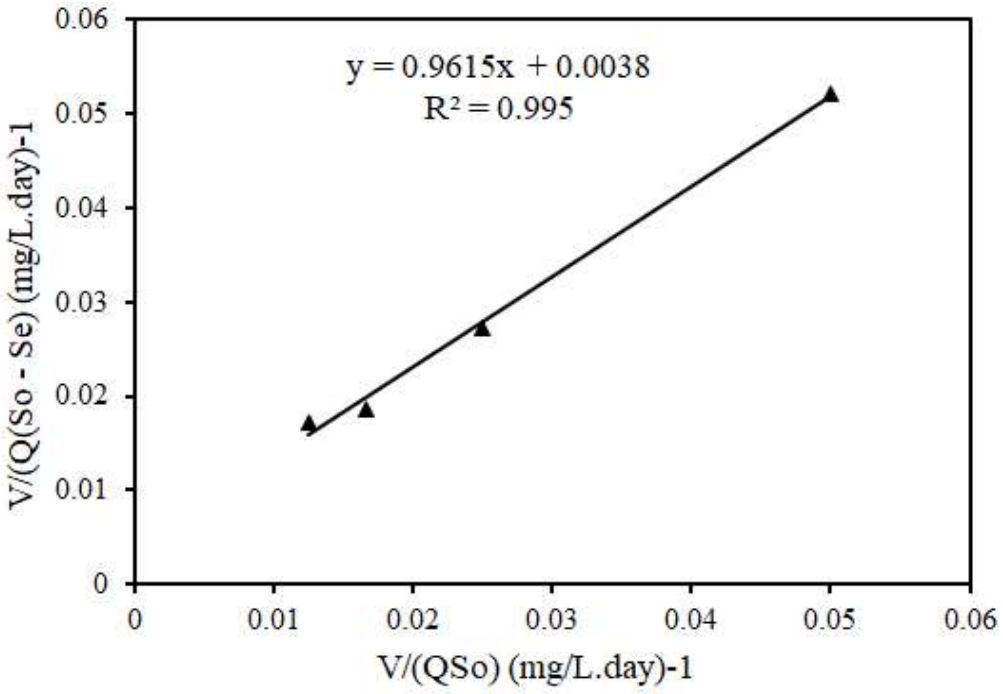


Figure 6.7. Stover–Kincannon model plots for the biodegradation of Congo red dye.

Table 6.7 Profile of kinetics constants calculated during biodegradation of Congo red dye.

Dye	U_{max} (g/L.day)	K_b (g/L.day)	R^2	RMSE
Congo red	0.263	0.253	0.995	0.002

RMSE: Root mean square error

6.3.10. Biodegradation analysis

The spectral profile of the control and biodegraded sample of Congo red dye is shown in **Figure 6.8**. A significant decrease in the absorbance of the Congo red degraded sample was observed and confirmed the degradation of dye in MBBR. The FT-IR spectrum of the control and biodegraded sample of Congo red is reported in **Figure 6.9**.

The FT-IR spectrum of control Congo red reveals several peaks; a peak at 678 cm^{-1} due to bending and vibration of C-H, a peak at 863 cm^{-1} due to p- distributed ring vibration, a peak at 1272 cm^{-1} due to vibration and stretching of primary aromatic amine with C-N, the peak at 1542 cm^{-1} and 1666 cm^{-1} due to stretching bending of azo group of N=N, a peak at 2345 cm^{-1} due to stretching vibration of C-C, and a peak at 3347 cm^{-1} due to presence of O-H group (**Figure 6.9a**). The FT-IR spectrum of Congo red biodegraded sample shows peaks at 1635 cm^{-1} for stretching bending of the azo group of N=N, at 2347 cm^{-1} for stretching of C-C, and a broad peak at 3455 cm^{-1} due stretching vibration of O-H. It was observed that some peaks were disappeared, whereas new peaks at 532 cm^{-1}, 663 cm^{-1}, and 2067 cm^{-1} have appeared (**Figure 6.9b**). These new peaks were appeared duo to reductive cleavage of azo group of N=N.

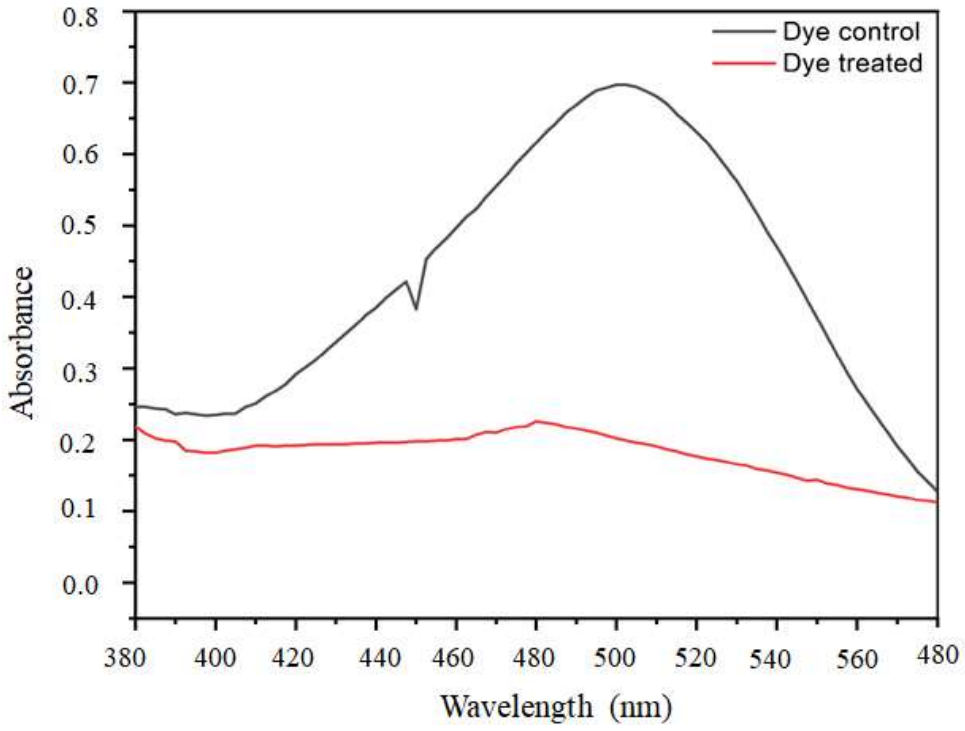


Figure 6.8. The spectral profile of control and biodegraded sample of Congo red dye in MBBR.

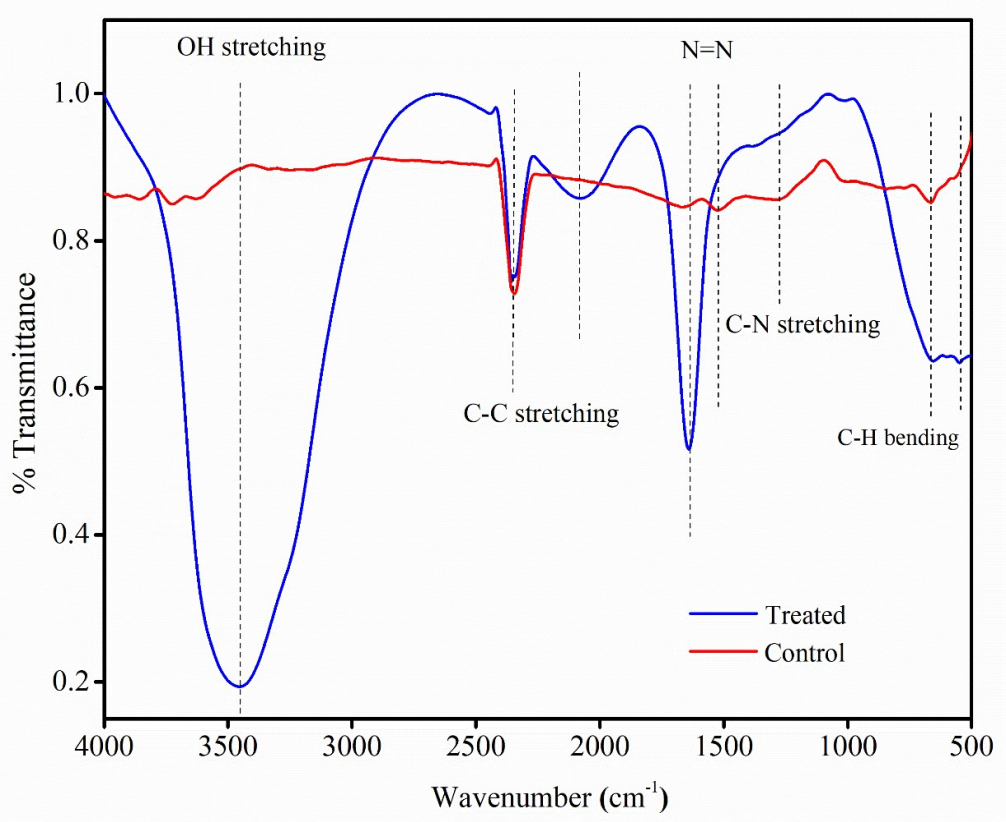


Figure 6.9. The FT-IR spectrum of (a) Congo red dye; (b) biodegraded sample of Congo red dye.

6.3.11. Phytotoxicity study

The *Vigna radiata* seeds were grown in distilled water (control), untreated and treated wastewater (**Figure 6.10**). The seeds grown in untreated wastewater supported 60% germination, 1.02 ± 0.21 cm of average root length, and 1.11 ± 0.12 cm of average shoot length. The seeds grown in treated wastewater show 90% germination, 5.05 ± 0.17 cm of average root length, and 4.12 ± 0.21 cm of average shoot length (**Table 6.8**). The seeds grown in distilled water indicates 95% of germination, 5.54 ± 0.13 cm of average root length, and 4.62 ± 0.19 cm of average shoot length. It can be clearly observed that seeds grown in treated wastewater show better growth in germination, shoot, and root length than the untreated wastewater.

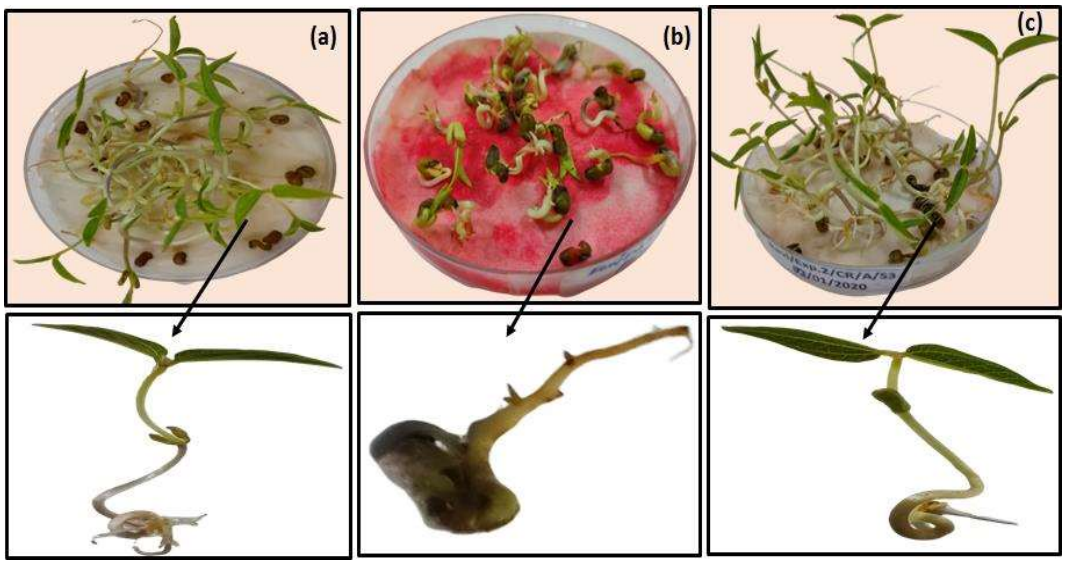


Figure 6.10. Images of *Vigna radiata* seeds germinated in a distilled water, (b) untreated dye solution, and (c) treated dye solution.

Table 6.8 Summary of phytotoxic analysis of *Vigna radiata* seeds grown in treated and untreated wastewater.

S.N.	Parameter	Distilled water	Untreated wastewater	Treated wastewater
1	Germination (%)	95	60	90
2	Root length (cm)	5.54 ± 0.13	1.02 ± 0.21	5.05 ± 0.17
3	Shoot length (cm)	4.62 ± 0.19	1.11 ± 0.12	4.12 ± 0.21

6.4. Conclusions

The biodegradation of Congo red dye was performed using polyurethane foam-polypropylene immobilized *Bacillus* sp. MH587030.1 in MBBR. The response surface methodology was used to optimize the process parameters, namely pH, Congo red concentration, and media filling ratio, and the optimum conditions were observed to be 7.0, 50 mg/L, and 45%, respectively in batch MBBR. The performance of a continuous MBBR was studied at various inlet loading rates, and more than 90% of RE and 57.6 mg/L.day of EC were found under optimum conditions. A modified Stover-Kincannon model examined the biodegradation kinetics of Congo red dye and proposed kinetic correlations can be used for the prediction of effluent (dye) concentration and reactor volume to scale-up of the process. Finally, the *Vigna radiata* seeds germinated in the dye treated wastewater showed better growth than untreated wastewater.