Chapter 3

Bioremediation of Congo red in an anaerobic moving bed bioreactor: Process optimization and kinetic modeling

3.1. Introduction

Rapid industrialization during the 20th century resulted in a very high-water requirement for the operation of different industries. Various industries discharge toxic compounds such as pesticides, dyes, aromatic compounds, heavy metals, etc., without proper treatment, adversely affecting the ecosystem (Sonwani et al., 2019; Vikrant et al., 2018; Hameed et al., 2018). Among these, many industries such as tanneries, carpets, textiles, cosmetics, and paper printing widely use azo dye in their operations (Bharti et al., 2019; Chen et al., 2018; Hsueh et al.,2007). Globally, greater than 1,00,000 tons/years of azo dye are manufactured, and approximately 40 % of it is released into the water bodies during the dying process (Mezohegyi et al., 2007). The run-offs discharged from textile industries contain these dyes, resulting in low dissolved oxygen levels and high chemical oxygen demand in the waterbodies. The photosynthesis process of aquatic plants inhibits due to the decrease of the penetration of sunlight into the water bodies in the presence of CR (Swain et al., 2021; Affam et al.,2014). In specific, CR dye is extensively used in textile, polymer, paper, leather industries, etc. The growth and fertility of the aquatic plants are severely decreased even at a low concentration of CR dye (Zamora et al., 2016; Van Tan et al.,2021). It has carcinogenic, teratogenic, and mutagenic effects on humans and animals (Kabbout et al.,2014; Asad. et al.,2007). The Environmental Protection Agency of the United States (USEPA) enlisted

the CR dye as a priority pollutant due to its persistent and toxic nature. Therefore, various physicochemical and advanced oxidation process (AOP) methods such as membrane separation, adsorption, coagulation, chemical oxidation, UV-Fenton oxidation, ozonation, and photocatalysis are extensively studied for the degradation of CR from the waste stream (Luo et al.,2014; Liang et al., 2017; Yadav et al.,2014; Swain et al.,2020). However, operating cost and environmental aspects are the main concern for the Physico-chemical and AOP methods. In addition, secondary waste generation, high energy and chemical cost, and toxic intermediates formation are the main disadvantages of these processes (Bharti et al., 2019; Bajaj et al., 2008; Derakhshan et al.,2018a, Pahlavanzadeh et al., 2018). However, biodegradation is considered an effective process to overcome the challenges faced in Physico-chemical and AOP processes. Further, cost-effective, complete mineralization and eco-friendliness are the advantages of the biological methods (Sonwani et al.,2020; Chen et al., 2018; Asad et al.,2007). Several researchers have used various types of microorganisms like "*Pseudomonas aeruginosa*, *Bacillus* sp., *Aeromonas hydrophila*, *Shewanellaoneidensis*, *Alcaligenes faecalis*, *Acinetobactorbaumannii*, *Klebsiella quasipneumoniae*, *Aeromonas sp.*, etc., for bioremediation of CR dye" (Kashefi et al., 2019; Bharti et al., 2019; Geed et al., 2018; Srinivasan and Sadasivam, 2018).

The biological method is mainly categorized into two types: free (suspension) and immobilized (attached) cell systems. The immobilized cell system is an effective technique as it involves the entrapment of the microorganisms on the solid matrix, which increases the removal efficiency and the accepting ability of microbes against adverse conditions (Sahoo and Panigrahy, 2018; Sonwani et al., 2019b). For the immobilization purpose, different types of packing materials such as high-density polyethylene, gravel, sugarcane bagasse, biochar, polyurethane foam, polyvinyl alcohol beads, and sodium alginate have been used (Martin et al.,2008; Bharti et al., 2019; Sonwani et al.,

2019a; Gomes et al., 2019). Various researchers have shown the advantage of immobilized systems for the biodegradation of CR dyes in different types of bioreactors (Bharti et al., 2019; Shalini and Setty, 2019; Cooper et al., 2016; Vikrant et al., 2018). However, very minimal work has been reported on the biodegradation of CR in an anaerobic moving bed bioreactor (AnMBBR).

The current work is focused on the optimization of process variables like bio carrier filling ratio (BFR), CR dye concentration, and agitation speed in an AnMBBR filled with *Lysinibacillus fusiformis* immobilized polyurethane foam-polypropylene (PUF-PP). Response surface methodology (RSM) combined with central composite design (CCD) was used to optimize the process variables. The behavior of an AnMBBR was evaluated for CR dye treatment under various influent flow rates. Further, the experimental data were used to assess the substrate utilization rate by applying the modified Stover–Kincannon (MSK) model.

3.2. Materials and method

3.2.1. Minimal salt medium and bacterial culture

Analytical grade CR (CAS number 573-58-0) and the chemicals for the minimal salt medium (MSM) preparation were purchased from Sigma Aldrich, India. The following chemicals (g/L) were used to prepare MSM solution: KH_2PO_4 (1.3); NaH_2PO_4 (2.6); $FeCl_3$ (0.02); $MnSO_4 \cdot H_2O$ (0.3); $MgSO_4$ (0.4); beef extract (1.8.); peptone (1.5); and glucose (2.0) (Sonwani et al., 2020; Chen et al., 2018). The desired amount of CR has been added to MSM to prepare the synthetic effluent.

The azo dye-contaminated soil sample has been collected from a textile industry at Bhadohi, near Varanasi, in Utter Pradesh, India (25° 23′ 14.1720″ N latitude and 82° 34’ 4.9116” E longitude). The sample (soil) was aseptically kept in a sterile plastic bottle at 4.0 °C in the lab. The

count of potential bacterial species was enhanced by adding 5.0 g of soil in a 250 mL flask containing 25 mg/L of CR in 100 mL MSM. The flask was incubated for ten days at 30 ± 2.0 °C and 120 RPM. Then, 20 mL of aliquot was taken from the previous flask, added into a new flask containing 50 mg/L of CR in MSM, and again incubated at similar operating conditions for 15 days. The above technique was replicated thrice by increasing the CR concentration by 25 mg/L in each batch. Further, the potential bacterial species were isolated from the final batch by the serial dilution method, and it was taken for the biodegradation of CR.

3.2.2. Detail description of bio carrier and bioreactor set-up

The bio-carriers were made of polypropylene (PP) and polyurethane foam (PUF). The PP (25 mm length and 12 mm diameter) used in the preparation of bio carriers was purchased from Biotech, New Delhi, India. The PUF sheets were acquired locally and cut into the cubical shape of 2.5 ± 0.3 cm. PUF-PP (bio carrier) was prepared by inserting PUF in the hollow space of PP. The density, surface area, and specific mass of PUF-PP bio carrier were measured and found to be 940 kg/m^3, 520 m^2/m^3 and 75 kg/m^3, respectively. These bio carriers were filled in the bioreactors for experimental study.

The present work is carried out in a lab-scale AnMBBR (TAE/1000 Pignat, France), as shown in Figure 3.1. The AnMBBR was associated with a rectangular tank (working volume of 30 L) made up of polyvinyl chloride (PVC). The synthetic wastewater was supplied into the AnMBBR from an influent tank (40 L) using a peristaltic pump (Watson Marlow 323E/D) through a rotameter (Figure 3.1). Initially, the AnMBBR was operated in batch mode with MSM containing glucose (2.0 g/L) as a carbon source and isolated bacterial inoculum for 30 days. The biofilm growing on the PUF-PP bio carrier was examined using a scanning electron microscope. After

immobilization, the AnMBBR was fed with synthetic wastewater containing CR to optimize the process variables like BFR, agitation speed, and CR concentration using the CCD of RSM.

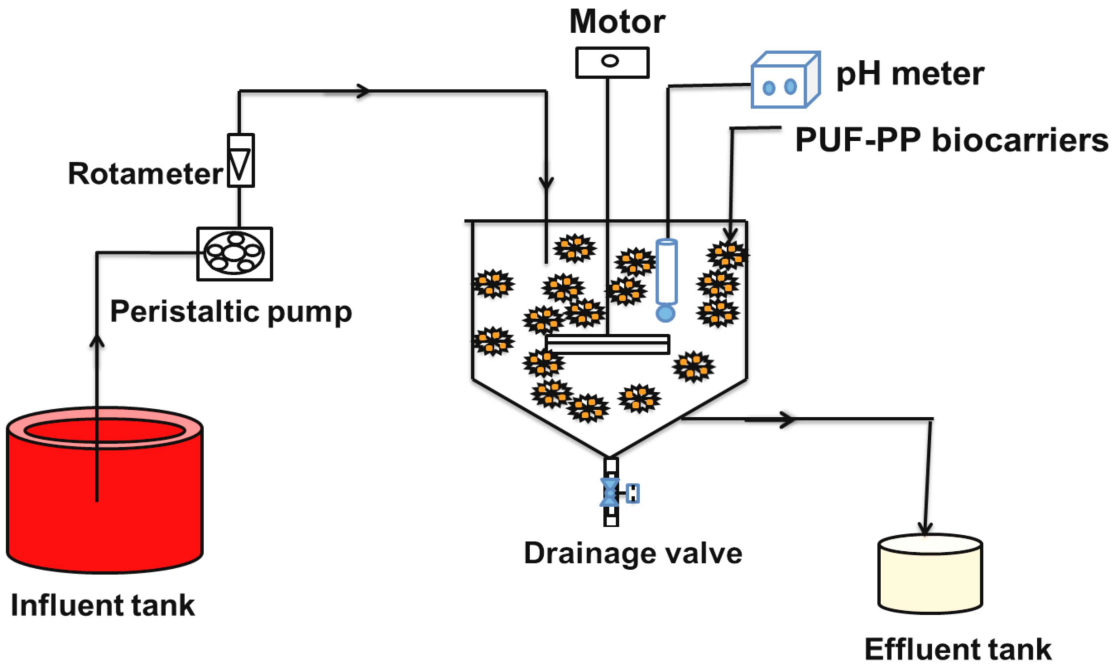

Figure. 3. 1. Schematic diagram of an anaerobic moving bed reactor (AnMBBR) for the biodegradation of Congo red.

3.2.3. Optimization of process parameters

RSM combined with CCD is an effective tool to establish a mathematical correlation between independent variables and response. It is more effective than the conventional optimization technique as it decreases the experimental cost and number of experiments. RSM-based optimization was done using the Design-Expert software system (Version 12, Stat–Ease Inc., Minneapolis, USA). The range of independent process variables used in the study is shown in

Table 3.1. A polynomial equation was applied that equation represents second-order to express the relevant model as follows:

$$Y = \alpha_0 + \alpha_i X_i + \alpha_j X_j + \alpha_{ij} X_{ij} + \alpha_{ii} X_i^2 + \alpha_{jj} X_j^2 + \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

where Y represents the CR removal efficiency (%), α denotes the correlation coefficient, and i and j are the multi-degree coefficients.

Table 3.1: The range of the independent process variables in the biodegradation of CR dye.

Factor	Name	Unit	Minimum	Mean	Maximum
A	CR concentration	mg/L	20	60	100
B	BFR	%	20	45	70
C	Agitation speed	RPM	40	70	100

CR: Congo red; BFR: Biocarrier filling ratio

3.2.4. Continuous study for removal of CR in the AnMBBR

After optimizing process variables, the performance of the AnMBBR was evaluated under continuous mode by changing the feed flow rate ranging from 10 – 60 mL/min for 120 days. The bioreactor was operated at each feed flow rate till the steady-state performance was achieved. The behavior of the bioreactor was studied in terms of the following performance parameters (Geed et al., 2017; Swain et al., 2021).

$$\text{Removal efficiency (RE, \%)} = \frac{S_i - S_f}{S_i} \times 100 \quad (3.2)$$

$$\text{Elimination capacity (EC, mg/L. d)} = \frac{(S_i - S_f) \times Q}{V} \quad (3.3)$$

$$\text{Inlet loading rate (ILR, mg/L. d)} = \frac{S_i \times Q}{V} \quad (3.4)$$

where S_i and S_f represent the influent and effluent concentrations of CR (mg/L), respectively, Q represents the feed flow rate (mL/h), and V represents the working volume of the bioreactor (L).

3.2.5. Analytical techniques

UV–vis spectrophotometer (ELICO SL-2012) and pH meter (pH Meter, SSI-303) were used to estimate the CR concentration and pH of the samples, respectively. Before the analysis, the effluent sample was centrifuged at 5000 rpm for 15 minutes, and the supernatant sample was used to measure residual CR concentration. The bio-carriers were dried in a vacuum oven at room temperature and coated with gold particles. Then, the morphological characteristic of the bio carrier was studied by a scanning electron microscope (EVO-18, ZESIS).

3.2.6. Kinetic of CR dye removal

Mathematical modeling is essential for designing and upscaling laboratory bioreactors to an industrial scale. The researchers developed several kinetic models to analyze the substrate utilization rate in the bioreactor. The Modified Stover- Kincannon (MSK) kinetic equation is one of the widely used models for studying the substrate utilization rate of the organic pollutants in an immobilized bioreactor (Hassani et al., 2014). In the present study, the AnMBBR was operated at optimized conditions (BFR of 45 %, agitation speed of 70 RPM, and CR concentration of 100 mg/L). The intermediate samples were collected to determine the concentration of residual CR in the effluent. The MSK model was used to predict the correlation between the substrate utilization rate and organic loading rate in the various reactors such as RBCs, MBBRs, and Upflow anaerobic reactors (Cesar et al., 2013; Sonwani et al., 2019). The following expression gives the substrate utilization rate.

$$\frac{dS}{dt} = \frac{Q}{V}(S_0 - S_f) = \frac{U_{max}\left(\frac{Q.\ S_0}{V}\right)}{K_B + \left(\frac{Q.\ S_0}{V}\right)} \quad (3.5)$$

where S_o and S_f (mg/L) are the initial and final concentrations of dye, U_{max} is the maximum substrate utilization rate (mg / L d), and K_B is the saturation constant (mg / L d).

Eq. (6) can be obtained by linearizing the Eq. (5) as

$$\frac{V}{(S_0 - S_f).Q} = \frac{K_B}{U_{max}}\left(\frac{V}{Q.\ S_0}\right) + \frac{1}{U_{max}} \quad (3.6)$$

To estimate the value of kinetic parameters, the slope and intercept were obtained from the straight-line graph plotted between $\frac{V}{(S_0 - S_f).\ Q}$ against $\frac{V}{Q.\ S_0}$

3.3. Results and Discussion

3.3.1. CR dye degrading bacterial species

The samples were sent to Triyat Scientific, Nagpur, India, to characterize and identify isolated bacterial species. The Polymerase Chain Reaction (PCR) was used to amplify DNA using forward (27F-5' AGAGTTTGATCTGGCTCAG 3') and reverse (1492R-5' TACGGTACCTTGTTACGACTT 3') primers. The PCR was operated at 90 ºC for 2 min, denaturation at 90ºC for 30 sec, annealing at 47 ºC for 30 sec, extension at 70 ºC for 2 min, and final extension at 70ºC for 10 min, respectively. The ABI 3730xl (Applied Biosystems) sequencer was used to sequence the PCR sample. The obtained 16s rRNA sequences were submitted to the NCBI database to identify the bacterial species based on similarity analysis (https://www.ncbi.nlm.nih.gov). MW599200 is the accession number of bacterial species. These species were identified as *Lysinibacillus fusiformis* KLM1, and the phylogenetic tree was

constructed using MEGA X software [3]. The phylogenetic tree of the bacterial species is depicted in Figure 3.2.

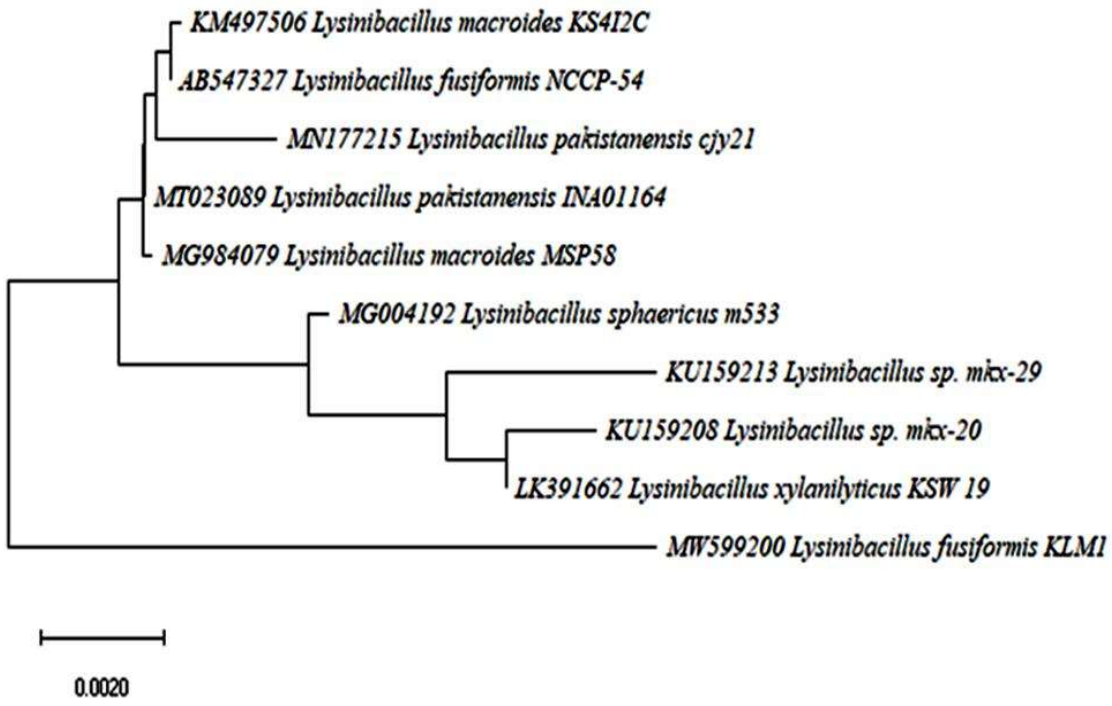

Figure. 3.2. The Phylogenetic tree of isolated bacterial species Lysinibacillus fusiformis KLM1 (MW599200).

3.3.2. Morphological study of PUF-PP bio-carrier

The morphology of the PUF-PP bio carrier before and after immobilization was studied using scanning electron microscope (SEM) images. The results clearly show several micropores on the surface of PUF-PP on the 0^{th} day (figure 3.3.). The pores provide a high specific surface area for the growth of the bacterial species. After 30^{th} days, a biofilm layer was observed on the surface of the bio carrier.

The biosorption impact on the removal of CR dye was investigated in batch experiments before the start-up of experiments (Swain et al., 2021). The immobilized bacterial cells were isolated from the PUF-PP surface using sonication and further autoclaved at 121 ℃ for 15 min. The batch experiments were performed to evaluate the effect of biosorption and biodegradation using non-living and living (immobilized) cells, respectively. It was found that the maximum CR dye RE of 5.8 % was obtained using non-living cells, whereas the immobilized cell was able to remove 88.76 % of CR dye. The above analysis revealed the insignificant role of biosorption in the removal of CR dye.

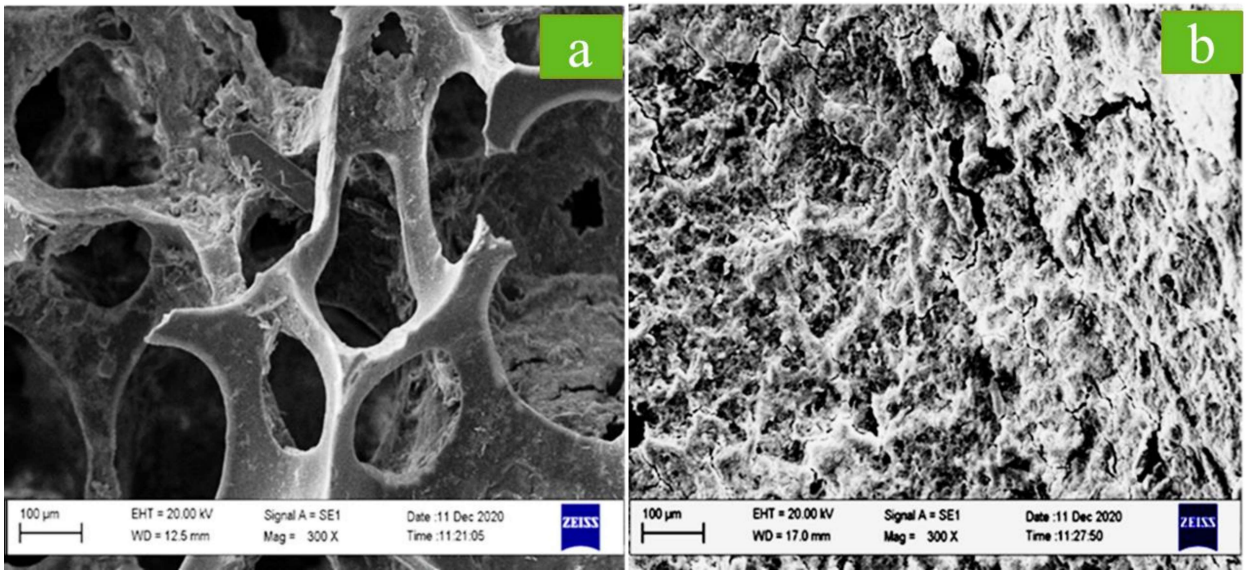

Figure. 3.3. SEM images of polyurethane foam (a) before immobilization (0th day), (b) after immobilization (30th day)

3.3.3. Optimization of process variables

The independent process variables like BFR (20-70%), CR concentration (20-100 mg/L), and agitation speed (40-100 rpm) were optimized in AnMBBR. A detailed description of the experimental runs and the response variable, such as CR dye removal (%), were presented in Table 3.2. As shown by Eq. 7, the quadratic model was well-fitted with the experimental data. The regression coefficients (R^2, predicted R^2, and adjusted R^2) for CR dye removal were evaluated and shown in Table 3.3. The value of R^2 predicted R^2 and adjusted R^2 were obtained as 0.988, 0.924, and 0.978, respectively. The high values (greater than 0.9) of the regression coefficients reveal the accuracy of the model to predict the experimental data. The quadratic model has predicted a correlation to establish the relationship between the response value and the independent variables.

$$\text{CR dye removal (\%)} = 85.14 - 4.66A + 9.56B + 7.33C + 0.0712AB + 1.45AC + 1.84BC + 0.7718A^2 - 13.76B^2 6.51C^2 \quad (3.7)$$

Further, ANOVA analysis has been carried out to justify the significance of the model, and the detail are shown in Table 3.3. A higher F-value and lower p-value (less than 0.05) signify the quadratic model's validity with the obtained data.

Table 3.2: Predicted and experimental CR dye removal using RSM

Run	*A*: CR concentration (mg/L)	*B*: BFR (%)	*C* Agitation speed (RPM)	CR removal (%)
1	20	20	100	64.31
2	20	20	40	57.73
3	60	45	70	86.03
4	100	70	40	60.94
5	20	70	40	73.44
6	20	45	70	89.28
7	60	70	70	79.64
8	20	70	100	87.04
9	60	45	70	84.25
10	60	45	70	85.91
11	100	70	100	80.7
12	100	20	40	45.3
13	60	45	70	86.4
14	60	45	100	88.45
15	60	45	70	86.49
16	60	45	40	67.17
17	100	45	70	80.9
18	100	20	100	57.33
19	60	20	70	61.47
20	60	45	70	85.07

Table 3.3: ANOVA analysis of RSM response

Source	Sum of Squares	*Df*	Mean Square	*F*-value	*p*-value	
Model	3317.85	9	368.65	95.37	< 0.0001	Significant
A-CR concentration	217.44	1	217.44	56.25	< 0.0001	
B-BFR	914.32	1	914.32	236.53	< 0.0001	
C-Agitation speed	536.56	1	536.56	138.80	< 0.0001	
AB	0.0406	1	0.0406	0.0105	0.9204	
AC	16.85	1	16.85	4.36	0.0634	
BC	27.20	1	27.20	7.04	0.0242	
A^2	1.64	1	1.64	0.4238	0.5297	
B^2	520.92	1	520.92	134.76	< 0.0001	
C^2	116.48	1	116.48	30.13	0.0003	
Residual	38.66	10	3.87			
Lack of Fit	34.89	5	6.98	9.26	0.0144	Significant
Pure Error	3.77	5	0.7532			
Cor Total	3356.50	19				

3.3.4. Effect of process variables

3.3.4.1. Effect of initial CR dye concentration and BFR

The interactive Effect of initial CR dye concentration and BFR on the CR dye degradation was depicted using surface and contour plots (Figure. 3.4). The results obtained using CCD combined with RSM were shown in (Figure.3.4) and validate the instantaneous effect of the initial concentration of CR and BFR on CR dye degradation. The CR dye removal was increased with the BFR and decreased with an increase in CR dye concentration. For illustration, the CR dye Removal response of 45.3% was obtained with a 20% carrier BFR and at 100 mg/L of CR dye concentration. Moreover, maximum CR dye removal of 89.28 % was obtained at a BFR of 45 % and 20 mg/L of initial CR dye concentration (Figure. 3.4 (a, b)). In AnMBBR, with the increase in BFR up to 70 %, the removal efficiency was found to be 87.04 % at 20 mg/L of CR dye concentration.

The reasons for the decrease in removal response at higher CR dye concentrations may be due to substrate inhibition, which inhibits the enzymatic activity of bacterial species and consequently deteriorates the biodegradation efficacy (Talha et al., 2018, Sonwani et al., 2020; Padmanaban et al., 2015). Bharti et al. (2019) have analyzed the efficacy of a packed bed bioreactor (PBBR) for the azo dye removal using *Pseudomonas aeruginosa* and reported that the performance of the bioreactor was decreased at a higher inlet loading rate.

3.3.4.2. Effect of initial CR concentration and agitation speed

The three-dimensional surface has shown the cooperating effect of process variables in Figure.3.4. These figures provide the required information regarding the experimental design of AnMBBR by CCD of RSM. The surface and graphs are shown in Figure.3.4 (c, d) to validate the effect of agitation speed and initial concentration to remove CR. When AnMBBR was operated with the initial CR concentration (20 mg/L) and agitation speed (70rpm), a maximum CR removal response of 89.28 % was obtained. Similarly, at a BFR of 40% and initial CR concentration of 100mg/L, and an agitation speed of 40 rpm, 45.3% CR removal response was obtained. The substantial reduction in % removal at high dye concentration is due to the substrate barrier (Talha et al., 2018).

3.3.4.3. Effect of BFR and agitation speed

The plot relates that the maximum CR removal was obtained at an average BFR (45%) and moderate agitation speed (70 rpm). The CR removal response (%) of CR dye increased from 45.3 % to 89.28 %, with a rise in agitation speed from 40 to 100 rpm. Again, as we increased agitation speed, the CR removal response significantly decreased. At moderate agitation speed, increasing the BFR (%), the CR removal was also increased. However, above 45% of the BFR, no more increment in CR removal was observed (Figure. 3.4 (e, f)).

Moreover, the higher BFR decreased free space in the bioreactor, which hinder the proper movement of the bio-carrier. A thin layer of biofilm was developed around the bio-carriers when increasing more BFR in AnMBBR, which leads to incompetent and substrate inside diffusion of oxygen the formed biofilm and hence affects the RE (Barwal and Chaudhary, 2015; Lopez-Lopez et al., 2012).

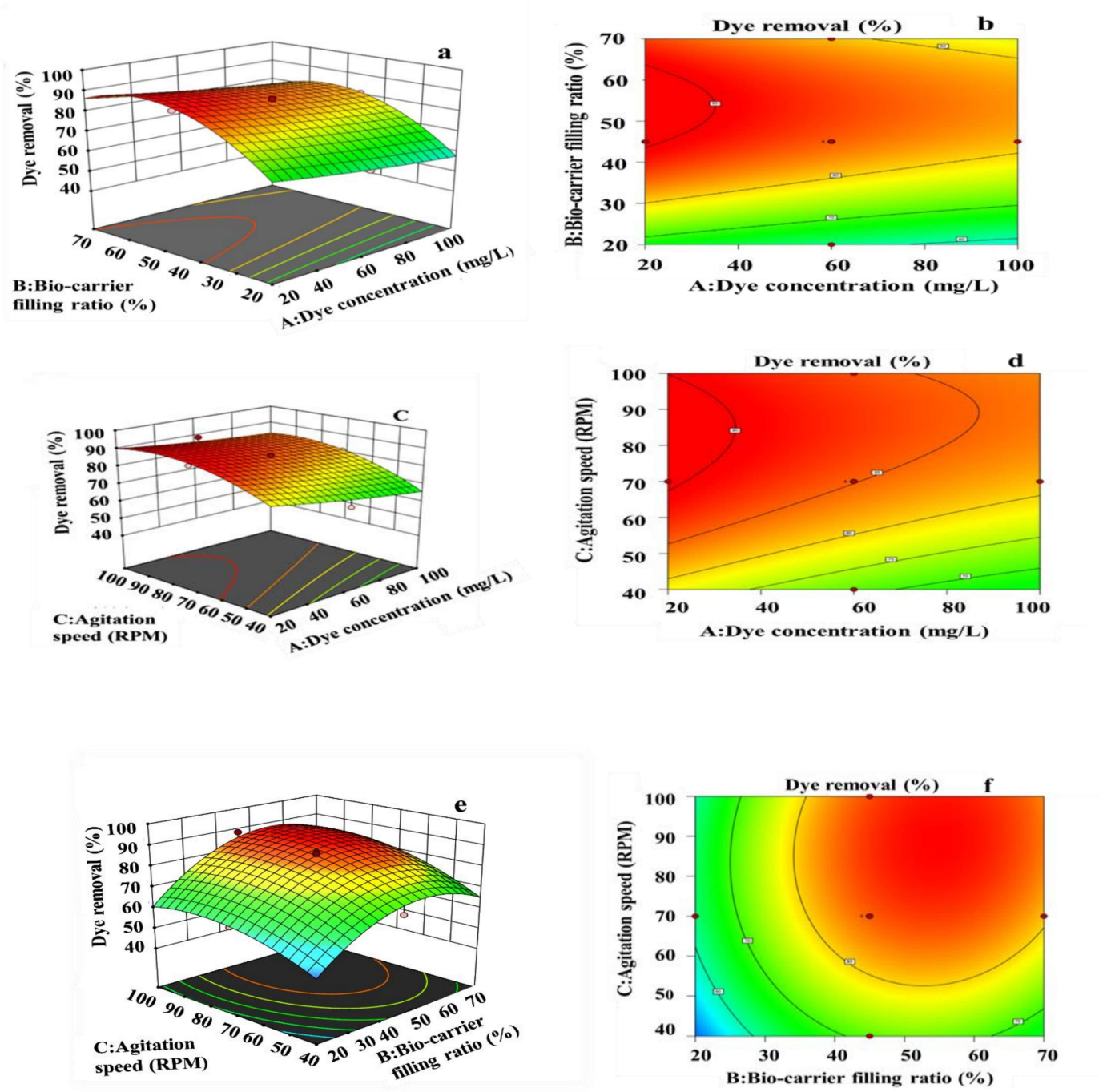

Figure.3.4: Surface and contour plots for the % removal of CR: (a, b) variation of% removal of CR with BFR and CR concentration; (c, d) variation of % removal of CR with agitation speed and CR concentration; (e, f) variation of % removal of CR with agitation speed and BFR (%).

3.3.5. Verification of the model

The optimum conditions for the maximum CR degradation were obtained using the RSM tool. The range of agitation speed (40-100 rpm), CR concentration (20-100 mg/L), and BFR (20-70%) were used, and optimum values were obtained as 70.48 rpm, 60 mg/L, 45.28%, respectively. The maximum removal of 89.28 % was obtained at the optimized conditions. To validate the model, the process variables such as agitation speed, BFR, and CR dye concentration were rounded off to 70 rpm, 45%, and 60 mg/L, respectively. The corresponding practical values were obtained for the maximum removal efficiency of 87.64 % of CR. The acquired experimental value obtained was in good agreement with the projected response. It exposed only 1.85 % of error with predictable results in Table 4. According to Dubey et al. (2016), an error of less than 5 % shows the validity and suitability of the model.

3.3.6. Kinetic study

In the present work, the modified Stover-Kincannon (MSK) model was used in an AnMBBR to evaluate the substrate utilization rate. The kinetic parameters were calculated from the graph plotted between the inverse of total organic loading rate [$V/(Q \times S_i)$] vs inverse of total organic loading removal rate [$V/(Q(S_i - S_f)$] shown in (figure 3.5). The kinetic values were obtained to be 0.23 g/L. d for saturation constant (K_B) and 0.21g/L. d for the maximum CR removal rate (U_{max}) from the slope and intercept, respectively. The high value (0.99) of the regression coefficient (R^2) represents that the model could be well predicted by the experimental data. The MSK model value has been reported: K_B and U_{max} (0.43 g/L.d) and (0.47 g/L.d), respectively, in the biodegradation of Reactive red 195 Kapda (2005). The moving bed biofilm reactor for industrial wastewater treatment containing ethylene glycol using an MSK model reported values of saturation constant

were (K_B = 7.67g/L. d), and the highest ethylene glucose removal rate (U_{max} = 8.43g/L. d) (Hassani et al., 2014). In the current work, K_B and U_{max} values have been obtained less than those reported by researchers (Hassani et al., 2014 and Sonwani et al., 2020). However, the researchers obtained different values of kinetic parameters (KB and Umax) due to the differences in process parameters and reactor configurations such as concentration, inlet loading rate of required pollutants, bacterial species, bioreactor design, and operating process parameters. Further, the essential volume of AnMBBR can be predicted from the following expressions as obtained U_{max} and K_B values putting in equations. (5) and (6), the CR dye concentration, and Eqs. (3.8) and (3.9), respectively.

$$S_f = S_i - \frac{0.21 S_i}{0.236 + \frac{Q \times S_i}{V}} \tag{3.8}$$

$$V = \frac{Q S_i}{\left(\frac{0.21 S_i}{S_i - S_f}\right) - 0.23} \tag{3.9}$$

The kinetics study of CR dye in the AnMBBR using the MSK model can be used effectively to scale up the bioreactor.

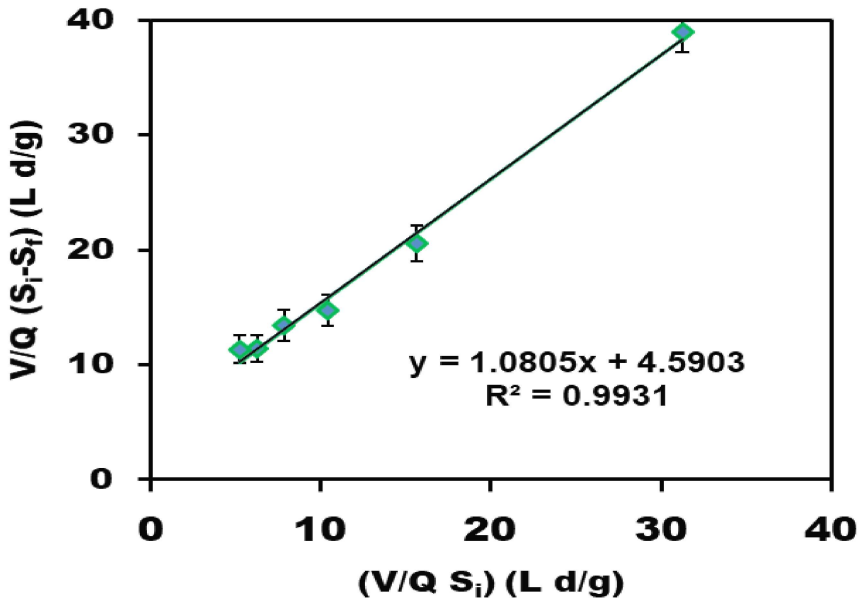

Figure.3.5: Modified Stover-Kincannon model for CR dye response

3.3.7. The continuous study in An MBBR for biodegradation of CR dye

The effect of the various flow rates (10–60 mL/min) on the performance of AnMBBR in continuous mode was evaluated under optimum conditions, and the various parameter was evaluated to investigate the efficacy of the bioreactor (Table 3.4). At the start of the operation, the feed flow rate was increased slowly up to 10 mL/min, corresponding to an ILR of 24 mg/L. d. The exponential rise and then maximum values of EC (21.0 mg/L. d) and RE (87.33 %) were achieved within 20 days. On the 21st day, with an increase in the flow rate up to 20 mL/min, the corresponding ILR was also increased to 48 mg/L. d. The RE and EC decreased initially but again started rising and achieved steady-state values of 76.12 % and 36.5 mg/L. d after the 37th day. On the 38th day, the flow rate was increased to 30 mL/min (ILR of 72 mg/L. d); again, a similar trend was observed. As shown in Figure.3. 6 and Table 3. 4, the steady-state RE continuously decreased with an increase in flow rate. Steady-state EC increased initially with a faster rate and then tending towards constant value. Maximum EC of 66.15 mg/L. d was obtained at a 60 mL/min flow rate, corresponding to RE of 45.64%. The rate-controlling mechanism plays a vital role in the performance of the bioreactor. It was observed that bioreactor performance reduced significantly at a high flow rate due to the change in the rate-controlling mechanism (Chung et al., 2003).

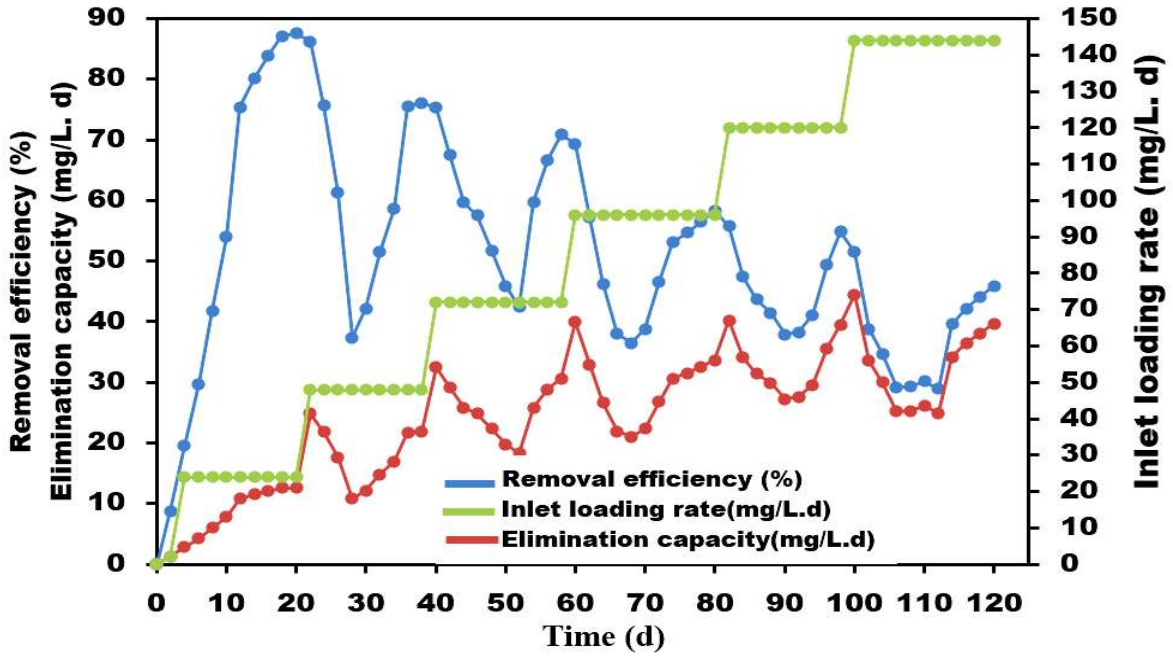

Figure.3.6: Congo red dye removal performance of a continuous AnMBBR

Table 3.4: Performance of the AnMBBR for the biodegradation of CR dye.

Process time (day)	Flow rate (mL/min)	ILR (mg/L. d)	EC (mg/L. d)	RE (%)
20	10	24	21.00	87.33
28	20	48	36.52	76.1
58	30	72	51.01	70.86
80	40	96	56.01	58.35
100	50	120	65.90	54.92
120	60	144	66.15	45.64

ILR: Inlet loading rate; EC: Elimination capacity; RE: Removal efficiency

3.3.8. FTIR analysis of CR dye before and after biodegradation

The FTIR spectrum analysis of CR dye before and after biodegradation in the AnMBBR was done using the KBr disc method to confirm the presence of functional groups and changes in the CR dye after biodegradation (Figure. 3.7). The spectrum of untreated (control) CR shows a broad peak at 3425 cm^{-1} due to stretching of O-H group, a peak at 1634 cm^{-1} due to stretching of azo group N=N, a peak at 1235 cm^{-1} due to C-N stretching of primary aromatic amines. The FTIR spectra of the treated CR dye show various peaks; a peak at 1647 cm^{-1} for N=N stretching of the azo group, a broad peak at 3434 cm^{-1} due to stretching of O-H, a peak at 1624 cm^{-1} along with another peak at 1076 cm^{-1}. The spectra analysis of the treated CR dye shows a major shift in the peaks, and the disappearance of peaks was observed at 540 cm^{-1} and 2090 cm^{-1}. The spectrum variation of the treated CR dye with the control sample observed in FTIR analysis revealed the successful biodegradation of CR dye in AnMBBR. Reduction of significant peaks FTIR analysis also confirms the effective biodegradation of CR dye

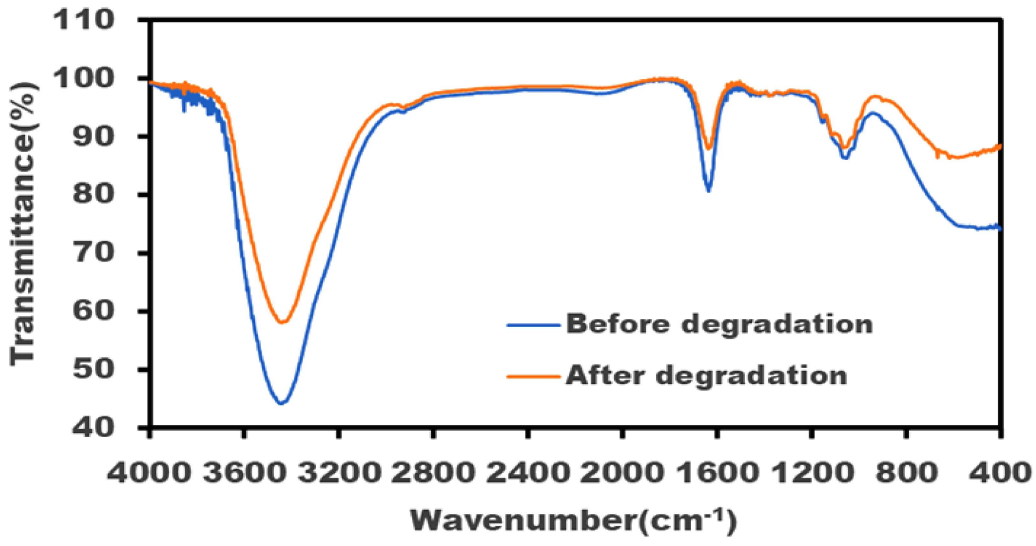

Figure. 3.7. The spectral profile (FTIR analysis) is shown in the graph before and after the degradation sample of CR in AnMBBR.

3.4. Conclusions

The present study successfully demonstrated the biodegradation of CR dye in the AnMBBR. It was evident that mathematical tools like RSM can be used to optimize the process parameters using very few experiments compared to the conventional optimization method. The maximum CR dye degradation was 87.33 % at optimum process conditions. The MSK model was successfully employed to calculate kinetic parameters: maximum CR removal rate (U_{max} of 0.21 g/L. d) and saturation constant (K_B of 0.23 g/L.d). The value of kinetic parameters indicates that the potential of AnMBBR is still underutilized, and there is further scope for improvement in the performance of AnMBBR