Chapter 5

Biodegradation of Congo red dye using polyurethane foam-based biocarrier combined with activated carbon and sodium alginate: Batch and continuous study

5.1. Introduction

More than 10^5 tons per year of azo dyes are generated worldwide due to increased demand and consumption of synthetic azo dyes in various industries (Maurya et al., 2021). The textile industries utilize more than 70% of azo dye in dying processes (Sudha et al., 2014). Moreover, the untreated wastewater released from these industries (dye and pigments) contains 10 to 25 % of synthetic dyes, which subsequently causes environmental pollution (Karim et al., 2018). Dye-containing wastewater decreases dissolved oxygen (DO) and sun-light infiltration of water, adversely affecting aquatic lives (Ong et al., 2015; Sonwani et al., 2020). Congo red (CR), a diazo dye, is broadly used in the textile, printing, and food processing industries (Gupta et al., 2013; Modak et al., 2016). It consists of sodium salt of 3,3′-([1,1′-biphenyl]-4,4′-diyl) bis (4-amino naphthalene-1-sulfonic acid) (Kofie et al. 2014). Azo bonds associated with the aromatic structure make the CR dye recalcitrant and persistent. The carcinogenic and mutagenic properties of CR dye raise concerns for researchers to develop an economical technique for its degradation. Physio-chemical (adsorption, membrane separation, ion exchange, etc.), advanced oxidation (UV/H_2O_2, Fenton, Ozonation, etc.,), and biological processes have been extensively studied for the degradation of azo dyes (Ahlawat et al., 2020; Srinivasan and Viraraghavan, 2010). Among these, various researchers consider the biological treatment of azo dyes as a cost-effective and environmentally friendly route (Popli and Patel, 2015; Sonwani et al., 2021; Tee et al., 2015). This technique utilizes microorganisms (bacteria, fungi, and algae) to degrade various pollutants. Biological treatment

has many advantages, including low operating cost, no or less toxic product generation, and low energy cost (Khan et al., 2021; Sudha et al., 2014; Zubair et al., 2018).

Several bacterial species, including *Pseudomonas* sp., *Bacillus* sp., *Shewanella oneidensis* sp., *Aeromonas* sp., *Aeromonas hydrophila, Lysinibacillus* sp., and *Alcaligenes faecalis* have been used for dye degradation (Gopinath et al., 2009; Talha et al., 2018; Zheng et al., 2017). Previously, Sari and Simarani (2019) have reported the high removal ability of *Lysinibacillus fusiformis* W1B6 during the treatment of methyl red dye. Similarly, the effectiveness of *Lysinibacillus* sp. has been studied for different azo dyes (Masarbo et al., 2019; Srinivasan et al., 2017). However, very few studies were reported on the efficacy of *Lysinibacillus* sp. for the biodegradation of CR dye. Moreover, the immobilized/attached cell system is considered as an effective route for the removal of organic pollutants than the free cell system (Geed et al., 2017; Swain et al., 2021). The immobilization of the microorganisms onto the solid matrix provides high removal efficiency of pollutants, chemical stability, and high biomass growth against adverse environmental conditions (Swain et al., 2020). Several attached growth bioreactors (e.g., packed bed bioreactor, moving bed biofilm reactor, fluidized bed bioreactor, trickling biofilter, and rotating biological contactor) were designed and analyzed for the biodegradation of azo dyes (Bharti et al., 2019; Vikrant et al., 2018). The bio-affinity, morphology, and chemical nature of the biocarrier play a significant role in the biodegradation process. Therefore, great attention has been given to the development of low-cost, porous, and durable biocarriers in the recent past.

In this direction, several carriers such as activated carbon (AC), sugarcane bagasse (SB), low-density polyethylene (LDPE), calcium alginate (CA), polyacrylamide, and polypropylene (PP) have been used to immobilize the microorganisms (Ong et al., 2015; Sonwani et al., 2019). It has been reported that polyurethane foam (PUF) is a superior-quality carrier for cell immobilization

due to its excellent chemical resistance, high porosity, and stability (Majul et al., 2020; Partovinia and Rasekh, 2018). The PUF offered a large specific surface area for the attachment of the microorganisms, which enhanced the Acid orange 7 dye biodegradation in a PBBR (Swain et al., 2021). Further, AC, a high carbon content residue prepared through pyrolysis using biomass, has excellent adsorption capacity and high specific surface area (Shaheen et al., 2018; Wang et al., 2020; Xu et al., 2021). Ong et al. (2015) performed the continuous biodegradation of orange II dye in a packed bed bioreactor (PBBR) packed with immobilized AC. They have reported that immobilized AC could remove the azo dye at 0.38 g/h of biodegradation rate at an initial dye concentration of 1150 mg/L. Moreover, the efficacy of the bacterial cell immobilized activated carbon in azo dye degradation was examined in previous studies (Xu et al., 2021; Zheng et al., 2017). Sodium alginate (SA) is a biocompatible and cheap biopolymer with high gelation ability, preventing biomass washout from the solid carrier (Do and Lee, 2013; Mallakpour and Behranvand, 2021). The literature review revealed that the immobilization of bacterial cells was carried out on either a single carrier or a combination of any two carriers, such as PUF, AC, and SA. However, very limited studies are available on the application of combined biocarriers (e.g., immobilized-PUF with both SA and AC) to treat azo dyes.

The present study has focused on the preparation of a composite bio carrier made up of *Lysinibacillus* sp. immobilized-PUF combined with AC and SA. The objectives of this study are as follows: (i) to develop a novel biocarrier composed of polyurethane foam (PUF), activated carbon (AC), and sodium alginate (SA); (ii) to optimize the process variable, namely process time, glucose concentration, and dye concentration; (iii) to investigate the performance of continuous PBBR for CR dye degradation; and (iv) to evaluate the growth inhibition kinetics of *Lysinibacillus fusiformis* by Monod and Andrew-Haldane models.

5.2. Material and methods

5.2.1. Minimal salt medium and bacterial culture

The Congo red (CR) dye (CAS number 573-58-0) and sodium alginate (SA) (CAS number 9005-38-3) were bought from Sigma Aldrich, India. The activated carbon (AC) was brought from Research Lab Fine Chemical Ind., India. The chemicals were used for minimal salt medium (MSM) were purchased from Merck, India. The MSM of the following compositions (g/L): K_2HPO_4 (1.5); KH_2PO_4 (2.8); $CaCl_2$ (0.05); $MnSO_4 \cdot H_2O$ (0.28); $MgSO_4$ (0.42); peptone (1.2) was prepared in distilled water. The wastewater was prepared with CR dye mixed in MSM.

The azo dye polluted (i.e., contaminated) soil samples were collected in a sterile polyethene bag from a local textile industry at Bhadohi, Varanasi, Utter Pradesh, India and stored at 4 °C. The acclimatization of the microorganisms was carried out as per the procedure described by Maurya et al. (2021). In detail, 10 g of soil sample was taken in a flask containing 100 mL of MSM containing 30 mg/L of CR dye. The flask was incubated at 32 °C and 100 rpm till more than 80 % CR dye removal was achieved. Then, 25 mL of supernatant was transferred to a new flask containing 60 mg/L of CR dye. Now, the flask was incubated under similar operating conditions. The acclimatization procedure was repeated four times more by increasing the CR dye concentration by 30 mg/L in each stage. Finally, the potential dye degrading microorganisms were isolated using the serial dilution technique, and then, the bacterial inoculum was stored in nutrient broth at 4 °C for further experimental study.

5.2.2. Description of the modified bio-carrier

The modified bio carrier was composed of bacterial immobilized-PUF, AC, and SA. Two types of bio carriers, namely PUF_1 (immobilized-PUF combined with AC, SA) and PUF_2 (immobilized-PUF combined with SA), were prepared and used in this study. The PUF was bought from the

resident market near IIT BHU Varanasi, Utter Pradesh. Initially, the PUF was cut into the cubic size of 1.0 cm^3 and washed with distilled water. The modified biocarriers were prepared using the following procedure (i) Initially, the PUF pieces were placed in a beaker containing distilled water and AC (4.0 g/L) followed by stirring for 12 h at 30 °C to load the AC on PUF; (ii) the loaded sponge was injected with the mixed liquor consisting of SA (4%, m:v) and the bacterial culture (2.0 %, m:v) in 1:2 ratio until the PUF was saturated; (iii) it kept into a calcium chloride (2.0 %) solution for crosslinking with SA. Finally, the modified biocarriers were dried at room temperature and stored for further use in PBBR.

5.2.3. Packed bed bioreactor setup and operation

Two identical cylindrical bioreactors ($PBBR_1$ and $PBBR_2$) were fabricated of borosilicate glass (60 cm in height and 5.5 cm in diameter). The total and working volumes of the reactor were 1.425 and 1.0 L, respectively. Three sampler ports were provided in each bioreactor for inlet, outlet, and drainage purposes. The $PBBR_1$ and $PBBR_2$ were filled up with PUF_1 and PUF_2 biocarriers, respectively (Figure.5.1). Peristaltic pumps (Watson Marlow 323E/D) were used to feed the wastewater into the PBBRs. The air compressor supplied air to the bioreactors to maintain aerobic conditions. The pH and dissolved oxygen (DO) of the wastewater were maintained to be 7.0 ± 0.2 and 4 ± 0.5 mg/L, respectively. All the experiments were conducted at room temperature (32 ± 3.0 °C). The process variables such as CR dye concentration (50 - 300 mg/L), time (1.0 -7.0 days), and glucose concentration (1.0 - 3.0 g/L) were optimized in PBBRs.

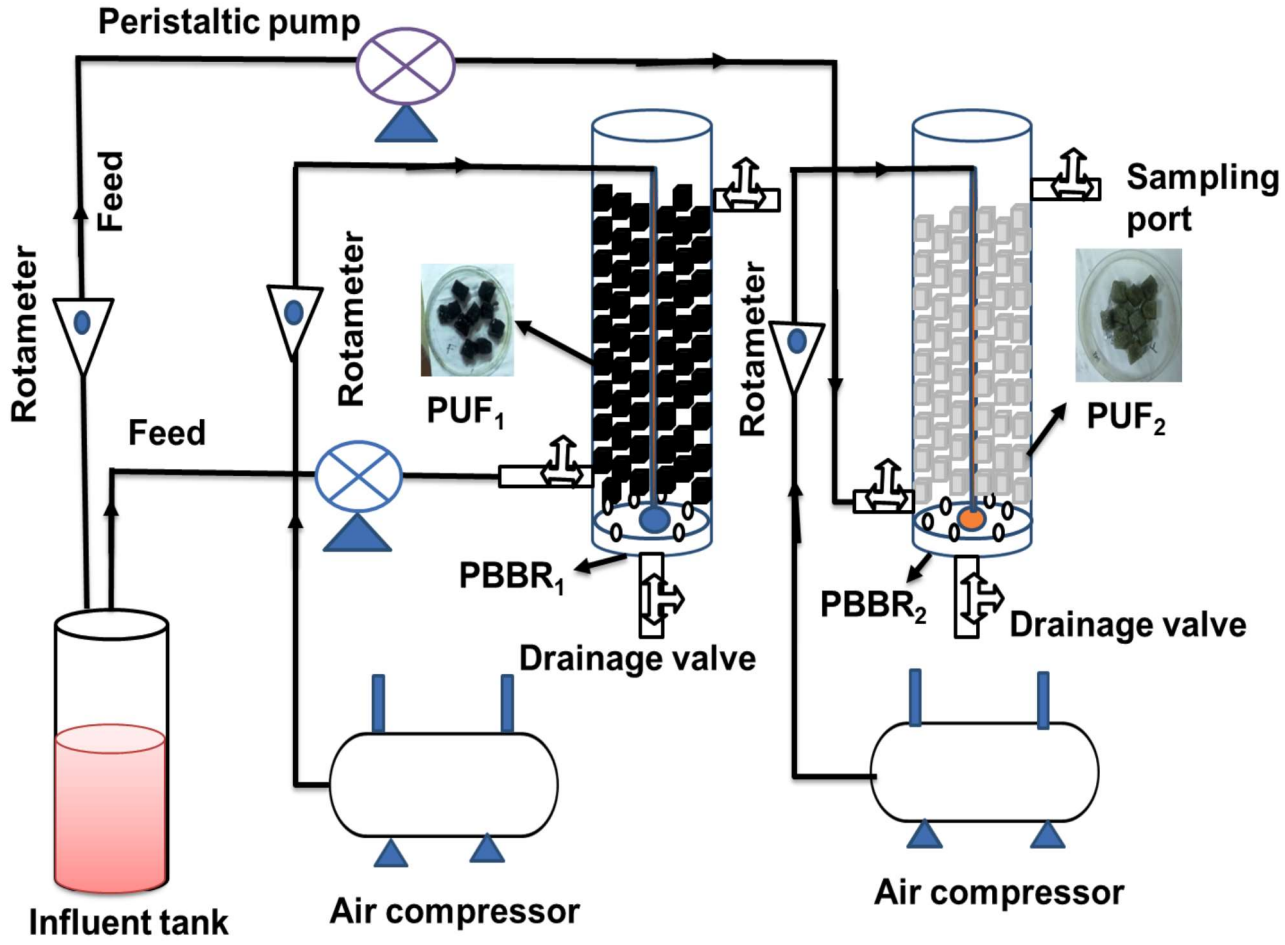

Figure. 5.1. A schematic representation of PBBRs used for Congo red dye biodegradation

5.2.4. Continuous study for dye biodegradation in packed bed bioreactor

After optimizing process parameters, the PBBRs were switched from batch to continuous mode by varying the inlet flow rate from 10 to 60 mL/h. The performance of the PBBRs was examined by calculating the removal efficiency (RE), elimination capacity (EC), and inlet loading rate (ILR) by using the following expressions:

$$\text{Removal efficiency (RE) (\%)} = \frac{F_{in}-F_{out}}{F_{in}} \times 100 \quad (5.1)$$

$$\text{Elimination capacity (EC) (mg/L.d)} = \frac{(F_{in}-F_{out}) \times Q}{V} \quad (5.2)$$

$$\text{Inlet loading rate (ILR) (mg/L.d)} = \frac{F_{in} \times Q}{V} \quad (5.3)$$

where F_{in} and F_{out} denote the inlet and outlet concentrations of CR dye (mg/L), respectively. In addition, Q denotes the feed flow rate (mL/h), and V denotes the working volume of the bioreactor (L).

5.2.5. Microbial growth kinetics

The presence of toxic compounds adversely affects the production of nucleic and organic acids, which causes inhibition of bacterial growth (Iyyappan et al., 2019). Moreover, bacterial growth depends upon substrate (CR dye) concentration and the utilization rate. Therefore, it is essential to estimate substrate inhibition, which may adversely affect the performance of the overall treatment system. In this direction, several mathematical kinetics, namely Monod and Andrew-Haldane models, were used in this study.

5.2.5.1. Monod model

The Monod model is well known to predict the growth kinetics of the microorganisms under substrate non-inhibition conditions, which can be expressed (Geed et al., 2017):

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{5.4}$$

where μ and μ_{max} represent the specific growth rate (h^{-1}) and the maximum specific growth rate of the bacterial species (h^{-1}). In addition, K_s and S represent the half-saturation constant (mg/L) and the limiting substrate concentration (mg/L), respectively.

This model is valid under the assumption that the specific growth of the microbial species remains intact irrespective of substrate concentration. Therefore, the bacterial growth should linearly increase with the substrate concentration.

5.2.5.2. Andrew-Haldane model

The Andrew-Haldane model can be applied to predict the growth kinetics of the bacterial species under substrate inhibition conditions (Swain et al., 2021). The correlation between the substrate concentration and the specific growth can be written as:

$$\mu = \mu_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}} \tag{5.5}$$

Where K_i represents the substrate inhibition constant (mg/L).

5.2.6. Analytical techniques

The UV–vis spectrophotometer (ELICO SL-2012) was used to determine the CR dye concentration. Before the analysis, the treated samples were centrifuged at 5000 rpm for 12 min, and the supernatant samples were used to measure the residual CR dye concentration. The

functional group analysis of the treated and untreated wastewater was carried out by using FTIR analysis (Nicolet iS5, THERMO Electron Scientific Instruments LLC, USA).

5.2.7. Statistical analysis

Each experiment was carried out in triplicates, and the response was presented as mean ± standard deviation. The ANOVA analysis was employed to estimate the statistical significance of the data.

5.3. Results and discussion

5.3.1. Identification and characterization of bacterial species

The isolated bacterial sample was sent to Triyat Scientific, Nagpur, India, for identification and characterization. The PCR (polymerase Chain Reaction) method was used for the amplification of genomic DNA by using forward (27F-5'AGAGTTTGATCTGGCTCAG3') and reverse primers (1492R5'TACGGTACCTTGTTACGACTT3'). Single-pass sequencing was performed using an ABI 3730xl sequencer. The obtained sequence of the bacterial species was blasted using the NCBI blast similarity search tool. The phylogeny analysis of the query sequence with the closely related sequence of blast results was performed, followed by multiple sequence alignment. MEGA X software was utilized to construct the phylogenetic tree of the bacterial sp. (*Lysinibacillus fusiformis* KLM1) (Figure.5.2).

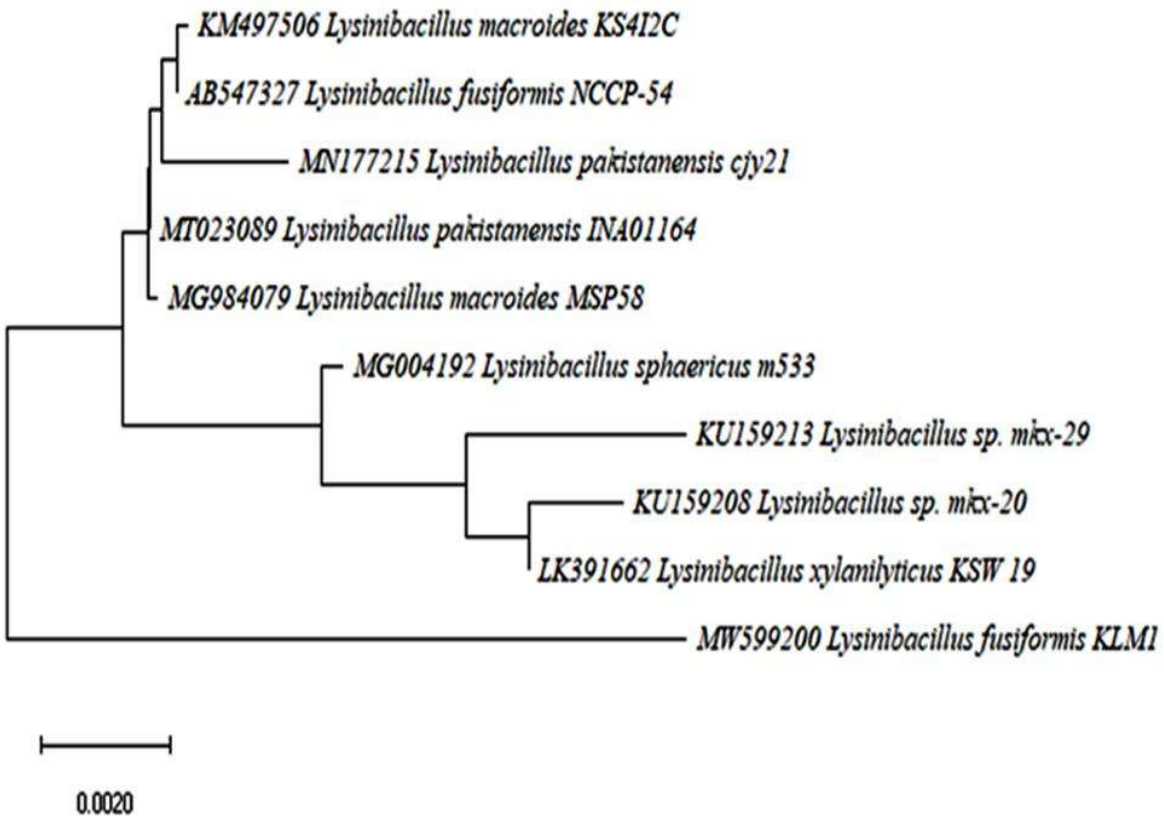

Figure. 5.2. Phylogenetic tree of isolated bacterial species *Lysinibacillus fusiformis* KLM1 (MW599200) by using the Neighbour-Joining method.

5.3.2. Process parameters optimization

5.3.2.1. The effect of process time

The process time plays a key role in biodegradation as the bacterial cells require a specific time period to utilise carbon sources. The effect of process time (1.0-7.0 days) on the removal efficiency (RE) of CR dye was evaluated by keeping the initial dye and glucose concentrations at 50 mg/L and 2.0 g/L, respectively. The RE was observed to be improved with the increase of process time. The REs of 91.21 % and 82.42 % were achieved by PUF_1 and PUF_2 biocarriers, respectively, within 4.0 days of operation (Table 5.1). However, no significant improvement in RE was observed with a further increase in process time. This was due to the low dye concentration gradient in the medium, for which RE remained almost constant after 4.0 days. Therefore, the optimum process time of 4.0 days was taken for further analysis. The maximum REs were measured to be 93.84 and 84.85 % for PUF_1 and PUF_2, respectively, on the 7^{th} day of operation.

Table 5.1. Effect of process time on biodegradation of CR dye (glucose concentration = 2.0 g/L and CR dye concentration = 50 mg/L).

	Process time(d)	**RE_1 (%)**	**RE_2 (%)**	**RE_3 (%)**	**Mean RE (%)**
	1	35.23	37.84	41.71	38.26 ± 3.26
	2	60.54	60.86	65.05	62.15 ± 2.51
$PBBR_1$	3	80.65	83.12	87.12	83.63 ± 3.26
	4	94.65	90.63	88.35	91.21 ± 3.18
	5	93.56	88.45	94.71	92.24 ± 3.33
	6	93.43	90.45	96.56	93.48 ± 3.05
	7	95.45	90.47	95.6	93.84 ± 2.91
	1	29.24	28.32	34.66	30.74 ± 3.4
	2	54.45	52.43	48.55	51.81 ± 2.99
	3	74.46	73.98	68.85	72.43 ± 3.10
$PBBR_2$	4	83.76	78.78	84.72	82.42 ± 3.18
	5	85.64	80.56	84.42	83.54 ± 2.65
	6	84.75	81.76	87.32	84.61 ± 2.78
	7	85.35	82.38	86.82	84.85 ± 2.26

RE: Removal efficiency

5.3.2.2. Effect of glucose concentration

The effect of glucose concentration (1.0-3.0 g/L) on the RE of CR dye was studied at an initial CR dye of 50 mg/L and an optimum process time of 4.0 days (Table 5.2). It was found that the addition of the glucose enhanced the RE, and the maximum REs were obtained to be 88.53 % and 80.25 % for PUF_1 and PUF_2, respectively (at 2.0 g/L of glucose concentration). The presence of glucose enhanced the RE as it acted as a supplementary carbon source for bacterial species, which helped degrade the complex dye molecule (Khan et al., 2021). However, no further enhancement of RE was observed with the increased glucose concentration after 2.0 g/L. The PUF_1 showed that the REs of 89.61% and 90.74% were obtained at glucose concentrations of 2.5 and 3.0 g/L, respectively. Similarly, PUF_2 could remove 81.37% and 82.54 % of CR dye at similar operating conditions.

Table 5.2. Effect of glucose concentration on biodegradation of CR dye (process time = 4.0 days and CR dye concentration = 50 mg/L).

	Glucose concentration (g/L)	RE_1 (%)	RE_2 (%)	RE_3 (%)	Mean RE (%)
$PBBR_1$	1	41.54	40.23	39.01	40.26 ± 1.27
	1.5	63.46	61.26	61.73	62.15 ± 1.16
	2	89.12	86.32	90.15	88.53 ± 1.98
	2.5	91.31	88.35	89.17	89.61 ± 1.53
	3	91.46	88.76	92	90.74 ± 1.74
$PBBR_2$	1	33.87	31.37	33.25	32.83 ± 1.30
	1.5	52.48	49.38	52.52	51.46 ± 1.80
	2	81.32	78.68	80.75	80.25 ± 1.39
	2.5	82.34	80.12	81.65	81.37 ± 1.14
	3	83.96	80.28	83.38	82.54 ± 1.98

RE: Removal efficiency

5.3.2.3. Effect of dye concentration

The effect of initial dye concentration (50-300 mg/L) on dye RE was analyzed at the optimum process time (4.0 days) and glucose concentration (2.0 g/L). The maximum REs were obtained to be 92.63% and 81.27% for PUF_1 and PUF_2, respectively, at 50 mg/L of initial dye concentration (Table 5.3). The RE decreased with a further increase of dye concentration due to the substrate

inhibition effect. The REs of 81.1%, 74.63%, 67.21%, and 63.24% were obtained at initial dye concentrations of 100, 150, 200, and 250 mg/L, respectively, for PUF_1 biocarrier. Similarly, 74.53, 66.46%, 61.58%, and 58.41% of REs were found for PUF_2 biocarrier under identical operating conditions. The lowest REs of 61.32% and 54.68% were observed for PUF_1 and PUF_2, respectively, at an initial dye concentration of 300 mg/L. Better RE was observed in PUF_1 compared to PUF_2 due to the activated carbon and sodium alginate bonding on PUF.

Table 5.3. Effect of initial CR dye concentration on the biodegradation of CR dye (process time = 4.0 days and glucose concentration = 2.0 g/L).

	CR dye Concentration (mg/L)	**RE_1 (%)**	**RE_2 (%)**	**RE_3 (%)**	**Mean RE (%)**
	50	92.34	90.29	95.26	92.63 ± 2.50
	100	78.54	80.46	84.45	81.15 ± 3.01
	150	76.45	75.23	72.21	74.63 ± 2.18
$PBBR_1$	200	69.78	66.43	65.42	67.21 ± 2.28
	250	64.34	65.32	60.06	63.24 ± 2.80
	300	63.23	62.24	58.49	61.32 ± 2.50
	50	82.34	81.54	79.93	81.27 ± 1.23
	100	73.76	72.34	77.49	74.53 ± 2.66
$PBBR_2$	150	67.65	68.43	63.3	66.46 ± 2.76
	200	60.23	63.42	61.09	61.58 ± 1.65
	250	59.26	60.22	55.75	58.41 ± 2.35
	300	55.37	56.35	52.32	54.68 ± 2.10

5.3.3. Performance evaluation of the continuous packed bed bioreactors

Two identical PBBRs ($PBBR_1$ and $PBBR_2$) packed with modified biocarriers (PUF_1 and PUF_2) were operated continuously at the optimized glucose (2.0 g/L) and CR dye concentration (50 mg/L) by varying the influent flow rate from 10 to 60 mL/h. Various parameters, i.e., removal efficiency (RE), inlet loading rate (ILR), and elimination capacity (EC), were measured at each flow rate, and overall performance is summarized in Table 5.4. Initially, the bioreactors were operated at a flow rate of 10 mL/h and ILR of 12 mg/L. d. The maximum REs were obtained to be 90.73% and 80.37% in $PBBR_1$ and $PBBR_2$, respectively, on the 12^{th} day of operation (Figure.5.3). The corresponding ECs were measured to be 10.89 and 9.64 mg/L. d. The flow rate was changed from 10 to 20 mL/h with an ILR of 12 to 24 mg/L.d, respectively, on the 13^{th} day of operation. A decrease in RE was observed in both the bioreactors. However, the bioreactor became stable, and the maximum REs of 89.73 and 77.33% were attained on the 22^{nd} day of operation. The corresponding ECs were evaluated to be 21.54 and 18.56 mg/L. d. The reduction of RE at a higher feed flow rate was due to the decrease in the contact time period between the immobilized cell and the substrate. The REs were further dropped to 73.21 and 60.37% when the flow rate was increased from 20 to 30 mL/h on the 23^{rd} day of operation. Then the REs were improved, and the bioreactors attained a maximum of 85.73% and 73.73% of RE in $PBBR_1$ and $PBBR_2$, respectively. However, the ECs were improved with flow rate due to the more substrate availability in the bioreactor. The corresponding ECs were found to be 30.86 and 26.54 mg/L. d. Similarly, the REs were drastically reduced with an increase in the inlet flow rate due to the increase in the loading rate. On the 50^{th} day of operation, the maximum REs of 79.53% and 65.53% were obtained for $PBBR_1$ and $PBBR_2$, respectively, for the influent flow rate of 40 mL/h. However, with a change of flow rate up to 60

mL/h, the efficacy of the bioreactors was suddenly reduced on the 68th day of operation. Then it recovered and achieved REs of 60.07 and 51.27%. Similarly, the corresponding ECs were found to be 43.25 and 36.91 mg/L.d for $PBBR_1$ and $PBBR_2$, respectively.

Table 5.4. Effect of the flow rate and inlet loading rate on removal efficiency and elimination capacity during biodegradation of CR dye in packed bed bioreactor (PBBR).

Flow rate (mL/h)	**Days of operation (days)**	**ILR (mg/L. d)**	**$PBBR_1$**		**$PBBR_2$**	
			RE (%)	**EC (mg/L. d)**	**RE (%)**	**EC (mg/L. d)**
10	0-12	12	90.73	10.89	80.37	9.64
20	13-22	24	89.73	21.53	77.33	18.56
30	23-38	36	85.73	30.86	73.73	26.54
40	39-50	48	79.53	38.17	65.53	31.46
50	51-67	60	66.88	40.128	60.9	36.54
60	68-88	72	60.07	43.24	51.27	36.91

EC: Elimination Capacity; RE: Removal Efficiency; ILR: Inlet loading rate

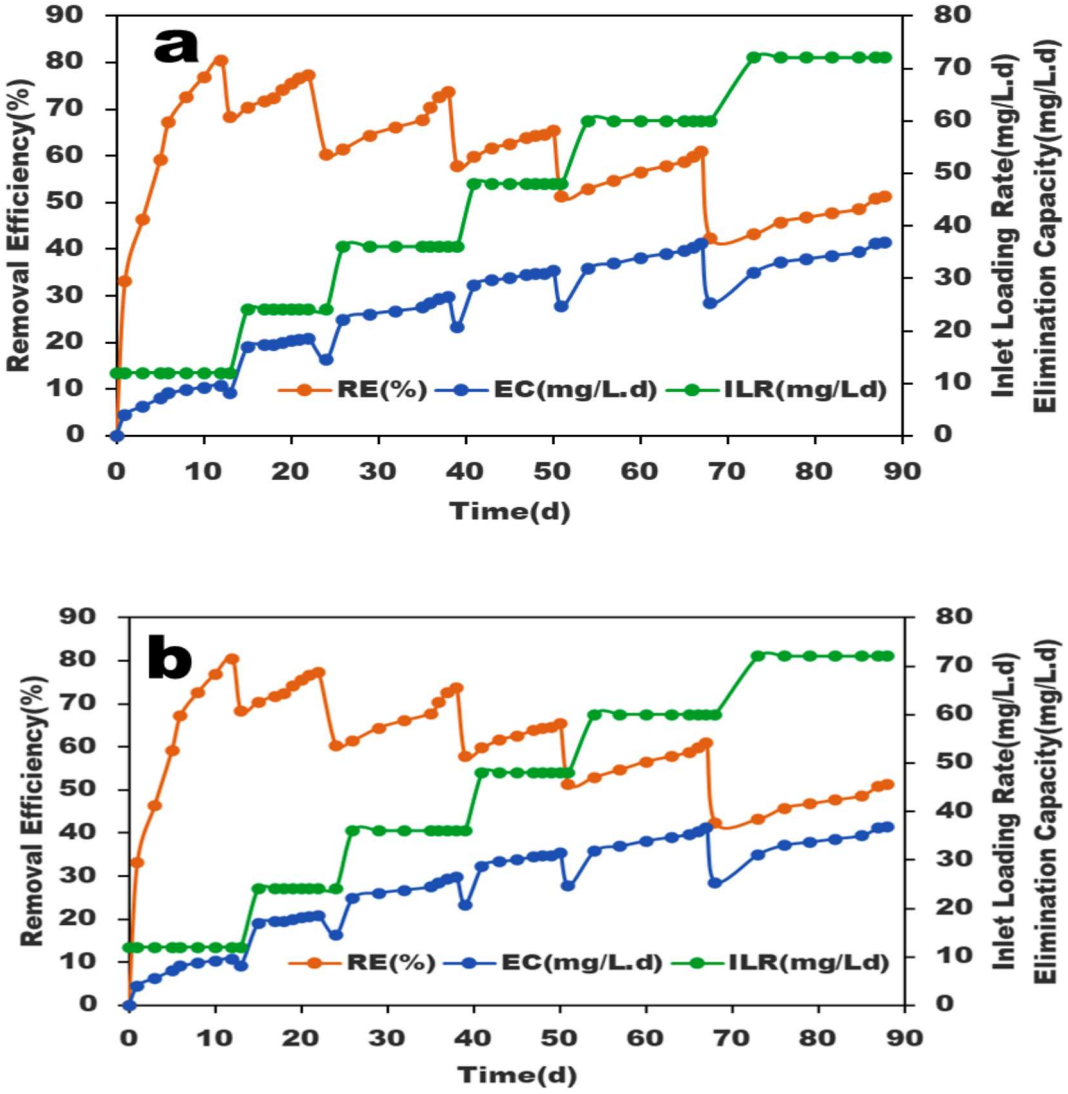

Figure.5.3. Performance of a continuous PBBR for (a) $PBBR_1$ (b) $PBBR_2$ showing the effect of flow rate and inlet loading rate on removal efficiency and elimination capacity.

Mohanty and Kumar (2021) investigated the efficacy of the PBBR for the biodegradation of Indanthrene Blue RS dye using immobilized biochar. They reported that RE had an inverse relation with the feed flow rate due to the washout of the dye molecule from the bioreactor without proper diffusion. Therefore, a suitable retention time is required for the surface diffusion of the pollutants and complete degradation by microorganisms (Swain et al., 2021; Yadav et al., 2014). Moreover, the present study revealed that $PBBR_1$ showed higher RE than $PBBR_2$ under identical conditions. The loading of AC in PUF_1 biocarrier provided a more specific surface area for developing active biofilm and subsequently achieved high degradation of CR dye.

5.3.4. Growth kinetic study

The growth kinetics of *Lysinibacillus fusiformis* KLM1 were studied at a wide range of CR dye concentrations (50-300 mg/L). The high regression coefficient (R^2 = 0.98) revealed that the Andrew-Haldane model accurately predicted the bacterial growth rate (Figure.5.4). The specific growth rate of the bacterial species was found to be decreased after 50 mg/L of CR dye concentration. The kinetic growth parameters such as maximum specific growth rate (μ_{max}), substrate inhibition constant (K_i), and half-saturation constant (K_s), were evaluated to be 0.295 h^{-1}, 50.32 mg/L, and 10.54 mg/L, respectively, by the Haldane-Andrew model. Similarly, the values of μ_{max} and K_s were evaluated as 0.194 h^{-1} and 97 mg/L, respectively, for the Monod model. The lower value of K_s and the high value of μ_{max} are always favorable in biodegradation (Yadav et al., 2014).

Yao et al. (2011) investigated the growth kinetics of *Lysinibacillus cresolivorans* during the biodegradation of m-cresol and found that the bacterial growth was inhibited with an increase of substrate (m-cresol) concentration. The values of μ_{max}, K_s, and K_i were evaluated to be 0.1875 h^{-1} 49.53 mg/L, and 133.32 mg/L, respectively, for *Bacillus* sp. during the treatment of Acid orange 7 dye (Sonwani et al., 2021) similarly, the growth kinetic behavior of *Bacillus* sp. S4 was investigated during the biodegradation of malathion (Geed et al., 2017). The maximum specific growth rate (μ_{max}) of the bacterial species was found to be 0.013 h^{-1}. In addition, bacterial growth inhibition (K_i) occurred at a substrate concentration of 594.75 mg/L. The comparative study of kinetic parameters obtained with other literature could not be possible due to the variation of bacterial species, carbon source, and operating conditions.

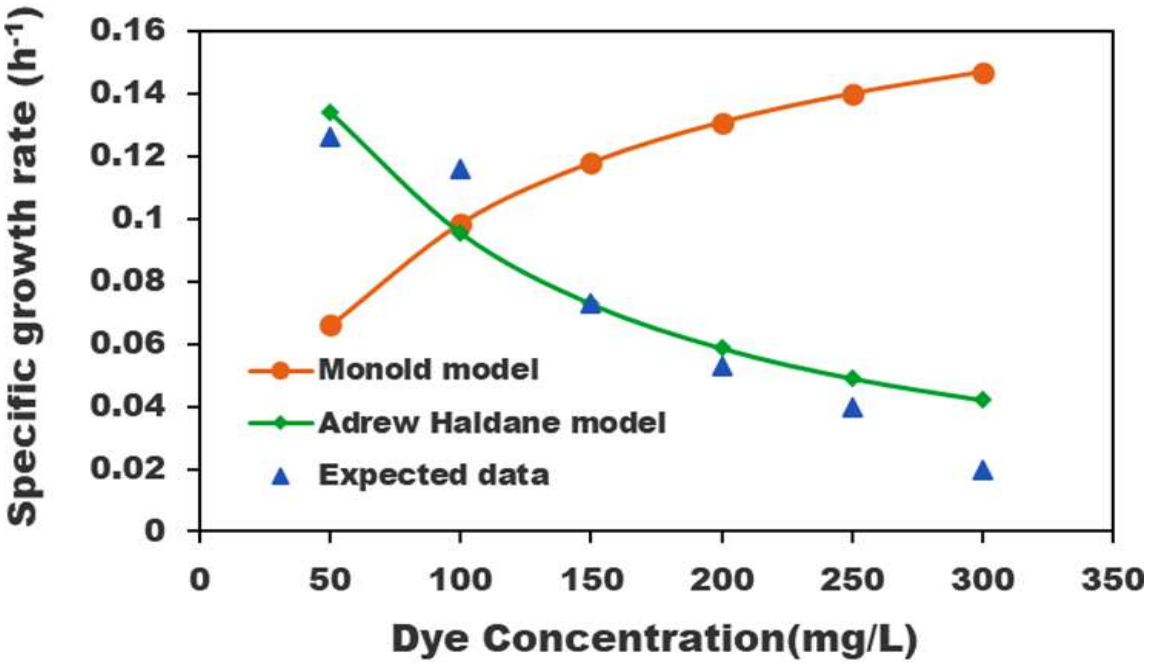

Figure. 5.4. Graph plotted between specific growth rates against dye concentrations using Monod and Andrew-Haldane models.

5.3.5. FTIR analysis

The FTIR analysis of CR dye (untreated and treated) samples was carried out to detect the changes in functional groups after degradation. The untreated CR dye sample spectrum showed different wave numbers, including a wide-range peak at 3468.83 cm^{-1} due to the stretching bond of the O-H group, a peak at 1616.06 cm^{-1} due to stretching of azo group N=N, a peak at 1051.98 cm^{-1} due to stretching S-O group. The FTIR spectra of the treated sample showed different peaks at 3440.39 cm^{-1} and 3446.65 cm^{-1} due to O-H group stretching, a peak at 1630.04 cm^{-1} and 1624.25 cm^{-1} due to stretching bending of the azo group N=N, peak at 1045.71cm^{-1} and 1051.98 cm^{-1} due to stretching S-O group shown in figure.5.5.

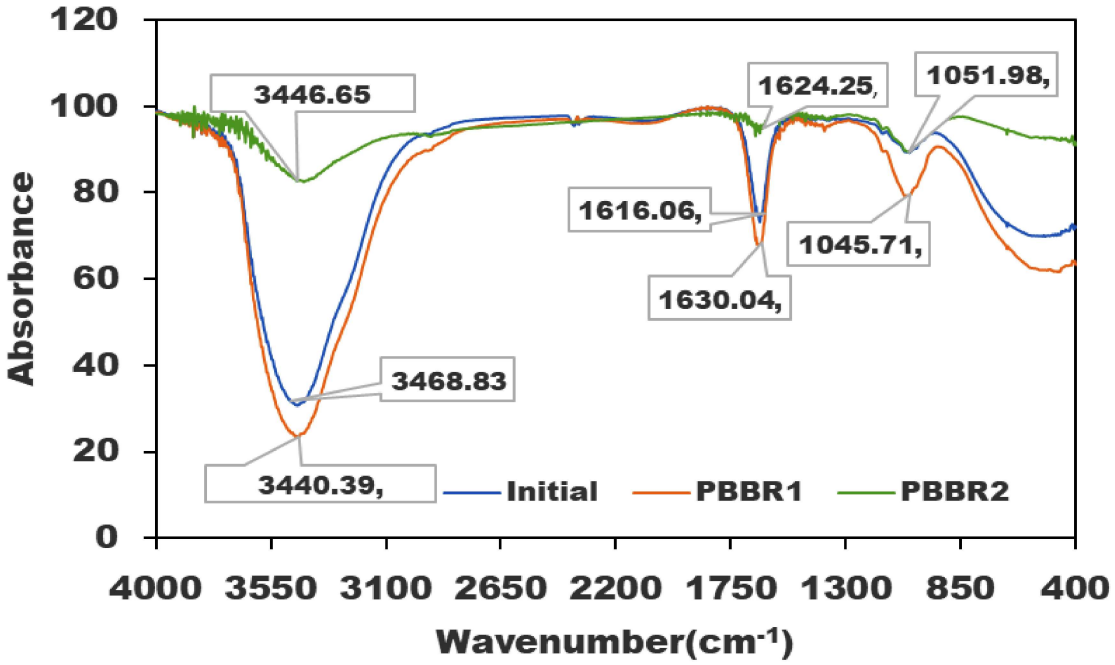

Figure. 5.5. The FTIR analysis of congo red dye intial and final (after dye degradation) sample for $PBBR_1$ and $PBBR_2$

5.3.6. Phytotoxicity

Untreated textile wastewater disposal into water bodies carries significant environmental and health problems. Determining the dye's phytotoxicity before and after degradation was, therefore extremely important. The mean root and shoot length of the vigna radiata plants were 1.0 ± 0.21and 1.32 ± 0.10 cm, respectively, after treatment with untreated congo red dye, while the mean root and shoot length were 4.41 ± 0.15 and 5.82 ± 0.23 cm after treatment with the treated effluent during 2 weeks, respectively. As a positive control, the plant that received distilled water displayed 14-day root and shoot lengths of 5.91 ± 0.12 and 6.23 ± 0.16cm, respectively shown in figure 5.6 and table.5.5. It is observed that after biodegradation sample plants have better growth compared to the initial sample of Congo red dye and seed germination percentage is greater (treated sample) compared to the influent sample.

Table 5.5: Summary of phytotoxic analysis of Vigna radiata seeds in distilled water, initial sample, and after degradation CR dye contaminated wastewater

Parameters	Initial Sample	After degradation sample	Distilled water
Germination (%)	73	86	93
Shoot length (cm)	1.32 ± 0.10	5.82 ± 0.23	6.23 ± 0.16
Root length (cm)	1.0 ± 0.21	4.41 ± .15	5.91 ± 0.12

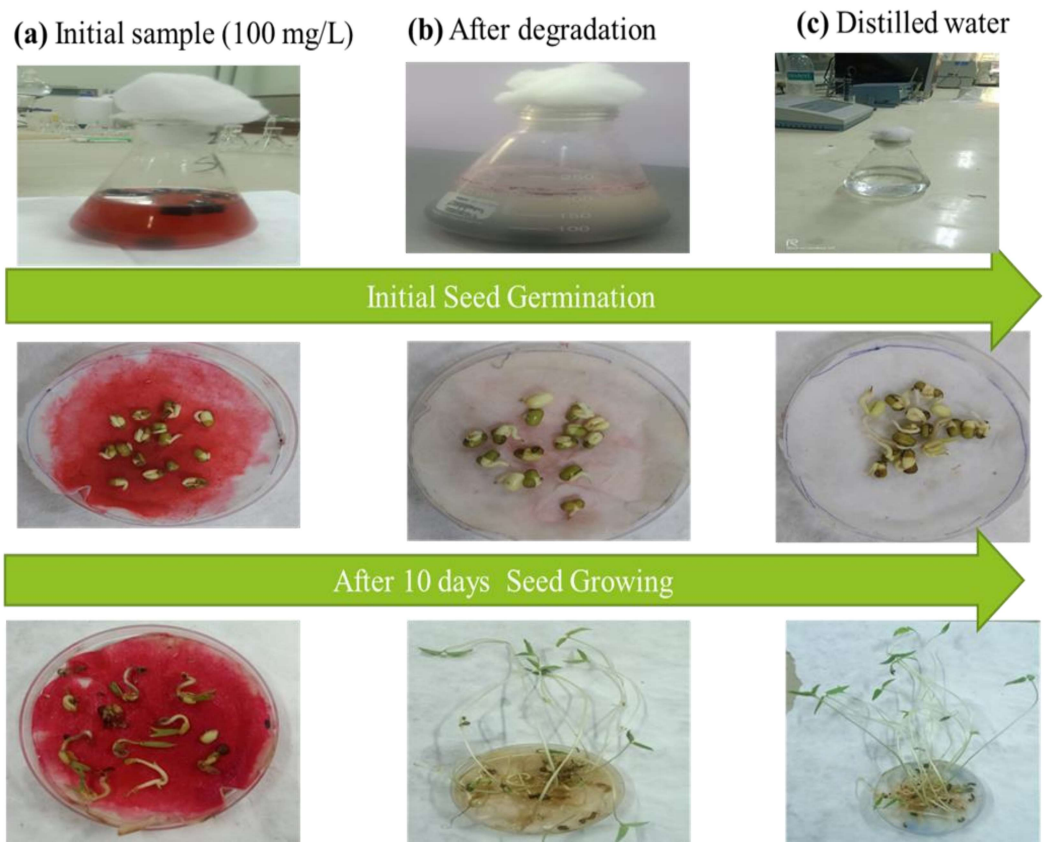

Figure. 5.6: Phytotoxic analysis of *Vigna radiata* seeds in (a) initial sample, (b) after degradation of CR dye contaminated wastewater and distilled water.

5.4. Conclusion

The optimization study suggested that the process variables (i.e., dye concentration, process time, and glucose concentration) significantly affect the performance of bioreactors. The $PBBR_1$ filled with PUF_1 (PUF combined with AC, SA, and bacterial sp.) showed higher performance than $PBBR_2$ (filled with immobilized PUF combined with SA). The $PBBR_1$ achieved the maximum CR dye RE of 90.73 %. However, the external mass diffusion across the biocarrier, the metabolic activity of the bacterial species under different conditions, and internal substrate transport phenomena control the biodegradation rate, which could be further analysed to enhance the present treatment method. The phytotoxicity analysis show the significant improvement in plant growth after treatment of the sample.