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Decolorization of Congo red dye in a continuously operated rotating biological contactor reactor

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ABSTRACT

A continuous study in a rotating biological contactor (RBC) reactor was carried out using polyurethane foam (PU) surface-immobilized with live fungal biomass of *Neurospora crassa* with wheat bran adsorbent/substrate for the removal of color from synthetic Congo red (CR) dye wastewater. The experiments were conducted at 303 K, pH 6, to study the effect of various operational parameters on the CR dye decolorization process. Experimental data confirmed that the amount of biomass produced and % color removal of CR increased with the increase in the number of discs, disc rotation speed, % disc submergence in the liquid medium, wheat bran dosage, and air flow rate. The maximum color removal of 90.15% was obtained using 50 mg L^{-1} inlet dyestuff concentration, 20 discs, 16 rpm, 40% disc submergence, 1.5 L min^{-1} air flow rate, 1 mL min^{-1} dye solution flow rate and 12.5 g L^{-1} wheat bran dosage. The activities of various extracellular enzymes of the fungus were measured during the decolorization of synthetic dye wastewater. The results reveal that the cellulase enzyme has a significant role in the decolorization process with its maximum activity and chemical oxygen demand removal being 1,284 U L^{-1} and 93.34%, respectively at the end of 240 h.

Keywords: Wastewater treatment; Color removal; Congo red dye; Polyurethane foam; Enzyme activity

1. Introduction

Nowadays, one of the key ecological issues confronting mankind is the ever-increasing pollution of freshwater systems with many modern synthetic organic compounds. The intensive development of industry has been accompanied by a decrease in environmental quality [1]. Environmental problems associated with the production and utilization of dyes have attracted considerable attention in recent days. Synthetic dyes are extensively used in textile dyeing, paper printing, plastic, and photography industries. As a result, they generate a considerable amount of colored wastewater [2]. Worldwide, the overall dye utilization of the textile industry is in excess of 10^7 kg y^{-1} and approximately 1 million kg of textile dyes are released as industrial wastewater per year [3]. On the other hand, about 10%–15% of textile dyes are lost in the dyeing process and 2%–20% are directly discharged as aqueous effluent in various ecological segments [4]. The discharge of dye wastewater from industry without adequate treatment can lead to an increase in the level of toxicity and chemical oxygen demand (COD) of the effluent [5].

Diazo dyes are considered to be highly toxic to the aquatic ecosystem and have been reported to be carcinogenic to humans. The breakdown of azo linkages present in the dye molecule forms carcinogenic products [6]. The synthetic dye Congo red is a water-soluble diazo anionic dye prepared by coupling tetra-azotized benzidine with two molecules of naphthionic acid. It is considered as highly toxic due to its

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metabolism to benzidine, a human carcinogen, and its exposure causes allergic dermatitis, skin, eye, and gastrointestinal irritation [7]. Even a low concentration of CR dye can cause various harmful effects such as difficulties in breathing, diarrhea, nausea, vomiting, abdominal, and chest pain, severe headache, etc. [8]. Therefore, there is a need to remove dyes from the effluent before it is discharged into the receiving water bodies. Wastewater containing azo dyes from the textile industry are very difficult to treat using conventional treatment methods (excluding adsorption and enzymatic treatment) because dyes are structurally complex and stable aromatic organic compounds [9]. Efforts should be made to reduce the cost of the treatment and increase the removal efficiency of the process [10]. Hence, the investigation of alternative and appropriate technologies for the removal of textile dyes is of great environmental importance.

Adsorption onto activated carbon is a widely used technique for the removal of dyes in wastewater. However, in view of the high cost and associated problems of regeneration and disposal of spent carbon, there is a need for alternative adsorbents, which are low in cost, easily available, and more effective [11]. These adsorbents include plant and agricultural waste such as wheat bran [12], papaya seeds, garlic peels, sugarcane bagasse, guava leaves, and neem leaves [13]. Biosorption is a technique, which is gaining significant consideration. The use of microbial biomass especially, fungal biomass as an adsorbent decolorize dye wastewater is gaining traction [14]. The use of live cells for large scale process utilization (reactor studies) has some problems such as low biomass growth rate, enzyme reutilization, and restriction of enzyme mobility. To ameliorate these problems, the live cells can be immobilized on the surface of the bio-support materials by weak van der Waals forces [15].

The proper realization of the live fungus in real wastewater treatment system is based on the selection of a suitable reactor. Among the various reactor systems present for wastewater treatment, aerobic rotating biological contactor (RBC) reactor is the most effective in treating complex substances in the effluents [15]. In the RBC reactor, degrading microorganisms are grown by surface attachment (immobilized) onto the rotating discs of the reactor [16]. Various studies on the removal of color from dye wastewater in a RBC reactor have been reported in the literature using several fungal species. It includes *Phanerochaete chrysosporium* [16], *Coriolus versicolor* [17], *Dichomittus squalens* [18], and *Trametes versicolor* [19]. Decolorization of selected toxic dye compounds with a few types of live fungal biomass and the use of agricultural by-product as a low-cost adsorbent of dye molecules has been studied [14,20]. The literature survey indicates that color removal from dye effluents using live biomass with agricultural waste is limited. However, studies on the removal of CR dye from wastewater in RBC reactor using *Neurospora crassa* live biomass with wheat bran adsorbent/substrate is an area that has not been explored much. Experiments need to be performed to make use of fungus, grown in a simple, inexpensive medium with higher growth rates, and maximum efficiency of color removal in the effluent. To the best of our knowledge, there are no RBC reactor studies that have been reported for describing the potential of surface-immobilized *N. crassa* with wheat bran adsorbent/substrate for the removal of CR dye from aqueous solutions. The combination of live biomass with wheat bran has better decolorization efficiency in the removal of color from dye wastewater as compared to the individual substances and the removal rate is rapid with live biomass and wheat bran [21]. The effect of various parameters on removal of CR from dye wastewater in RBC reactor studies followed the already published papers. But not for the combination of CR dye and *N. crassa* live biomass with wheat bran. Studies need to be conducted to make the use of *N. crassa* live biomass with wheat bran for the removal of color from CR dye wastewater in a RBC reactor. Therefore, an effort has been made to remove color from CR dye from wastewater using live biomass with wheat bran in a RBC reactor. *N. crassa* (the common pink bread mould) is a filamentous non-pathogenic ascomycete fungus that easily grows in a nutrient (broth) medium [22,23]. Wheat bran is the outer shell of wheat grain, and an agricultural by-product of the wheat milling operation [12]; furthermore, wheat bran is an economically viable and widely available natural material in India. Therefore, the present paper focusses on an economical treatment process to remove CR color from an aqueous solution using polyurethane foam surface-immobilized live fungal biomass of *N. crassa* with wheat bran in an RBC reactor.

2. Materials and methods

2.1. Chemicals required

An anionic dye Congo red (dye content ≥35%, molecular formula = $C_{32}H_{22}N_6Na_2O_6S_2$, molecular weight = 696.66, λ_{max} = 498 nm) supplied by Sigma Aldrich, India was used in the study. The dye is of analytical reagent grade, and of 99.8% purity. The analytical grade malt extract broth and malt extract agar were obtained from Himedia, India. All other chemicals used are of analytical grade (Himedia and Merck, India Limited).

2.2. Preparation of CR dye stock solution

A stock solution of 1,000 mg L^{-1} was prepared by dissolving an accurate quantity of CR dye powder in distilled water. The detailed procedure for the evaluation of optimized values of pH and wheat bran dosage for CR dye removal is given in previous batch studies published earlier [21].

2.3. Analytical measurements

The pH of the dye solution is observed using a digital pH-meter (Systronics 335, India). A pre-calibrated double-beam UV/visible spectrophotometer (Shimadzu UV-1800, Japan) operating at a wavelength (λ_{max}) of 498 nm is used to determine the unknown residual concentration of CR dye solution.

2.4. Preparation of agricultural by-product wheat bran and N. crassa live biomass

Wheat bran was procured from M/s Ganesh Flourmill Industries, Kolkata, India. It was washed with distilled water to remove soluble impurities. The detailed procedure for the preparation of wheat bran is taken from a previous

study [21]. The fungus *N. crassa* (Microbial Type Culture Collection, MTCC 1852) used in this study was obtained from the Institute of Microbial Technology, Chandigarh, India, and was stored at 277 K. The detailed procedure for preparation of live fungal biomass is taken from a previous study [21].

2.5. Experimental setup of the RBC reactor

A laboratory-scale RBC reactor was constructed using a polymethyl methacrylate (PMMA) sheet of 10 mm thickness. The RBC reactor (tank) is 74 cm in length, 21.4 cm in diameter, and 19 cm in height. The tank was cut into two portions with the top portion acting as the lid, and the bottom portion was closed at the ends to form a reactor vessel. For sample withdrawal, two ports are provided at the bottom of the vessel with a spacing of 36 cm between the ports. The RBC reactor has 20 circular discs (14 cm diameter) equally spaced at a distance of 2 cm and it was made using a 10 mm thick Perspex sheet. The discs are covered with 5 mm thickness PU as the bio-support material and firmly attached to the aluminum rod by means of screws to enable rotation of the discs inside the vessel. The discs are mounted on a 3.5 cm diameter aluminum rod supported with bearings at both ends of the reactor through a hole at the center of the discs. A plate is kept in the middle of the tank to support the aluminum rod in the reactor. It contains small circular holes to maintain a uniform level of the medium/dye solution in the reactor. The reactor has one compartment (stage) with 20 discs. The discs are made to rotate at the desired speed inside the reactor and the aluminum rod is connected to a 0.5 HP variable speed DC motor (1–20 rpm) mounted outside the main unit, maintaining 30%–40% disc submergence in the liquid portion of the reactor. For the immobilization of fungal biomass, freshly prepared malt extract broth (MEB) medium is added to the reactor and inoculated with 2% (w/v) *N. crassa* fungal spores. The air volume is enriched with the aid of an air pump giving a continuous flow of air (1.5 L min^{-1}) to the reactor. The air is led into and out from the reactor through sterile filters (10 μm) in order to avoid contamination. The reactor is operated at an ambient temperature of 303 K under batch mode. The fresh nutrient medium was refilled every 4 d until fungal biomass was seen prominently attached onto the PU covered discs (thick layer of fungal biomass was observed on the discs). After biofilm formation on the discs, the suspended biomass and liquid medium are removed from the reactor through the sampling port. The mass of attached active biomass per unit area of bio-disc in the nutrient medium was noted [15,16,18,24]. Table 1 lists the design specifications of the RBC reactor and the experimental setup is shown in Fig. 1.

2.6. Decolorization of synthetic dye wastewater in the continuously operated RBC reactor with PU surface immobilized live fungal biomass and wheat bran

A continuous study in a laboratory scale RBC reactor was carried out using PU surface immobilized live fungal biomass of *N. crassa* with wheat bran for the removal of color from synthetic CR dye wastewater at 303 K. The experiments were conducted to study the effect of various parameters such as number of discs, disc rotation speed, % disc submergence in liquid medium, flow rate of dye solution, air flow rate, wheat bran dosage, and inlet dyestuff concentration on CR dye decolorization. Continuous decolorization experiments were carried out by varying the level of one factor and keeping the level of other factors constant on the other hand. After attachment of sufficient fungal biomass on the discs, the liquid phase was removed and replaced with a fresh medium containing nutrients (MEB, 10 g L^{-1}) and dyestuff of required concentration [15].

An aqueous CR dye solution of known concentration at pH 6 is pumped through the top of the reactor by a peristaltic pump at the required flow rate. The flow rate of dye solution is maintained constant throughout the experiments. A steady-state was achieved by measuring the flow rate at the top and bottom of the reactor. The required concentration of wheat bran (12.5 g L^{-1}) was added in the reactor. The appropriate air flow rate is provided continuously at another end on the top of the reactor [25]. The small fine mesh filter (10 μm opening) is connected to the bottom sampling port of the reactor; this is to prevent the release of wheat bran from dye solution in the reactor. The treated

Table 1
Design specifications of the RBC reactor

S. No	Specifications	Value
1	Total volume of reactor	30 L (74 cm × 21.4 cm × 19 cm)
2	Number of stages	1
3	Number of discs	20
4	Diameter of each disc	14 cm
5	Spacing between each disc	2 cm
6	Working volume of liquid	12 L
7	Disc submergence level	40% of total liquid volume
8	Disc rotation speed	1–20 rpm
9	Airflow rate	0–5 L min^{-1}
10	DC motor	0.5 HP
11	Peristaltic pump speed	0–160 rpm
12	Air pump capacity	Maximum output 70 L min^{-1}, pressure 0.035 Mpa

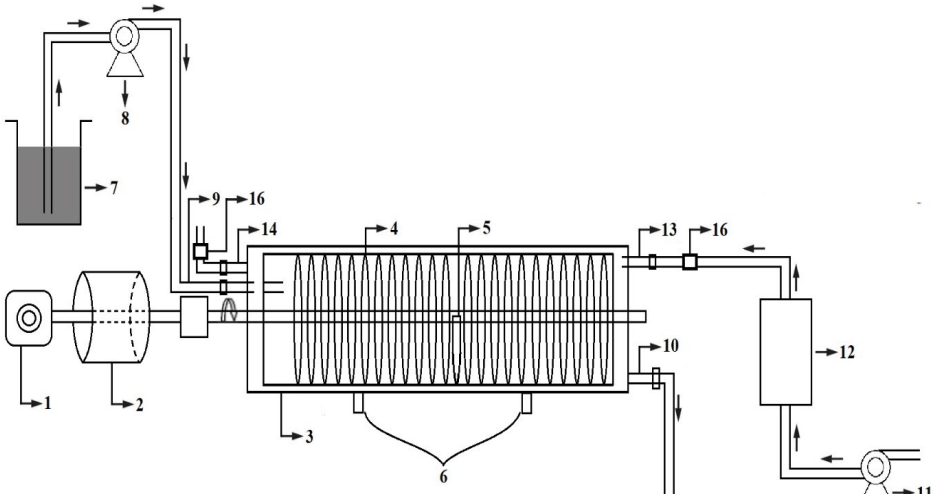

Fig. 1. Schematic diagram of the experimental setup showing the rotating biological contactor (RBC) reactor. (1) AC–DC rectifier with speed regulator, (2) DC motor, (3) reactor vessel, (4) rotating discs, (5) aluminum rod, (6) sampling ports, (7) feed solution, (8) peristaltic pump, (9) medium inlet, (10) medium outlet, (11) air pump, (12) air flow meter, (13) air inlet, (14) air outlet, (15) solution outlet, and (16) sterile filter.

dye solution samples were collected from sampling ports (medium outlet ports) of the reactor at uniform time intervals using a peristaltic pump with the flow rate same as the feed stream. The samples collected were centrifuged at 12,000 rpm for 15 min at 277 K in a cooling centrifuge. The clear supernatant liquid was analyzed using a UV/visible spectrophotometer to measure the unknown residual dye concentration in the solution based on the value of absorbance [15]. After decolorization experiments, each disc was carefully removed from the aluminum rod, then the mass of the attached active biomass per unit area of bio-disc in the dye medium was noted. Net amount of attached biomass per unit area of bio-disc (X_a) is the difference between the total amount of attached biomass per unit area of bio-disc in dye medium (X_d) and MEB medium (X_n). The concentration of attached active biomass in the dye medium (X_f) is calculated as [26]:

$$X_f = \frac{X_a A_w}{V} \tag{1}$$

where A_w is the total surface area of the bio-disc (m^2) and V is the volume of liquid in the reactor (L). The % CR color removal is determined by the following Eq. (2) [27]:

$$\%\ \text{CR color removal} = \frac{\left(S_0 - S\right)\times 100}{S_0} \tag{2}$$

where S_0 and S (mg L^{-1}) are the influent and effluent CR dye (substrate) concentrations at initial time and at time t, respectively. The yield coefficient of attached active biomass is found using the expression [28]:

$$Y_a = \frac{X_f - X_0}{S_0 - S} \tag{3}$$

where X_f and X_0 are the final and initial concentration of attached active biomass in the dye medium (mg L^{-1}), respectively.

2.7. Enzyme assays during the decolorization of synthetic CR dye wastewater

The hydrolytic enzymes such as polygalacturonase, cellulase, xylanase, and protease were produced by solid state fermentation using wheat bran as the solid substrate [29]. The fungus *N. crassa* will secrete various extracellular enzymes such as cellulose [30], endoglucanase [31], and endoxylanase [32] in the liquid medium containing the mixture of synthetic dyestuff (50 mg L^{-1}), nutrients (MEB, 10 g L^{-1}), wheat bran (12.5 g L^{-1}) and glucose (2 g L^{-1}) in the reactor. Decolorization experiments were carried out for 10 d at ambient temperature (303 K). For analysis of enzyme activity, samples collected were centrifuged at 12,000 rpm for 15 min at 277 K in a cooling centrifuge to remove the fungal

biomass. After the cells were separated, clear supernatant liquid containing the enzymes was assayed using a UV/visible spectrophotometer [15]. Cellulase activity in the supernatant was measured based on the change in absorbance caused by the degradation of cellulose to D-glucose at 540 nm. The total cellulase activity was determined using a Whatman No. 1 filter paper. Measurement of released reducing sugars was accomplished by using the DNS (Dinitrosalicylic acid) method using D-glucose standard [33]. Endoglucanase activity in the supernatant is calculated by measuring the degree of hydrolysis of carboxymethyl cellulose to D-glucose [30]. The degree of hydrolysis can be determined by measuring the changes in the absorbance of the reducing sugars at 540 nm. The reducing end concentration of D-glucose is measured by the DNS method using D-glucose standard. The activity of endoxylanase is measured using the substrate PNPG (p-nitrophenyl-β-D-glucoside). The release of PNP (p-nitrophenol) from the substrate is determined by measuring the increase in absorbance at 400 nm using p-nitrophenol standard [32].

2.8. Decolorization of textile industrial CR dye effluent in the continuously operated RBC reactor with PU surface immobilized live fungal biomass and wheat bran

The industrial CR dye effluent was collected from Bright Traders, Erode District, Tamil Nadu State, India. The method of analysis of physico-chemical parameters of real industrial CR dye effluent was given in previous continuous studies of fixed-bed adsorption column published earlier [34]. The industrial raw effluent was centrifuged to separate the salt content, and dispersed solids, which were present because of the high COD value of the effluent. Decolorization experiments were conducted with industrial dye effluents using PU surface-immobilized live biomass and wheat bran. The optimal values of various factors that were obtained from continuous studies were used for the estimation of % removal of COD from industrial dye effluent. The treatment of industrial dye effluent was carried out to study the effect of glucose concentration (0–5 g L^{-1}). The % COD removal of the industrial effluent was found from the equation [24]:

$$\%\text{COD removal} = \left(\frac{\text{Initial COD of the effluent} - \text{Final COD of the effluent}}{\text{Initial COD of the effluent}} \right) \times 100 \quad (4)$$

3. Results and discussion

3.1. Analysis of various parameters on continuous decolorization of synthetic dye wastewater in RBC reactor using PU surface-immobilized live biomass with wheat bran

3.1.1. Effect of number of discs (N_D)

The effect of number of discs on continuous decolorization of CR was examined by varying the number of discs from 8 to 20 and the results are reported in Table 2. It depicts that the decolorization of CR increases from 57.70% to 75.52% with an increase in the number of discs (Fig. S4). This is because, the concentration of biomass (X_f) increased from 72.91 to 99. 85 mg L^{-1} with an increase in the number of bio-discs in a mixture of dye and nutrient medium with wheat bran. The increase in the value of X_f is due to the

Table 2
Effect of number of discs (N_D), disc rotation speed (N), % disc submergence level in liquid medium (D_{SL}), air flow rate (Q_{air}), flow rate of dye solution (F), wheat bran dosage (M_{WB}), and inlet dyestuff concentration (S_0) on CR dye decolorization in a RBC reactor

N_D	N (rpm)	D_{SL} (%)	Q_{air} (L min^{-1})	F (mL min^{-1})	M_{WB} (g L^{-1})	S_0 (mg L^{-1})	% Color removal	X_a (mg m^{-2})	X_f (mg L^{-1})	Y_a
8	16	40	1.5	1	12.5	200	57.70	1,987.53	72.91	0.6318
14	16	40	1.5	1	12.5	200	65.57	1,297.90	83.64	0.6534
20	16	40	1.5	1	12.5	200	75.52	1,107.96	99.85	0.6612
20	4	40	1.5	1	12.5	200	49.31	702.63	63.42	0.6430
20	8	40	1.5	1	12.5	200	61.19	881.90	79.60	0.6504
20	16	30	1.5	1	12.5	200	60.07	828.00	99.57	0.6220
20	16	35	1.5	1	12.5	200	68.46	965.38	99.64	0.6434
20	16	40	0.5	1	12.5	200	67.02	971.38	87.67	0.6437
20	16	40	2.5	1	12.5	200	78.36	1,133.88	102.34	0.6604
20	16	40	1.5	3	12.5	200	61.67	882.73	79.67	0.6524
20	16	40	1.5	6	12.5	200	40.14	571.56	51.58	0.6428
20	16	40	1.5	1	4	200	40.65	438.67	39.59	0.6418
20	16	40	1.5	1	8	200	55.28	713.45	64.39	0.6534
20	16	40	1.5	1	12.5	50	90.15	328.90	29.68	0.6585
20	16	40	1.5	1	12.5	100	83.36	597.48	53.92	0.6469
20	16	40	1.5	1	12.5	150	79.24	846.86	76.43	0.6367
20	16	40	1.5	1	12.5	500	45.24	1,566.02	141.34	0.6249

increase of the surface area of bio-supporting material yielding better immobilization and growth [35].

3.1.2. Effect of disc rotation speed (N)

The effect of disc rotation speed on growth of attached active biomass for CR dye decolorization was studied by varying the speed from 4 to 16 rpm. Fig. 2 shows that the color removal of CR increases from 49.31% to 75.52% with an increase in disc rotation speed from 4 to 16 rpm. This may be due to the value of X_a increasing from 702.63 to 1,107.96 mg m^{-2} with an increase in disc rotation speed from 4 to 16 rpm (Table 2). The increase in value of X_a at higher rotational speed is due to an increase in contact between immobilized biomass and the liquid phase, and hence better treatment efficiencies can be obtained. Higher rotational speed can provide better aeration, resulting in increased biomass growth leading to a better decolorization efficiency [17]. In order to achieve oxygen saturation and the production of thicker biofilms, higher rotational speeds are required [19].

3.1.3. Effect of % disc submergence in liquid medium (D_{SL})

The effect of % disc submergence in the liquid medium on growth of attached active biomass for CR dye decolorization was analyzed by varying the submergence level from 30% to 40% of the total volume of the reactor. Table 2 illustrates that the decolorization efficiency of CR increased from 60.07% to 75.52% with an increase in % disc submergence level from 30% to 40%. This is due to the value of X_a increasing from 828 to 1,107.96 mg m^{-2} with an increase in the discs submergence level. The increase in value of X_a is due to an increase in the contact between the liquid medium and surface area of bio-supporting material that gives better immobilization and growth [36].

3.1.4. Effect of air flow rate (Q_{air})

Oxygen is essential for the growth of fungus which in turn facilitate an increase in decolorization. The effect of air flow rate on growth of attached active biomass for decolorization efficiency of CR was studied by varying the air flow rate from 0.5 to 2.5 L min^{-1}. Fig. S5 shows that the color removal of CR increased from 67.02% to 78.36% with an increase in air flow rate from 0.5 to 2.5 L min^{-1}. This is because, the value of X_a increased from 971.38 to 1,133.88 mg m^{-2} with an increase in air flow rate from 0.5 to 2.5 L min^{-1} (Table 2). Increased aeration may be due to the high levels of dissolved oxygen in the reactor allowing more biomass growth rate, hence resulting in higher % decolorization. At higher flow rate of air, the liquid phase may be saturated with oxygen [19].

3.1.5. Effect of wheat bran dosage (M_{WB})

The effect of wheat bran dosage on growth of attached active biomass for % color removal of CR was studied by varying the wheat bran dosage from 4 to 12.5 g L^{-1}. Fig. 3 depicts that the removal efficiency of CR increased from 40.65% to 75.52% with an increase in amount of wheat bran in the mixture of dye and nutrient medium from 4 to 12.5 g L^{-1}. This may be due to the value of X_a increasing from 438.67 to 1,107.96 mg m^{-2} (Table 2). This indicates that the attached fungal biomass can utilize the wheat bran as a nutrient source for its growth.

The decolorization efficiency increased with the increase in the number of discs, disc rotation speed, % disc submergence in the liquid medium, air flow rate, and wheat bran dosage which yields a higher amount of live biomass attached on the discs. The increase in the amount of live biomass results in greater available surface area and therefore an increase in the number of binding sites accessible for the adsorption of CR dye molecules [37]. Thus the competition

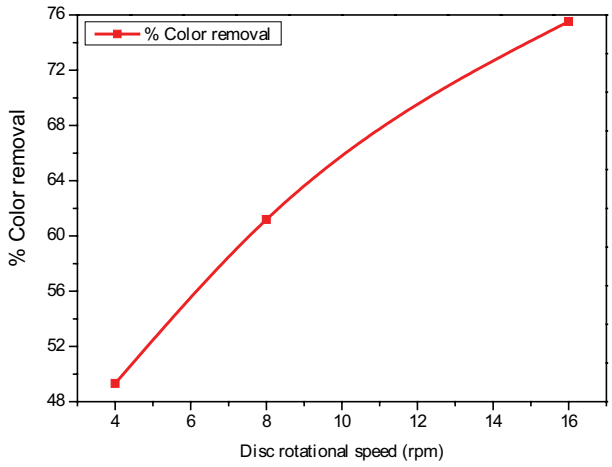

Fig. 2. Effect of disc rotational speed on CR dye decolorization in RBC reactor. (Initial pH: 6; initial dye concentration: 200 mg L^{-1}; wheat bran dosage: 12.5 g L^{-1}; flow rate of dye solution: 1 mL min^{-1}; air flow rate: 1.5 L min^{-1}; number of discs: 20; disc submergence level in liquid medium: 40%; temperature: 303 K; effluent treatment time: 48 h; volume of liquid in the reactor: 12 L).

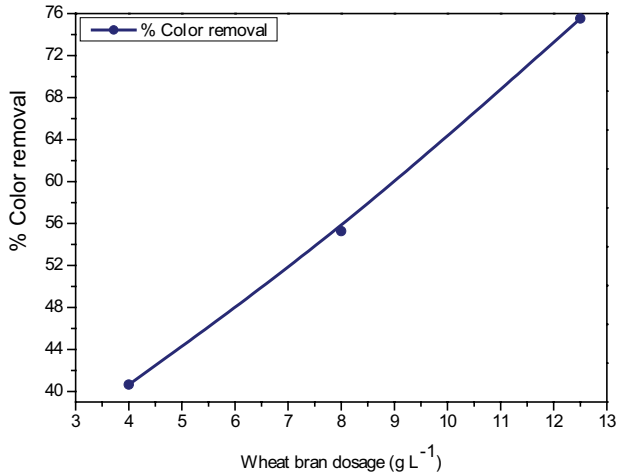

Fig. 3. Effect of wheat bran dosage on CR dye decolorization in RBC reactor. (Initial pH: 6; initial dye concentration: 200 mg L^{-1}; flow rate of dye solution: 1 mL min^{-1}; air flow rate: 1.5 L min^{-1}; number of discs: 20; disc rotation speed: 16 rpm; disc submergence level in liquid medium: 40%; temperature: 303 K; effluent treatment time: 48 h; volume of liquid in the reactor: 12 L).

for the availability of active sites for the adsorption of dye decreases with the increase in the live biomass growth/wheat bran dosage [38]. Therefore, an increase in live biomass growth/wheat bran dosage results in a decrease in the dye concentration in the solution.

3.1.6. Effect of flow rate of dye solution (F)

The effect of flow rate on the % decolorization of CR was analyzed by varying the flow rate of dye solution fed into the reactor from 1 to 6 mL min^{-1}. Fig. S6 shows that at lower flow rate (1 mL min^{-1}) yields better decolorization as compared to a higher flow rate. From Table 2, it can be seen that as the flow rate increases from 1 to 6 mL min^{-1}, the % color removal of CR decreases from 75.52% to 40.14%. This may be due to the value of X_a decreasing from 1,107.96 to 571.56 mg m^{-2}. Hence, these results suggest that a low flow rate of dye solution is needed to allow sufficient contact between immobilized biomass and the liquid medium for decolorizing the effluents especially at optimum inlet dyestuff concentration [34]. The % color removal decreased due to the decrease in the amount of live biomass produced on the discs, which in turn may be due to the reduced time for CR dye molecules to come in contact with the live fungal biomass-wheat bran particle surface and diffusional limitation of the dye molecules into the pores of the wheat bran [39].

3.1.7. Effect of inlet dyestuff concentration (S_0)

The effect of the inlet dyestuff concentration on growth of attached active biomass for CR dye decolorization was analyzed by varying the feed concentration from 50 to 500 mg L^{-1} and the results are shown in Fig. 4. It shows that the color removal of CR decreased from 90.15% to 45.24% with an increase in dyestuff concentration from 50 to 500 mg L^{-1}. Table 2 exhibits that the value of X_a increased from 328.90 to 1,566.02 mg m^{-2} with an increase in dyestuff concentration of feed solution from 50 to 500 mg L^{-1}. But there was a lack of significant attached growth found on the bio-disc in dye solution of various concentrations. Therefore, for efficient decolorization of CR by immobilized *N. crassa* live fungal biomass with wheat bran, the inlet dyestuff concentration should be optimum with suitable nutrient source (glucose, fructose, sucrose, etc.). In other words, the decrease in % color removal with increase in inlet dyestuff concentration may be due to available binding sites of wheat bran and live biomass decreasing, due to accumulation of CR dye molecules and competition between more dye anions at the positively charged fixed binding sites of the wheat bran surface (i.e., enhances the interaction between dye molecules and active sites on the live biomass and wheat bran particle surface, therefore, lack of available sites) [37]. The yield coefficient of attached active biomass on the effect of several process parameters at various operating conditions is in the range between 0.62–0.66. The experimental setup of attached active live fungal biomass on PU covered discs in RBC reactor before and after decolorization of CR dye wastewater are shown in Figs. S7 and S8

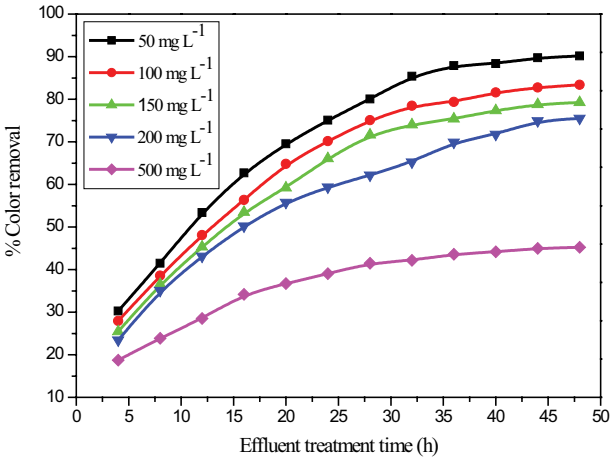

Fig. 4. Effect of inlet dyestuff concentration on CR dye decolorization in RBC reactor. (Initial pH: 6; wheat bran dosage: 12.5 g L^{-1}; flow rate of dye solution: 1 mL min^{-1}; air flow rate: 1.5 L min^{-1}; number of discs: 20; disc rotation speed: 16 rpm; disc submergence level in liquid medium: 40%; temperature: 303 K; effluent treatment time: 48 h; volume of liquid in the reactor: 12 L).

3.2. Analysis of enzyme activities during the decolorization of synthetic CR dye wastewater

The activities of the various enzymes were measured and the results are shown in Table 3 and Fig. 5. From Fig. 5, it is observed that, at the initial stage of the decolorization process, activities of various enzymes are found to be zero. After 36 h, the enzymes activity increased with increase in the operation period. Moreover, superior activity was obtained by the enzyme cellulase which may indicate a major role of cellulase in the decolorization process. The maximum activities of cellulase, endoglucanase, and endoxylanase were 1,284; 794; and 436 U L^{-1}, respectively. Wheat bran is an excellent source of hemicellulose and it is a good inducer of the cellulolytic enzyme system. It consists of cellulose (25%), hemicellulose (33%), starch (19%), crude protein (18%), and lipids (5%) [40]. Table 3 compares the activity of various enzymes and COD removal efficiency with an operational period. It shows that the % COD removal of synthetic dye effluent increased with an increase in operation period. The maximum COD removal observed at 240 h was 93.34%. It shows that the decolorization of CR may be due to both biosorption and the enzymatic decolorization process. The agricultural by-product wheat bran acts as an adsorbent and it also may act as a substrate for the growth of fungus which will improve the decolorization efficiency. It also means that the first 2 d, biosorption is active, followed by the enzymatic decolorization process for the rest of the days. This may indicate the accumulation of CR onto the surface of wheat bran and live fungal biomass, and action of various enzymes produced by the immobilized fungus *N. crassa* in the influent substrate medium. Moreover, the acidic pH of the substrate medium in the range of 5.5–6 is the most favorable environment for the growth of surface-immobilized live fungal biomass on PU covered discs in the mixture of dye and nutrient medium with wheat bran and glucose [41].

Table 3
Comparison of various extracellular enzyme activity as a function of operational period

Operation period (days)	% COD removal	Enzyme activity (U L^{-1})		
		Cellulase	Endoglucanase	Endoxylanase
0.5	51.37	–	–	–
1	74.26	–	–	–
1.5	82.90	19	8	5
2	86.35	45	34	12
3	87.74	190	98	56
4	88.36	264	154	84
5	89.28	433	262	116
6	90.54	582	336	195
7	91.48	816	478	256
8	92.82	942	540	306
9	93.18	1,160	715	394
10	93.34	1,284	794	436

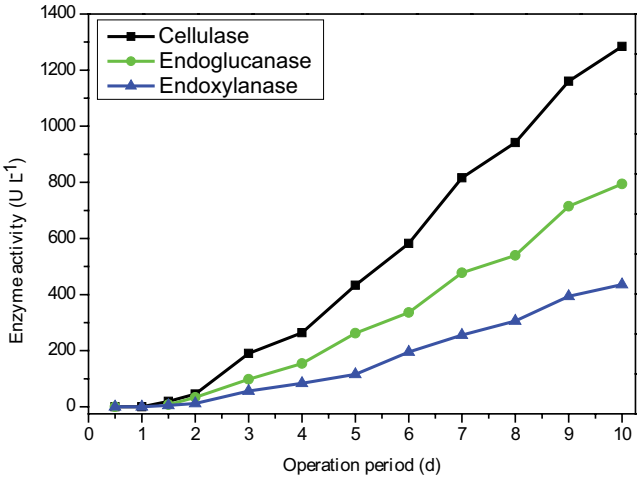

Fig. 5. Effect of various enzymes activities on CR dye decolorization in RBC reactor. (Initial pH: 6; initial dye concentration: 50 mg L^{-1}; inlet COD: 486 mg L^{-1}; wheat bran dosage: 12.5 g L^{-1}; glucose concentration: 2 g L^{-1}; flow rate of dye solution: 1 mL min^{-1}; air flow rate: 1.5 L min^{-1}; number of discs: 20; disc rotation speed: 16 rpm; disc submergence level in liquid medium: 40%; temperature: 303 K; volume of liquid in the reactor: 12 L).

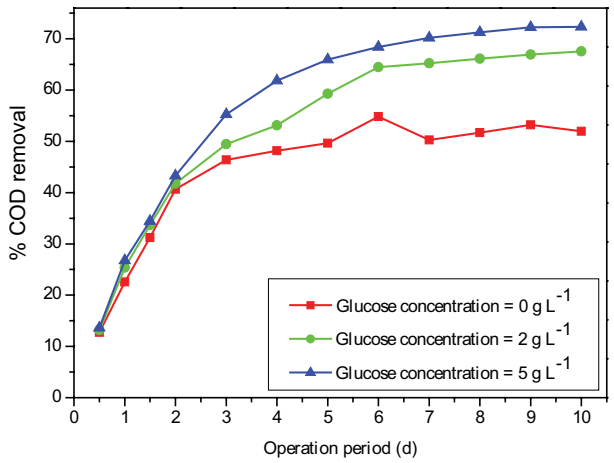

Fig. 6. Effect of glucose concentration on decolorization of industrial CR dye effluent. (Initial pH: 6; inlet COD: 1,824 mg L^{-1}; wheat bran dosage: 12.5 g L^{-1}; malt extract broth dosage: 10 g L^{-1}; flow rate of dye solution: 1 mL min^{-1}; air flow rate: 1.5 L min^{-1}; number of discs: 20; disc rotation speed: 16 rpm; disc submergence level in liquid medium: 40%; temperature: 303 K; effluent treatment time: 240 h; volume of liquid in the reactor: 12 L).

3.3. Analysis of RBC reactor studies with real textile industrial CR dye effluent

The effect of glucose concentration on the growth of attached active biomass for industrial CR dye effluent decolorization in RBC reactor was studied by varying the glucose concentration from 0 to 5 g L^{-1}. The experimental data obtained at various glucose concentrations are reported in Table S2. It shows that the COD removal of industrial CR dye effluent increased from 54.84% to 72.34% with an increase in glucose concentration from 0 to 5 g L^{-1} at the end of 240 h. On the other hand, the % COD removal was low when substrate containing glucose concentration was less than 5 g L^{-1}. In the absence of glucose concentration, the COD removal efficiency was effective up to 6 d (54.84%) and started decreasing from next 4 d (51.96%) (Fig. 6). This may be due to the lack of enzyme activity without glucose concentration [42]. The immobilized fungus *N. crassa* may have adapted to the process environmental factors at high glucose concentration. A similar observation has been reported elsewhere [24]. Furthermore, the intensity of the peaks of industrial CR dye effluent were measured before and after treatment. After treatment (10 d), the intensity of the CR peak had diminished with a glucose concentration of 5 g L^{-1} (Fig. S9). The experimental setup of attached active

live fungal biomass on PU covered discs in RBC reactor after decolorization of industrial CR dye effluent is shown in Fig. S10.

4. Conclusion

The surface immobilized live fungal biomass of *N. crassa* with wheat bran may be used as an effective material for the removal of CR from aqueous solution in an RBC reactor. The amount of attached active biomass per unit area of bio-disc (X_a) and decolorization efficiency was found to vary with various process parameters. The amount of biomass produced and % color removal of CR increased with an increase in the number of discs, disc rotation speed, % disc submergence in the liquid medium, air flow rate, and wheat bran dosage. The decolorization efficiency decreased with an increase in the flow rate of dye solution and inlet dyestuff concentration. Enzyme activity studies showed that the activities of various enzymes increased with an increase in the operation period. Moreover, superior activity was obtained by the enzyme cellulase which may indicate that cellulase plays a major role in the decolorization process. It shows that the decolorization of CR may be due to both the biosorption and enzymatic decolorization process. The decolorization of solute from real industrial CR dye effluent studies shows that the COD removal efficiency increased with an increase in glucose concentration. After treatment, the intensity of CR peak disappeared completely at high glucose concentration of 5 g L^{-1} and the maximum COD removal efficiency was 72.34% at the end of 240 h. The experimental results showed that the PU surface-immobilized live fungal biomass of *N. crassa* with wheat bran adsorbent/substrate may be used effectively to remove other anionic dyes from industrial effluents. A better decolorization efficiency of industrial dye effluent suggests that *N. crassa* live fungal biomass with wheat bran can be used effectively in wastewater treatment.

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Supplementary information

S1. Introduction

Currently more than 1×10^5 dyes are commercially available having various applications with global annual production in excess of 7×10^5 million tons [S1]. A literature survey indicates that on an average 150–200 L of water is consumed and about 125 L of effluent is generated per kg of finished textile produced in India [S2]. It is about 1×10^6 L of effluent is discharged per day by an average-sized textile mill having a daily production of 8,000 kg of finished products [S2]. Color is the first contaminant to be recognized in wastewater. Generally, color is visible in the effluents from textile-dyeing processes when the dye concentration is greater than 1 mg L^{-1} and at an average concentration of 300 mg L^{-1} [S3]. Most of the azo dyes have been reported to be the main cause for bladder cancer in humans, splenic sarcomas, hepatocarcinomas, and chromosomal aberration in mammalian cells [S4].

The synthetic dye Congo red has a strong affinity for cellulose fibers and is widely used in textile processing industries [S5]. It has been investigated as a mutagen and reproductive effector. It may affect blood factors such as clotting, and induce somnolence, and respiratory problems [S6]. Furthermore, industrial effluents containing synthetic dyes can significantly affect the dissolved oxygen concentration, photosynthetic activities of aquatic flora, and thereby severely affecting aquatic organisms due to reduced light penetration [S7]. The effluents must be treated to bring down the concentration of dyes present in it to permissible and bearable limits before discharging into water bodies as required under the environmental regulation act [S8].

Wastewater containing azo dyes from the textile industry are very difficult to treat using conventional treatment methods (excluding adsorption and enzymatic treatment) such as chemical precipitation, ion exchange, membrane filtration, electrochemical oxidation, photo-catalytic degradation, ozonation, Fenton process, and sonication because dyes are structurally complex and more stable aromatic organic compounds [S9]. However, the usual treatment methods have several drawbacks such as high capital and operating cost, the complexity of the treatment processes, sludge disposable problem, and the need for chemicals, which may, in turn, pollute the water [S10].

Immobilized cells may produce more biomass growth rate as the active site of the biomass is typically unaffected even after adsorption and nearly full activity can be retained. RBC reactor is an established technology and yields good performance for large-scale wastewater treatment applications. A large volume of effluent can be treated continuously using RBC reactor systems which results in a better decolorization efficiency [S11]. It has several advantages such as low-shear environment, low energy requirements, simple construction, and operation, requires less space, low maintenance cost, easy scale-up, high biomass concentration, efficient mixing, short hydraulic retention time, low accumulation of removed biofilm, and channeling and clogging problems are eliminated [S12,S13]. Moreover, it provides a greater interfacial area generated

in the rotating disc to initiate good contact between the live biomass and toxic substance in the wastewater [S14]. Some bio-support materials that can be used for fungal immobilization are polyurethane foam [S15], polyether foam [S16], natural loofa sponge [S17], metal mesh [S18], plastic discs [S18], plastic mesh [S19], scouring web [S13], polystyrene mesh [S15], nylon fiber mesh [S20], and jute twine [S20].

S2. Materials and methods

S2.1. Immobilization of the Neurospora crassa live cells by surface adsorption method

Various bio-supporting materials such as polyurethane foam, polyether foam, natural loofa sponge, metal mesh, plastic sheet (acrylic sheet), plastic mesh, polystyrene mesh, scouring web, nylon fiber mesh, and jute twine were obtained locally in Udupi district, Karnataka State, India. The materials were then washed with water to remove fine dirt particles. The washed materials were then dried in a hot air oven at 333 K for 24 h. The mycelium carriers of different structure and surface properties are cut into small slices (3 cm length × 12 cm height × 0.5 cm thickness) [S21]. Various materials are tested for the evaluation of fungal biomass growth activity. The Erlenmeyer flasks containing each support material in malt extract broth medium were inoculated with 0.5% w/v filamentous fungus *Neurospora crassa* under sterile conditions. The fungi were allowed to grow for one week in a shake flask in an incubator shaker rotated at a speed of 120 rpm at 298 K to obtain the maximum amount of fungal biomass. Enzyme activities are reported in units (U) per liter of the enzyme of solution. One unit of enzyme is defined as 1 μmol of product produced per minute per liter of enzyme solution consumed at specified pH and temperature [S22].

S3. Results and discussions

S3.1. Evaluation of suitable carrier material for immobilization of live cells

The suitable carrier material for the immobilization of live cells was selected based on its binding capacity. The amount of dry biomass produced on each carrier material per unit mass of dry supporting material (yield factor) is shown in Fig. S1 and Table S1. There was a sufficient growth found on various bio-supporting materials such as polyurethane foam, polystyrene mesh, polyether foam, scouring web, and nylon fiber mesh. Most of the fungal biomass growth was attached on the surface of the supporting materials and it was partially suspended in the liquid phase. Based on live biomass growth, the yield factor was determined to be in the following order: polyurethane foam (0.294 g g^{-1}) > polystyrene mesh (0.235 g g^{-1}) > polyether foam (0.193 g g^{-1}) > scouring web (0.156 g g^{-1}) > nylon fiber mesh (0.091 g g^{-1}) > plastic mesh (0.058 g g^{-1}) > loofa sponge (0.039 g g^{-1}) > metal

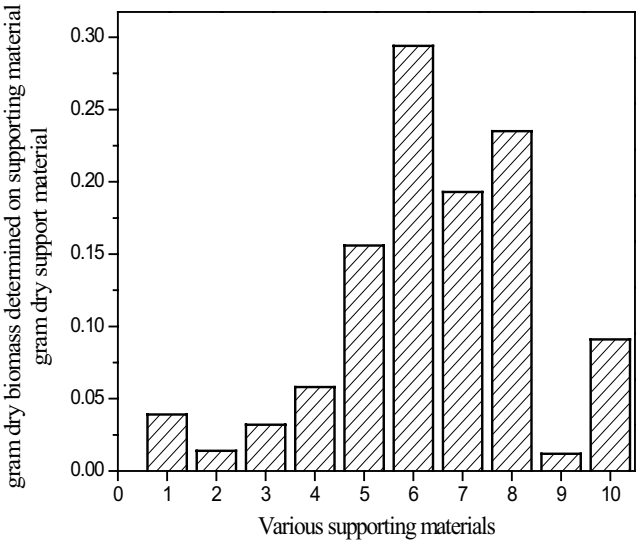

Fig. S1. Selection of suitable supporting material for the immobilization of live cells (dimension of each mycelium carrier material: 3 cm length × 12 cm height × 0.5 cm thickness). (1) Loofa sponge, (2) plastic disc, (3) metal mesh, (4) plastic mesh, (5) souring web, (6) polyurethane foam, (7) polyether foam, (8) polystyrene mesh, (9) jute twine, and (10) nylon fiber mesh.

Table S1
Evaluation of suitable supporting material for the immobilization of live cells

S. no	Supporting materials	gram dry biomass determined on supporting material / gram dry supporting material
1	Loofa sponge	0.039
2	Plastic disc	0.014
3	Metal mesh	0.032
4	Plastic mesh	0.058
5	Scouring web	0.156
6	Plolyurethane foam	0.294
7	Polyether foam	0.193
8	Polystyrene mesh	0.235
9	Jute twine	0.012
10	Nylon fiber mesh	0.091

mesh (0.032 g g^{-1}) > plastic disc (0.014 g g^{-1}) > jute twine (0.012 g g^{-1}). Therefore, out of ten different supporting materials options, the polyurethane foam was found to exhibit better results and was considered for further analysis. The other supporting materials were relatively less effective in favoring the growth of live biomass. A similar observation has been reported elsewhere [S20].

S3.2. Effect of initial pH on the decolorization of CR by the supporting material PU

The effect of initial pH on the removal of CR was analyzed by varying the aqueous pH from 6 to 12 with an initial dye concentration of 100 mg L^{-1}. It was studied by using the fixed dimension of PU material with the absence of live biomass and wheat bran. As shown in Fig. S2, the decolorization of CR was very poor and it was decreased from 8.36% to 1.08% with the increase in pH from 6 to 12. The decrease in decolorization efficiency with increasing pH is may be due to the repulsive forces between the functional groups present on the surface of the PU material and the CR dye molecules. The poor adsorption is due to the absence of live biomass and wheat bran dual adsorbent in the aqueous solution. After performing the experiment, the stability of supporting material PU was observed at various pH. The material was not denatured at various operating conditions. This study again proved that the material PU was found to exhibit better stability and hence chosen for further analysis.

S3.3. Analysis of various parameters on continuous decolorization of synthetic dye wastewater in RBC reactor using PU surface immobilized live biomass with wheat bran

The continuous CR dye decolorization experiments were conducted for 48 h. The intensity of the peaks of synthetic CR dye effluent was measured before and after treatment. During these 48 h, the peak wavelength (λ_{max}) of

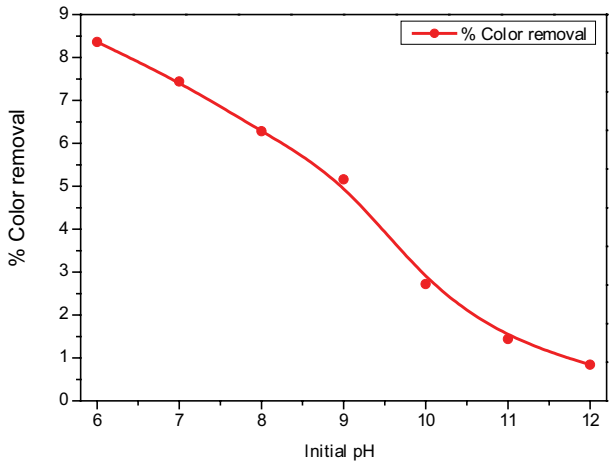

Fig. S2. Effect of initial pH on decolorization of CR by PU. (Initial pH: 6; initial dye concentration: 100 mg L^{-1}; mass of PU dosage: 0.5 g; volume of dye solution: 100 mL; agitation speed: 180 rpm; temperature: 303 K; contact time: 10 h).

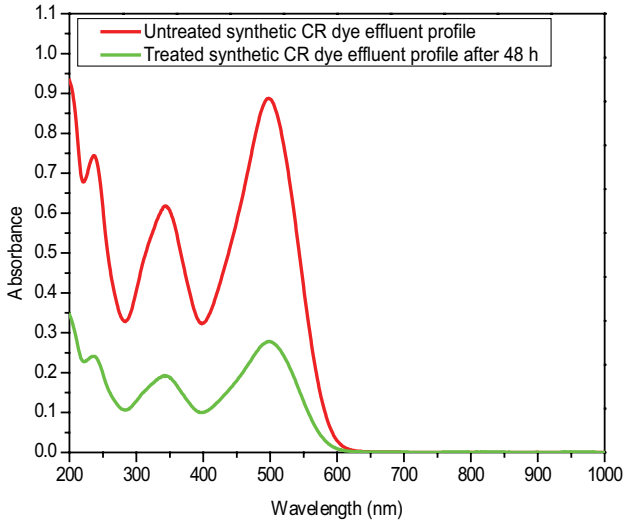

Fig. S3. Synthetic CR dye effluent decolorization profile obtained at 48 h as compared with untreated effluent. (Initial pH: 6; initial dye concentration: 200 mg L^{-1}; wheat bran dosage: 12.5 g L^{-1}; flow rate of dye solution: 1 mL min^{-1}; air flow rate: 1.5 L min^{-1}; number of discs: 20; disc rotation speed: 16 rpm; disc submergence level in liquid medium: 40%; temperature: 303 K; effluent treatment time: 48 h; volume of liquid in the reactor: 12 L).

Fig. S4. Effect of number of discs on CR dye decolorization in RBC reactor. (Initial pH: 6; initial dye concentration: 200 mg L^{-1}; wheat bran dosage: 12.5 g L^{-1}; flow rate of dye solution: 1 mL min^{-1}; air flow rate: 1.5 L min^{-1}; disc rotation speed: 16 rpm; temperature: 303 K; effluent treatment time: 48 h; disc submergence level in liquid medium: 40%; volume of liquid in the reactor: 12 L).

treated synthetic CR dye effluent was similar to the original untreated CR dye wavelength of concentration 200 mg L^{-1}. Fig. S3 illustrates that the intensity of the peaks declined considerably after treatment which indicates that the CR dye molecules did not biodegrade in 48 h (CR dye molecules were adsorbed on the surface of live biomass and wheat bran in 48 h). Therefore, absorbance values of all the experiments were measured at 498 nm before and after

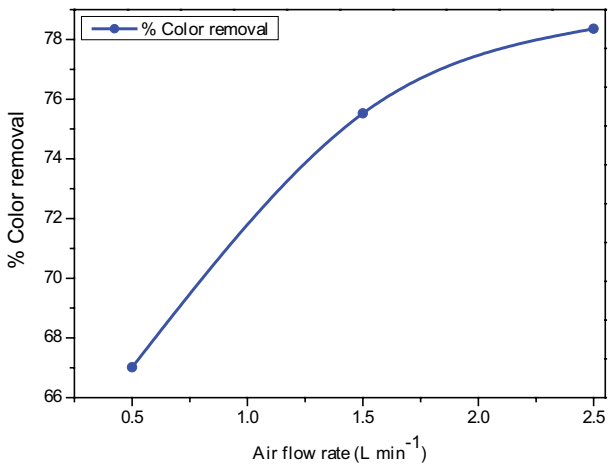

Fig. S5. Effect of air flow rate on CR dye decolorization in RBC reactor. (Initial pH: 6; initial dye concentration: 200 mg L^{-1}; wheat bran dosage: 12.5 g L^{-1}; flow rate of dye solution: 1 mL min^{-1}; number of discs: 20; disc rotation speed: 16 rpm; disc submergence level in liquid medium: 40%; temperature: 303 K; effluent treatment time: 48 h; volume of liquid in the reactor: 12 L).

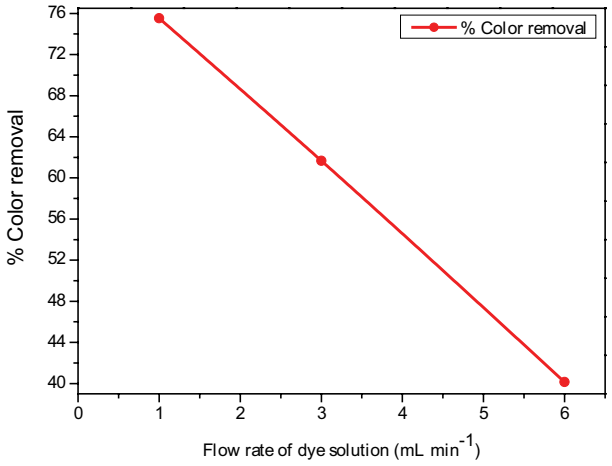

Fig. S6. Effect of flow rate of dye solution on CR dye decolorization in RBC reactor. (Initial pH: 6; initial dye concentration: 200 mg L^{-1}; wheat bran dosage: 12.5 g L^{-1}; air flow rate: 1.5 L min^{-1}; number of discs: 20; disc rotation speed: 16 rpm; disc submergence level in liquid medium: 40%; temperature: 303 K; effluent treatment time: 48 h; volume of liquid in the reactor: 12 L).

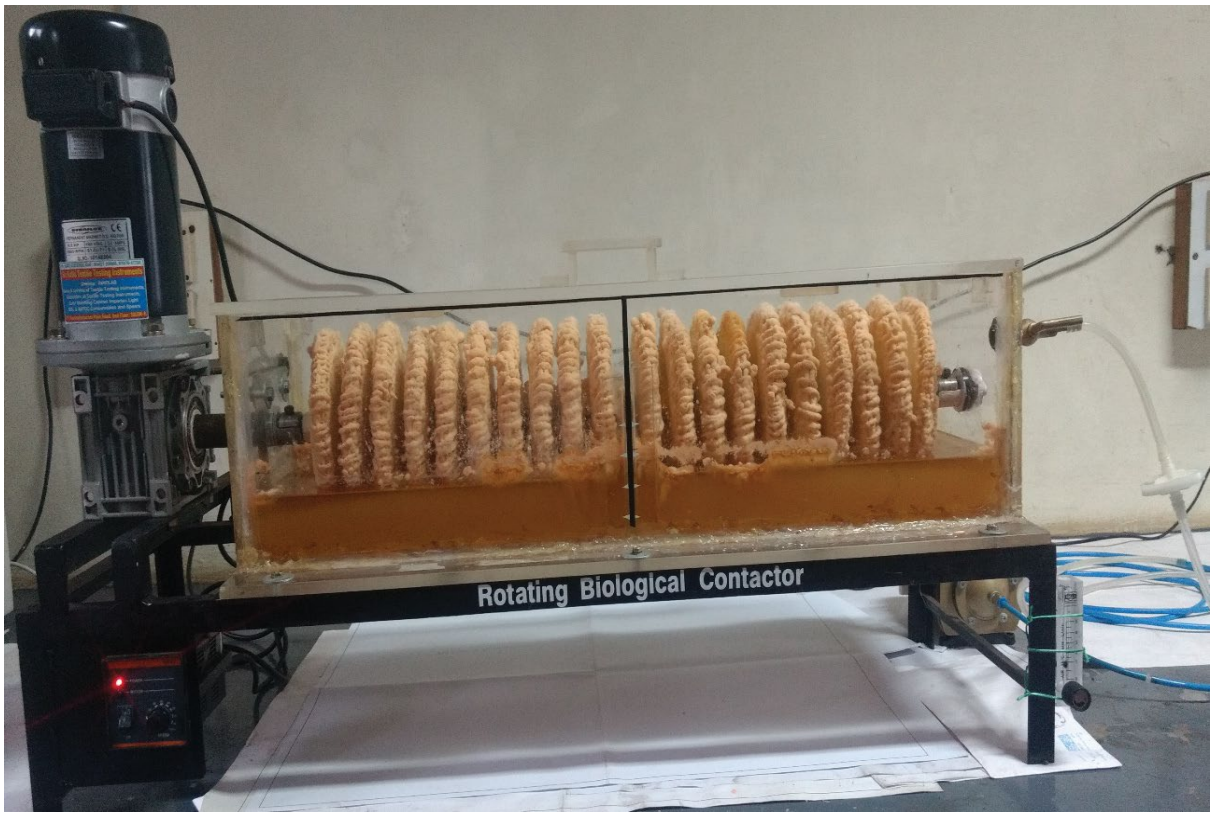

Fig. S7. Experimental setup of attached active live fungal biomass on PU covered discs in RBC reactor (before decolorization of CR dye wastewater).

Table S2
Effect of glucose concentration on decolorization of industrial CR dye effluent

Operation period (days)	% COD removal with various glucose concentration		
	Glucose concentration 0 g L^{-1}	Glucose concentration 2 g L^{-1}	Glucose concentration 5 g L^{-1}
0.5	12.72	13.20	13.64
1	22.56	25.38	26.75
1.5	31.24	33.67	34.43
2	40.68	41.76	43.31
3	46.39	49.45	55.28
4	48.18	53.13	61.84
5	49.66	59.28	65.95
6	54.84	64.47	68.42
7	50.25	65.24	70.18
8	51.68	66.12	71.27
9	53.22	66.94	72.26
10	51.96	67.54	72.34

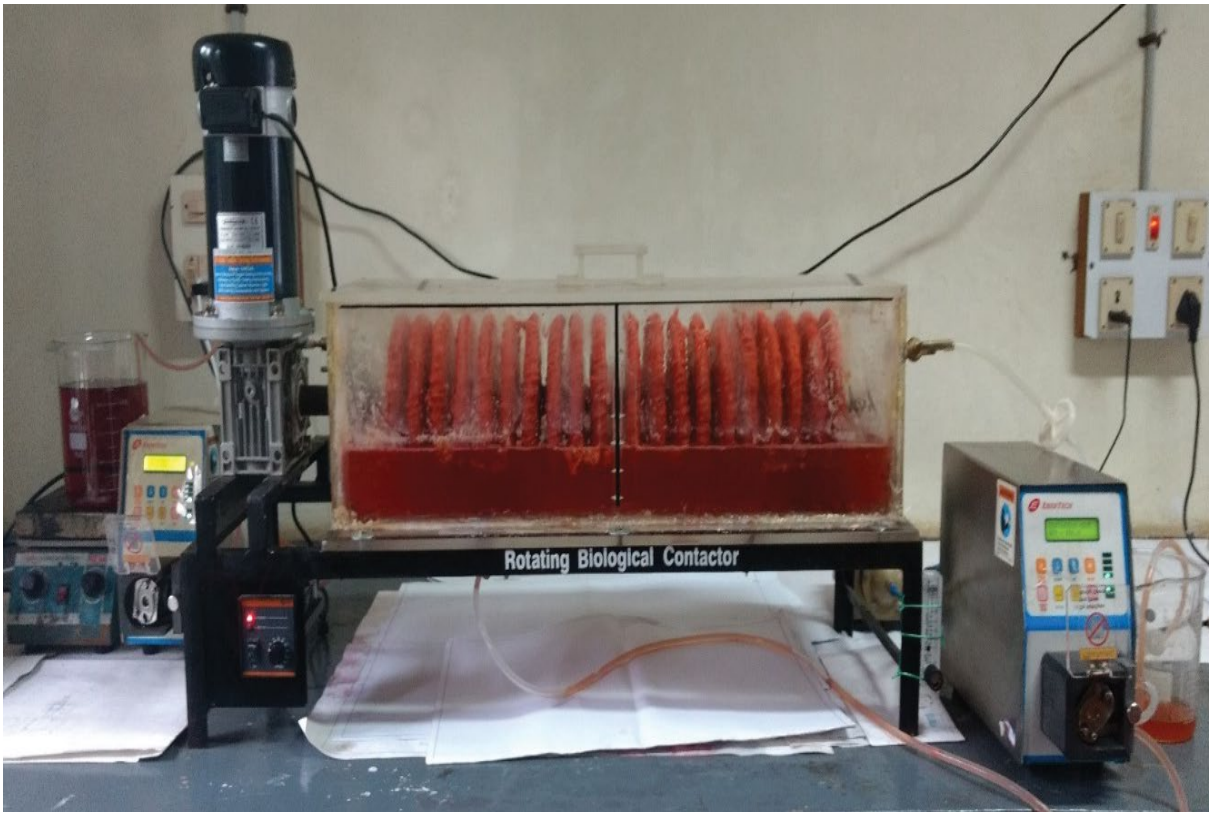

Fig. S8. Experimental setup of RBC reactor (after decolorization of synthetic CR dye effluent). (Initial pH: 6; initial dye concentration: 200 mg L^{-1}; wheat bran dosage: 12.5 g L^{-1}; flow rate of dye solution: 1 mL min^{-1}; air flow rate: 1.5 L min^{-1}; number of discs: 20; disc rotation speed: 16 rpm; disc submergence level in liquid medium: 40%; temperature: 303 K; effluent treatment time: 48 h; volume of liquid in the reactor: 12 L).

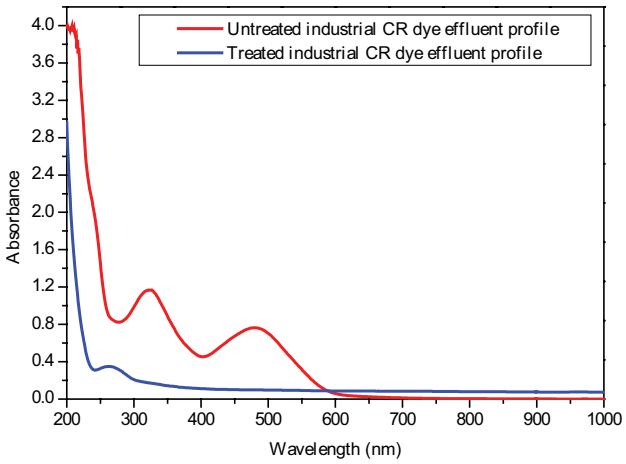

Fig. S9. Industrial CR dye effluent decolorization profile obtained in RBC reactor studies with untreated effluent profile. (Initial pH: 6; inlet COD: 1,824 mg L^{-1}; wheat bran dosage: 12.5 g L^{-1}; malt extract broth dosage: 10 g L^{-1}; glucose concentration: 5 g L^{-1}; flow rate of dye solution: 1 mL min^{-1}; air flow rate: 1.5 L min^{-1}; number of discs: 20; disc rotation speed: 16 rpm; disc submergence level in liquid medium: 40%; temperature: 303 K; effluent treatment time: 240 h; volume of liquid in the reactor: 12 L).

treatment for 48 h. The results of the effect of number of discs, disc rotation speed, % disc submergence in the liquid medium, air flow rate, wheat bran dosage, flow rate of dye solution, and inlet dyestuff concentration on continuous decolorization of CR were reported in Table 2.

S3.3.1. Effect of number of discs (N_D)

More number of discs were gradual response for the growth of biomass yields to increase in the % color removal of CR dye from aqueous solutions. The maximum growth was observed when the number of discs is 20.

S3.3.2. Effect of inlet dye stuff concentration (S_0)

Table 2 shows that the decolorization efficiency was more than 45% when the dyestuff concentration was in the range of 50–500 mg L^{-1}. However, with 500 mg L^{-1} dyestuff concentration, the color removal efficiency dropped to 45.24%.

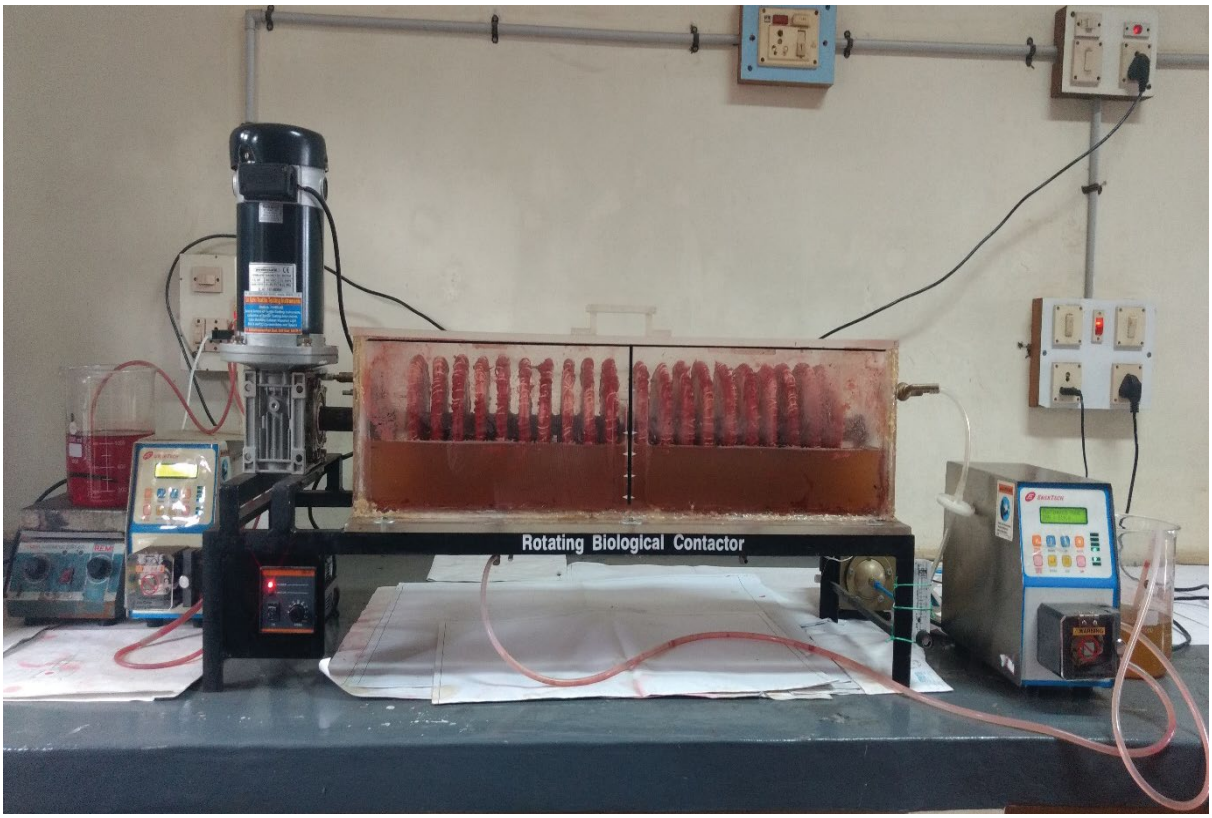

Fig. S10. Experimental setup of RBC reactor (after decolorization of industrial CR dye effluent). (Initial pH: 6; inlet COD: 1,824 mg L^{-1}; wheat bran dosage: 12.5 g L^{-1}; malt extract broth dosage: 10 g L^{-1}; glucose concentration: 5 g L^{-1}; flow rate of dye solution: 1 mL min^{-1}; air flow rate: 1.5 L min^{-1}; number of discs: 20; disc rotation speed: 16 rpm; disc submergence level in liquid medium: 40%; temperature: 303 K; effluent treatment time: 240 h; volume of liquid in the reactor: 12 L).

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