Biotreatment of azo dye containing textile industry effluent by a developed bacterial consortium immobilised on brick pieces in an indigenously designed packed bed biofilm reactor

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Abstract

This study highlights the development of a lab-scale, indigenously designed; Packed-Bed Biofilm Reactors (PBBR) packed with brick pieces. The developed biofilm in the reactor was used for the decolourisation and biodegradation of the textile industry effluent. The PBBR was continuously operated for 264 days, during which 301 cycles of batch and continuous treatment were operated. In batch mode under optimised conditions, more than 99% dye decolourisation and ≥92% COD reduction were achieved in 6 h of contact time upon supplementation of effluent with 0.25 g L^{-1} glucose, 0.25 g L^{-1} urea, and 0.1 g L^{-1} phosphates. A decolourisation rate of 133.94 ADMI units h^{-1} was achieved in the process. PBBR, when operated in continuous mode, showed ≥95% and ≥92% reduction in ADMI and COD values. Subsequent aeration and passage through charcoal reactor assisted in achieving ≥96 % reduction in COD and ADMI values. An overall increase of 81% in dye-laden effluent decolourisation rate, from 62 mg L^{-1} h^{-1} to 262 mg L^{-1} h^{-1}, was observed upon increasing the flow rate from 18 mL h^{-1} to 210 mL h^{-1}. Dye biodegradation was determined by UV-Vis and FTIR spectroscopy and toxicity study. SEM analysis showed the morphology of the attached-growth biofilm.

Highlights

Packed-Bed Biofilm Reactor with an indigenous consortium was developed for textile effluent treatment.

The use of developed consortia and optimisation resulted in more than 92% of COD and 99% of dye removal.

FTIR analysis was conducted for the detection and quantification of the dye metabolites.

SEM analysis was used to examine the morphology of the attached-growth biofilm.

The developed reactor was successfully run for 264 d with 301 cycles.

Introduction

Indian dyestuff industry fulfills more than 95% of the domestic requirements, out of which the textile industry consumes more than 60% of the dyes, and the remaining is used collectively by paper, leather, and other consumer industries (Agrawal et al., 2016). More than 50% of all the colourants used worldwide are azo dyes which cause severe environmental pollution problems by releasing toxic and potentially carcinogenic compounds into the aqueous phase and impose adverse effects on aquatic life (Ito et al., 2018; Koupaie et al., 2013; Talarposhti et al., 2001). It is also responsible for the potential danger of bioaccumulation that may eventually affect human beings through the food chain (Balapure et al., 2016).

Hence, dye remediation is a must for a safe and healthy environment. So far, different methods like adsorption, filtration, ozonation, coagulation, Photo-Fenton process, UV/NaOCl, ultrasonic irradiation, electrochemical oxidation, and UV/H_2O_2, etc. are in practice for dye remediation. Nevertheless, these

methods are either inefficient to degrade dye molecules completely or generate secondary pollutants (Joshi et al., 2020; Nouren et al., 2017; Palanivelan et al., 2019). Additionally, the diversity and complex molecular structures of dyes make wastewater from textile industries challenging to be treated by conventional aerobic and anaerobic technologies because compounds such as toxic aromatic amines can be generated (Torres-Farrada et al., 2018).

Cost-effectiveness and ecological compatibility make bioremediation of such effluents the most promising technology (Kumari et al., 2016; Unnikrishnan et al., 2019). Dye biodegradation mediated by bacterial species seems to be an eco-friendly and greener approach to environmental pollution problems. The dye degrading microbes are usually isolated from industrial effluents containing organic dyes or from the analogous and harsh environment surrounding the dye industry (Kabeer et al., 2019).

Microbial consortia have proved superior to pure isolates in some bio-refractory wastewater treatments. The target pollutants are expected to be entirely and rapidly mineralised by the possible co-metabolism amongst various types of microbial constituents of the consortium (Lu et al., 2009). In the case of the microbial consortia, the presence of various organisms can complement each other and collectively carry out biodegradation that cannot be achieved using pure cultures (Chan et al., 2012).

Many researchers have reported the aerobic and anaerobic processes for the treatment of dye effluent, and the anaerobic process has shown better results than aerobic processes (Ong et al., 2017). Previous investigations have already shown that azo dyes can be completely degraded and some intermediates such as aromatic amines with side groups ($-SO_3$, $-OH$, $-COOH$, $-Cl$) containing metabolites were quantitatively detected (Kolekar et al. 2012).

Many studies on biodegradation of the dye wastewater had been performed using different types of anaerobic reactor configurations, for instance, sequential batch reactors, fluidised beds, up-flow anaerobic sludge blanket reactors, rotating biological contactor, and fixed/packed bed reactors; and high decolourisation and degradation efficiencies were obtained (You et al., 2010). Fixed film processes have proven to be advantageous over suspended growth reactors because of their more responsiveness to the removal of xenobiotics, higher biomass retention, and maintenance of slow-growing microbes and activated sludge in terms of higher hydraulic loading rates and more excellent stability against shock loadings (Agrawal et al., 2019). However, the majority of the available data on the treatment of reactive dye-laden wastes describes the usage of various reactors for the treatment of either with single dye solution or simulated dye wastewater or dyeing effluent mixed with simulated municipal wastewater or actual dyeing effluent containing low concentrations (10–100 mg L^{-1}) of respective dye (Sheth and Dave, 2010).

Sharma et al. (2004) showed that the naturally available brick pieces could be utilised as an inert support matrix for developing and immobilizing bacterial biofilms for biodegradation and decolourisation of azo dyes in an immobilised cell bioreactor. Numerous microbial activities are mediated by bacterial biofilms, in natural as well as biotechnological circumstances and are thus successfully applicable to wastewater

treatment. The biofilm systems facilitate greater mechanical stability, stable operating performance, the potential for repeated use of the biomass, and excellent decolourisation abilities. Additionally, biofilms promote cell-cell interactions between cells of the same species and/or different species. This, in turn, enables biofilms to perform more efficient, diverse, and economically critical metabolic activities than their free-living counterparts (Anjaneya et al., 2013).

This article aims to investigate the performance of Packed-Bed Biofilm reactors (PBBR) packed with the pieces of refractory bricks for the treatment of azo dye-containing industrial wastewater, which to our knowledge: has not been addressed previously. For this purpose, COD and dye removal efficiencies were monitored. The FTIR and UV–Vis spectra of the treated and untreated effluent samples were compared to detect changes in the molecular structures of the dye present in the effluent. Furthermore, morphological observations were also performed, and the bacterial biofilm structure grown on surfaces of the packing medium was compared.

Materials And Methods

Chemicals, dyestuff, and industrial effluent used

Commercial grade triazo acid dye Acid Black 210 (AB210), Acid Black 194 (AB194), Reactive Red 223 (RR223), Reactive Orange 122 (RO122), Acid Yellow 23 (AY23), Reactive Blue 25 (RB25), Reactive Red 111 (RR111), Reactive Red 2 (RR2) were procured from the local market, Indore, M.P., India. Other dye samples Direct Yellow 44 (DY44), Direct Red 254 (DR254), and Acid Brown 15 (AB15), were procured from Harsha dyeing, Ahmedabad, Gujarat, India. Bushnell Hass broth, glucose, and yeast extract were obtained from HiMedia, India, and other chemicals were obtained from SD Fine Chemicals, India. All the chemicals used were of analytical grade.

The highly coloured effluent of the textile dyeing plant in Pithampur, Indore, containing various dyes, was collected in a sterile, airtight plastic container and was kept tightly stoppered. The collected effluent was transported to the laboratory and filtered through sterile Whatman filter paper no. 1 under the aseptic condition to remove large suspended particles and stored at 4 ± 1°C until processing to prevent contamination of non-indigenous microbes.

Acclimatisation and development of dye decolourising microbial consortium

In the present day, bioremediation relies upon the pollutant degrading capacities of the naturally occurring microbial consortia in which bacteria play crucial roles. Microbial consortia are usually used without analysing the constituent microbial populations for environmental remediation, and the complexity of the microbial consortium enables them to act on a variety of pollutants (Asgher et al., 2007).

Five effluent samples designated as WW-I to WW-V were collected from Textile and Dyeing Industries near Indore, M.P. India. They were mixed in equal volumes to prepare a composite sample. For the development and acclimatisation of the microbial consortium capable of dye decolourisation, the sample was inoculated in the Bushnell and Hass basal medium (BHM) of pH 7.0 containing the following ingredients in g L^{-1}: $MgSO_4$, 0.2; $CaCl_2$, 0.02; KH_2PO_4, 1.0; K_2HPO_4, 1.0; $(NH_4)_2NO_3$, 1.0; $FeCl_3$, 0.05; 4% NaCl supplemented with glucose 1.0 g L^{-1} and yeast extract 1.0 g L^{-1} as carbon and energy source with 10% v/v the industrial effluent. The process was repeated for ten sequential transfers. The developed acclimatised and stabilised mixed microbial cultures were used for further studies.

Decolorisation of different dyes by the consortium

A series of 250 mL Erlenmeyer flasks were taken containing Bushnell Hass broth supplemented with glucose 1.0 g L^{-1}, yeast extract 0.5 g L^{-1}, and 50 mg L^{-1} of eleven different dyes individually. The different dyes used were namely; AB210 (triazo, trisulfonated), AB194 (monoazo, monosulfonated), RR223 (monoazo, tetrasulfonated), RO122 (monoazo, tetrasulfonated), AY23 (monoazo, monosulfonated), RB25 (metal complex, phthalocyanine), RR111 (diazo, vinyl sulfone), DY44 (diazo, monosulfonated), DR254 (diazo, disulfonated), AB15 (diazo, disulfonated), RR2 (monoazo, disulfonated). The text in the brackets indicates the number of azo bonds present in individual dye molecules and dye sulfonation. All the flasks flask containing different dyes were inoculated with the 10% v/v (containing ~ 10^8 cells/ml) of the activated culture of the indigenous consortium and incubated at 32 ± 2 ºC under static conditions. Uninoculated controls for each dye sample were also incubated under similar conditions. The extent of dye decolourisation by the developed activated consortium was determined spectrophotometrically at the maximum absorbance of individual dye in the cell-free supernatant, obtained after centrifugation at 6000*g* for 10 min (Agrawal et al., 2014).

Designing of the bioreactor

PBBRs permit high volumetric biomass concentration to facilitate the biodegradation of toxic and recalcitrant organic compounds and provide stability against shock loadings. However, the PBBR experiences some severe drawbacks, such as the non-uniform distribution of oxygen and nutrients across the reactor bed (Agrawal et al., 2019).

Specifications are as shown in Table 1; an indigenously designed laboratory-scale PBBR vessel, composed of a polyvinyl chloride (PVC) column, was set up to study biodecolorisation and biodegradation efficiency of the developed consortium with real textile industry effluent. The column established was with a height of 40 cm, an inner diameter of 4.16 cm, and a total volume of 500 mL. The lower end of the reactor vessel was provided with an inlet, and the upper outlet was sealed with rubber cork bored at the centre as an outlet. The cork was fitted with a rubber tube and an adapter for collecting the treated sample. The reactor was mounted perpendicularly to the tabletop. The flow rate was adjusted with the help of a peristaltic pump affixed to the inlet tube.

Table 1
Details of the indigenously designed PBBR for the treatment of textile industry effluent.

Specification	Dimension/quantity	
	PBBR	Charcoal filter
Inner diameter (cm)	4.16	5.56
Outer diameter (cm)	5.18	6.12
Height (cm)	40	24
Total volume (mL)	500	650
Void volume (mL)	200	250
Packing material	Brick pieces	Charcoal
Size (cm)	0.4-1.0	0.4-1.0
Height occupied by packing material (cm)	35	20
No. of pieces	300	656

The pieces (0.4-1 mm size) of refractory brick obtained from a local brick kiln were used as a support matrix to immobilise the developed bacterial consortium due to their high porosity and inertness. The brick pieces were thoroughly washed with tap water to get rid of salt/soil deposited in the cavities. The brick pieces were then dried, at 45 ± 2º C for 24 h, before filling into PBBR column, leaving a total void volume of 200 mL.

The laboratory-scale PBBR was operated in batch as well as continuous mode. The reactor specifications are described in Table 1, and Fig. 1 shows the diagrammatic representations of the reactor.

The activated culture of the indigenously developed consortium in the BH broth was added to the PBBR to form the biofilm and incubated at room temperature (32 ± 2°C) for 15 d. The spent medium was removed from the PBBR, and the fresh sterile medium was added every 48 h of intervals. The experiments were repeated in the same manner, after 15 days of incubation, the developed biofilm was acclimatised to increasing concentration of dye-containing textile industry wastewater added to the nutrient broth, every day from 10% with an increment of 10% at each addition till it reached to100% textile industry effluents, with an incubation of 48 h between successive additions. After the establishment of active biofilm till the 35th day, the working volume of the PBBR was replaced with fresh BHM (supplemented with glucose 1.0 g L^{-1} and yeast extract 0.5 g L^{-1}) for 7 d (42nd day) for acclimatisation and adaptation of biofilm to inorganic nutrients, followed by successively increasing concentration of textile industry wastewater added to BHM every day from 10% and next time with an increment of 10% till the addition reached to100% textile industry effluents wastewater on 52nd day.

Batch decolourisation of dye effluent in the PBBR

The performance of active biofilm was assessed as a decrease in the colour intensity of the effluent coming out from the bioreactor compared to the colour intensity of the effluent fed into the reactor. On decolourisation of added effluent, 200 mL of the system volume was again replaced with 200 mL of the undiluted effluent. The process was repeated for 28 cycles till stable decolourisation and COD reduction was observed. After stabilisation, the reactor was fed with fresh wastewater after complete decolourisation, and in this manner, the reactor was operated for 60 days.

Increasing concentrations of glucose, urea and an equimolar mixture of K_2HPO_4 and KH_2PO_4 were added with three successive fresh additions of the effluent to enhance the decolourisation rate and substantial reduction in COD in the system.

The treated dye manufacturing industrial effluent from the PBBR was then subjected to agitation in a 500 mL Erlenmeyer flask at 150 rpm at 32 ± 2°C for 12 h. After aeration, the effluent was passed through a column filled with furnace charcoal (average particle size 0.4-1 mm diameter) for adsorption of the remaining colour and dissolved solids before it was discharged. Therefore, the effluent from PBBR was trickling through a column packed with charcoal. The details of the column are also listed in Table 1. The effluent coming out of this column was also analysed for COD and colour reduction.

Continuous decolourisation of dye-containing industrial effluent in a packed bed reactor

A laboratory-scale continuous packed bed biofilm bioreactor (PBBR) was operated in this study. The decolourisation of dye-containing industrial effluent was achieved in a PBBR at different flow rates to give desired hydraulic retention time (HRT). The decolourisation of the effluent, having a COD value of 9937 mg L^{-1} and ADMI value of 3100, was conducted at an HRT of 6 h, which corresponds to a flow rate of 35 mL h^{-1} and an organic loading rate of 1250 mg L^{-1} h^{-1}. The packed bed biofilm bioreactor was operated at room temperature and pH 7.2 ± 0.2. The decolourisation process was carried out by the continuous supply of the dye-containing effluent supplemented with desired nutrients through the top of the column with the help of a peristaltic pump. The effluent was continuously collected from the sidearm situated just below the packed bed and analysed to calculate the decolourisation activity and COD reduction (51 days, a total of 301 days), followed by agitation and aeration in a 500 mL Erlenmeyer flask at 150 rpm at room temperature (32 ± 2°C) for 12 h.

Aeration and Charcoal reactor

The anaerobic process could reduce the azo bond of the dye molecule and the intermediate compounds that are generated could be further mineralized through the aerobic process. Thus, a sequential anaerobic followed by aerobic treatment technique is considered very efficient in the mineralization of dye effluent. The combination of anaerobic and aerobic processes can be realized either using two separate treatment tanks or in a single treatment tank Ong et al., (2017).

The treated dye manufacturing industrial effluent from the PBBR was then every time subjected to agitation in a 500 mL Erlenmeyer flask at 150 rpm at room temperature (32 ± 2°C) as described by Chan et al., (2012) and Ong et al., (2017), but only for 12 h, instead of 48h and 3 h, respectively. After aeration, the effluent was then passed through a column filled with furnace charcoal (0.4-1 mm diameter) for adsorption of the remaining colour and dissolved solids before it was discharged. Therefore, the effluent from PBBR was trickling through a column packed with charcoal as described by Dave and Dave, (2009); the details of the column are also listed in Table 1. The effluent coming out of this column was also analysed for COD and dye reduction.

Effect of flow rate

The column was attached to a reservoir of effluents through which effluent was pumped into the reactor with the help of a peristaltic pump. The decolourisation process was carried out by the continuous supply of the effluents through the upper end of the column at different flow rates (18–210 mL h^{-1}). The treated effluent was continuously collected from the sidearm below the packed bed and analysed to calculate the decolourisation activity. The dilution rates are calculated as follows:

$$Dilution\ Rate\ (D) = \frac{Flow\ rate\ of\ influent\ into\ the\ packed\ bed\ bioreactor\left(^{mL}/_{h}\right)}{Volume\ of\ influent\ into\ the\ packed\ bed\ bioreactor}$$

Toxicity study

The use of untreated and treated dyeing effluents in agriculture has a direct impact on the fertility of the soil. Thus, it was of concern to assess the toxicity of the dye before and after its degradation.

Phytotoxicity

The plant seeds, namely *Cicer arietinum* L. (chickpea) and *Vigna radiata* L. (green gram) seeds were purchased from local markets, Indore, India. Phytotoxicity was studied at room temperature. Ten seeds each of *Cicer arietinum* L. and *Vigna radiata* L. were treated separately with 10 mL of the PBBR treated, PBBR-charcoal reactor treated, untreated textile effluent, and control (distilled water) in three separate systems to each respective seed and after 7 d, germination (%), length of radical (cm) and plumule (cm) were measured (Patel et al. 2018).

Microbial toxicity

The microbial toxicity of the PBBR treated, PBBR-aerated-charcoal reactor treated, untreated textile effluent, and control (distilled water) was carried out by agar-well diffusion method, in three separate systems, using *Bacillus subtilis*, *Escherichia coli*, *Azotobacter* sp. and phosphate solubilising strains separately, and the mean of inhibition zone (diameter in cm) was recorded after 24 h of incubation at 30 ± 2◦C (Agrawal et al., 2014a). Microbial cultures of *E. coli*, *B. subtilis*, *Azotobacter*, and phosphate solubilizers were isolated from the soil.

Analytical studies

Decolorisation assay

The colour removal was reported as percent decolourisation and was expressed as Eq. 1:

Decolorization (%) = $[(A_0 - A_t) / A_0] \times 100$ (1)

where A_0 is the absorbance of the dye solution initially and A_t is the absorbance of the dye solution at the time (t) (Agrawal et al., 2014a).

Chemical oxygen demand (COD) of the influent and effluent samples of the bioreactor was measured by the procedures outlined in standard methods (APHA 1998; Sheth and Dave, 2009). The American Dye Manufacturers' Institute (ADMI 3WL) tristimulus filter method was used to measure the decolourisation of the textile industry effluent. To observe the reduction in ADMI value in the textile industry effluent and reactor effluent after biotreatment, an aliquot of 3 mL was withdrawn at an interval of 24 h, and percent transmittance at 590 nm, 540 nm, and 438 nm wavelengths was measured. ADMI removal percent (%) is the ratio between the ADMI removal value at any contact time and the initial ADMI value described by Agrawal et al. (2015; 2016a).

SEM analysis

The refractory brick pieces were collected from the reactor after biofilm formation by the consortium and washed twice with 50 mM phosphate buffer (pH 6.8) for 20 ± 1 min. Subsequently, the washed biofilms were dehydrated in a gradient of ethanol solutions (20, 40, 60, 80, and 100% ethanol) for 10 min each and stored in 10% ethanol till analysis. Gold coating of the dried brick pieces was carried out in a vacuum by evaporation in order to make the samples conducting. The control refractory brick pieces devoid of the biofilm were also prepared similarly. The gold-coated samples were examined for SEM (Zeiss, Germany) images at 20 kV (Anjaneya et al., 2013) to detect attached microorganisms.

Organic characterization of biofilm

The biofilm developed on the surface of brick pieces was analysed for the exopolysaccharide content and ability to retain cells as described by Anjaneya et al., (2013). The EPS formed by the consortium biofilm present on the surface of brick pieces (10 g) was dissolved in 50 mL distilled water and centrifuged at 10,000 g for 20 min at 4°C to eliminate insoluble materials and the supernatant was used for EPS analysis. The reducing sugar and protein content of EPS was determined by using the DNS (Miller, 1959) and Lowry et al., (1951) method.

Additionally, the leakage of planktonic cells from the consortium biofilm was analysed by the plate count method. The leaked cells during the operation of the batch as well as continuous packed bed bioreactor, which was operated at a flow rate of 35 mL h^{-1}, 200 µL of the serially diluted effluent sample were plated on nutrient agar plates and CFU was determined by the standard plate count method.

UV–Visible and FTIR analysis

UV-visible spectral analysis and FTIR studies were carried out on the model azo dye AB210 to ensure the decolourisation and degradation capabilities of the developed consortium. A 250 mL Erlenmeyer flasks containing 100 mL Bushnell Hass broth (pH 7.0) as a growth medium supplemented with glucose 1.0 g L^{-1}, yeast extract 0.5 g $L^{-,1}$, and 50 mg L^{-1} of AB210 dye was inoculated with 10% of the activated inoculum contacting 10^8 cells ml^{-1} of the consortium and were incubated at 32 ± 2 °C for 24 h. All the flasks were incubated at 32 ± 2 °C under static conditions. UV-Vis and FTIR analysis were studied by analysing the dye-containing system and control (without dye) after centrifugation at 10,000*g* for 10 min at 4 °C. The peak differences between untreated and treated dye were recorded using a UV-Vis spectrophotometer as described in Agrawal et al. (2014).

The decolourised broth after cell separation was extracted twice using double volumes of ethyl acetate and concentrated in a rotary vacuum evaporator and was determined as described earlier by Patel et al. (2018). The FTIR (Fourier Transform Infra-Red) spectra of the dye AB210 and extracted metabolites were obtained in the mid-IR region (400–4000 cm^{-1}) at 16-scan speed (Shimadzu, FTIR-8400S, Japan) to study the structural transformations in the dye molecules present in the effluent during the process of dye biodegradation.

Statistical analysis

One-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparison tests, using MS-Excel (Free version), was used to analyse data. Readings were considered significant when the *p*-value was ≤ 0.05.

Results And Discussion

Decolorisation of different dyes by the consortium

Decolorisation studies were conducted with eleven different dyes, namely; AB210, AB194, RR223, RO122, AY23, RB25, RR111, DY44, DR254, AB15, and RR2; inoculated with the developed consortium showed ≥ 60% decolourisation of different dyes (Fig. 2). The maximum decolourization and degradation were observed with the dye DY44 (~ 99.66%), whereas the minimum decolourization effect was seen on RO223 dye (~ 89.66%). The observed variation in the decolourisation of different dyes by the developed indigenous consortium might be attributable to the structural diversity of the dyes and due to the various enzymatic activities of the consortium.

The dyes with simple structures and low molecular weights were decolourised faster than the dyes with complex structures and/or high molecular weight. The presence of sulfonates in the azo dyes further increases resistance to dye decolourisation. In our study, the indigenously developed consortium decolourise dyes containing one or two azo bonds and lesser sulfonate groups (RO122, RR223, AY23, RR2, and AB194). However, RO122 and RR223 have four sulfonate groups and thereby have a decreased extent of decolourisation. RB25 has a copper metal complex, so its rate of decolourisation was also less. However, this study shows the efficiency of the developed consortium to decolourise varieties of dyes due

to adaptation and selection of microbial population and demonstrated the suitability of the developed consortium to treat textile industry effluents which are usually laden with different types of dyes.

UV–Vis analysis of model dye

The UV–Vis spectrum of the treated and untreated model azo dye AB210 and the extracted metabolites are presented in Fig. 3. The characteristic absorbance peak of the AB210 dye at 605 nm was almost completely removed in samples from both the reactors and the UV-region absorbance area was increased after the anaerobic reaction. This indicates that the azo bond reduction through an anaerobic reaction phase was the primary mechanism for the decolourisation of the dye. The contribution of the physical adsorption in decolourisation was negligible.

FTIR spectral analysis of model dye

A comparison of the FTIR spectrum of AB210 (Fig. 4a) with degraded metabolites (after 24 h) indicated the biodegradation of the dye by the consortium. Peaks in the control spectrum showed an aromatic O-H stretch at 3435.26 cm^{-1}, and the peak at 1618.33 cm^{-1} showed an N = N stretch (indicates the presence of azo bonds in dye). The presence of N-H deformation of secondary amines at 1552.30 cm^{-1}, O-H deformation of phenols was observed at 1411.31 cm^{-1}, S = O stretch was observed at the peak of 1318.39 cm^{-1}, 1211.21 cm^{-1}, 1045.26 cm^{-1} (supports that the dye contains the sulfonated compound), peak at 1498.33 cm^{-1} indicates C = C bond stretching of multiple aromatic bonds. A peak indicates c-Cl stretching at 671.43 cm^{-1} and 892.51 cm^{-1}. The peaks at 834.50 cm^{-1} and 798.47 cm^{-1} indicate C-H deformation and ring stretching of trisubstituted benzene.

The FTIR spectra of metabolites (Fig. 4b), extracted after treatment with the consortium, showed the disappearance of the sharp peak at 1618.33 cm^{-1} specific for azo compounds, which confirms the cleavage of azo bonds of the dye. The peak at 3368.78 cm^{-1} indicates the O-H stretching of alcohol. The peaks at 2958.73 cm^{-1}, 2927.71 cm^{-1}, and 2857.76 cm^{-1} show the C-H stretching of aldehydes. A sharp peak at 1727.71 cm^{-1} with a supporting peak at 1384.76 cm^{-1} indicates O-H stretching/deformations of aromatic alcohols, while the peak at 1273.73 cm^{-1} indicates O-H deformation of secondary alcohols. The peak at 1460.76 cm^{-1} and 1073.75 cm^{-1} indicates the C = C stretching of multiple aromatic bonds. A peak indicates C-OH stretching of aromatic alcohol at 1038.75 cm^{-1}. The peak at 1123.75 cm^{-1} shows C-H deformation of substituted benzene, and 771.80 cm^{-1}, 743.80 $cm^{-1,}$ and 704.81 cm^{-1} indicate C-H deformations of substituted benzenes.

The appearance of new peaks in degraded metabolites and the disappearance of the peak of the control spectrum confirmed the degradation of dye.

Scanning electron microscopy

SEM was used to study the morphological features and surface characteristics of the adsorbent materials. The brick pieces used had a highly irregular and porous surface, providing a larger surface area

facilitating immobilisation and biofilm formation by microbial cells (Fig. 5a). The obtained SEM images revealed the formation of bacterial biofilm on the surface of the brick pieces. It is observed that the bacterial biofilm cells are well distributed on the surface of brick pieces that would favour the formation of biofilm. The bacterial biofilm consisted of a heterogeneous population of short and long rods, cocci, and filamentous organisms. Clusters of bacterial spheres embedded in an extracellular polymeric matrix were evident (Fig. 5b-d).

Naturally available seashells have also served as good matric material to form a bacterial biofilm system to decolourise the Acid Violet-17 by a bacterial consortium in an up-flow immobilised cell bioreactor (Sharma et al. 2004). Similarly, degradation of Reactive Red 120 using *Bacillus cohnii* RAPT1 immobilised on polyurethane was studied by Padmanaban et al. (2016). Bioremediation of Congo Red dye in immobilised batch and continuously packed bed bioreactor by *Brevibacillus parabrevis* using coconut shell bio-char is also reported (Talha et al. 2018). Azo dye decolourisation under high alkaline and saline conditions by *Halomonas* sp. was studied (Montañez-Barragán et al. 2020) with volcanic rocks as packing material in a PBR.

Biofilm organic analysis

The protein and carbohydrate content of the EPS formed by the consortium biofilm present on the surface of brick pieces is shown in Table 2. The protein and carbohydrate composition of EPS formed on the surface of the brick pieces used in the column during the batch phase was found to be 11.3 mg L^{-1} and 6.5 mg L^{-1} respectively in 10 g biofilm containing brick pieces after the decolourization of effluent in PBBR batch process, whereas, they were 14.6 mg L^{-1} and 9.1 mg L^{-1} in a continuous process. The EPS plays an important role in the attachment of cells to the surface of brick pieces, positively correlated with biofilm development and possibly by protecting the individual cells in the biofilm against the detrimental environment.

Table 2
Organic characterization of the matrix extracted from biofilms.

Parameter	Batch phase	Continous phase
Biomass (mg/g of brick pieces)	37.6 ± 0.22	38.3 ± 0.61
Protein (mg/L)	11.3 ± 0.81	14.6 ± 0.17
Carbohydrate (mg/L)	6.5 ± 0.43	9.1 ± 0.49
Leakage of cells (CFU/mL)	10.45×10^6	12.01×10^8

Similar studies were conducted by Anjaneya et al., (2013) on the biofilm developed by a consortium on sheep bone chips filled in a packed bed bioreactor. The total amount of EPS and proteins are found to be 80 mg L^{-1} and 135 mg L^{-1} respectively in 30 g of consortium bone chips after the decolourization of Amaranth dye in a packed bed reactor.

The cell leakage studies in PBR revealed that 1.04 x 10^8 CFU mL^{-1} of cells were released when the reactor was operated with a decolourization medium in batch mode, whereas 1.2 x 10^{10} CFU mL^{-1} of cells were released in the effluent when the reactor was operated under continuous mode with a flow rate of 35 mL h^{-1} (Table 2). From these results, it was evident that the adhered cells to the surface of brick pieces were continuously involved in the formation of biofilm in a packed bed reactor.

The cell leakage studies on packed bed reactor established by Anjaneya et al., (2013), on the biofilm developed by a consortium on sheep bone chips for the decolourization of Amaranth dye, revealed that 18.75x10^9 CFU ml^{-1} of cells were released when the reactor is operated with decolourization medium containing peptone, yeast extract, and dye at a flow rate of 50 mL h^{-1}.

Biodegradation and decolourisation in PBBR in the batch process

The variation of COD removal efficiency and dye decolourisation rates during the whole operation period of the PBBR are presented in Fig. 6. The COD and ADMI removal efficiency of the reactor in the first cycle was 69.16–84.78% from the effluent having a COD value of 7500 mg L^{-1} and ADMI value of 2674. On the 31st day of the batch operation, the reactor showed signs of stability. Further studies were conducted upon stabilisation of the reactor under steady-state conditions when the bioreactor performance parameters like COD, pH, and colour removal were relatively constant. According to the results shown in Fig. 6a, the maximum fraction of COD was removed from the anaerobic biofilm reactor stage. The COD removal efficiency (averaged at the 5 days of the operation period) of the reactor was 47.56–82.11% after 120 days. The average reduction in COD and decolourisation was 55 ± 8% in 22 h (Fig. 6b) for the first five cycles. The average percent decolourisation of the effluent increased during successive cycles, as evident from the values calculated over every subsequent 5 cycles. The decolourisation finally reached 93.99%, and COD reduction was 82.11% after 60 days of the batch operation. The wastewater COD and ADMI value which was 7500 mg L^{-1} and 3135 respectively, initially was reduced to 1341.75 mg L^{-1} and 188.41 mg L^{-1}, respectively during the last five cycles of operation for 60 days.

The degradation of reactive dyes was reported to be maximum in the system involving anaerobic and aerobic phases; the efficiency of the degradation depends on the microaerophilic condition provided by the matrix (Padmanaban et al., 2016). Bioremediation of Congo Red dye by immobilised *Brevibacillus parabrevis* using coconut shell bio-char packed bed bioreactor in batch mode was studied by Talha et al. (2018). The immobilisation of bacteria on coconut shell biochar gave a 63.23% removal for up to 300 mg L^{-1} of Congo Red dye concentration.

A reticulated sponge from the dried fruit of Loofa was used as a carrier for the immobilisation of *Lysinibacillus* sp. RGS to decolourise sulfonated azo dye Blue HERD and 50% actual textile effluent in an up-flow column bioreactor in batch mode. Another study showed 200 mg L^{-1} Blue HERD (flow rate of 40 mL h^{-1}) and effluent completely decolourised at 50% concentration in Waste Yeast Biomass Medium with a flow rate of 15 mL h^{-1} (Bedekar et al., 2014).

Effect of nutrient addition on reactor performance

Supplementation with the nutrients (Table 3) resulted in a marked improvement in the decolourisation rate and a reduction in the average time required to achieve the desired results. For 93.99% decolourisation and 79.16% COD removal, the required time was reduced from 22 h to 9 h for the successive 18 cycles with the addition of 0.25 g L^{-1} glucose as a nutrient. However, any further increase in the glucose concentration did not improve the dye removal efficiency in the PBBR system. The reduction in the reactor performance at lower glucose levels might be due to the inadequate availability of glucose to the microorganisms located deep inside the biofilm present in the pores of the brick pieces, leading to nutrient and electron donor deprivation conditions for these microorganisms.

Table 3
Effect of nutrient addition on effluent decolourisation and COD reduction in PBBR by the indigenously developed consortium.

Nutrient concentration ($g\ L^{-1}$)	COD reduction (%)	Time for complete decolourization (h)	Decolorization rate ($mg\ L^{-1}\ h^{-1}$)
Glucose only			
0.0	82.11 ± 1.6	22	227.25
0.1	82.96 ± 1.0	12	416.62
0.2	83.32 ± 2.3	10	499.95
0.25	84.76 ± 1.6	9	555.50
0.3	81.98 ± 0.9	9	552.74
0.35	79.16 ± 1.1	9	549.36
Glucose + Urea			
0.25 + 0.1	84.76 ± 1.9	9	555.50
0.25 + 0.2	86.98 ± 2.2	8	624.94
0.25 + 0.25	91.03 ± 1.6	7	714.21
0.25 + 0.3	91.11 ± 0.7	7	712.65
0.25 + 0.4	91.35 ± 2.3	7	714.87
Glucose + Urea + (KH_2PO_4 + K_2HPO_4)			
0.25 + 0.25 + 0.10	92.43 ± 2.6	6	833.25
0.25 + 0.25 + 0.20	92.51 ± 1.6	6	834.77
0.25 + 0.25 + 0.30	93.03 ± 2.9	6	836.92

The addition of urea (0.25 g L^{-1}) and glucose facilitated a maximum of 91.35% COD removal and 95.86% decolourisation of the effluent in 7 h. It is observed that the addition of nitrogen at 0.3 and 0.4 g L^{-1} showed ~ 91% reduction in COD with insignificant change in decolourization time and decolourization rate. The optimum level leads to a rapid decrease in percentagedecolourizationn. The decrease in percentage decolourization might be due to the reduction reaction involving the nitrogen in the medium and the nitrogen in the dyes (Karthikeyan et al., 2010).

Similarly, the addition of 0.25 g L^{-1} glucose, 0.25 g L^{-1} urea, and 0.1 g L^{-1} phosphates facilitated 92.43% COD removal and 99.70% decolourisation in 6 h. Adding glucose, urea, and phosphorus alone to higher

concentrations did not support higher COD removal and decolourisation of the effluent. The reactor with this optimised nutrient content was further operated for 25 days with 3 cycles each day.

During the initial cycles, the culture in the biofilm gradually acclimatised to the constituents of the dye-laden effluent, which led to a gradual decrease in the decolourisation time. The observed improvement after adding nutrients could be obviously due to the availability of carbon, nitrogen, and phosphorus sources responsible for faster growth of the organisms and metabolic activities which resulted in improved dye decolourisation rate (Sheth and Dave, 2010). Another reason for the improved dye decolourisation rate might be the stimulation of the electron transfer processes through the oxidation-reduction reactions (Santos et al., 2016) in the PBBR.

PBBR continuous process

After stabilization of the PBBR in the batch process, the wastewater treatment was carried out in continuous mode for 51 cycles for an effluent having a COD value of 8713 mg L^{-1} and an ADMI value of 3100. The COD of the effluent increased to 9937 mg L^{-1} upon the addition of nutrients. Figure 7 (a,b) shows the reduction in COD, ADMI values, final effluent COD, and dye concentration values, for 51 cycles averaged over every third cycle. During the continuous mode of operation the PBR showed stabilized and consistent performance with an average reduction of 92.59% and 95.13% reduction in COD and ADMI values, respectively. The COD and ADMI values of the effluent after treatment in PBBR during the period of continuous operation were 736.16 mg L^{-1} and 147.98 units, respectively.

The effluent treated in PBBR was then subjected to aeration and passed subsequently through the charcoal column. Figure 8 (a,b) shows the reduction in COD, ADMI values, final effluent COD, and dye concentration values, for 51 cycles averaged over every third cycle. An additional 4.66 ± 1.08% reduction in COD and 0.77 ± 2.37% reduction in ADMI units (averaged over 51 cycles), was observed. The COD of the ready-to-discharge effluent after this three-step treatment was 240.36 ± 94.10 mg L^{-1} and 124.03 ± 73.36 ADMI units (averaged over 51 cycles). A very insignificant decrease of 2.45%±0.76 in the efficiency of the charcoal reactor was noticed during the operation of the 51 cycles.

The COD value of the untreated waste, after nutrient addition, was around 9937 mg L^{-1} and 3100 ADMI units, whereas that of the waste treated in PBBR was as low as 736.16 mg L^{-1} and 147.98 units mg L^{-1} only and it was further reduced to a COD of 262.09 mg L^{-1} upon aeration and passage through the charcoal reactor. Similarly, the dye content in the untreated wastewater was 3100 units which was reduced to 124.03 units, and finally to 110.01 ADMI units after sequential passage through PBBR, aeration, and charcoal reactor. The three-step biological treatment including PBBR, aeration, and activated charcoal, given to the effluent, the latter resulted in a 97.29% reduction in COD and a 96.45% reduction in ADMI units in the textile industry wastewater. Even the treatment of aeration and activated charcoal was found to be equally efficient; however, the cost of activated charcoal is the main constraint for its application.

Thus, activated charcoal could be used after biological treatment so that more waste could be treated with almost 98% decolourization. As the COD of the biologically treated waste is very less than the untreated waste, the requirement for activated charcoal could be reduced by 10-fold. Thus, it could make the dye bioremediation process economically viable.

The degradation of Reactive Red 120 using *Bacillus cohnii* RAPT1 immobilized on polyurethane was studied by Padmanaban et al. (2016). Under optimum conditions (36 h, initial inoculum size $3x10^8$ colony-forming units per mL, 8.0 pH, and 35°C) 100% of 200 mg L^{-1} of the dye was removed within 4 h.

A synthetic, simulated mixed dye waste (Basic Yellow 28, Basic Yellow 21, Basic Red 18.1, Basic Violet Red 16, Basic Red 46, Basic Blue 16, Basic Blue 41) representing a known waste from a fiber production factory was investigated for colour removal using a two-phase anaerobic packed bed seeded with anaerobic digesting domestic sewage sludge. The results of this study showed that a 2-stage mesophilic anaerobic up-flow packed bed reactor can remove up to 90% of the colour from a mixed cationic dye-containing 1000 mg L^{-1} of dye (Talarposhti et al., 2001).

Similarly, degradation of simulated textile wastewater containing 6 different azo dyes (COD 7200 mg L^{-1} and dye concentration 300 mg L^{-1}) was studied in a microaerophilic fixed film reactor using a pumice stone as a support material under varying hydraulic retention time and organic loading rate (OLR). The intense metabolic activity of the inoculated bacterial consortium in the reactor led to 97.5% COD reduction and 99.5% decolourization of simulated wastewater operated under OLR of 7.2 kg COD m3 d^{-1} and 24 h of HRT (Balapure et al., 2015).

Effect of flow rate

Results of continuous mode operation of PBBR and charcoal reactor with variations in flow rate are shown in Fig. 9. More than 90% decolourization of the influent was obtained. However, with an increase in flow rate, a successive decrease in COD and ADMI reduction efficiency was noticed. A 37.71% and 43.43% reduction in COD and ADMI value, respectively, was observed at a flow rate of 210 mL h^{-1} with an HRT of 1 h. However, an overall increase in the dye decolourization rate, from 62 mg L^{-1} h^{-1} to 262 mg L^{-1} h^{-1}, was observed with an increase in flow rate from 18 mL h^{-1} to 210 mL h^{-1}. This could be due to the decreased contact time between the active biofilm in the reactor and the influent wastewater. The effect of flow rate on the performance of the PBBR in continuous mode is shown in Fig. 9.

Many researchers have successfully developed various microbial systems for the effective decolourization of several dyes with different bacteria. However, the majority of the studies concentrate on the usage of either single dye or simulated dye effluent or real dye effluent with complex media. However, the packed bed bioreactor described in this study is a promising alternative method for an economical method of treating the actual textile dye effluent supplemented with simple nutrients. The bacterial consortium used in this study is capable of withstanding non-sterile conditions present at the start-up and operating cycles. The anoxic conditions in a packed bed bioreactor provided a suitable

environment for the decolourization of azo dyes, as the chromophore of azo dyes is susceptible to reduction under anaerobic conditions.

Toxicity study

Phytotoxicity of the textile industry effluent, the effluent treated in PBBR and charcoal reactor was studied and it was observed that, because of the toxicity of the untreated textile effluent, germination rate, root length and shoot length were less in the case of *Cicer arietinum* L. (chickpea) and *Vigna radiata* L. (green gram) seeds exposed to the textile effluent exposed to PBBR treated textile effluent and distilled water. At the same time, the seeds exposed to treated effluent from the charcoal reactor effluent showed a similar germination rate, root length, and shoot length as those that were exposed to distilled water (Table 4).

Table 4
Toxicity study of real textile effluent and effluent treated in PBBR and PBBR-charcoal reactor.

Analysis	Parameter analyzed	Control	Textile dye effluent		
			Untreated	PBBR treated	PBBR-aerated-charcoal treated
Phytotoxicity					
	Cicer arietinum L				
	Germination (%)	100	0	90	100
	Root (cm)	3.10 ± 0.42	-	2.86 ± 0.33	3.01 ± 0.27
	Shoot (cm)	3.06 ± 0.37	-	2.14 ± 0.39	2.91 ± 0.32
	Vigna radiata L.				
	Germination (%)	100	0	90	100
	Root (cm)	4.14 ± 0.41	-	3.01 ± 0.25	3.92 ± 0.39
	Shoot (cm)	2.19 ± 0.28	-	1.98 ± 0.18	2.16 ± 0.31
Microbial Toxicity	Zone of Decolourization				
	E.coli	0.80 ± 0.01	1.60 ± 0.02	0.91 ± 0.03	0.81 ± 0.03
	Azotobacter sp.	0.80 ± 0.02	1.56 ± 0.05	0.94 ± 0.03	0.88 ± 0.03
	Phosphate-solubilizer	0.80 ± 0.01	1.53 ± 0.01	0.90 ± 0.01	0.81 ± 0.01
	Cellulase degrader	0.81 ± 0.03	1.71 ± 0.04	0.92 ± 0.04	0.83 ± 0.04

#: No germination and rotting of seeds. Values are the mean of three experiments and SD (±) is significantly different from the control at, *P < 0.001, by one-way analysis of variance (ANOVA) with Tukey–Kramer comparison test.

Similar results have been observed by Patel et al., (2018) while studying the reduction in toxicity of two simulated wastewaters containing 1:1 metal complex acid dyes namely, Acid blue 158 and Acid black 52, separately, by a developed bacterial consortium. Toxicity was found negligible in the case of seeds of *Sorghum bicolor* as 100% seed germination was observed whereas, in the case of *Cicer aeriatinum*, seed germination was inhibited by 20 and 60% with untreated AB158 and AB52, respectively.

Microbial toxicity study

The microbial toxicity study showed that the biggest zones of inhibition in all the tested four bacterial cultures (*E. coli*, *B. subtilis*, *Azotobacter* sp., and phosphate solubilizers) were observed with untreated textile industry effluent. Compared to untreated effluent the PBBR-aerated-charcoal reactor treated effluent did not exhibit any growth inhibition, which showed the formation of non-toxic metabolites after degradation of the dyes present in the textile industry effluent and removal of the residues during aeration and passage through the charcoal column (Table 4).

Both microbial toxicity and phytotoxicity assays revealed the less toxic nature of PBBR-aerated-charcoal reactor-treated effluent. This indicated that the treated effluent, when discharged to water bodies, might not cause any harmful effects to its surroundings.

Conclusions

The dyes, pigments, and auxiliary chemicals are extensively used in the textile industries, and their effluent discharge is of significant health and environmental concern. The presented investigation was focused on the preparation of bacterial biofilms on brick pieces, decolourisation, and remediation of textile industry effluent by consortial biofilm and its potential application for the decolourisation and degradation of azo dyes. The brick pieces used in this study are suitable matrix materials for developing bacterial biofilms. The developed process was highly efficient for treating the dyeing industry's waste containing high COD and ADMI values. It functioned successfully without the aseptic condition even after 264 d and a total of 301 treatment cycles and without further addition of inoculum. Even at the termination of the treatment, it showed an 82.11% COD reduction without nutrient addition as compared to a 92.11% reduction with nutrients. Batch mode of operation showed as high as 99.0% COD reduction within 6 h contact time. A drop of 98.28% in ADMI values was observed upon treatment of the dye effluent with PBBR and charcoal reactor. Developed indigenous PBBR process showed potentiality for direct treatment of textile industry waste.

Additionally, many studies have been conducted earlier on various reactors, either with the simulated textile industry or a mixture of some dyes. Therefore, this study is promising as it is performed with real textile industry effluent and the PBBR and charcoal reactor demonstrated a substantial reduction in ADMI and COD values.

Declarations

Conflicts of interests

The authors declare that there are no conflicts of interest in this work.

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Figures

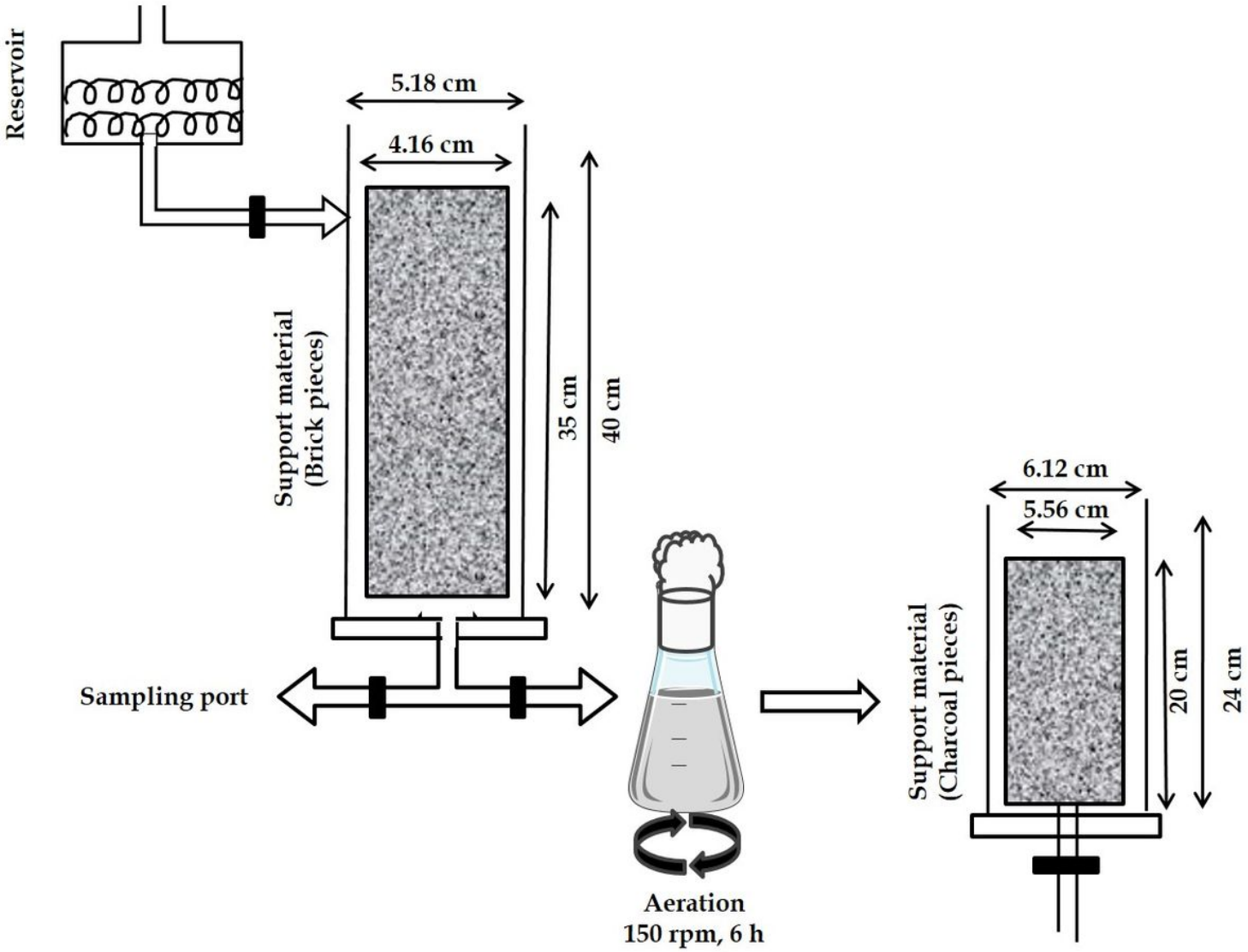

Figure 1

Diagrammatic representation of the indigenously designed bioreactor for the biotreatment of textile industry effluent (not to scale).

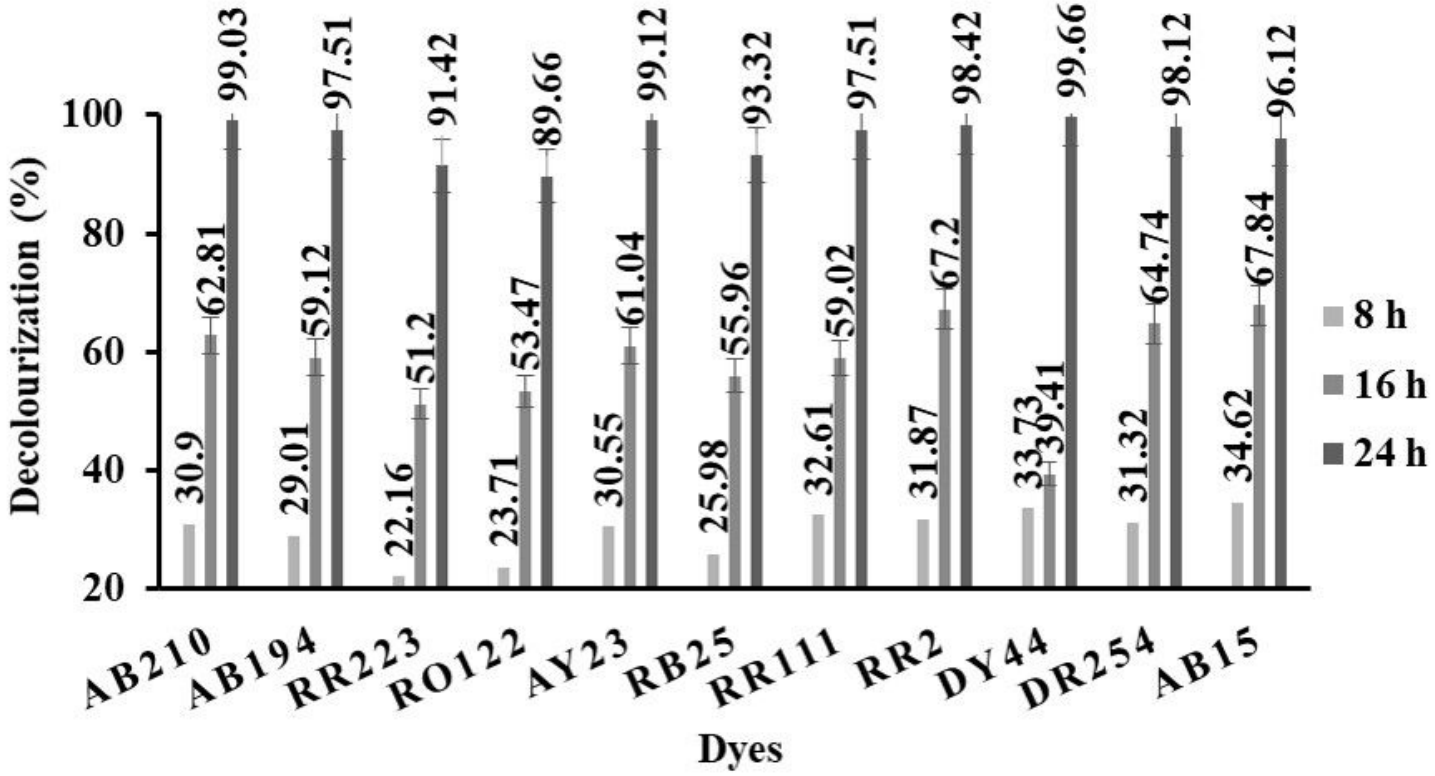

Figure 2

Decolorisation of different dyes by the indigenously developed consortium

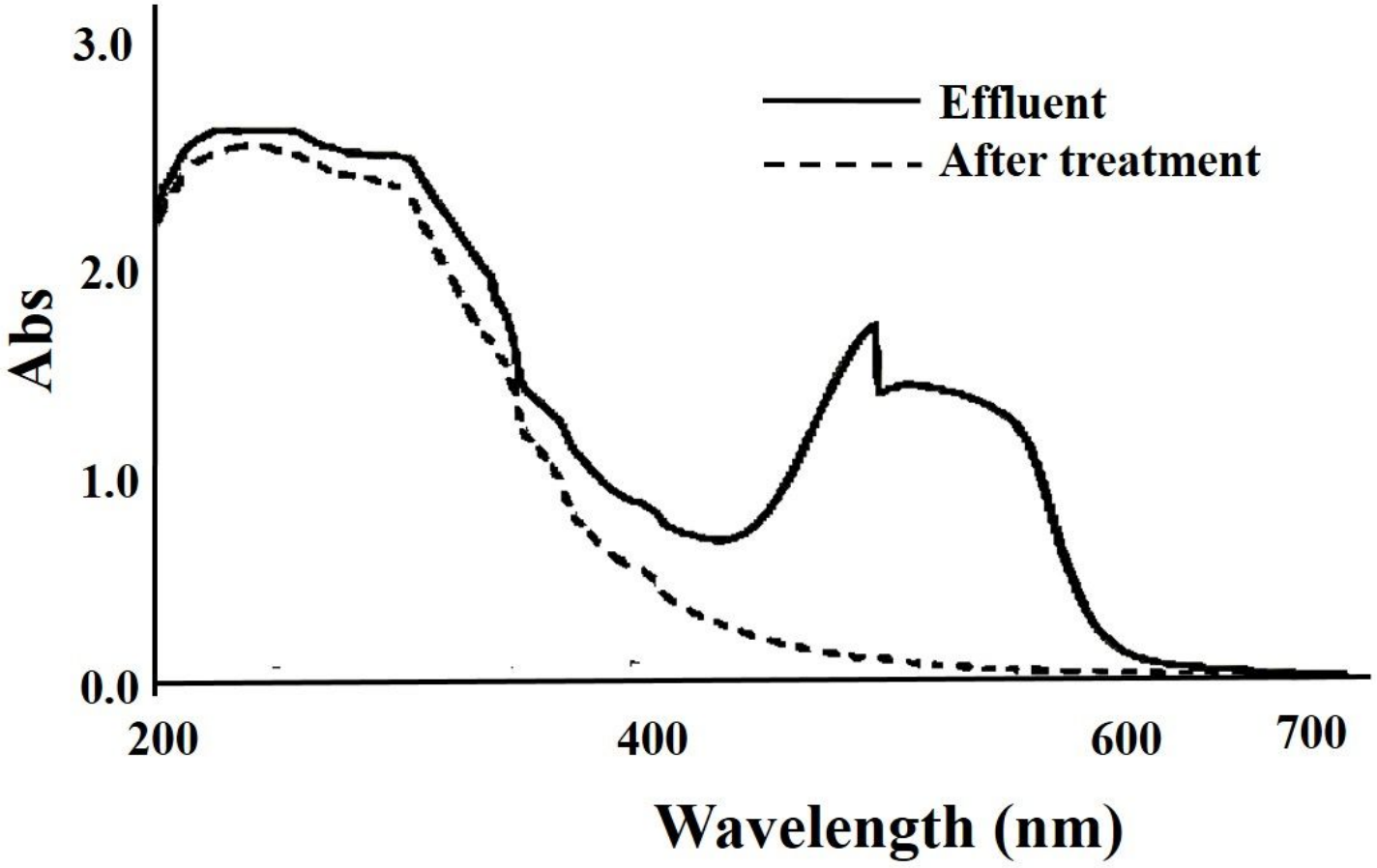

Figure 3

Changes in the UV-visible spectrum of AB210 and degraded metabolites after treatment with the developed consortium.

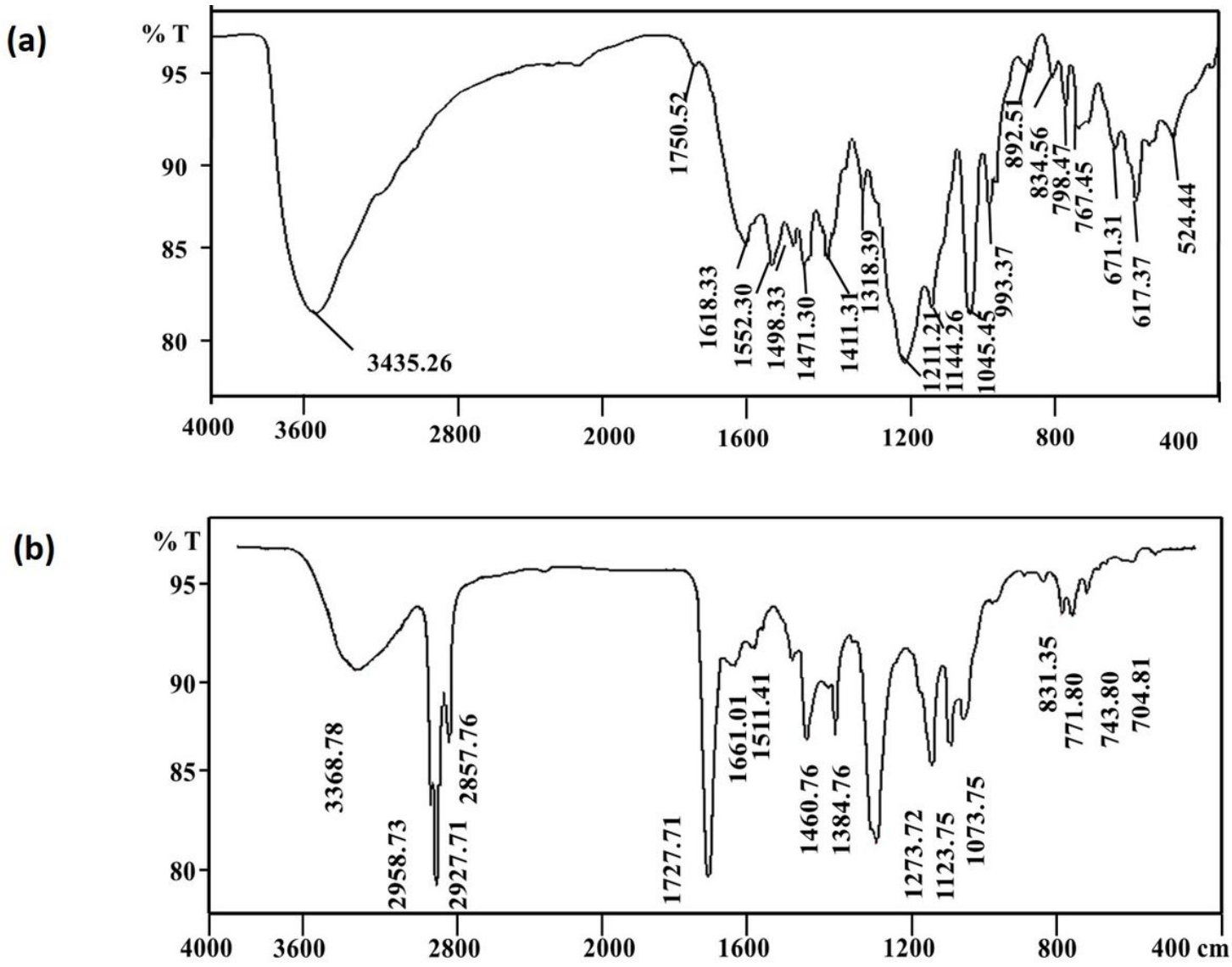

Figure 4

Change in FTIR spectra of AB210 (a); and degraded metabolites (b); and degraded metabolites.

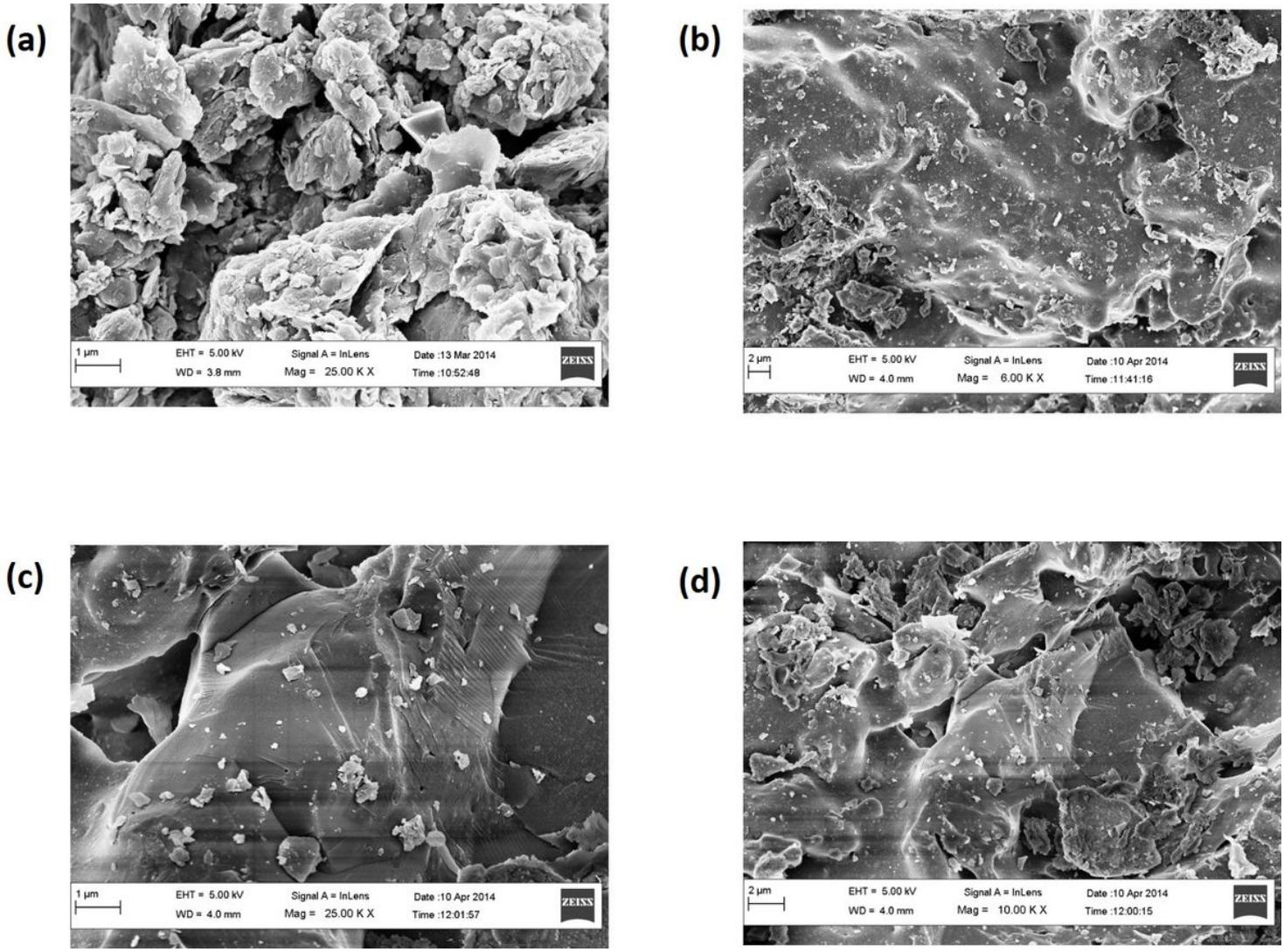

Figure 5

SEM analysis of brick pieces packed in the bioreactor (a) before the development of biofilm and (b) after the development of consortial biofilm on brick pieces (b, c, d).

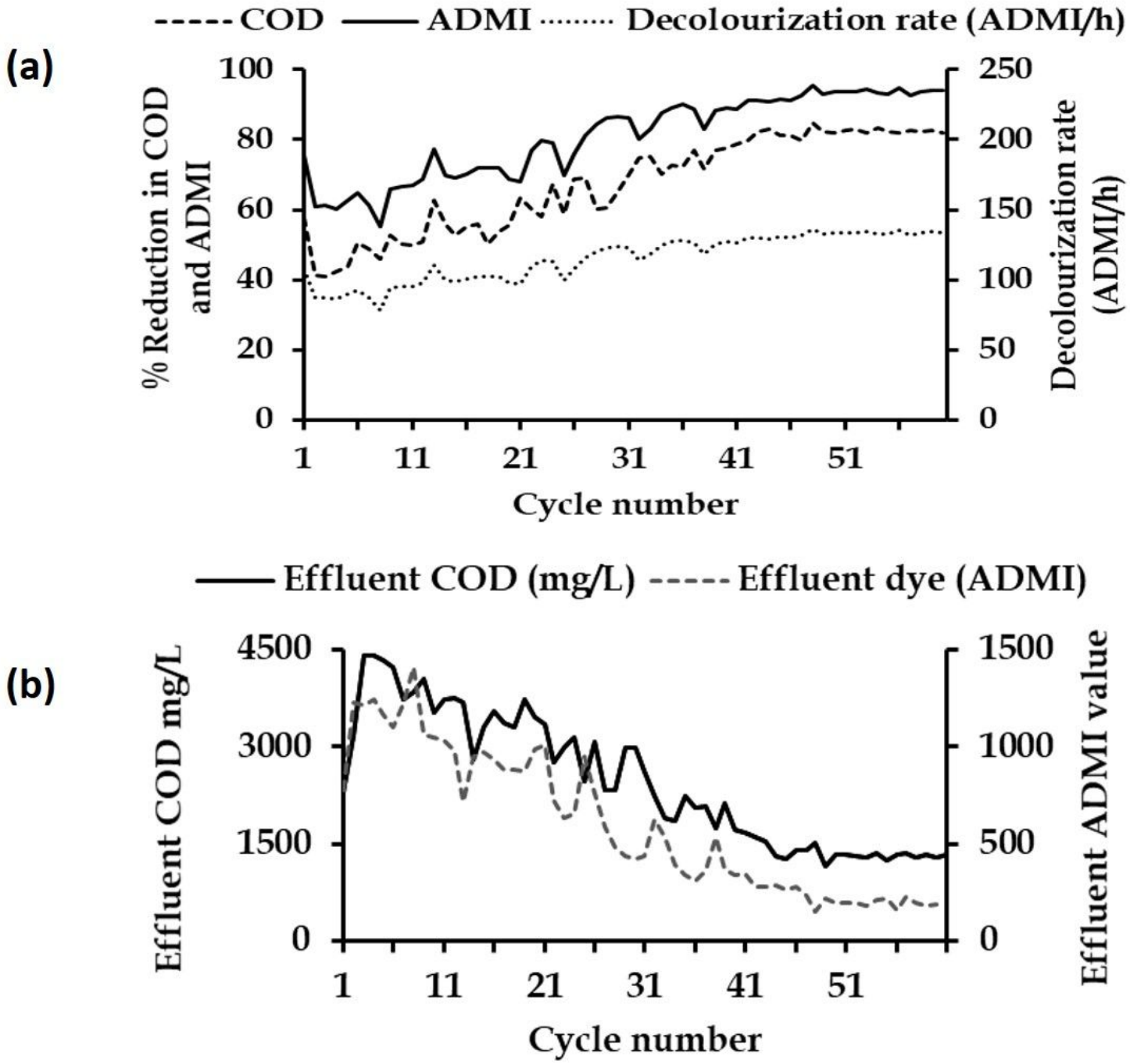

Figure 6

Performance of PBBR in batch mode: (a) effluent dye decolourisation and COD removal efficiency; (b) effluent dye concentration and COD.

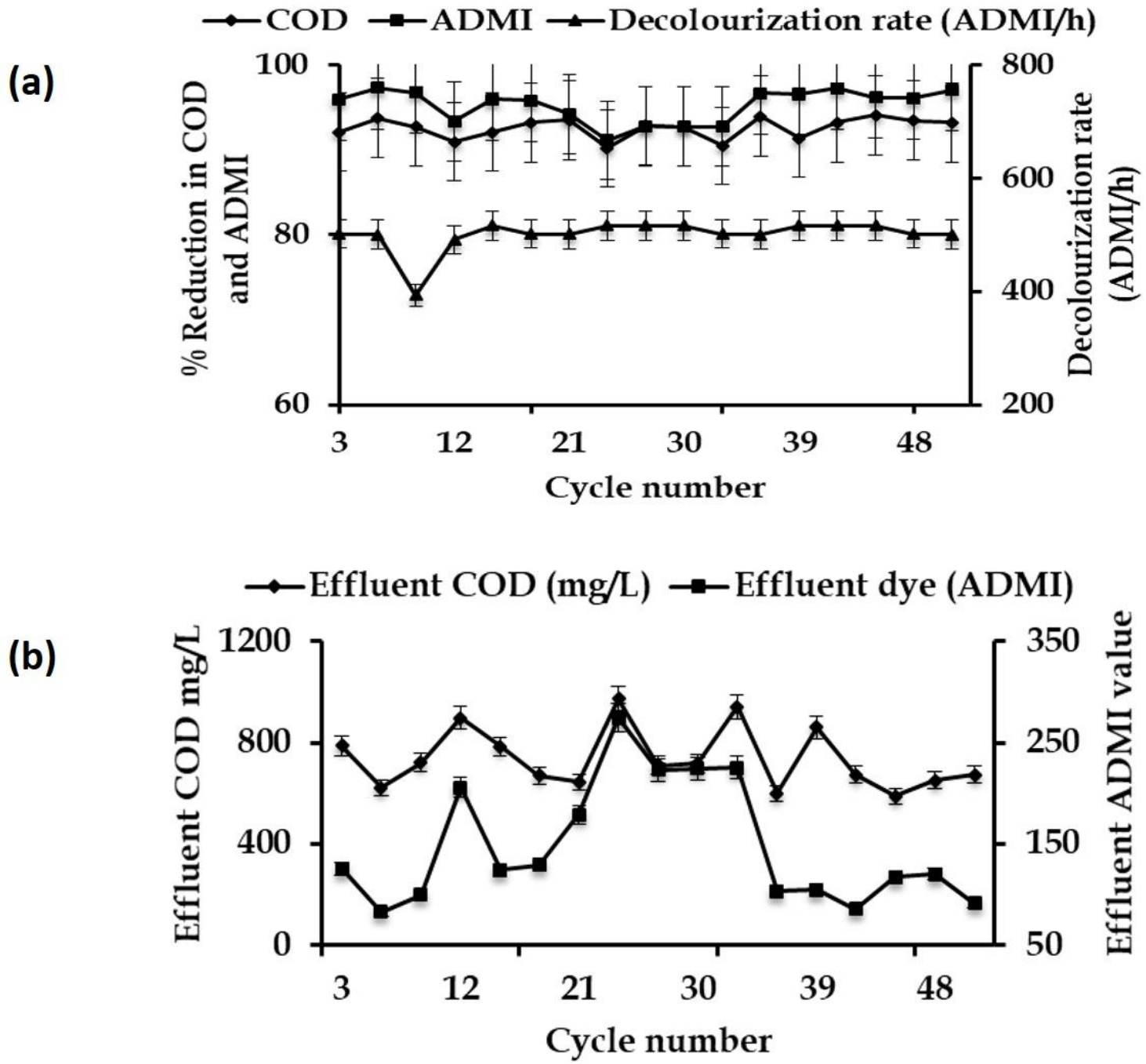

Figure 7

Performance of PBBR in continuous mode COD and dye removal: (a) effluent dye decolourisation and COD removal efficiency; (b) effluent dye concentration and COD.

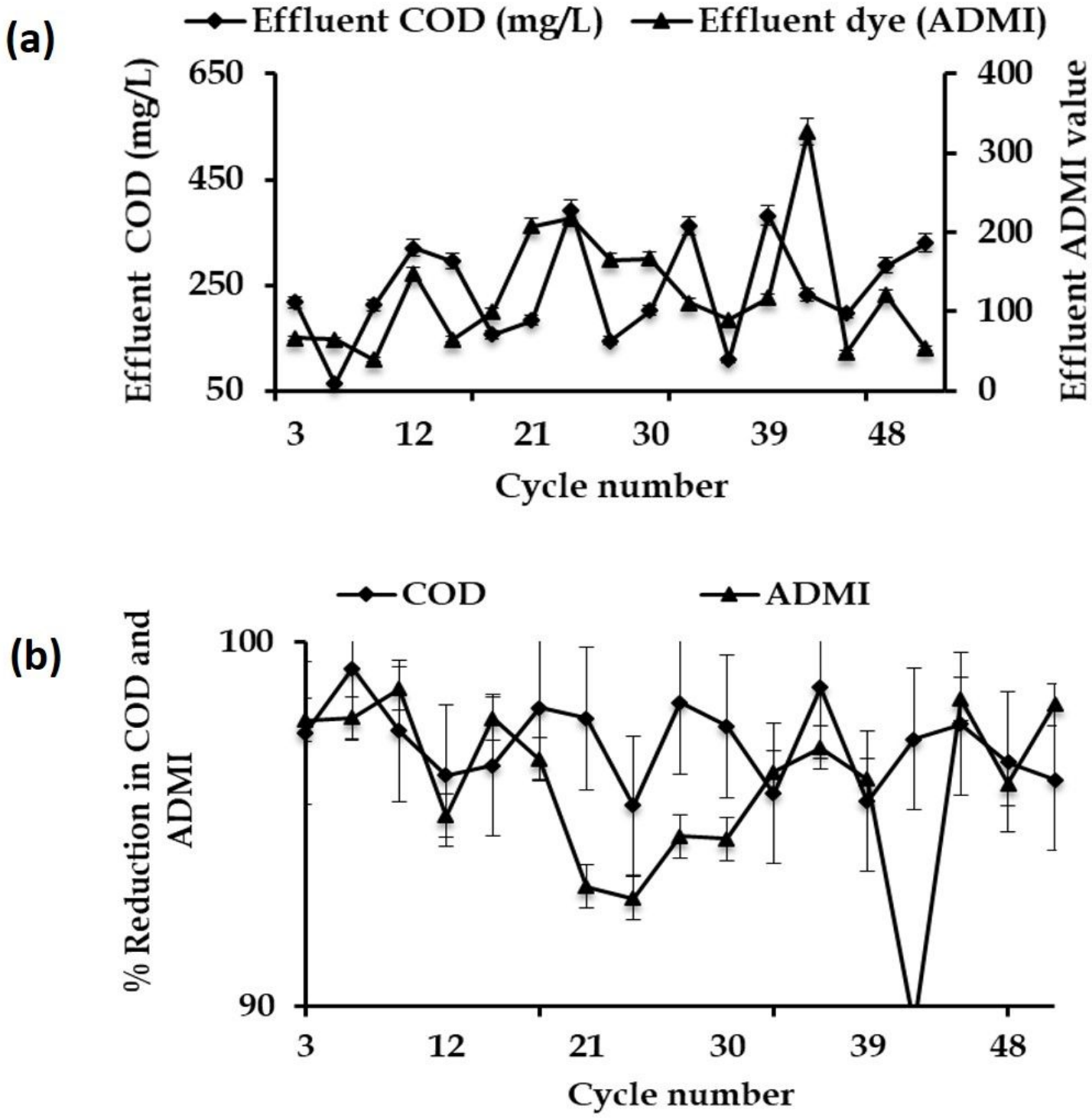

Figure 8

Performance of charcoal reactor during continuous mode in COD and dye removal: (a) COD removal efficiency and decolourisation efficiency, (b) effluent COD concentration and effluent dye concentration.

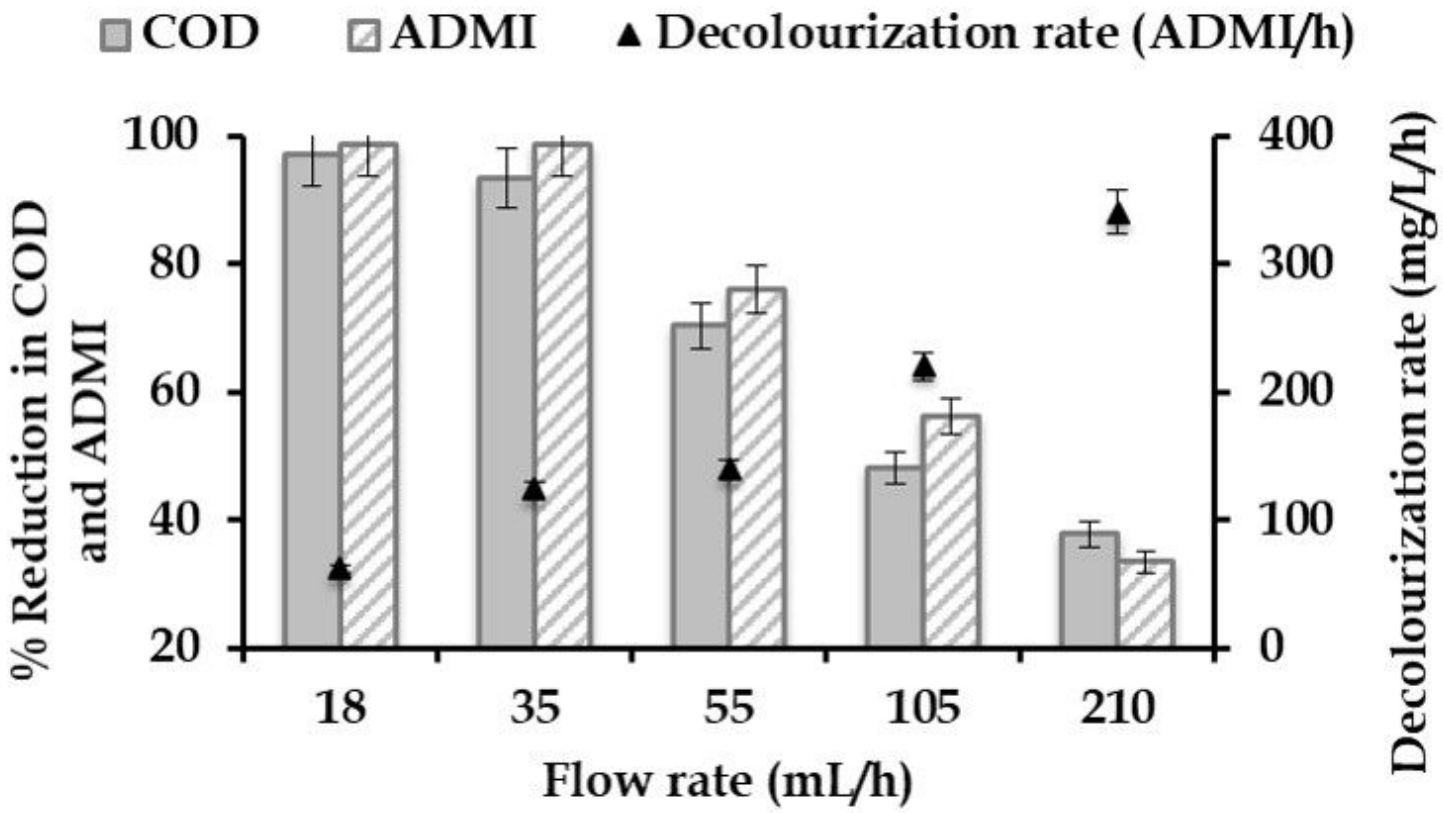

Figure 9

Effect of different flow rates on PBBR augmented with the indigenously developed consortium in continuous mode.