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An evaluation of decolorization mechanism of synthetic dyes belonging to the azo, anthraquinone, and triphenylmethane group, as a sustainable approach, by immobilized *CB8* strain (*Trametes versicolor*)

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ABSTRACT

The accumulation of different kinds of synthetic dyes in the environment poses a global ecological threat. White rot fungi play an important role in dye decolorization, but there is a scope for developing improvised dye decolorization methods and analysing its mechanism. In the present study, we used free and immobilized biomass (on two solid synthetic supports) of the *Trametes versicolor* (*CB8*) strain to decolorize five of the most utilized synthetic dyes belonging to three different classes, namely triphenylmethane (Brilliant Green, Crystal Violet), azo (Congo Red, Evans Blue), and anthraquinone (Remazol Brilliant Blue R) in a wide range of initial concentration (100, 200, 300 and 400 mg·L^{-1}). The best removal (more than 90%) was observed for three dyes (Evans Blue, Remazol Brilliant Blue R, Crystal Violet) in case of free and immobilized biomass at all concentrations whereas immobilization did not prove beneficial for elimination of Congo Red. The UV-Vis spectrum analysis proved the dye decolorization was performed by biodegradation method. Through the desorption study, the individual participation of physical sorption in the decolorization of dye was investigated. It was concluded that biochemical decolorization played a major role in dye decolorization. Further research is indicated to optimize the degradation process by immobilized *CB8* strain.

Keywords: Decolorization; Azo dye; Anthraquinone dye; Triphenylmethane dye; Biosorption; *Trametes versicolor* (*CB8*)

1. Introduction

Dyes are an important organic compound being extensively utilized in commercial products of textile, leather, pharmaceutical, plastic, paper, and food industry. The wide variety of availability of over 100,000 dyes makes it possible to meet requirements of different types of industries and as a proof, a large annual production of these dyes is observed which counted to approximately $8 \times 10^5 - 9 \times 10^5$ tons [1,2]. Among these, the textile industry is the only consumer of 20% of dye and noticed that 10%–15% of dyes are discharged into wastewater [3]. The effluents of these industries are strongly colored and show disturbing levels of other physico-chemical properties of water, such as temperature, pH, biochemical oxygen demand, chemical oxygen demand [4]. Generally, the color imparts its effect in water even at a low concentration like 1 mg·L^{-1}. However, it has been reported that the effluents of the textile manufacturing process show an average concentration of 300 mg·L^{-1} [5]. When effluent is not properly treated prior to disposal, it has direct

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effect on the water ecosystem and ended up deteriorating the environmental quality. According to the Sustainable Development Goals of the United Nations Development Program, the recurring scarcity of pure water is projected and hence treatment technologies for restoration of the purity of water system is urged to serve better water quality for future generations.

Synthetic dyes have common property to absorb light in the visible region. The basic dyes are cationic, disperse dyes are non-ionic, while acid, direct and reactive dyes are anionic [6]. Congo Red dye is classified as direct dye which is popular in the paper industry because they tend for have a high affinity to cellulose fibres due to its linear molecular structure and a system of conjugated double bonds. This dyes usually also exhibits good wet fastness properties with the addition of a fixative [4,5]. Evan's Blue is Direct Blue 53 appears as blue crystals with a greenish-bronze luster or a black powder. Evans Blue is an organic sodium salt that is the tetrasodium salt of 6,6′-{(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis[diazene-2,1-diyl]}bis(4-amino-5-hydroxynaphthalene-1,3-disulfonate). It has a role as a histological dye, a fluorochrome, a teratogenic agent and a sodium channel blocker. Overall, they are water-soluble, intensely colored, recalcitrant, and xenobiotic compounds prevalent in industrial wastewater. Reductive azo cleavage can result in generation of toxic amines in effluents, whereas anthraquinone (such as Remazol Brilliant Blue R) and triphenylmethane contain complex aromatic structure, which is more resistant to degradation. The Crystal Violet and Brilliant Green dyes are some of the oldest synthetic cationic dyes that belong to the triphenyl group and have the most important amino group donor. The accumulation of these dyes is a major source of water pollution near industrial area as pure substances and intermediates of their transformation can cause different health problems in humans and other organisms related to acute toxicity and/or genotoxicity [7,8].

Various physical and chemical approaches like flocculation, flotation, precipitation, oxidation, reduction have been employed for the treatment of wastewater containing harmful dye [9,10]. But it faced problems such as expensive chemicals, ineffectiveness against diverse groups of dye, and sludge generation [11,12]. With advancement in biotechnological tools and techniques, it can provide a cheap, efficient, and eco-friendly alternative for removal of dyes. The classic approach to the remediation of textile dyes is considered as a biological strategy using microorganism like bacteria, yeast, algae [4,13–16]. The mechanism underlying the dye removal process is the biodegradation/biotransformation and/or sorption of pollutants by microbial biomass.

Among biological agents, mycoremediation using white rot fungi (WRF) has several advantages for dye removal mechanism. The decolorization of the dye by white rot fungi such as *Pleurotus ostreatus*, *Trametes versicolor*, *Irpex lacteus* is the result of a cumulative mechanism such as the adsorption and absorption of dye by the mycelium fungi, the action of degradative extracellular and intracellular enzymes secreted by fungi [17,18]. The previous study focuses on dye decolorization via degradative enzymes such as laccases, lignin peroxidase and manganese peroxidase [19,20]. *Trametes versicolor* served several advantages in dye degradation. The enzymatic pathway in WRF represents mainly metabolic dependency for the dye removal mechanism. On the contrary, the biosorption process represents a metabolically independent adsorptive process. It is divided into two types: physisorption, in which the bonds between the adsorbent and the adsorbate are weak reversible forces, and chemisorption, in which the adsorbent and the adsorbate are bound by generally strong irreversible bonds [21].

Moreover, the decolorization process is also influenced by many physico-chemical parameters such as the interaction between dye and fungi, the chemical nature of dye, pH, and temperature. Many researchers have tried to incorporate immobilization of microbial cells or enzymes to improve the dye decolorization capacity as it is noted that immobilized cells have advantage over the free system when it is fixed to a natural or synthetic support [22]. Immobilized microorganisms serve a wide area of application as they could be used in continuous and semicontinuous treatment process, increase reusability of the biocatalyst, decrease the requirements for biomass and liquid separation [23]. Until now, different types of natural and synthetic adsorbents such as apple peel, peanut shell, sawdust have been used to treat dye containing wastewater [24–26]. However, the process faced problems with low adsorption capacity and, as a result, required a large amount of adsorbent. Hence, it is worth developing low-cost and efficient immobilized fungal biomass which combine physical, chemical and biological functions together for dye decolorization [27]. The use of inexpensive and easily available solid inert carrier, such as polyethylene sponges used for washing (dishwasher) and sponge, for immobilization of fungal biomass makes a good choice for an attractive, cheap, convenient, eco-friendly and more acceptable tool for wastewater treatment [22].

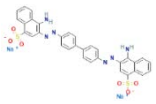
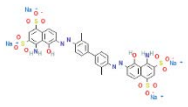
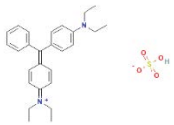
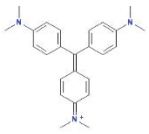
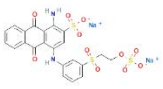
The purpose of this study was to develop an effective immobilized biological system by using *Trametes versicolor* (*CB8*) fungal strain which would be able to decolorize five structurally different dyes belonging to different classes (azo, triphenylmethane, and anthraquinone) at a wide range of initial concentration, the maximum being 400 mg·L^{-1}. The effect of immobilization on synthetic solid supports was also evaluated in order to improve the decolorization process and investigate the mechanism of dye degradation. The desorption behaviour of free and immobilized mycelium along with supports was investigated to evaluate the effectiveness of the sorption process.

2. Methodology

2.1. Preparation of the test dye solution

Five dyes, namely Brilliant Green (BG), Remazol Brilliant Blue R (RBBR), Congo Red (CR), Evans Blue (EB), Crystal Violet (CV), were selected for decolorization study. The details of the compound studied are given in Table 1. The dye solutions were prepared as a stock solution (1,000 mg·L^{-1}) in deionized water, autoclaved at 121°C for 15 min and stored in the dark at 4°C. The pH of dye was measured using the CP-105 ELMETRON pH meter and was recorded 4.36 for BG, 5.77 for RBBR, 7.90 for CR, 7.71 for EB, and 4.35 for CV. All chemicals used were analytical grade.

Table 1
Physico-chemical properties of dyes along with classification

Name of dye	Congo Red	Evans Blue	Brilliant Green	Crystal Violet	Remazol Brilliant Blue R
Class	Azo	Azo	Triphenylmethane	Triphenylmethane	Anthraquinone
Molecular formula	$C_{32}H_{22}N_6Na_2O_6S_2$	$C_{34}H_{24}N_6Na_4O_{14}S_4$	$C_{27}H_{34}N_2O_4S$	$C_{25}H_{30}N_3Cl$	$C_{22}H_{16}N_2Na_2O_{11}S_3$
Molecular weight (g·mol^{-1})	696.67	960.79	482.65	407.98	626.54
C.I.	CAS. 573-58-0	CAS Number: 314-13-6	C.I. 42040; CAS Number: 633-03-4	C.I. 42555	61200
Absorbance maxima (nm)	492	598	623	584	596
Structure					

2.2. Collection of fungal strain and culture condition

The pure culture of WRF-*Trametes versicolor* (strain *CB8*) was collected from the depository of Fungal Strain Collection of Environmental Biotechnology Department, The Silesian University of Technology, 44–100 Gliwice, Poland. The isolation and identification of fungal species is described in Jureczko et al. [28]. For propagation of *Trametes versicolor* (*CB8*) mycelium, 2 cubes of mycelium from MEA plates were transferred to 250 mL Erlenmeyer flask containing 150 mL of liquid organic medium (glucose – 5 g/L, peptone – 1 g/L, $MgSO_4{\cdot}7H_2O$ – 1 g/L and KH_2PO_4 – 1 g/L, pH 5.7).

2.3. Preparation of immobilized fungal biomass and free fungal biomass

A week pre-cultivation of fungal biomass was carried out in the liquid organic medium containing glucose – 5 g/L, peptone – 1 g/L, $MgSO_4{\cdot}7H_2O$ – 1 g/L and KH_2PO_4 – 1 g/L (pH 5.7) and the cultivated biomass was transferred to 250 mL Erlenmeyer flask containing 150 mL of liquid organic medium having 15 pieces of 10 mm^3 size of support S1 (dishwasher, polypropylene) or S2 (sponge, polyester). The immobilized *CB8* biomass on dishwasher was denoted as *CB8*/S1 whereas immobilized *CB8* biomass on sponge was denoted as *CB8*/S2. Free fungal biomass was cultivated in same medium without support, and it was marked as *CB8*. All culture flasks were incubated on a rotary shaker at 150 rpm for 7 d at room temperature (20°C ± 2°C). The controls were prepared and incubated in the same manner without inoculation.

2.4. Evaluation of decolorization efficiency at different dye concentrations

The appropriate concentration of each dye (100, 200, 300, and 400 mg·L^{-1}) was prepared by diluting stock solution of the dye (1,000 mg·L^{-1}) with culture medium. The one piece of 7-day-grown mycelium pellets with immobilizer was transferred to 2 mL of medium containing an appropriate concentration of each dye and incubated in static condition at room temperature (20°C ± 2°C). The free fungal biomass was homogenized using a Bagmixer prior to transferring. The control samples of solid supports without biomass were incubated under the same conditions. The decolorization experiment was repeated three times. The decolorization of the dye was observed regularly and after 96 h of incubation, the absorbance of the decolorized medium was recorded using a Hitachi U-1900 UV-Vis spectrophotometer using a wavelength scan (200–1100 nm) at a scan speed of 200 nm/s. The maximum absorbance λmax for dyes BG (623 nm), CV (584 nm), CR (492 nm), EB (598 nm), and RBBR (596 nm) were determined by performing wavelength scanning (200 – 1100 nm). The UV-Vis wavelength scan was further utilized to study dye degradation mechanism. The standard curve was prepared for each dye in the range of 1 to 100 mg·L^{-1} concentration. On the basis of the recorded absorbance, the concentration of dye in control samples and biomass-containing samples were calculated by using a standard curve and applying the appropriate dilution factor. The total percentage of the decolorization (DP) of dye was calculated using the Eq. (1).

$$\mathrm{DP}(\%) = \frac{C - S}{C} \times 100 \tag{1}$$

where C is the current concentration of dye in a control sample (without biomass) with only support or only medium (mg·L^{-1}), and S is the current concentration of dye in samples with immobilized or free fungal biomass (mg·L^{-1}).

2.5. Determination of the sorption proportion during decolorization

The physical sorption of dye by fungal mycelium during decolorization was measured according to [29] with little modification. To evaluate the desorption of dye from free

and immobilized biomass, 2 mL of 70% (v/v) Methanol solution was used. Firstly, the fungal pellets were separated from culture medium and then pellets were soaked for 24 h in a 2 mL 70% (v/v) Methanol solution in static condition at room temperature (20°C ± 2°C) to recover the physically adsorbed dye. The desorbed dye was measured by checking the absorbance of the desorbed medium at λmax for each dye; BG (624nm), CV (584nm), CR (501nm), EB (598nm), RBBR (596nm). The calculations for the estimation of desorption were performed by slightly modifying the equation used by [30]. The percentage of dye desorption (DS%) was calculated by using Eq. (.2)

$$\mathrm{DS}(\%) = \frac{C_s}{C_c} \times 100 \tag{2}$$

where DS [%] is the concentration of percentage of desorption, C_S is the desorb dye in sample with fungal biomass ($mg \cdot L^{-1}$), C_C is the concentration of desorb dye in control sample without biomass ($mg \cdot L^{-1}$).

The proportion of chemisorption and biodegradation was collectively named as Biochemical Decolorization (BD%). It was useful to estimate the biochemical decolorization to get idea about mechanism of dye removal [eq. (3)].

$$\text{Biochemical decolorization}(\mathrm{BD}\%) = \text{Total Decolorization}(\mathrm{DP}\%) - \mathrm{DS}\% \tag{3}$$

where, BD is biochemical decolorization, DP% represents total decolorization and DS% is sorption percentage.

3. Result and discussion

The biological removal of synthetic dye, as a wastewater treatment, depends on the biosorption and biodegradation capacity of the chosen organism. In this study, we conducted primary research of decolorization of five structurally different dyes by white rot fungi (*Trametes versicolor CB8* strain). The ability to remove the dye by free fungal biomass and immobilized fungi on solid supports was evaluated at a range of initial concentration of dye. Improvement in decolorization efficiency was tried by providing solid support for immobilization of the fungal mycelium. It was basic research to check the capacity of the *CB8* strain in free and immobilized form, so the result will provide a basic idea about its universal usage for the degradation of various dyes and will help in the development of bioreactors using immobilized fungal biomass. In addition, the desorption study was performed using 70% Methanol to recover physically adsorbed dyes by fungal hyphae, which gave insight into the role of physisorption and degradation in the dye removal mechanism.

3.1. Evaluation of immobilization support

The growth of immobilized fungal biomass on two different solid supports and free fungal biomass was performed by visual observation, which is presented in Table 2. The most intensive and even growth of *T. versicolor* (*CB8*) was observed on sponge. Each sponge piece was evenly coated compared to other support (dishwasher) when it was grown on constant shaking conditions. The main problem was associated with the dishwasher, as only 30% to 40% of the pieces were colonized by fungi. The remaining pieces were not efficiently colonized by fungi and hence wasted. As a result, a greater number of dishwashers were needed to carry out the dye degradation experiment. In case of free fungal biomass, under shaking conditions, mycelium pellets were formed (self-immobilized whole cell pellets). It was homogenized using Bagmixer before mixing it with dye solution. The mycelium pellets having larger surface area may help in better sorption of dye by fungi, as the sorption is observed in the initial phase of dye degradation and may influence on final removal including enzymatic degradation. Control samples with appropriate supports were also incubated under similar condition. It showed no growth in it. The dry weight of free fungal *CB8*, *CB8*/S1 and *CB8*/S2 biomass were 0.011 ± 0.003 g, 0.019 ± 0.005 g and 0.022 ± 0.006 g, respectively.

Both supports were tested for dye absorption capacity too, and since it was obvious that the sponge was able to absorb more dye than the dishwasher. The structural properties of the sponge allowed more absorption. Congo Red dye and RBBR dye were recorded to show maximum absorption capacity on sponge ranging from 30% to 50% (data not shown here) and showed increase in absorption as the initial concentration of dye increases, while the dishwasher barely removed 1% to 6% of the dye. In case of Crystal Violet, Brilliant Green, and Evans Blue, the absorption on sponge was observed in deceasing order with increasing dye concentration.

3.2. Efficiency of fungi-mediated dye decolorization

The isolated *T. versicolor CB8* strain showed great potential for decolorization of all dyes tested to a greater or lesser extent, particularly Evans Blue, Crystal Violet, and RBBR was removed with higher efficiency (more than 90%) at all initial dye concentration. It showed great efficiency of *CB8* strain for dye decolorization in both free and immobilized form.

In comparison of dyes belonging to the Azo group, the *CB8* strain was able to remove Evans Blue dye to a greater extent than Congo Red dye. Evans Blue removal efficiency, at highest tested initial concentration tested, was higher when the biomass was immobilized in the sponge as a solid support (Fig. 1b). The *T. versicolor* (*CB8*) strain was more suitable to decolorize Evans Blue at concentrations ranging from 100 to 400 $mg \cdot L^{-1}$. Based on the literature survey,

Table 2
Growth analysis of immobilized and free fungal biomass after 7 d (Growth condition: 150 rpm, pH 5.7, temperature 20°C ± 2°C)

Culture condition	Dishwasher (S1)	Sponge (S2)	No support
T. versicolor (*CB8*)	+ +	+ + +	+ +
Control (Medium)	–	–	–

–, lack of growth (0%); + +, medium growth (50%–70%); + + +, intensive growth (70%–100%)

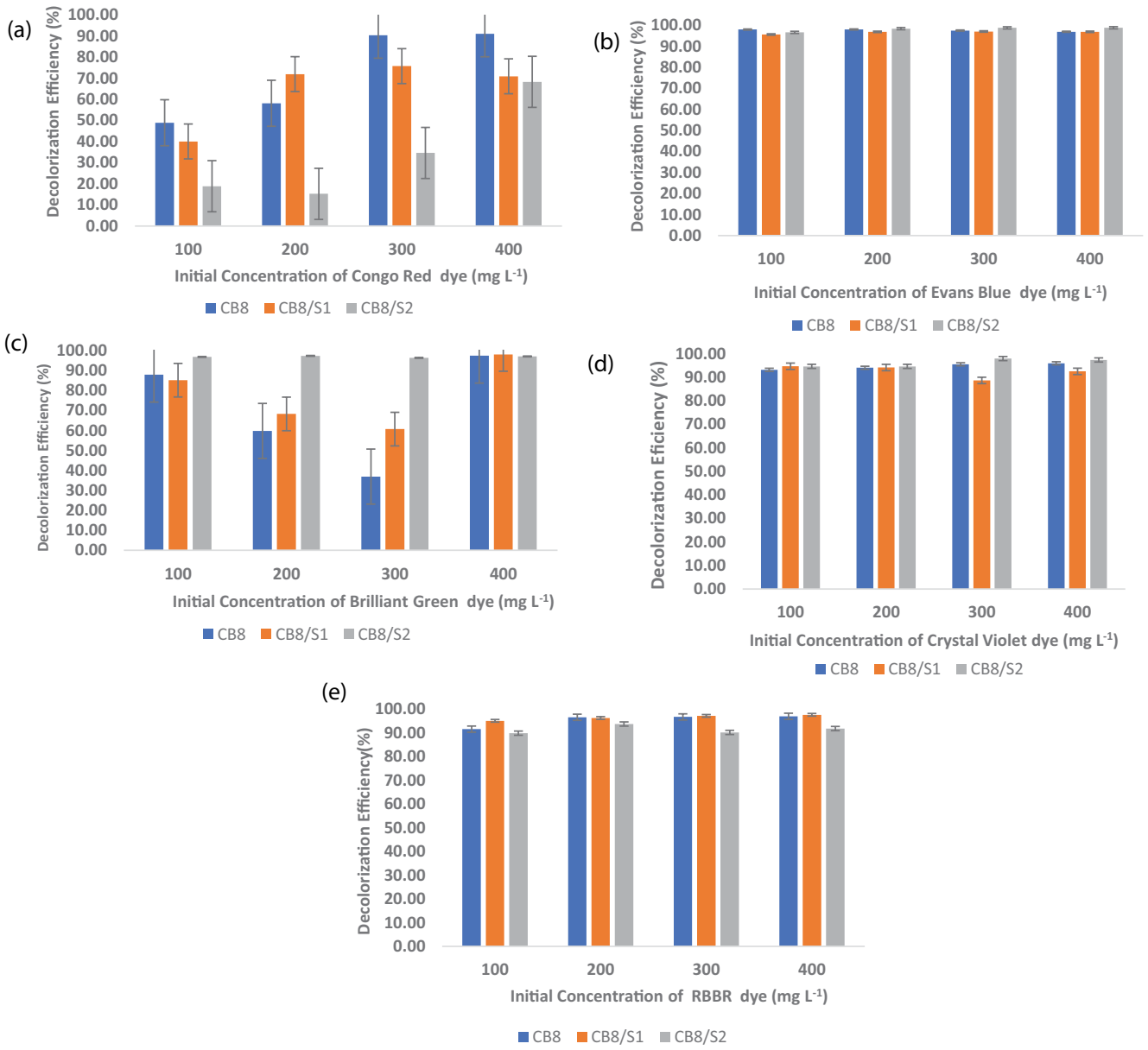


Fig. 1. Decolorization efficiency (%) of *T. versicolor* (*CB8*), immobilized *CB8* on dishwasher (*CB8*/S1) and on sponge (*CB8*/S2) for dye (a) Congo Red, (b) Evans Blue, (c) Brilliant Green, (d) Crystal Violet and (e) Remazol Brilliant Blue R (Parameters: $T = 20°C \pm 2°C$, $t = 96$ h, static condition).

the observed removal capacity of the *CB8* strain is indeed higher in response to the initial concentration of Evans Blue dye. In the case of Congo Red dye, the free fungal biomass was more suitable to decolorize this dye compared to fungi immobilized on solid supports. As mentioned previously, Congo Red was removed more efficiently by an inert support sponge but when the sponge was coated with *CB8* strain, it showed a diminishing decolorization efficiency. As the initial concentration of CR dye increased, the dye decolorization proportion was also increased to 91.05% (Fig. 1a). Our finding is in correlation with the previous report [31] that the WRF fungi were able to remove the CR dye at a concentration of 400 mg·L^{-1} compared to lower concentration like 100 and 200 mg·L^{-1} at a 30 h incubation time. The CR removal rate also increasing with concentration (0.28–18.01 mg/L h), but only up to 600 mg·L^{-1}. Subsequently, at 800 and 1,000 mg·L^{-1}, the dye decolorization rate was observed 10.86–20.21 and 10.52–16.59 mg/L h, respectively. They have checked the removal efficiency of CR dye in broad range of pH (3–7) and reported the best decolorization efficiency (89%) at relatively higher pH (8), which attributed to the fact that [H$^+$] ion concentration, therefore a pH is influential factor in the removal of pollutant [31,32]. While studying UV-Vis spectrum for Congo Red dye, one extended peak was observed at 330 nm, which may due to the interaction between aromatic hydrocarbon or polycyclic aromatic hydrocarbon groups and other chromophore and another at 495 nm, which had a

relation to azo double bond and large conjugated system for the whole dye molecule [33].

Brilliant Green dye (triphenylmethane group) showed the best removal (97%) in the full range of dye concentrations when fungi were immobilized on sponge. A quite different trend was observed for free biomass and fungi immobilized in support 1. It is clear from Fig. 1c that the BG dye was more efficiently removed at the lowest (88% in free fungal biomass (FFB) and 85.17% in immobilized fungal biomass (IFB), respectively) and highest (97.51% in FFB and 98.07% in IFB, respectively) tested concentration and showed less efficiency at intermediate concentration. IFB on dishwasher and FFB gave similar kind of pattern for decolorization of dye at different concentrations. The UV-Vis absorbance spectral profile indicated mineralization of BG dye as after degradation, there was complete disappearance of absorbance peak at 623 nm and no other new peak was generated. Similar results were observed when BG dye was degraded in recirculating packed bed bioreactor. As it was mentioned by Kumar et al. [34] with reference to control BG dye, the samples taken at 4th, 8th and 16th day showed decreasing absorbance at 630 nm and at day 20, it was very low.

Another dye from this triphenyl group, Crystal Violet, was removed efficiently in all combinations tested (Fig. 1d). Among them, the combination of fungi with support 2 was the best, as it gave the highest color removal (97.36%) at the highest concentration (400 mg·L^{-1}). We utilized Crystal Violet at natural pH, and it showed maximum absorbance at 585nm while gave two relatively small peaks at 299 and 246 nm. While studying the UV-Vis spectrum, it was noted that the sponge immobilized *CB8* biomass showed only one peak (at 567 nm) after dye degradation whereas FFB and dishwasher immobilized *CB8* showed two peaks (at 567 and 220 nm) after same time interval. The dyes did not changed color after decolorization process. The authors Asgher et al. [35] reported that Crystal Violet dye give maximum absorption peak at 584 nm and three smaller peaks at 210, 245 and 295 nm in neutral condition (pH 7) but it changes it peak to 420 nm in strong acidic condition. The few papers reported the *N*-demethylation pathway in *Aspergillus* sp. for triphenylmethane group containing methyl violet dye [34].

The dye of the anthraquinone group, Remazol Brilliant Blue R, indicated dye removal with a high efficiency range between 89.85% and 97.59% for each designed variable. Here, in this case, the combination of fungi with support 1 (dishwasher) provided higher removal capacity (Fig. 1e). The absorbance maxima at 596 nm were observed in control dye samples which just disappear in samples with fungal biomass after 96 hr incubation which indicate the breakdown of chromophoric group of dye. There was no new peak or shift in peaks observed. The connection between the effect of the initial dye concentration and the percentage of decolorization remains unclear. As in dyes such as EB, CV, and RBBR, the decolorization capacity was similar as the dye concentration whereas CR and BG gave different patterns for decolorization. The biosorption and degradation of dye are due to heteropolysaccharide and lipid components of the cell wall and also a result of the enzyme action on functional groups of dyes. *Trametes versicolor* has the potential to degrade a wide range of xenobiotics and recalcitrant compounds with help of complex enzyme system that includes extracellular and intracellular [35]. The *CB8* strain exhibited significant laccase enzyme activity as a lignin modifying enzyme in the culture filtrate when observed for two anticancer drugs. The noteworthy amount of 25 and 20 U/l was observed for the control and vincristine samples, respectively [36]. Therefore, in this study, laccase could have played an important role in dye degradation in addition to biosorption of dye molecules. The *CB8* strain proved its best decolorization efficiency for removal of dyes having different chemical composition. Table 3 comprise different fungus capacity to degrade similar dyes which we used in our study. And it also indicates that *CB8* strain in self immobilized (FFB) and solid support immobilization form gave better decolorization efficiency in shorter time and even at higher concentrations such as 400 mg·L^{-1}. So indeed, it is beneficial to optimize and use this strain in real life implications.

The solid support provides stability for efficient dye decolorization. Microbial cell immobilisation refers to the systems or techniques in which 'there is a physical confinement or localisation of microorganisms that permits their economic reuse'. Whole cell immobilization provides

Table 3
Decolorization efficiency of various fungus reported

Biological agent	Pollutant-dye (Conc. mg·L^{-1})	Experimental condition	Decolorization efficiency, removal time t	References
Phanerochaete chrysosporium	Reactive Black 5 (100 mg·L^{-1})	Bioreactor with *P. chrysosporium* immobilized on sunflower seed shell, Temp. = 25°C, aeration rate = 0.5 vvm	90.3%, *t* = 72 h	[39]
Aspergillus niger	Crystal Violet (10–40 ppm)	Static condition, Temp. = 30°C, pH-5.5	up to 84.6%, *t* = 10 d	[35]
White rot fungus, strain KRUS-G	Remazol Brilliant Blue R (100 ppm)	Static condition (120 rpm), Temp. = 28°C, pH-4	89%, *t* = 4 d	[40]
Aspergillus niger	Congo Red (200 mg·L^{-1})	Static condition (120–150 rpm), Temp. = 28°C, pH-5, biomass dosage-2 g	97%, *t* = 6 d	[33]
Cerrena sp.	Malachite Green	pH-6, Temp. = 25°C	91.6%, *t* = 2.87 h	[41]

several advantages over enzyme immobilization. As enzyme immobilization requires skills to purify the enzyme, less cost effectiveness [37]. The synthetic support provides an added advantage in the long term in bioreactor studies, as its structure did not show fast degradability. So far, many natural supports such as peanut shell, pistachio shell, coconut mesocarp, saw dust have been utilized for improvised dye degradation capacity of fungi [22,36,37]. There is no doubt that these organic materials are an environmentally friendly way of managing waste, but their degradability make them prone to loss of supporting material for fungi and also responsible for blocking the waste stream [5].

3.3. Comparative analysis of dye sorption and biodegradation by fungi

The desorption studies aimed to evaluate the involvement of the sorption procedure during the decolorization of dye and is also useful for understanding recycling and reusability of utilized immobilized fungal system [38]. When the appropriate desorbing agent is utilized for dye desorption, it should desorb dye without destruction of the structure and functional groups of the biomass and applied after each consecutive cycles, the mycelium pellets can be reused for up to four or five cycles. After five sorption/desorption cycles, the sorption efficiency of the Methylene Blue dye of brown macroalga, *Nizamuddinia zanardinii* decreased from 96.99% ± 0.90% to 48.16% ± 1.98%, and the dye desorption efficiency decreased from 68.70% ± 2.03% to 46.83% ± 1.49% [38,39]. Different physical and chemical tests such as autoclaving, the addition of 1 M HCl or 1 M NaOH had been utilized to check the stability of the sorption process after decolorization [31]. Desorption can be achieved through the ion exchange reaction using a relatively inexpensive eluent [42]. One of the most widely used is methanol and ethanol. It also gives insight about the interaction between functional groups of dye with respect to the ionic charge prevalent on the cell surface of fungal mycelium.

In this study, fungal hyphae were stained darkly after incubation with dye-containing medium. It indicated the strong involvement of the sorption process in the decolorization of the dye by fungi. The porous surface of a solid support such as a sponge also possesses great sorption capacity. In Brilliant Green dye, the desorption order was observed like *CB8* > *CB8* with support 1 > *CB8* with support 2 (Fig. 2c). It is co-related with the stable chemisorption of dye on sponge-containing fungi. In samples with free biomass, the percentage of desorption was higher (approx. 46%) at lower initial dye concentration (100 and 200 mg·L^{-1}), but when dye concentration was increased (400 mg·L^{-1}), the desorption percentage were decreased. FFB showed more possibility of irreversible binding during sorption of BG dye on fungal biomass. When biomass was immobilized on sponge, a low percentage of desorption range (0.36%–0.72%) was noted irrespective of the difference in initial dye concentration (Fig. 2c). It also suggests the stability of enzymatic degradation in the immobilized fungal system [5]. Brilliant Green is a basic dye. The authors observed that the desorption of Rhodamine B, a basic dye, increased with increase in ethanol concentration from 0% to 80%. It reached 99.7% desorption efficiency at the highest concentration of ethanol [43].

Congo Red is an anionic dye azo and is known as Direct Red 28. The CR dye showed an increase in the proportion of desorption (30.44% and 59.34%, respectively) at higher initial concentration of dye (300 and 400 mg·L^{-1}) (Fig. 2a). This trend is opposite to Brilliant Green (triphenylmethane) dye. The interaction between the cell wall of the fungi and the dye depends on the structure of the dye, the nature of the solid support for immobilization [14,22]. More anion exchange may happen at higher dye concentration and resulted in increased desorption of dye into the medium. It indicated the requirement to try different solid supports for immobilization of fungal biomass to optimize the decolorization process, as well as to optimize the different eluent and its volume to observe best desorption rate.

In samples with anthraquinone dye (RBBR), the lowest percentage of sorption (6.55%–11.09%) was observed for fungi immobilized in the dishwasher. This combination showed the highest decolorization by means of a biochemical process. The order of desorption for RBBR dye was recorded as: fungi > fungi with support 2 > fungi with support 1 (Fig. 2e). In the conducted experiment, we have made an observation that in FFB, the desorption percentage increased with increase in dye concentration. It indicates that sorption was higher at a higher dye concentration. It found that the Loofa sponge immobilized *Phanerochaete chrysosporium* has 18.60% dye removal and biosorption capacity with an increase in dye concentration compared to free biomass [44]. When the biomass was immobilized in the dishwasher, the main removal of the dye was observed by biochemical pathway. The chemisorption results in irreversible binding of dye molecule to fungi, which show less desorption upon addition of methanol. In the study conducted for sorption, the desorption proportion was estimated at different pH values. They have also observed that chemisorption has played an important role in the removal of Brilliant Blue dye as the maximum desorption was only 32% in the pH range 4–10 [38].

The desorption efficiency was different in case of Evans Blue and Crystal Violet dye. In case of free fungal biomass, it showed quite higher dye desorption compared to immobilized biomass (Figs. 2b and d). For the Crystal Violet dye desorption was quite high, indicating a higher proportion of irreversible binding of dye. There might be a possibility of any reaction between the fungi enzyme and the dye molecule that imparted much color to the solution. It was also previously reported [22]. The number of positively charged groups on the biomass surface increases under acidic conditions. It increased the desorption efficiency of Methylene Blue dye due to electrostatic repulsion between positively charged sites in biomass and cationic dye [40]. The biosorption process could be possible because of interaction of cell wall and the dye molecule. Different kinds of metabolism and pollutant sorption reaction are responsible. Extracellular accumulation of pollutants, intracellular accumulation, physical sorption, ion exchange, cell surface sorption, physical sorption are the causes of the biosorption of pollutants by WRF [45]. After the addition of 70% methanol, the physically adsorbed dye was removed from the hyphae and solid supports covered by mycelium. Compared to each other, overall, the highest desorption was achieved for free *CB8* hyphae, particularly at higher initial dye concentration. However,

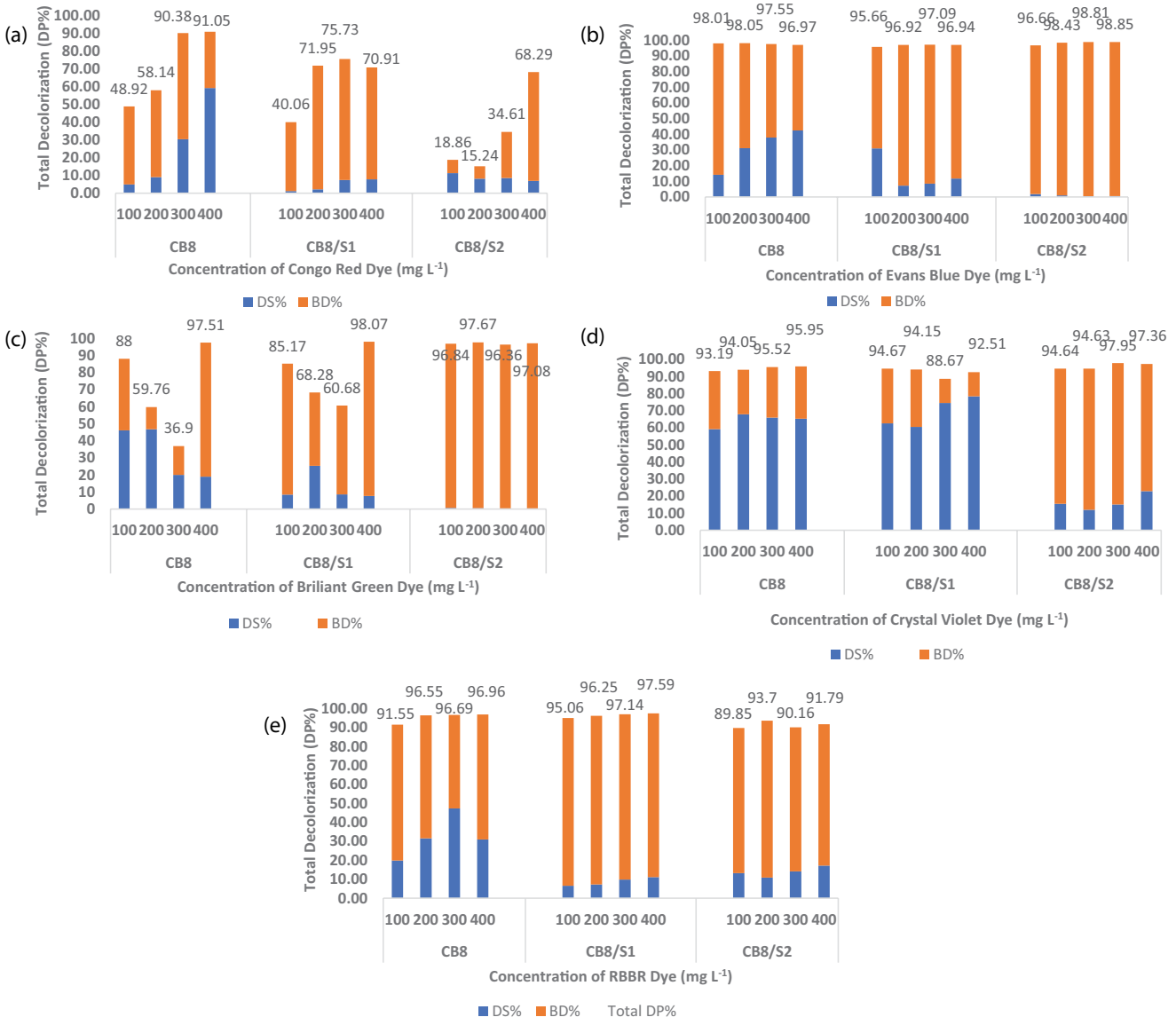


Fig. 2. Comparative analysis of desorption (DS%) and biochemical decolorization (BD%) from total decolorization (DP%) of (a) Congo Red, (b) Evans Blue, (c) Brilliant Green, (d) Crystal Violet and (e) Remazol Brilliant Blue R dye by immobilized and free *CB8* biomass with respect to initial concentration of dye (Desorbing agent-70% methanol, static condition, $T = 20°C \pm 2°C$, $t = 24$ h).

when the fungi were grown on solid supports, it shows a less physical desorption of dye, and more removal was observed by chemical or biochemical process. The fungi immobilized on sponge showed very stable sorption, since the dye was not extracted from the mycelium. The use of supports for immobilization of fungal biomass was beneficial to provide stable interaction between fungal hyphae and dye. The corrugated parallel bundle model (CPBM) have utilized to analyse the effectiveness of immobilized cells and supported the argument that cell immobilization could improve the operational life of the system. This experiment was useful in investigating the sorption process of dye molecule with different dye concentrations. Adsorption models are also available to study the sorption mechanism and kinetics of dye removal. However, this study provides novel insight and differentiation of the possible involvement of physisorption and chemisorption in the overall dye removal mechanism. The study performed represents *T. versicolor* strain *CB8* as a strong candidate for the removal from aqueous solution. It is a sustainable approach for the removal of dye belonging to different classes in higher concentrations. The supports used for immobilization of fungal biomass impart greater stability to dye – cell wall – support complex, which is beneficial for discard. The complex enzyme system of white rot fungi and morphology of fungi played crucial role in different types of removal of pollutant from environment, but it requires further research to study the underlying molecular mechanism of WRF-mediated bioremediation [46].

4. Conclusions

The efficiency of decolorization by *Trametes versicolor* (*CB8*) strain was analysed for five structurally different dyes belonging to three classes. The immobilized and free fungal biomass was highly effective in treating all dyes with great decolorization potential. The removal capacity of more than 95% at such high concentration of dye (400 $mg \cdot L^{-1}$), provides the solution for biological treatment of wastewater containing dyes belonging to different classes at higher concentration. The study has also shed light on the mechanism of dye removal, as the sorption process and biochemical decolorization play an important role in decolorization of the recalcitrant compound. The good desorption capacity of methanol gave the main possibility for the reuse of fungal biomass up to several cycles of decolorization. It also gives a higher chance for safe disposal of biomass after it is utilized in treatment of aqueous dye solution. The result indicates the great capacity of *Trametes versicolor* (*CB8*) strain to be used in wastewater treatment for different dye removal. The further research is indicated to optimize various physico-chemical parameters for improvised dye decolorization efficiency and study metabolite being generated. The current study also indicates the need to develop good methods for studying physical sorption of dye by fungal mycelium. The fungal bioremediation is the best solution to achieve sustainable development goals for water purification contaminated with industrial effluents.

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Competing interest

The authors declare that they have no known competing financial interests or personal relationships that influence the work reported in this paper.

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