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Research Article

Influence of Surfactants on Dye Removal and Growth of *Aspergillus versicolor* – an Effective Way to Decolorize Textile Dye

This study examined the reactive dye decolorization activities of sodium dodecyl sulfate (anionic), cetylpyridinium chloride monohydrate cetylpyridinium chloride monohydrate (CPC) (cationic) surfactants, and their influence on fungal growth. The effect of CPC on Remazol Blue (RB) bioremoval by *Aspergillus versicolor* was investigated in low-cost molasses medium. The optimum pH (3–7) and CPC (0.1–1 mM) concentrations for fungal RB decolorization were achieved in molasses medium with 50 mg/L RB. It was observed that the CPC effectively removed RB due to electrostatic interactions and it was well tolerated by fungus. Maximum decolorization in the presence of CPC occurred at pH 6. Increasing the toxic CPC concentrations (0, 0.1, 0.25, 0.5, 1 mM) decreased fungal growth from 5.08 to 2.87, 1.40, 0.21, and 0.07 mg/L dry biomass, respectively, but enhanced fungal decolorization, respectively (63.2, 78.7, 92, 95.75, and 98.9%). As the augmentation of CPC concentration reduced energy costs by reducing effective decolorization period from 96 to 24 h, surfactant stimulated fungal decolorization method may provide highly efficient, inexpensive, and time-saving procedure in treatment of textile effluents.

Keywords: Decolorization; Fungus; Molasses; Reactive dye; Surfactant

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1 Introduction

Azo-Reactive dyes are commonly preferred in textile industry because of their bright color, ease of application, and low energy consumption [1]. Reactive dyes have azo-based chromophores combined with different types of reactive groups such as vinyl sulfone, chlorotriazine, trichloropyrimidine, and difluorochloropyrimidine. Vinyl sulfone (Remazol) reactive dyes are among the pollutants whose presence in aqueous systems is undesirable [2]. Remazol Blue (RB) belongs to anionic azo-reactive dye class which is found in wastewater at higher concentrations than other dye classes. Since, the reactive dyes are mainly found in hydrolyzed form and cannot be removed efficiently by conventional wastewater treatment systems [3]. Removing the reactive dyes from textile effluents is a challenging issue because these dyes can cause considerable environmental pollution. Moreover, they bear serious health-risk factors [4, 5].

There are some chemical and physical methods to decolorize textile wastewater such as coagulation/flocculation, ozonation, oxidation, ion exchange, irradiation, precipitation, and adsorption

which have financial and methodological disadvantages [2]. In addition to these; they are time-consuming and mostly ineffective [6]. Biological wastewater treatment methods are alternative to decolorize textile effluents because of their inexpensive and eco-friendly nature [7]. Fungi are low-cost biosorbent materials for the removal of reactive dyes due to their high growth rate. Furthermore, fungi cells can be easily cultivated into inexpensive growth media, and they are a readily available source of biomass that has potential for bioremoval of dyes [8]. To date, many filamentous fungal strains have been reported to be effective in decolorization of reactive dyes by growing and dead cells [1, 4, 9–13].

Surfactants are surface active agents that adsorb the interfaces between phases by lowering interfacial tensions [14]. Surfactants are found as monomers in low concentrations [15]. Critical micelle concentration (CMC) is the minimum surfactant concentration to form micelle [15]. Each surfactant monomer has a hydrophilic head and a hydrophobic tail. The head of the molecule is the charged part. Surfactants are classified according to their charge as anionic, nonionic, cationic, and amphoteric [16]. Surfactants are widely used in various industries such as petroleum, food, cosmetic, and textile industries [8, 17]. There are some studies that showed the toxic effect of surfactants on fungal cultures [18, 19]. Surfactants are also used as levelling, dispersing, and wetting agents for improving dyeing process in textile industry [14, 20]. Therefore, the textile industry wastewater also contains surfactants with dyes.

Currently numerous studies have focused on the use of surfactant modified adsorbents for the effective treatment of colored effluents contained reactive dyes [21–25]. There was a study that reported the

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Abbreviations: CMC, critical micelle concentration; CPC, cetylpyridinium chloride monohydrate; DBS, dodecyl benzene sulfonate; DTAB, dodecyl trimethyl ammonium bromide; RB, Remazol Blue; SDS, sodium dodecyl sulfate

efficient cationic dye (Methylene Blue) removal by dead *Rhizopus arrhizus* cells (as low-cost biosorbent) in the presence of anionic surfactant sodium dodecyl sulfate (SDS) [8]. Gül and Dönmez [26] have recently shown that dodecyl trimethyl ammonium bromide (DTAB) cationic surfactant enhanced the anionic dye removal capacity of growing *R. arrhizus* strain. Using growing cultures in bioremoval has an advantage of avoiding a separate biomass production process (e.g. cultivation, harvesting, drying, processing, and storage prior to use) [27]. The potential of metabolically active *Aspergillus versicolor* biomass for reactive dye accumulation has been reported previously [13]. However, there is no report on the dye removal activity of growing *A. versicolor* in the presence of anionic and cationic surfactants to the best of our knowledge. The major objectives of this study are to investigate the potential of using low toxic surfactants with a simple and inexpensive fungal biomass to treat textile effluents and to develop an effective reactive dye removal system due to the enhancement of surfactants. It was also intended to use inexpensive media such as molasses, a waste product of sugar refineries, within this framework, considering their extensive usage as a carbon source in microbial growth media.

2 Materials and methods

2.1 Preparation of dye and surfactant solutions

RB (λ_{max} : 600 nm) was obtained from Aytemizler Textile, Turkey, in pure form. The dye stock solution was prepared by dissolving the powdered dyestuff in distilled water for a final concentration of 2% w/v. The structure of the RB dye is not in the public domain [28]. The anionic surfactant SDS ($\text{C}_{12}\text{H}_{25}\text{SO}_4\text{Na}$, MW: 288.4 g/mol) and cationic surfactant cetylpyridinium chloride monohydrate (CPC) ($\text{C}_{21}\text{H}_{38}\text{ClN}\cdot\text{H}_2\text{O}$, MW: 358.07 g/mol) were supplied by Fluka. Stock surfactant solutions were prepared from surfactants, at 1 g/L concentration by dissolving weighed amount in double-distilled water. Appropriate volumes of the stock solutions were added to the media.

2.2 Microorganism and growth conditions

The filamentous fungus *A. versicolor*, which was isolated from the soil samples of Batman (Turkey) [13], was used in this study. The pure cultures (*A. versicolor*) were kept at 4°C and were transferred to molasses media containing dye every three months, immediately after their arrival to the laboratory.

The growth medium was composed of molasses solution (approximately equivalent to 10 g/L sucrose), 1 g/L $(\text{NH}_4)_2\text{SO}_4$, and 0.5 g/L KH_2PO_4 . The pH was adjusted to desired value with 0.1 M NaOH. The medium was autoclaved (121°C for 15 min) and then a defined quantity of dye and surfactant solutions with a known concentration were added to the growth medium.

The fungal cells were inoculated twice into 250 mL Erlenmeyer flasks containing 100 mL of molasses medium at 25°C on a rotary shaker (New Brunswick Scientific Innova 4230) at 100 rpm.

2.3 Decolorization assays

Decolorization activity of surfactants (SDS and CPC) were examined in 250 mL flasks containing 100 mL molasses medium with 0.5 mM surfactant (144.2 mg/L SDS or 179.035 mg/L CPC), and 50 mg/L RB at pH 6 after 24 h of incubation. In order to determine the effect of SDS and CPC on the fungal growth, the fungus was

inoculated into 250 mL flasks containing 100 mL molasses medium with 50 mg/L RB, 0.5 mM each of SDS (144.2 mg/L) and CPC (179.035 mg/L). The dry weight of the fungus was measured after 96 h of incubation period. To investigate the CPC surfactant on dye bioremoval activity of fungus, the cultures were inoculated in 100 mL molasses medium contained 50 mg/L RB in the absence and presence of 0.1 mM CPC at pH 6 and incubated 96 h. The flasks contained molasses medium with 0.1 mM CPC and 50 mg/L RB were used as control.

The effect of pH on fungal bioremoval in the absence and presence of 0.5 mM CPC was determined at pH values of 3, 4, 5, 6, and 7 in 100 mL molasses with 50 mg/L RB. In order to investigate the influence of CPC concentration on dye bioremoval and fungal growth, *A. versicolor* was grown in 100 mL molasses medium including 0.1, 0.25, 0.5, and 1 mM CPC and 50 mg/L RB. To determine the influence of CPC concentrations (0.1, 0.25, 0.5, and 1 mM CPC) on fungal growth in 100 mL molasses medium contained 50 mg/L RB, dry weight of fungus was measured at the end of incubation period. To examine the dye surfactant interactions, the effect of CPC concentration on absorption intensity of RB was determined in flasks contained molasses medium supplied with 0, 0.1, 0.25, 0.5, 1, and 2 mM CPC and 50 mg/L RB after 30 min incubation.

Experiments were performed at a constant temperature of 25°C to be representative of environmentally relevant conditions. All the experiments were carried out at least twice. The values used in calculations were mostly the arithmetic average of the experimental data.

2.4 Analytical methods

A 3 mL sample was taken daily from each flask and centrifuged at 10000 rpm at 15 min to remove suspended biomass during the incubation period. The concentration of RB was measured spectrophotometrically by reading the absorbance values at 600 nm. In our previous study, the influence of cationic surfactant on the absorbance of RB dye was already examined on the samples with surfactant concentrations of 0, 0.1, 0.5, 1, and 2 mM in the spectral range of 350–650 nm. The maximum dye absorbance occurred at 600 nm in the samples with or without the surfactant, the wavelength was not significantly changed by the addition of the surfactant, so 600 nm was used to analyze dye removal rates in all experimental series [26]. Cell-free molasses medium was used as the blank, dry weight of fungal biomass was measured at the end of the incubation period to determine the fungal growth. Dry weight of the fungal biomass was obtained by filtering the contents of each flask through pre-weighed filter paper and washed two times with distilled water. The liquid medium was removed and the filtered biomass was dried to a constant weight at 80°C at one night and measuring the dry weight of biomass. Dry weight was expressed in terms of g biomass/L culture.

Shimadzu UV 2001 model spectrophotometer and Medical Instruments MPW-351 R model centrifuge were used for absorbance measurements and centrifugation, respectively.

3 Results and discussion

Dye removal activities of SDS (anionic) and CPC (cationic) were determined and the effects of these surfactants on fungal growth were examined. The influence of CPC on dye removal properties of *A. versicolor* were investigated in a batch system as a function of initial pH, and surfactant concentrations. The percentage bioremoval

of dye was calculated from:

$$\text{Dye decolorization (\%)} = \frac{C_0 - C_f}{C_0} \times 100 \quad (1)$$

In this equation, C_0 and C_f represent the initial and final dye concentrations (mg/L), respectively.

3.1 Decolorization activity of SDS (anionic) and CPC (cationic) surfactants

To determine the most active surfactant in decolorization of dye in molasses medium, the flasks contained molasses medium (pH 6) with 50 mg/L RB and 0.5 mM surfactants (an anionic surfactant SDS and a cationic surfactant CPC) were prepared and analyzed after 24 h incubation period. The cationic surfactant CPC (85.65%) removed more anionic dye than anionic surfactant SDS did (1.5%) (Fig. 1). Simoncic and Span [17] have shown that there was not any interaction between an anionic dye C.I. Acid Orange 7 and an anionic surfactant SDS but a cationic surfactant dodecyl pyridinium chloride strongly interacted with anionic dye because of electrostatic attractive interactions. In this study anionic surfactant SDS removed 1.5% of 50 mg/L anionic RB due to repulsive electrostatic interactions which prevented SDS-RB interactions and dye decolorization. There were electrostatic interactions between anionic RB and cationic CPC and these interactions led dye removal activity of surfactant.

3.2 The influences of SDS (anionic) and CPC (cationic) surfactants on fungal growth

The influences of anionic and cationic surfactants on fungal growth were examined to measure the dry weight of biomass. *A. versicolor* was inoculated to flasks included molasses medium supplied with 50 mg/L dye and 0.5 mM surfactant and then the dry weight of fungus was measured after 96 h incubation. The fungus did not grow in the presence of anionic SDS, but fungal growth was observed in presence of cationic CPC as 0.59 mg/L after 96 h of incubation. Gül and Dönmez [26] have studied with a filamentous fungus *R. arrhizus* and they observed fungal growth both in the presence of anionic surfactant dodecyl benzene sulfonate (DBS) and cationic surfactant DTAB, but the amount of biomass was decreased in the presence of DBS and DTAB. Anionic DBS was more toxic than cationic DTAB for fungus. Garon et al. [18] have tested the toxicity effect of anionic and non ionic surfactants on various fungal strains. Anionic surfactant SDS

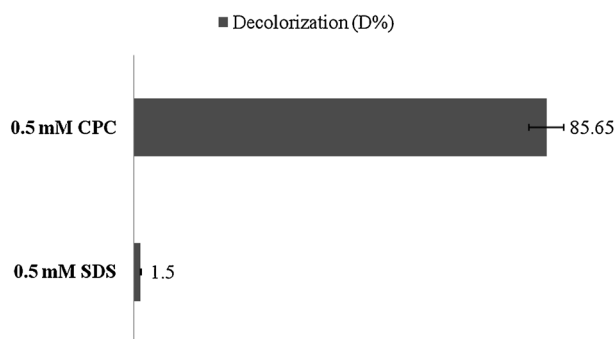


Figure 1. Decolorization ($D\%$) of surfactants in molasses medium with 50 mg/L RB at pH 6 ($T: 25 \pm 1^\circ\text{C}$; stirring rate: 100 rpm; incubation period: 24 h).

inhibited fungal growth, while nonionic surfactants (Triton X100 and Tween 80) were well tolerated by fungi. The toxicity of SDS was related with the physicochemical interactions between surfactants and fungal surface structures. Tanuja et al. [19] have recently shown that the cationic surfactants such as cetyl dimethyl ammonium bromide, DTAB and the anionic surfactant (SDS) completely inhibited spore germination of *A. parasiticus* (NRRL 2999) at 0.01% concentration. In this study, the growth of *A. versicolor* strain was completely prevented at 0.5 mM SDS concentration but the fungus tolerated and grew in the presence of 0.5 mM CPC. It was observed that dry weight of *A. versicolor* in the presence of 0.5 mM CPC was 0.21 mg/L after 24 h of incubation (Tab. 1). There was no fungal growth in the presence of SDS. The cationic surfactant CPC was low toxic than SDS. Previously, it was reported that the surfactants enhanced the removal of dyes by using adsorbents [21–26]. It is assumed that the addition of less toxic surfactants for fungus may be a useful tool to accelerate fungal bioremoval of dyes.

3.3 The influence of CPC on fungal bioremoval

In order to examine the influence of CPC on fungal decolorization, *A. versicolor* was inoculated in flasks contained molasses medium with 50 mg/L RB at pH 6 in the absence and presence of 0.1 mM CPC. The dye removal of only CPC was also tested in the same conditions. Maximum dye removal occurred in flasks contained both fungus and 0.1 mM CPC as 78.7% after 96 h incubation period (Fig. 2). Anionic surfactant SDS inhibited fungal growth and dye removal of fungus,

Table 1. The influence of CPC concentration on fungal decolorization ($D\%$) and growth (dry weight – X_m) in molasses medium at pH 6

	C_{ORB} (mg/L)	$D\%$	X_m (mg/L)	Time (h)
A.v.	50	63.2	5.08	96
A.v. + 0.1 mM CPC (total)	50	78.7	2.87	96
0.1 mM CPC	50	34.6	–	96
A.v. + 0.25 mM CPC (total)	50	92	1.40	96
0.25 mM CPC	50	83	–	96
A.v. + 0.5 mM CPC (total)	50	95.75	0.21	24
0.5 mM CPC	50	85.65	–	24
A.v. + 1.0 mM CPC (total)	50	98.9	0.07	24
1.0 mM CPC	50	98	–	24

C_{ORB} , initial RB concentration; A.v., *A. versicolor*; $T, 25 \pm 1^\circ\text{C}$; stirring rate, 100 rpm.

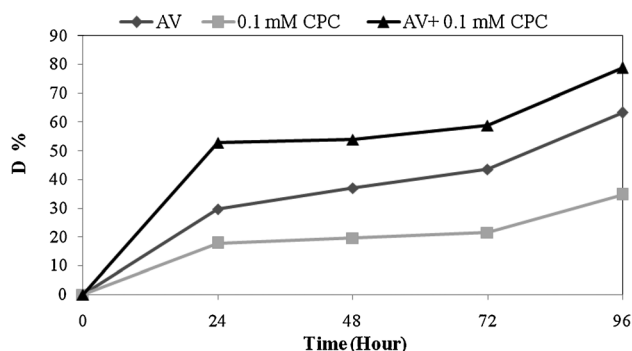


Figure 2. Decolorization ($D\%$) of fungus in the absence and presence of CPC in molasses medium with 50 mg/L RB at pH 6 (A.v.: *A. versicolor*; $T: 25 \pm 1^\circ\text{C}$; stirring rate: 100 rpm).

but cationic CPC induced dye adsorption on fungal surface. It was known that the *A. versicolor* strain, which was used in this study, produced an extracellular laccase enzyme which was responsible for decolorization activity [29]. Surfactants can promote the secretion of extracellular enzymes from microorganisms or modify enzyme conformation in a manner that alters enzyme activity, stability and/or specificity [15]. It was assumed that CPC enhanced enzymatic fungal decolorization because there is no relationship between decolorization activity and fungal growth in the presence of surfactant. The surfactant can help substrat (dye) availability of enzyme by arranging electrostatic interactions.

3.4 The influence of pH on fungal bioremoval

To investigate the influence of pH on decolorization activity of fungus with CPC, 2 mL fungal culture inoculated in to the flasks contained molasses medium with 50 mg/L RB and 0.5 mM CPC at pH values of 3, 4, 5, 6, and 7. The flasks contained molasses medium including 50 mg/L RB and 0.5 mM CPC at the same pH values were used as control. In the presence of 0.5 mM CPC, *A. versicolor* showed maximum dye removal activity at pH 6 (Fig. 2). Recently, Eren et al. [30] have shown that increasing of pH value resulted in decreasing adsorption of dye due to electrostatic interactions. Gül and Dönmez [26] have reported that the filamentous fungus *R. arrhizus* performed maximum anionic dye removal at pH 3 as 75.3% in the absence of DTAB but at pH 6 as 85.9% in the presence of 0.5 mM DTAB. The surface of *R. arrhizus* was charged positively in low pH values such as pH 3 and the colored anions of RB interacted with the fungal surface electrostatically. The anionic functional groups on the surface of fungus were activated at pH 6 and repulsed the colored anions of RB. The addition of cationic surfactant (DTAB) enhanced dye removal efficiency to maintain electrostatic interactions. In the presence of 0.5 mM CPC, RB removal activity of *A. versicolor* was 95.75% after 24 h incubation period at pH 6 (Fig. 3). It is considered that the CPC cations performed as a linkage between dye anions and negative charged fungal surface at pH 6. There were electrostatic interactions between negative charged dye and positive charged surfactant. These interactions allowed removal of dye. Bernard and Latge have reported that the filamentous fungus *A. fumigatus* has chitin and chitosan polymers in its cell wall [31]. Chitin/chitosan was a component of fungal cell wall and the major site for dye adsorption [1]. Chatterjee et al. [32] have shown that chitosan removed an anionic dye Congo Red by the ionic interactions between

Congo Red and chitosan. Tunali Akar et al. [33] stated that the surface of *A. parasiticus* became negative at pH 6 like the surface of chitosan [32]. Özdemir et al. [25] have shown that the negative charged zeolite surface interacted with cationic surfactant hexadecyltrimethylammonium bromide (HTAB) and the quaternary amines of surfactant interacted with anionic dye.

3.5 The influence of CPC concentration on fungal bioremoval and growth

The influence of initial surfactant concentration on fungal bioremoval and growth was examined in more detail in the following series of experiments, in which initial CPC concentrations were varied as 0, 0.1, 0.25, 0.5, and 1 mM, whereas the RB concentration was kept constant at 50 mg/L. Moreover, the experiments were repeated without fungus. The dry weight of fungal biomass was decreased from 5.08 to 0.07 mg/L with the CPC concentration increasing from 0 to 1 mM (Tab. 1). On the other hand maximum decolorization occurred in the flask contained both surfactant and dye in all surfactant concentrations. Augmentation of CPC concentration (0–1 mM) increased decolorization rate from 63.2 to 98.9%. Aksu et al. [8] have shown that maximum cationic Methylene Blue (50 mg/L initial dye concentration) dye removal of *R. arrhizus* was increased as 12.4, 67.2, and 85.2% by the augment of anionic surfactant SDS concentrations (0, 0.01, and 1 mM). Yousefi et al. [24] have recently reported that CPC surfactant modified wheat straw effectively decolorized 83.15% of 50 mg/L Reactive Black 5 and increasing surfactant modified adsorbent dose enhanced dye adsorption. In this study, maximum dye removal was observed in the flask contained fungus and 1 mM CPC as 98.9%. In addition to this, the increasing surfactant concentration (0–1 mM CPC) resulted in decreasing of decolorization time period from 96 to 24 h (Tab. 2).

3.6 The influence of CPC concentration on dye surfactant interactions

In order to examine the interactions between dye and surfactant, the absorbance changes of dye (50 mg/L) was determined after adding 0, 0.1, 0.25, 0.5, 1, and 2 mM CPC and is given in Fig. 4. The absorption intensity of RB was decreased while the surfactant concentration was increasing up to 1 mM. The surfactant monomers and dye molecules interacted electrostatically surfactant and formed dye–surfactant complex and this formation caused a decrease in absorption intensity of dye [17, 20, 34, 35]. Further augment of CPC concentration such as 2 mM resulted in significant increase of RB absorbance (Fig. 4). In low surfactant concentrations, the surfactant molecules formed aggregates named micelles and dye molecules bound to opposite charged micelles [8]. The formation of dye and surfactant aggregates caused to increase absorption intensity of dye. Spectral characteristics of Direct Yellow with the addition of the cationic surfactant were tested by Bielska et al. [35] and in low surfactant concentrations such as 0.025–0.075 CMC a sharp decrease of the absorption intensity resulting from dye–surfactant complex formation was reported. In our study, it was observed that in low surfactant concentrations (0.1–1 mM) the absorbance was decreased. Decreasing absorption intensity of dye indicated the formation of dye–surfactant complex [8]. Özdemir et al. [25] have shown that adsorption of anionic dyes on hexadecyltrimethylammonium bromide (cationic) surfactant modified zeolite was enhanced because of electrostatic interactions

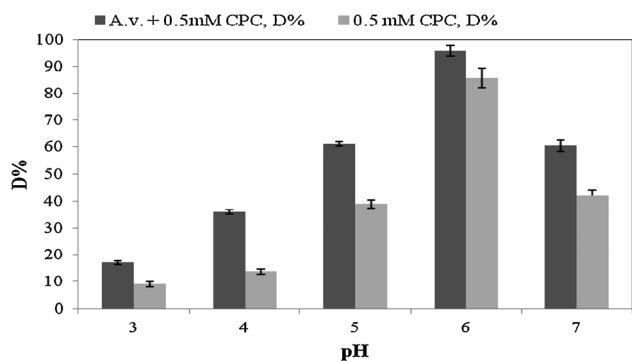
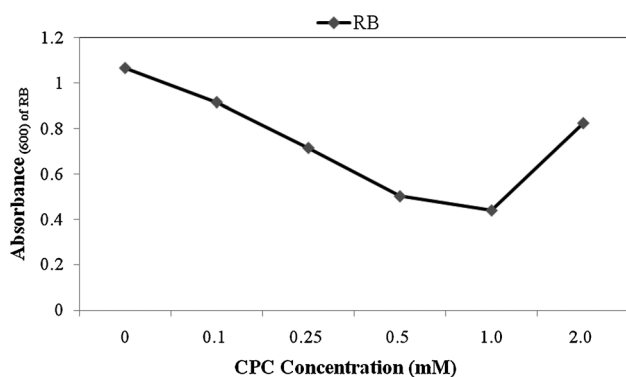


Figure 3. The influence of pH on RB decolorization ($D\%$) in molasses medium (pH 6) with 50 mg/L dye and 0.5 mM surfactant (A.v.: *A. versicolor*; T: $25 \pm 1^\circ\text{C}$; stirring rate: 100 rpm; incubation period: 24 h).

Table 2. Decolorization ($D\%$) of Reactive Blue dyes in the presence and absence of surfactant by other species from the literature

Fungal strain	S	D	C_{0D} (mg/L)	C_{0S} (mM)	$D\%$	Time (h)	Biomass type	Reference
<i>A. versicolor</i>	–	RB	123	0	95.1	168	Growing	[14]
<i>C. tropicalis</i>	–	RB	91.3	0	98.8	184	Growing	[36]
<i>S. cerevisiae</i>	–	RB	78.9	0	91.2	264	Growing	[37]
<i>R. arrhizus</i>	–	RB	100	0	39.2	120	Growing	[25]
<i>R. arrhizus</i>	DTAB	RB	100	0.5	85.9	120	Growing	[25]
<i>R. arrhizus</i>	DTAB	RB	100	1	97.1	120	Growing	[25]
<i>A. versicolor</i>	–	RB	63.1	0	63.2	96	Growing	This study
<i>A. versicolor</i>	CPC	RB	59.5	0.5	95.75	24	Growing	This study
<i>A. versicolor</i>	CPC	RB	57.1	1	98.9	24	Growing	This study

S, surfactant; D, dye; C_{0D} , initial dye concentration; C_{0S} , initial surfactant concentration; $D\%$, decolorization rate.

**Figure 4.** The influence of CPC concentration on absorbance of 50 mg/L RB (T : $25 \pm 1^\circ\text{C}$; stirring rate: 100 rpm; incubation period: 30 min).

between dye and surfactant. It is assumed that anionic dye RB molecules and cationic surfactant CPC monomers electrostatically interacted; this interaction enhanced the dye removal of fungus.

A comparison has been made between decolorization rate of Reactive Blue dyes in the presence and absence of surfactant by other species from the literature (Tab. 2). *Aspergillus versicolor* decolorized high amount of dye in long incubation periods. Oppositely charged surfactants enhanced dye decolorization of fungus and maximum dye decolorization occurred in the presence of CPC and *A. versicolor*. It was shown that the system contained 1 mM CPC and *A. versicolor* decolorized 98.9% of dye in a very short period of time as 24 h. There were a few studies about using surfactant as an enhancement in decolorization of textile dyes by fungal biomass (Tab. 2). The results of this study showed the maximum decolorization rate after short incubation period while comparing other studies.

4 Concluding remarks

The influence of anionic and cationic surfactants on fungal dye removal and fungal growth were determined in an inexpensive molasses medium. There was no fungal growth in the presence of anionic surfactant SDS. The cationic surfactant CPC affected fungal growth negatively but fungal decolorization positively. The electrostatic interactions between opposite charged surfactant and dye enhanced decolorization activity of fungal strain. The decolorization rates of *A. versicolor* strain were 95.75 and 98.9% in the presence of 0.5 and 1 mM CPC after 24 h incubation period. The usage of surfactants shortened the incubation period to save energy and lessen energy

costs (such as the required energy for treatment methods) in biological wastewater treatment process according to the results of this study.

It can be concluded that *A. versicolor* can effectively decolorize high amount of dye by the enhancement of cationic CPC. Surfactant enhanced fungal bioremoval technology is one of the feasible approaches to decolorize textile effluents.

The authors have declared no conflict of interest.

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