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Application of mixed fungal biomass for effective reactive dye removal from textile effluents

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ABSTRACT

Reactive Remazol Blue (RB) removal properties of growing *Aspergillus versicolor* and *Rhizopus arrhizus* were investigated as a function of initial pH (3–7) and dye concentration (50–800 mg/L) in low-cost molasses medium. Decolorization activity of mixed (*A. versicolor* and *R. arrhizus* together) culture in the absence and presence of (0.5 and 1 mM) dodecyl trimethyl ammonium bromide (DTAB) was examined at optimal conditions. *A. versicolor* and *R. arrhizus* exhibited maximum decolorization at pH 6 as 89.4% and at pH 3 as 69.23% at 100 mg/L dye concentration in six days. The decolorization of *A. versicolor* depended on fungal growth but *R. arrhizus* was related with its positively charged surface. Decolorization by both fungi decreased with increasing dye concentration up to 800 mg/L. The mixed culture decolorized 86.5% and 100% of 100 mg/L RB in the absence and presence of 0.5 mM DTAB in three days. Decolorization activities by *R. arrhizus* (10.06%), *A. versicolor* (15.79%), the mixed culture (26.59%), the mixed culture with 0.5 mM (61.33%) and 1 mM DTAB (73.19%) were compared at 800 mg/L RB in three days. The results of this study show that the systems contained mixed culture with surfactant that decolorized high level of reactive dye concentration effectively.

Keywords: Reactive dye; Decolorization; Fungi; Mixed culture; Surfactant

1. Introduction

Textile industries extensively use reactive dyes due to their favorable characteristics such as bright color, waterfastness, and simple application techniques [1]. In order to color textile products, they also consumed substantial volumes of water with dyes. Reactive dye-containing wastewater discharged from textile effluents have to be treated because of their impact on water bodies, and growing public concern over their toxicity and carcinogenicity [2]. The removal of reactive dyes is more difficult than other dye classes [1]. As a result, reactive dyes are found at higher concentrations than other dyes [3].

Recently, the interest on natural or eco-friendly biosorbents that removed dyes from wastewater is increased [4,5]. New methods are developed to remove dyes from textile effluents including use of micro-organisms such as fungi, bacteria, and algae [6,7]. Fungi are inexpensive and promising materials for removal of reactive dyes due to their high growth rate, and fungal cells can be easily cultivated into inexpensive growth media and are a readily available

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source of biomass that has potential for bioremoval of dyes [8]. Fungi, in common with other microbial groups, can remove dyes from their external environment by the means of physico-chemical and biological mechanisms. They are used for treatment of dye wastewater in two ways. The first one is mycelial adsorption of dyes by using living or dead fungal cells [9,10], while the second one involves the using of growing cells and their extracellular enzymes [11,12]. The major mechanisms are biodegradation for growing fungal cells due to enzymatic decolorization and biosorption involving physicochemical interactions such as adsorption, deposition, and ion exchange for dead fungal cells [9].

The potential for effective dye removal by growing and dead biomass of Aspergillus species has been documented [13–16]. To date, *Rhizopus arrhizus* strain has been reported to be effective in biosorption of dyes by dead cells [17–21]. However, decolorization activity of growing fungal biomass has been less frequently reported. The previous study emphasized that using growing cultures in bioremoval process has an advantage of avoiding a separate biomass production process (e.g. cultivation, harvesting, drying, processing, and storage prior to use) [22].

Pure fungal culture has been used to develop bioremoval of reactive dyes [13–21]. However, a long growth cycle and moderate decolorization rate still limit the performance of the fungal decolorization system [23]. The dye removal activity of fungal decolorization decreased while the dye concentration was increased due to toxic effect of dye on fungal growth [24,25]. Mixed culture has the advantage because of its synergistic effects on micro-organisms, therefore, it may quickly tolerate the toxic effects [26]. The previous studies have showed that the fungal biomasses of *R. arrhizus* and *Aspergillus* species have potential for use in the treatment of dye-contaminated wastewaters. However, there is no information available about the decolorization of reactive dyes by the mixed cultures of *Aspergillus versicolor* and *R. arrhizus*.

In recent years, a number of studies have focused on fungal biomass that removed reactive dyes efficiently by the enhancement of surfactants [24,25]. Textile wastewater also contains surfactants which are used as leveling, dispersing, and wetting agents for improving dyeing process in textile industry [27].

The present paper reports on application and potential of mixed *A. versicolor* and *R. arrhizus* cultures for treating reactive dye-containing wastewater. For this purpose, both *A. versicolor* and *R. arrhizus* cultures were inoculated into the molasses medium contained reactive Remazol Blue (RB) dye in the absence and presence of cationic dodecyl trimethyl ammonium bromide (DTAB) surfactant. The main objective of this study was to examine the potential of using such a simple and inexpensive mixed fungal biomass to treat textile wastewaters and developed an effective decolorization system by the enhancement of surfactants. Molasses is used as a carbon source because of its low-cost, ready availability, and ease of storage.

2. Materials and methods

2.1. Preparation of dye and surfactant solutions

Remazol Blue (λ_{max}: 600 nm) was obtained from Aytemizler Textile Co., 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 cationic surfactant dodecyl trimethyl ammonium bromide (DTAB) ($C_{12}H_{22}N(CH_3)_3Br$, MW: 308.34 g/mol) was purchased from Fluka. Stock surfactant solutions were prepared from surfactant, at 1.0 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) [28], 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 fungal cells were acclimated in the media that contained dye by transferring twice before measuring dye removal and 2 mL of activated fungal biomass was inoculated into 250 mL Erlenmeyer flasks containing 100 mL of molasses medium with adequate dye concentration and at 25 ± 1°C on a rotary shaker (New Brunswick Scientific Innova 4230) at 100 rpm at six days of incubation.

Another filamentous fungus *R. arrhizus*, was obtained from the US Department of Agriculture Culture Collection, was used in this study. The fungal cells were inoculated into experiment tubes contained 5 mL molasses media with dye and incubated in two days at 25 ± 1°C to activate fungal biomass. The active fungal biomass transferred into 250 mL Erlenmeyer flasks containing 100 mL of molasses medium with adequate dye concentration at 25 ± 1°C at six days of incubation.

The mixed *A. versicolor* and *R. arrhizus* culture were prepared by inoculating activated both fungal (*A. versicolor* and *R. arrhizus*) biomasses into 250 mL Erlenmeyer flasks containing 100 mL of molasses medium with adequate dye concentration at 25 ± 1°C

on a rotary shaker (New Brunswick Scientific Innova 4230) at 100 rpm at three days of incubation.

The growth medium was composed of beet molasses solution (approximately equivalent to 10 g/L sucrose), 1.0 g/L $(NH_4)_2SO_4$, and 0.5 g/L KH_2PO_4. The pH was adjusted to desired value with 0.1 M NaOH using pH meter (Consort). The medium was autoclaved (121˚C for 15 min) and then a defined quantity of dye and surfactant (for mixed culture) solution with a known concentration was added to the growth medium.

2.3. Decolorization assays

The effect of initial medial pH on RB decolorization was investigated in the molasses medium contained 100 mg/L RB at pH 3, 4, 5, 6, and 7. The active *A. versicolor* strain and *R. arrhizus* strain were inoculated into media separately to examine the effect of pH on each culture decolorization activity at six days of incubation.

To examine the effect of initial dye concentration on RB decolorization, initial dye concentrations were varied as 50, 100, 200, 400, and 800 mg/L in molasses medium at pH 6. The active *A. versicolor* strain and *R. arrhizus* strain were inoculated into media separately. After six days of incubation, the dry weight of fungal biomasses was also measured.

To investigate the decolorization activity of mixed culture, *A. versicolor* and *R. arrhizus* cultures were inoculated together into Erlenmeyer flasks contained molasses medium (pH 6) with100 mg/L RB. The effect of cationic surfactant DTAB on mixed culture was determined by adding 0.5 and 1 mM DTAB into Erlenmeyer flasks.

In order to determine the decolorization activity of individual *R. arrhizus*, individual *A. versicolor*, mixed (*A. versicolor* and *R. arrhizus* togather) culture and mixed culture with surfactant (0.5 and 1 mM DTAB) in high dye concentration, the cultures inoculated into Erlenmeyer flasks contained molasses medium with 800 mg/L RB.

The studies were performed at a constant temperature of 25 ± 1˚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

During the incubation period, a 3-mL sample was taken daily from each flask and centrifuged at 10000 rpm at 15 min to remove suspended biomass. The concentration of Remazol Blue was determined by measuring the absorbance at 600 nm. The previous study reported that the absorbance of Remazol Blue was maximum at 600 nm in the absence and presence of 0.5 and 1 mM DTAB surfactant in molasses media [24]. Cell-free molasses medium was used as the blank. For the measurement of microbial growth, the biomass concentration was determined by measuring dry weight at the end of the incubation period. Dry weight of the fungal biomass was obtained by filtering the contents of each flask through pre-weighed filter paper, drying 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 of biomass per liter of culture. Two control flasks were prepared. First control medium contained both dye and molasses without growing of fungus to observe any reaction of molasses with dye. Second control molasses medium contained both dye and surfactant to examine any reaction between dye and surfactant.

3. Results and discussion

Reactive dye RB removal properties of growing filamentous fungi *A. versicolor* and *R. arrhizus* were investigated as a function of initial pH and dye concentration. Decolorization activity of mixed (*A. versicolor* and *R. arrhizus* togather) culture in the absence and presence of (0.5 and 1 mM) DTAB was examined at optimal pH and dye concentration.

The percentage decolorization of dye was calculated from Eq. (1):

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

The bioremoval capacity of dye is the concentration of dye in the biomass and can be calculated based on the mass balance principle from Eq. (2):

$$q_m = (C_o - C_f)/X_m \quad (2)$$

In these two equations, q_m (the maximum specific dye uptake) represents the maximum amount of dye per unit dry weight of fungus (mg/L), X_m the maximum dried cell mass (g/L), and C_o and C_f the initial and final concentrations (mg/L), respectively.

3.1. The effect of pH on decolorization of fungi

The reactive dye decolorization of growing *A. versicolor* and *R. arrhizus* cultures were performed in various pHs such as 3, 4, 5, 6, and 7 in molasses media with 100 mg/L RB after six days of incubation separately. *A. versicolor* decolorized maximum 89.4%

of RB at pH 6, on the other hand *R. arrhizus* removed maximum 69.23% of RB at pH 3 (Fig. 1(a). Both *A. versicolor* and *R. arrhizus* are eurkaryotic filamentous fungi and belong to Ascomycota [2]. Although the scientific classification of these fungal strains was similar; they performed maximum decolorization activity in different pH values. It was assumed that the difference in decolorization performance was related with the decolorization mechanism.

The previous studies have showed that the maximum biosorption of reactive dyes such as Reactive Orange 16 [18] and Gemazol Turquise Blue-G [19] by dead *R. arrhizus* biomass occurred at lower pH values because in acidic conditions the functional groups on the fungal surface charged positively and interacted with dye anions electrostatically. Recently, Cardoso et al. (2012) have showed that the filamentous fungus *R. arrhizus* has chitin and Chitosan polymers in the structure of its cell wall [29]. Chitin/Chitosan was a component of fungal cell wall and the major site of sorption [9]. Chatterjee et al. (2007) have showed the anionic dye Congo Red sorption capacity of chitosan and ionic interaction between Congo Red and Chitosan [30]. The dye removal capacity of Chitosan decreased while the initial pH value was increasing [30]. Adsorption due to the electrostatic attraction could be the primary mechanism for the biosorption

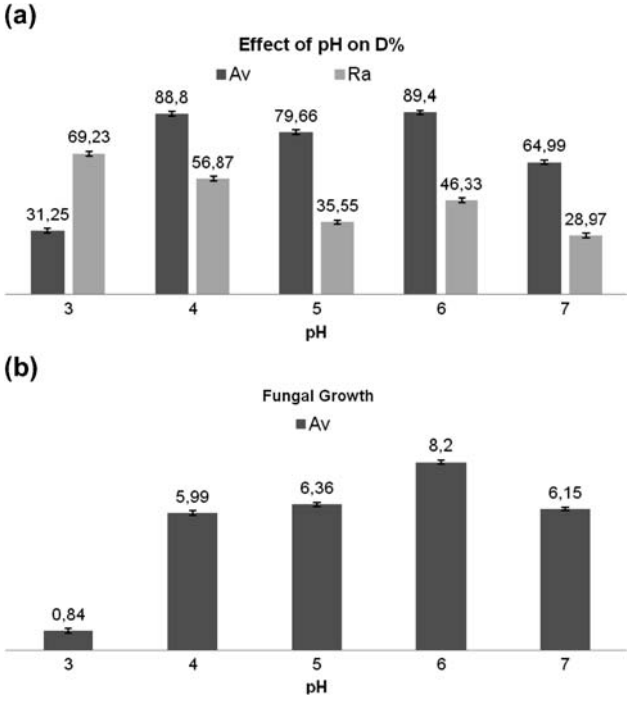

Fig. 1. (a) The effect of pH on decolorization (D%) by growing cultures and (b) the effect of pH on fungal growth of *A. versicolor* (Av: *A. versicolor*; Ra: *R. arrhizus*; C_{oRB}: 100 mg/L; incubation period: six days; *T*: 25 ± 1 °C).

of anionic dye on dried *R. arrhizus* surface at lower pH values [20]. Similarly in this study, it was observed that the dye removal activity of growing *R. arrhizus* culture increased in decreasing pH conditions.

Fu and Viraraghavan (2002) have reported that the maximum biosorption of anionic dye (Congo Red) by dead *Aspergillus niger* occurred at pH 6 [13]. Telke et al. (2009) purified laccase enzyme from growing filamentous fungus *Aspergillus ochraceus* NCIM-1146. Laccase was responsible for decolorization of textile dye and high laccase activity maintained at pH 5–7 [14]. Laccases can decolorize azo dyes related with the mechanism of free phenoxy radical formation which can end up in the polymerization [31]. Laccases destroy azo dyes' chromophoric assemblies to modify azo dye structure and phenoxyl radicals are generated in the reactions [32]. In this study growing *A. versicolor* showed maximum decolorization activity at pH 6 and it was known that *A. versicolor* produced extracellular laccase enzyme in order to remove textile dyes [unpublished data].

The fungal growth was determined by measuring the dry weight of fungal biomass at the end of incubation period. Fig. 1(b) shows the dry weight (g/L) of *A. versicolor* in the presence of 100 mg/L RB after six days of incubation. *A. versicolor* showed maximum dye removal at pH 6 (Fig. 1(a)), which was also the optimal value for fungal growth (Fig. 1(b)). So the decolorization of *A. versicolor* depended on fungal growth and also fungal enzyme production. On the other hand, *R. arrhizus* showed maximum decolorization activity at pH 3 (Fig. 1(a)) but maximum growth at pH 4–6 [unpublished data]. The dye removal activity was not depend on fungal growth but related with the positive charge of functional groups on fungal cell wall due to acidic conditions.

The dye removal capacity of *A. versicolor* was higher than *R. arrhizus* and maximum decolorization of *A. versicolor* occurred at pH 6. Chatterjee et al. (2009) have showed that the cationic surfactant CTAB enhanced the anionic dye decolorization activity of Chitosan and maximum removal occurred at pH 5 in the presence of CTAB [33]. Although only Chitosan showed maximum Congo Red removal at pH 2–3 [30]. In the presence of DTAB, *R. arrhizus* [24] and *A. versicolor* [25] showed maximum decolorization at pH 6. The optimal pH was selected as pH 6 for the mixed culture.

3.2. The effect of initial dye concentration on decolorization of fungi

To examine the effect of dye concentration on decolorization activity of *A. versicolor* and *R. arrhizus*,

the fungi inoculated to molasses media containing 50, 100, 200, 400, and 800 mg/L RB reactive dye at pH 6 and incubated six days. The dry weight of fungal biomasses was also measured at the end of incubation period. Both of the fungal strains decolorization activity decreased by increasing dye concentration (Fig. 2). It was reported that higher dye concentration strongly inhibited microbial decolorization because of toxic effects [34,35]. Recently, Namdhari et al. (2012) have examined the effect of dye concentration on decolorization of Reactive Blue MR by *Aspergillus* species and reported that *A. allhabadii* and *A. sulphureus* showed maximum activities as 95.1% and 93.01% with 200 mg/L dye and *A. niger* performed maximum 83.14% dye removal with 100 mg/L dye after 10 days of incubation [16]. In this study *A. versicolor* decolorized 100%, 89.4%, and 92.09% dye with 50, 100, and 200 mg/L dye after six days of incubation (Fig. 2).

The effect of dye concentration on dye removal capacity (q_m) of fungi was also investigated and given at Table 1. *A. versicolor* performed higher dye removal capacity and activity than *R. arrhizus* did. Maximum dye removal capacity of *A. versicolor* was observed in the presence of 800 mg/L RB, as 183.9 mg/g (Table 1). The difference in dye removal capacity of fungal strains was related with the dominant mechanism that they used to decolorize dye. *R. arrhizus* removed dye by the electrostatic interactions between dye and fungal surface. *A. versicolor* decolorized dye by using enzyme. It was observed that the growing cultures, which were metabolically active, produced enzymes in order to remove dye effectively.

The previous studies have compared dye removal of growing and dead (autoclaved at 121°C for 15 min) cells of *R. arrhizus* [24] and *A. versicolor* [25]. The growing cells showed maximum dye bioremoval and decolorization may mainly depend on fungal metabolic activity [24,25].

Table 1
The effect of initial dye concentration on dye removal capacity (q_m) of fungi (Av: *A. versicolor*; Ra: *R. arrhizus*; pH: 6; incubation period: six days; *T*: 25 ± 1°C)

C_{oRB} (mg/L)	$q_{m\ Av}$ (mg/g)	$q_{m\ Ra}$ (mg/g)
50	6.9	6.1
100	25.8	10.2
200	55.0	13.4
400	79.6	22.6
800	183.9	34.4

3.3. Total decolorization activity of mixed culture and surfactant

3.3.1. Total decolorization activity of mixed culture and surfactant in low dye concentration

In order to examine the decolorization activity of mixed culture, *A. versicolor* and *R. arrhizus* cultures were inoculated together into Erlenmeyer flasks contained 100 mg/L reactive dye at pH 6. The effect of cationic surfactant DTAB on mixed culture was investigated by adding 0.5 and 1 mM DTAB into Erlenmeyer flasks contained 100 mg/L RB at pH 6. Decolorization activity of *R. arrhizus* and *A. versicolor* 46.33% and 89.4% after six days of incubation and the total dye removal of mixed culture was 86.5% after three days of incubation in same conditions (Fig. 3). The decolorization efficiency was increased while the incubation period was shortened. Recent studies reported that DTAB enhanced fungal dye removal and *R. arrhizus* with 0.5 mM DTAB removed 85.9% of 100 mg/L RB after five days of incubation [24] and *A. versicolor* with 0.5 mM DTAB decolorized 98.9% of 100 mg/L RB after three days of incubation [26]. The total decolorization of mixed *A. versicolor* and *R. arrhizus* with 0.5 mM DTAB was maximum as 100% after

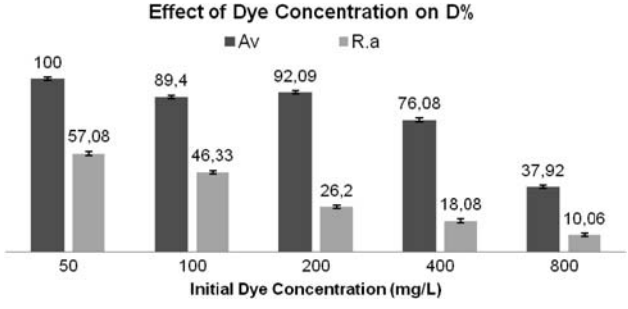

Fig. 2. The effect of initial dye concentration on decolorization (D%) (Av: *A. versicolor*; Ra: *R. arrhizus*; pH: 6; incubation period: six days; *T*: 25 ± 1°C).

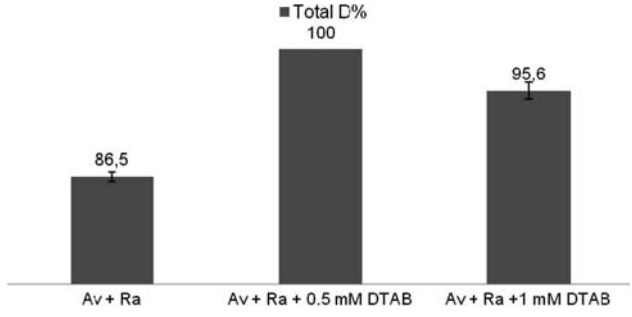

Fig. 3. Total decolorization (D%) activity of mixed culture (Av + Ra: mixed *A. versicolor* and *R. arrhizus*; DTAB: dodecyl trimethyl ammonium bromide; C_{oRB}: 100 mg/L; incubation period: three days; *T*: 25 ± 1°C).

three days of incubation (Fig. 3). In the literature, we encountered no report about using mixed fungal cultures of *A. versicolor* and *R. arrhizus* together in order to decolorize dye-containing wastewater.

3.3.2. Total decolorization activity of mixed culture and surfactant in high dye concentration

To investigate and compare the decolorization activity of *R. arrhizus*, *A. versicolor*, mixed culture, and surfactant in high dye concentration, the cultures inoculated into Erlenmeyer flasks contained molasses medium with 800 mg/L RB at pH 6. The mixed *A. versicolor* and *R. arrhizus* culture performed more dye removal activity than only *A. versicolor* and *R. arrhizus* did (Fig. 4). Saranjaj et al. (2010) have showed that *A. niger* and *Aspergillus flavus* removed maximum 40% and 60% of Direct Sky Blue (DSB, C_{oDSB}: 800 mg/L), which is a direct azo dye [15]. Comparing other dye classes such as direct azo dyes, reactive dye removal is the most difficult [3]. The previous studies have reported maximum decolorization activity of *R. arrhizus* with 1 mM DTAB as 52.8% [24] and *A. versicolor* with 1 mM DTAB as 67.7% [25] at 800 mg/L RB. In this study, dye removal activity of mixed culture with 1 mM DTAB reached 73.19% in 800 mg/L RB concentration (Fig. 4). The mixed culture resisted high dye concentration as 800 mg/L and dye removal efficiency of mixed culture was very high while comparing pure cultures performance.

It was observed that the decolorization activity of mixed culture was maximum at low dye concentration (100 mg/L RB) in the presence of 0.5 mM DTAB but minimum at high dye concentration (800 mg/L) in the presence of 1 mM DTAB. The competition of anionic dye ions and surfactant ions inorder to interact with two fungal surfaces may affect the sorption of dye ions which were less. For dye removal of mixed

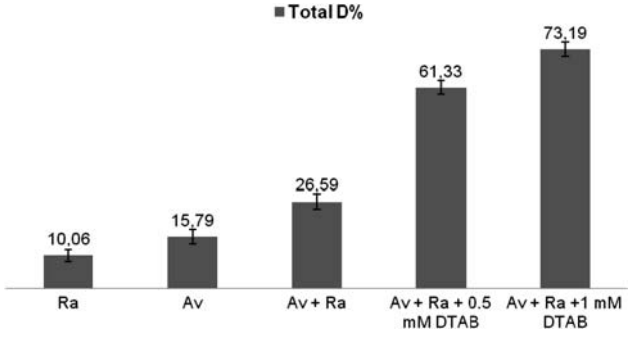

Fig. 4. Total decolorization (D%) activity (Av: *A. versicolor*; Ra: *R. arrhizus*; Av + Ra: mixed *A. versicolor* and *R. arrhizus*; DTAB: dodecyl trimethyl ammonium bromide; C_{oRB}: 800 mg/L; incubation period: three days; T: 25 ± 1 ℃).

culture, low surfactant concentration was more effective than high surfactant concentration at low dye concentration (Fig. 3) but high surfactant concentration (1 mM DTAB) enhanced more decolorization of high than low dye concentration (Fig. 4). The anionic dye and cationic surfactant (positive head group) associated because of electrostatic interactions but the limitation of dye concentration (100 mg/L) resulted in decreasing the interactions and also dye removal.

4. Conclusion

The application of economical and ecofriendly techniques using fungi gains importance to treat textile effluents. Since the removal of reactive dyes is the most difficult, it is important to develop fungal decolorization systems for reactive dye removal.

The following conclusions can be drawn from the experimental data:

- *R. arrhizus* performed maximum RB removal at pH 3 because the dominant decolorization mechanism is associated with electrostatic interactions between negative charged dye and positive charged fungal surface in acidic conditions.
- *A. versicolor* showed maximum RB decolorization at pH 6 due to the major dye removal mechanism, which is probably related with enzymatic reaction of laccase.
- The dye removal capacity of growing *A. versicolor* was more efficient than *R. arrhizus*. It owes to difference in dominant decolorization mechanisms. It is assumed that the growing cultures, which are metabolically active, produce enzymes in order to remove dye effectively.
- The total dye decolorization activity of mixed *A. versicolor* and *R. arrhizus* cultures were more effective than individual culture.
- DTAB surfactant enhanced dye removal activity of mixed *A. versicolor* and *R. arrhizus* cultures.

The results of this study show that the systems contained mixed culture and surfactant decolorized high level of reactive dye concentrations effectively.

Nomenclature

C_o — initial dye concentration (mg/L)
C_f — final dye concentration (mg/L)
q_m — the maximum amount of dye per unit dry weight of fungus (mg/L)
X_m — dried cell mass (g/L)
C_{oRB} — initial Remazol Blue concentration (mg/L)
T — temperature (℃)

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