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Removal of textile dye reactive black 5 by the cold-adapted, alkali- and halotolerant fungus *Aspergillus flavipes* MA-25 under non-sterile conditions

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ABSTRACT

The aim of the present study was to investigate the potential of isolated fungus *Aspergillus flavipes* MA-25 to decolorize the textile dye reactive black 5. The fungus was found to be capable of removing the dye from the culture in a wide pH range (not only acidic but also neutral and alkaline pHs) at high salt concentrations (0–50 g/L) and low temperatures (535°C) under non-sterile conditions. It was also observed that the fungus could tolerate the dye at high initial concentrations up to 1 g/L. Especially, no significant inhibitory effect on dye removal and fungal growth was seen at the initial dye concentrations of ≤0.4 g/L. At initial dye concentration of 0.2 g/L, complete removal of dye was achieved with 4.71 g/L biomass production in 80 h. The bioadsorption was the principle mechanism involved in dye removal by the fungus. Therefore, it was assumed that the fungus had a great potential for the treatment of dye-bearing textile effluents which are alkaline, cold, and saline.

Keywords: *Aspergillus flavipes* MA-25; Textile dye; Reactive black 5; Dye removal; Cold-adapted

1. Introduction

There are more than 10,000 types of commercially available dyes with over 700 tons of dyestuff produced annually worldwide and used extensively in textile, dyeing, and printing industries. The textile industry is one of the greatest producers of liquid effluent pollutants due to high quantities of water used in the dyeing processes. It is estimated that about 10–15% of the dyes were lost in industrial effluents [1,2].

Among the various classes of dyes, reactive dyes are among the most recalcitrant synthetic dyes against biodegradative processes and are considered as a worldwide problem. Their pollution hazard is primarily based on carcinogenic or toxic components, such as aromatic amines and related compounds. Also, due to light absorption, they could significantly reduce photosynthetic activity in water bodies [3].

Decolorization of textile dyes are possible using physical and chemical methods such as adsorption, oxidation, coagulation–flocculation, chemical degradation, and photodegradation [4], but these methods are usually inefficient, costly, and not adaptable to a

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wide range of dye wastewaters [5]. Conversely, biological processes have received increasing interest due to their low cost, effectiveness, ability to produce less sludge, and environmental benignity [6,7]. In this context, several investigations have been performed on the biological decolorization of synthetic dyes using bacteria, fungi, and algae [8–13]. However, it has been reported that the efficiency of microbial dye decolorization is greatly influenced by several parameters such as dye structure, dye concentration, aeration, temperature, pH, redox potential, as well as micro-organism kind and its nutritional requirements [10,11,14]. Wastewater of textile industries is generally characterized by neutral or alkaline pH (approximately 7–11) [15]. As a result, the optimum pH for microbial dye removal from this wastewater is reported to be neutral or slightly alkaline pH values and the rate of decolorization tends to decrease rapidly at strongly acidic or strongly alkaline pH values [14]. On the other hand, it has been reported that wastewater of textile industry contains high concentrations of salts, such as NaCl [16]. Various types of salts are added to dye baths to improve the dyeing efficiency. Usually, sodium nitrate is used to improve the fixation of dyes on textile fibers while sodium sulfate is added for the adjustment of ionic strength of the dye baths. Presence of salt residues in textile effluents, particularly sodium ions leads to the inhibition of most microbial activities [17]. Therefore, after dying, bioremediation of textile effluents requires the use of alkalophilic and halophilic micro-organism which is capable of growing under such harsh conditions [18]. Temperature is another important factor affecting dye removal efficiency in bioremediation systems. The temperature of systems can decrease or increase the rate of dye removal by affecting significantly growth performance and enzyme activity of micro-organisms [14]. However, additional energy consumption is required in order to adjust the temperature of textile effluents to the desired temperature for micro-organisms. To solve this problem, cold-adapted micro-organisms (psychrophilic or psychrotolerant) may be directly used in bioremediation systems for decolorization of textile effluents with low temperature. However, there is only one report on dye-decolorization potential of cold-adapted micro-organisms [3].

Therefore, the present study focused on investigating the potential of actively growing biomass of cold-active fungus *Aspergillus flavipes* MA-25 to decolorize the reactive black 5 dye under harsh conditions (alkaline, salty, and low temperatures) at laboratory scale.

2. Materials and methods

2.1. Dye

Reactive black 5, which is commonly used in cotton textile industry in Turkey, was obtained from AYTEMİZ Textile Co., Turkey, in pure form and used without further purification.

2.2. Isolation of dye-decolorizing fungi

The fungus used in this study was isolated from the soil around Tuz (Salt) Lake, Konya (Turkey). In brief, approximately 1 g of the salty soil was suspended in 10 mL sterile saline water. One milliliter of this mixture was spread on the agar plate of isolation medium. The isolation medium consisted of (g/L) glucose 20, NaCl 10, ammonium sulfate 1, KH_2PO_4 1, $MgSO_4{\cdot}7H_2O$ 0.5, $CaCl_2$ 0.01, dye 0.2, and agar 20 (pH 7.0). The inoculated petri dishes were aerobically incubated at 15˚C for 144 h. The dye-decolorization capacities of isolates growing on agar medium were assessed by the formation of clear zone around the colonies. Among total 35 fungal isolates, six isolates displaying wider clear zone diameter were subcultured, purified and then, used for the following experiments. Six isolates were screened for the determination of the best dye-decolorizing one. The screening experiments were performed in non-sterile basal medium. Unlike the isolation medium, the basal medium did not contain agar. But, the other components of isolation medium existed in the basal medium. For the screening experiments, one milliliter of spore suspension of each fungal isolate was used for the inoculation of 100 mL basal medium in the flask. The flasks were agitated at 15˚C in an orbital shaker (ZHWY-200B, Zhicheng Analytical Co., Shanghai, China) at 150 rpm for 60 h. Screening experiments exhibited that the best dye-decolorizing isolate was MA-25. Therefore, the isolate MA-25 was selected for the subsequent dye-decolorizing experiments. Taxonomic identification of the isolate MA-25 was performed according to Ellis [19] by evaluation of the macromorphological and micromorphological characteristics. The fungal preparations were mounted in lactophenol cotton blue. They were observed and photographed using an Olympus BX51 microscope. The isolate MA-25, which was identified as *A. flavipes*, was maintained on PDA slants at 4–6˚C and subcultured bimonthly.

2.3. Preparation of inoculum

The isolates were initially grown at 15˚C on potato dextrose agar (PDA) slants. At the end of incubation

period of 6 d, 10 mL of sterile saline water (0.9% NaCl) was added to culture slant of each isolate and the slant was vortexed. The final concentration of each spore suspension was adjusted to 10^6 /mL spores with sterile saline water. Then, 1 mL of this spore suspension was directly used for the inoculation of dye-decolorization medium [10].

2.4. Decolorization experiments with A. flavipes MA-25 in liquid medium

The decolorization experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of the decolorization medium (basal medium) described above. The flasks containing non-sterile basal medium was supplemented with 0.2 g/L non-sterile dye and then inoculated with 1 mL of the homogenized spore suspension. To determine the most favorable initial pH for dye decolorization and fungal growth, pH range of 3–10 was screened. The optimization of temperature was studied at 5–40 °C, and the optimization of shaking speed was 60–200 rpm. The influence of different NaCl concentrations on dye decolorization was tested at 5–60 g/L levels. The optimization experiments were carried out at an initial dye concentration of 0.2 g/L for 60 h. Finally, effect of different dye concentrations (0.2–1 g/L) on dye removal and fungal growth was studied at different incubation times.

2.5. Decolorization and biomass assays

Five milliliter sample taken from culture broth was firstly centrifuged at 5,000 rpm for 10 min to separate the supernatant and mycelium from each other, and 3 mL of obtained supernatant was then used for the determination of the degree of decolorization in liquid culture medium. Percent decolorization was determined by measuring the absorbance of culture supernatant at 595 nm by means of a UV–Vis spectrophotometer (Shimadzu UV-160A). Control experiments were performed using the same medium but without the fungus (control flask). Percentage removal of dye in liquid medium was determined using the following formula: % decolorization (% dye removal) = (initial absorbance − observed absorbance)/initial absorbance × 100. The concentration of fungal biomass produced was determined according to dry weight method. For this, the obtained pellets or mycelia were washed three times with distilled water. The washed biomass was then dried up to constant weight at 80 °C for 24 h.

Laccase activity was determined using ABTS as the substrate [20]. The reaction mixture contained 0.5 mL ABTS (5 mM) dissolved in 100 mM acetate buffer (pH = 4.5) and 0.5 mL of culture supernatant (after diluted). It was then incubated at 37 °C and 120 rpm. Oxidation of ABTS was monitored by an increase in absorbance at 420 nm (ε_{420} = 36,000/M cm). One unit laccase activity was defined as the amount of enzyme required to oxidize 1 μmol of ABTS/min. Manganese peroxidase activity was determined at 468 nm using dimethoxyphenol (DMP) as the substrate [21]. Lignin peroxidase (LiP) activity was determined by the oxidation of veratryl alcohol at 310 nm [22].

2.6. Statistical analysis

Each experiment was repeated at least three times in two replicates. The analysis of variance was conducted using one-way ANOVA test using SPSS 13.0 for Microsoft Windows, and means were compared by Duncan test at the 0.05 level of confidence.

3. Results and discussion

3.1. Isolation and screening of reactive black 5-decolorizing fungi

Selecting a salt and alkaline tolerant micro-organism is usually considered as a major aspect for improvement in bioremediation studies, since the textile effluents contained high concentrations of salts and are generally characterized by neutral or alkaline pH. With this regard, several investigators have paid a lot of attention to the isolation of salt and alkaline tolerant micro-organisms from various sources [10,18,23]. On the other hand, use of cold-adapted micro-organisms in bioremediation studies may be accepted as a new approach, especially for decolorization of textile effluents with low temperature. Therefore, the first step of the current study focused on isolation of a new fungal strain having three properties (cold-active, alkaline, and salt tolerant). For the isolation of salt-tolerant fungi, soil samples taken around Tuz (Salt) Lake (Konya, Turkey) were used as isolation source, and the isolation medium contained NaCl at a high concentration of 10 g/L. On the other hand, the pH of the isolation medium was adjusted to the alkaline value (pH 8.0) for the selection of alkaline tolerant ones. The isolation experiments were performed at a low temperature (15 °C) in order to select both cold-active and alkaline-tolerant ones among the salt-tolerant fungal strains. At the end of the isolation experiments, 35 fungal strains capable of decolorizing reactive black 5 dye under these severe conditions could be isolated from the soil. These fungal isolates were then screened for the determination of the most

efficient isolate. The screening experiments showed that only six isolates (MA-4, MA-9, MA-10, MA-21, MA-25, and MA-33) possessed noteworthy potential to decolorize the dye. Therefore, dye removal rates obtained for only six isolates were given in Table 1. The data given in this table clearly show that the maximum dye decolorization was achieved with the fungal isolate MA-25 in 60 h. This isolate was identified as *A. flavipes* and then used in the following dye-decolorization experiments.

3.2. Effects of pH and temperature on dye decolorization and mycelial biomass

The experiments demonstrated that although the maximum dye removal (84%) and biomass production (5.39 g/L) was achieved at pH 6.5, the fungus had the potential both decolorize dye and growth in a wide pH range from 4 to 9 (Table 2). This result was in good agreement with the fact that *Aspergillus* species may possess alkali-tolerant character [23–25]. On the other hand, it was seen that color removal rates were much closed to each other between pH 5.5 and 8. Even, the fungus possessed high ability to decolorize dye at neutral and slightly alkaline pHs. For example, although the maximum dye removal was achieved at pH 6.5, the fungus was able to remove 74% of the total dye at pH 8.0 within 60 h. These results will be very interesting for microbial remediation systems. This is because that pH of textile effluents is reported to be neutral or slightly alkaline pH value [14]. Although no microbial growth was observed at strong acidic (pH 3 and 3.5) and alkaline pHs (pH 9.5 and 10), a slight dye removal was detected at these pHs. For example, a dye removal rate of 6% was detected at pH 3.0. This situation may be explained by the fact that strong acidic or alkaline pHs have a potential to degrade textile dyes. Based on the present results, the following experiments were performed at a high alkaline pH of 8.0, which the fungus could effectively decolorize the dye.

As seen from Table 3, the fungus was capable of growing and decolorizing the dye in the range of temperature 5–35˚C, however, the maximum biomass production (4.81 g/L) and dye removal (83%) rate were achieved at the temperature of 20˚C. Cell growth and dye removal were not observed at 40˚C. These results

Table 1
Screening of dye-decolorizing fungal isolates screening

Isolate code	Dye removal (%)	Biomass (g/L)
MA-4	58 ± 1.35^{d}	3.91 ± 0.16^{d}
MA-9	65 ± 1.39^{c}	3.94 ± 0.25^{cd}
MA-10	51 ± 1.51^{e}	4.33 ± 0.38^{bc}
MA-21	70 ± 1.58^{b}	4.76 ± 0.24^{a}
MA-25	74 ± 2.15^{a}	4.41 ± 0.22^{b}
MA-33	62 ± 1.95^{c}	4.03 ± 0.18^{bcd}

All values are the means ± standard error of six determinations ($n = 6$). Alphabet letters with the same letters in the same column are not significantly different at $p < 0.05$. Initial pH = 8.0, shaking speed = 150 rpm, temperature = 15˚C, and incubation time = 60 h.

Table 2
Effect of initial pH on dye-decolorization potential of *A. flavipes* MA-25

Initial pH	Dye removal (%)	Biomass (g/L)
3.0	7 ± 0.12^{g}	No-growth
3.5	6 ± 0.10^{g}	No-growth
4.0	12 ± 0.15^{f}	1.13 ± 0.06^{g}
4.5	23 ± 2.2^{c}	1.61 ± 0.10^{f}
5.0	43 ± 3.4^{d}	2.65 ± 0.05^{d}
5.5	73 ± 4.6^{c}	4.48 ± 0.22^{c}
6.0	81 ± 4.2^{ab}	5.18 ± 0.18^{b}
6.5	84 ± 2.0^{a}	5.39 ± 0.14^{a}
7.0	79 ± 3.3^{b}	5.11 ± 0.13^{b}
7.5	75 ± 2.1^{c}	4.52 ± 0.12^{c}
8.0	74 ± 3.2^{c}	4.41 ± 0.26^{c}
8.5	22 ± 1.1^{e}	2.04 ± 0.08^{e}
9.0	12 ± 0.5^{f}	1.05 ± 0.05^{g}
9.5	4 ± 0.25^{g}	No-growth
10	5 ± 0.2^{g}	No-growth

All values are the means ± standard error of six determinations ($n = 6$). Alphabet letters with the same letters in the same column are not significantly different at $p < 0.05$. Salt concentration = 10 g/L; shaking speed = 150 rpm; temperature = 15˚C; and incubation time = 60 h.

Table 3
Effect of temperature on dye-decolorization potential of *A. flavipes* MA-25

Temperature (˚C)	Dye removal (%)	Biomass (g/L)
5	21 ± 1.02^{f}	2.02 ± 0.08^{e}
10	50 ± 1.82^{d}	3.82 ± 0.2^{c}
15	74 ± 2.20^{b}	4.41 ± 0.11^{b}
20	83 ± 3.4^{a}	4.81 ± 0.14^{a}
25	58 ± 2.7^{c}	4.21 ± 0.26^{b}
30	43 ± 1.5^{e}	3.14 ± 0.12^{d}
35	18 ± 0.8^{f}	1.91 ± 0.10^{e}
40	0	No growth

All values are the means ± standard error of six determinations ($n = 6$). Alphabet letters with the same letters in the same column are not significantly different at $p < 0.05$. Initial pH = 8.0, salt concentration = 10 g/L. Shaking speed = 150 rpm; and incubation time = 60 h.

confirmed that the fungus had a psychrotolerant character. This is because that obligate psychrophiles are not able to grow at temperatures above 20˚C, whereas facultative psychrophiles (psychrotolerant) are able to grow at or below 5˚C and tolerate temperatures above 20˚C [26]. This finding can make the fungus an important tool in bioremediation studies. When the dye-decolorization process is performed at the low temperature using this cold-adapted fungus, there will be no requirement to heat textile effluents. Thanks to this, energy saving will be possible.

When effect of agitation speed on dye removal was investigated, it was seen that dye removal percentage was maximum (95%) with 4.48 g/L biomass production at a shaking speed of 120 rpm (Table 4). When shaking speed was increased from 120 to 200 rpm, biomass production increased but dye removal rate decreased. This result could be attributed to the increasing oxygen concentration in the culture depending on the increase in shaking speed. Some researchers have reported that if the extracellular environment is aerobic, oxygen, a high redox potential electron acceptor, may inhibit dye reduction mechanism. Namely, electrons liberated from oxidation of electron donors are preferentially used to reduce oxygen rather than the azo dye in microbial systems, and the reduction product, water, is not a reductant. Therefore, it has been suggested that high levels of aeration and agitation, which increase the concentration of oxygen in solution, should be avoided for efficient color removal [14,27,28].

High salt concentration of wastewater of textile industry is another important factor, which restricts the dye removal capacity of micro-organisms [17,29,30]. Therefore, effect of salt concentration on dye removal potential of the fungus was also studied in detail. The fungal strain was able to effectively decolorize dye at salt concentrations of 0–30 g/L NaCl, while salt concentrations above 30 g/L significantly decreased the dye-decolorization potential of the fungus (Table 5). For example, only 10% dye decolorization was achieved in 60 h in the medium containing 50 g/L NaCl. No dye decolorization and fungal growth was observed at an NaCl concentration of 60 g/L. Subsequently, the following experiments were performed at a high NaCl concentration of 30 g/L, at which the fungus showed high dye-decolorization potential.

Table 4
Effect of shaking speed on dye-decolorization potential of *A. flavipes MA-25*

Shaking speed (rpm)	Dye removal (%)	Biomass (g/L)
60	50 ± 2.3^{d}	2.50 ± 0.3^{e}
90	72 ± 3.3^{c}	3.22 ± 0.2^{d}
120	95 ± 2.5^{a}	4.48 ± 0.24^{c}
150	83 ± 3.0^{b}	4.81 ± 0.4^{bc}
180	70 ± 2.7^{c}	5.19 ± 0.4^{ab}
200	49 ± 1.75^{d}	5.62 ± 0.3^{a}

All values are the means ± standard error of six determinations ($n = 6$). Alphabet letters with the same letters in the same column are not significantly different at $p < 0.05$. Initial pH = 8.0; salt concentration = 10 g/L; temperature = 20˚C; and incubation time = 60 h.

3.3. Effect of initial dye concentration on dye removal and fungal growth

The experiments exhibited that initial dye concentration affected not only fungal growth (Fig. 1) but also dye decolorization (Fig. 2). Fungal growth was not significantly inhibited by initial dye concentrations of ≤0.4 g/L. At all the initial dye concentrations tested, fungal growth continued up to 80 h of incubation period and further incubation periods did not increase biomass concentration. Biomass concentrations were determined as 4.71, 4.58, 3.62, 2.41, and 1.72 g/L at initial dye concentrations of 0.2, 0.4, 0.6, 0.8, and 1 g/L at the end of 80 h, respectively. In the case of fungal growth, no significant inhibitory effect on dye removal was observed at the initial dye concentrations of 0.2 g/L. At this initial dye concentration, the complete removal (100%) of dye was achieved with 4.71 g/L biomass production in 80 h. Dye removal potential and mycelial growth of the fungus were slightly inhibited at an initial dye concentration of 0.4 g/L. Even so, dye removal efficiency reached up to 58% (0.232 g removed dye) with 4.53 g/L of biomass production in 100 h and no further removal was detected. In contrast to initial dye concentrations of ≤0.4 g/L, initial dye concentrations above 0.4 g/L significantly inhibited the dye removal potential of the fungus. In contrast to fungal growth, no dye removal was detected in 30 h at the initial dye concentrations of 0.6 and 0.8 g/L. On the other hand, no further dye removal was detected at 0.6 and 0.8 g/L initial dye concentrations after 80 h of growth. At 1 g/L concentration of dye, no dye removal occurs within the first 40 h of growth. Similarly, no further dye uptake was detected at this dye concentration after 80 h of growth. Therefore, dye removal rate was only 5% at initial dye concentrations of 1 g/L in 100 h. These results showed that the dye removal potential of the fungus was affected by initial dye concentrations, as reported by the previous studies [23,24,31,32]. The present results also exhibited that dye removal was faster within the first 60 h of growth and then gradually slowed down. Hence, it was assumed that young biomass was more effective than old fungal

Table 5
Effect of NaCl concentration on dye-decolorization potential of *A. flavipes* MA-25

NaCl concentration (g/L)	Dye removal (%)	Biomass (g/L)
5	98 ± 1.8^{a}	4.55 ± 0.3^{a}
10	95 ± 1.5^{b}	4.48 ± 0.2^{a}
20	91 ± 1.0^{c}	4.31 ± 0.2^{ab}
30	88 ± 1.0^{d}	4.11 ± 0.1^{b}
40	41 ± 1.4^{e}	2.02 ± 0.1^{c}
50	10 ± 0.6^{f}	1.13 ± 0.08^{d}
60	0	No-growth

All values are the means ± standard error of six determinations ($n = 6$). Alphabet letters with the same letters in the same column are not significantly different at $p < 0.05$. Initial pH = 8.0; shaking speed = 120 rpm; temperature = 20°C; and incubation time = 60 h.

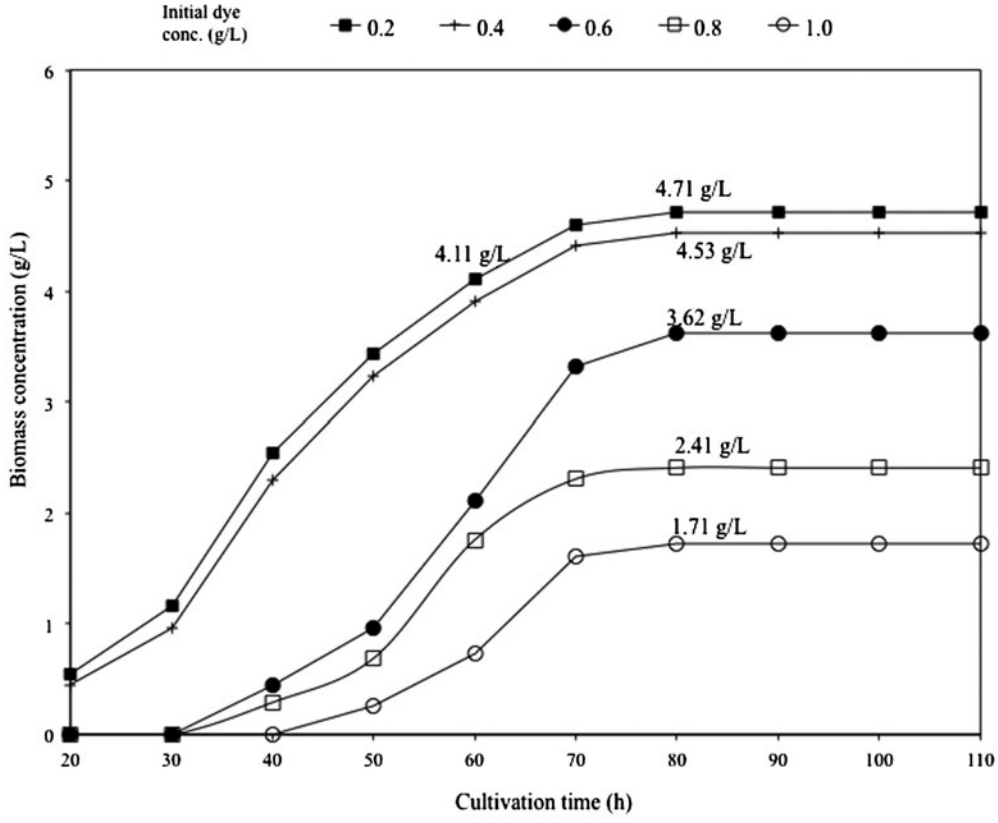

Fig. 1. Effect of initial dye concentration and incubation time on fungal growth. Initial pH = 8.0; shaking speed = 120 rpm; temperature = 20°C; and salt concentration = 30 g/L.

biomass for dye removal, as reported by Taskin and Erdal [10].

3.4. Investigation of dye removal mechanism

Two main dye-decolorization mechanisms of actively growing micro-organisms are biodegradation and bioadsorption. Adsorption using biomass occurs by ion exchange [33]. It has been reported that azo dyes adsorbed by fungal biomass can be effectively recovered through extraction with methanol. If dye can be recovered from fungal biomass with methanol treatment, dye decolorization mechanism is termed as bioadsorption [34]. Therefore, the methanol extraction method was applied to the colored fungal biomass in order to elucidate the dye-decolorization mechanism.

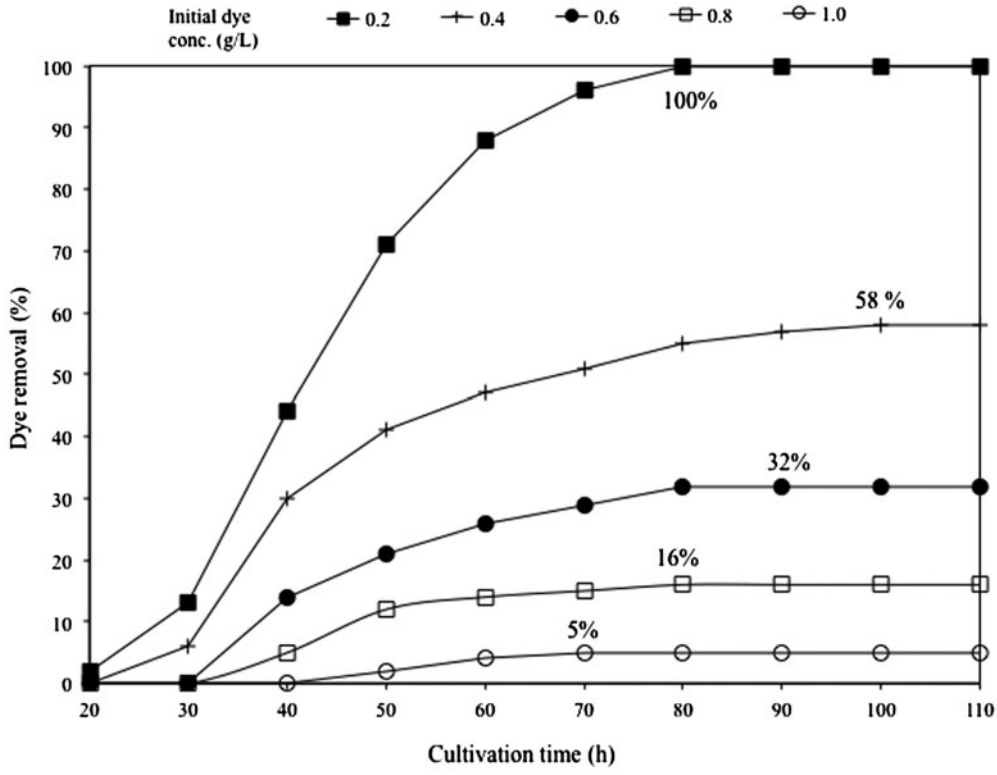

Fig. 2. Effect of initial dye concentration and incubation time on dye removal. Initial pH = 8.0; shaking speed = 120 rpm; temperature = 20°C; and salt concentration = 30 g/L.

The methanol treatment was applied to biomass several times until biomass became entirely colorless. However, spectrophotometric analyses demonstrated that about 94% of the total dye (0.2 g/L) could be recovered from biomass. Biodegradative abilities of fungi are depended on their extracellular enzymes such as laccases, lignin peroxidases, and manganese peroxidases [2,33]. Therefore, it was assumed that unrecovered part (only 6%) of the total dye might be converted to by-products in the medium through the biodegradation mechanism. The activities of the enzymes were analyzed to elucidate whether enzymatic biodegradation mechanism was or not effective in the dye decolorization. The experiments demonstrated that the activities of the three biodegradative enzymes were too low in the dye-containing medium during the growth period. No lignin peroxidase and manganese peroxidase activities were detected in the medium after completion of dye removal. There was very little laccase activity at all the initial dye concentration tested. Only fungal laccase activity of 16 U/L was determined in the medium after the complete decolorization of 0.2 g/L dye. The activity of *A. flavipes* MA-25 laccase was too lower than those reported for the same enzyme in the previous studies [2,13,35]. Based on the present results, we concluded that the dye removal mechanism was mainly associated with hyphal uptake mechanism (which is also known as bioadsorption) rather than enzymatic-decolorization mechanism (which is also known as biodegradation) [36,37].

4. Conclusions

The essential characteristics of micro-organisms used in dye removal process are reported to be tolerant and have high decolorization potential. The present study showed that the fungus was able to decolorize the high concentrations of the dye under alkaline, saline, and low temperatures. The fungus could be directly used as bioadsorbent in the dye-containing medium under non-sterile conditions. Undesired contaminations could be prevent, since the medium contained high salt concentration and had low temperature. Therefore, we think that the local fungal isolate *A. flavipes* may be an excellent tool for use in the treatment of industrial effluents that contain residual textile dyes.

References

[1] M.T. Sulak, H.C. Yatmaz, Removal of textile dyes from aqueous solutions with eco-friendly biosorbent, Desalin. Water Treat. 37 (2012) 169–177.

[2] T. Hadibarata, A.R.M. Yusoff, R.A. Kristanti, Decolorization and metabolism of anthraquionone-type dye by laccase of white-rot fungi *Polyporus* sp. S133, Water Air Soil Pollut. 223 (2012) 933–941.

[3] J.I. Rovati, H.F. Pajot, L. Ruberto, W.M. Mac Cormack, L.I.C. Figueroa, Polyphenolic substrates and dyes degradation by yeasts from 25 de Mayo/King George Island (Antarctica), Yeast 30 (2013) 459–470.

[4] G. Donmez, Bioaccumulation of the reactive textile dyes by *Candida tropicalis* growing in molasses medium, Enzyme Microb. Technol. 30 (2002) 363–366.

[5] I.M. Banat, P. Nigam, D. Singh, R. Marchant, Microbial decolorization of textile-dyecontaining effluents: A review, Bioresour. Technol. 58 (1996) 217–227.

[6] Y. Fu, T. Viraraghavan, Fungal decolorization of dye wastewaters: A review, Bioresour. Technol. 79 (2001) 251–262.

[7] Z. Aksu, Application of biosorption for the removal of organic pollutants: A review, Process Biochem. 40 (2005) 997–1026.

[8] B. Balu, K.V. Radha, Kinetic study on decolourization of the dye Acid Orange using the fungus *Phanerochate chrysosporium*, Mod. Appl. Sci. 3 (2009) 38–47.

[9] A.R. Khataee, M. Zarei, M. Pourhassan, Application of microalga *Chlamydomonas* sp. for biosorptive removal of a textile dye from contaminated water: Modelling by a neural network, Environ. Technol. 30 (2009) 1615–1623.

[10] M. Taskin, S. Erdal, Reactive dye bioaccumulation by fungus *Aspergillus niger* isolated from the effluent of sugar fabric-contaminated soil, Toxicol. Ind. Health 26 (2010) 239–247.

[11] R.G. Saratale, G.D. Saratale, J.S. Chang, S.P. Govindwar, Bacterial decolorization and degradation of azo dyes: A review, J. Taiwan Inst. Chem. Eng. 42 (2011) 138–157.

[12] N.F. Ali, R.S.R. El-Mohamedy, Microbial decolourization of textile waste water, J. Saudi Chem. Soc. 16 (2012) 117–123.

[13] M. Asgher, Q. Yasmeen, H.M.N. Iqbal, Enhanced decolorization of solar brilliant red 80 textile dye by an indigenous white rot fungus *Schizophyllum commune* IBL-06, Saudi J. Biol. Sci. 20 (2013) 347–352.

[14] C.I. Pearce, J.R. Lloyd, J.T. Guthrie, The removal of colour from textile wastewater using whole bacterial cells: A review. Dyes Pigments 58 (2003) 179–196.

[15] M.I. Jahmeerbacus, N. Kistamah, R.B. Ramgulam, Fuzzy control of dyebath pH in exhaust dyeing, Color. Technol. 120 (2004) 51–55.

[16] S. Vanhulle, M. Trovaslet, E. Enaud, M. Lucas, S. Taghavi, D. Van Der Lelie, B. van Aken, M. Foret, R.C.A. Onderwater, D. Wesenberg, S.N. Agathos, Y.J. Schneider, A.M. Corbisier, Decolourisation, cytotoxicity and genotoxicity reduction during a combined ozonation/fungal treatment of dye contaminated wastewater, Environ. Sci. Technol. 42 (2008) 584–589.

[17] A. Khalid, F. Kausar, M. Arshad, T. Mahmood, I. Ahmed, Accelerated decolorization of reactive azo dyes under saline conditions by bacteria isolated from Arabian seawater sediment, Appl. Microbiol. Biotechnol. 96 (2012) 1599–1606.

[18] A.A. Pourbabaee, S. Bostani, M.A. Amozzegar, R. Naddaf, Decolorization of Cibacron Black w-55 under alkaline conditions by new strain of *Halomonas* sp. isolated from textile effluent, Iran. J. Chem. Chem. Eng. 30 (2011) 63–70.

[19] M.B. Ellis, Dematiaceous Hyphomycetes, Commonwealth Mycological Institute, Kew, London, UK, 1971.

[20] H. Forootanfar, A. Moezzi, M. Aghaie-Khozani, Y. Mahmoudjanlou, A. Ameri, F. Niknejad, M.A. Faramarzi, Synthetic dye decolorization by three sources of fungal laccase, Iran. J. Environ. Health. Sci. Eng. 9 (2012) 27.

[21] J.A. Field, E. De Jong, G. Feijoo-Costa, J.A.M. De Bont, Screening for ligninolytic fungi applicable to the biodegradation of xenobiotics, Trends Biotechnol. 11 (1993) 44–49.

[22] M. Tien, T.K. Kirk, Lignin peroxidase of *Phanerochaete chrysosporium*, Methods Enzymol. 161 (1988) 238–249.

[23] P. Kaushik, A. Malik, Alkali, thermo and halo tolerant fungal isolate for the removal of textile dyes, Colloids Surf., B 81 (2010) 321–328.

[24] T. Anthony, K. Chandra Raj, A. Rajendran, P. Gunasekaran, High molecular weight cellulase-free xylanase from alkali-tolerant *Aspergillus fumigatus* AR1, Enzyme Microb. Technol. 32 (2003) 647–654.

[25] E.F. Sharaf, E. Alharbi, Removal of heavy metals from waste water of tanning leather industry by fungal species isolated from polluted soil, Afr. J. Biotechnol. 12 (2013) 4531–4535.

[26] M. Rossi, P. Buzzini, L. Cordisco, A. Amaretti, M. Sala, S. Raimondi, C. Ponzoni, U.M. Pagnoni, D. Matteuzzi, Growth, lipid accumulation, and fatty acids composition in obligate psychrophilic, facultative psychrophilic and mesophilic yeasts, FEMS Microbiol. Ecol. 69 (2009) 363–372.

[27] J.S. Chang, Y.C. Lin, Fed-batch bioreactor strategies for microbial decolorization of azo dye using a *Pseudomonas luteola* strain, Biotechnol. Prog. 16 (2000) 979–985.

[28] E.S. Yoo, J. Libra, L. Adrian, Mechanism of decolorization of azo dyes in anaerobic mixed culture, J. Environ. Eng. 127 (2001) 844–849.

[29] C.M. Carliell, S.J. Barclay, C. Shaw, A.D. Wheatley, C.A. Buckley, The effect of salts used in textile dyeing on microbial decolorisation of a Reactive Dye, Environ. Technol. 19 (1998) 1133–1137.

[30] B. Yan, C. Du, M. Xu, W. Liao, Decolorization of azo dyes by a salt-tolerant *Staphylococcus cohnii* strain isolated from textile wastewater, Front. Environ. Sci. Eng. 8 (2012) 293–304.

[31] R.K. Sani, U.C. Banerjee, Decolorization of triphenylmethane dyes and textile and dyestuff effluent by *Kurthia* sp., Enzyme Microb. Technol. 24 (1999) 433–437.

[32] B.D. Tony, D. Goyal, S. Khanna, Decolorization of textile azo dyes by aerobic bacterial consortium, Int. Biodeterior. Biodegrad. 63 (2009) 462–469.

[33] R. Khan, P. Bhawana, M.H. Fulekar, Microbial decolorization and degradation of synthetic dyes: A review, Rev. Environ. Sci. Biotechnol. 12 (2013) 75–97.
[34] M.C. Brahimi-Horn, K.K. Lim, S.L. Liang, D.G. Mou, Binding of textile azo dyes by *Myrothecium verrucaria*, J. Ind. Microbiol. 10 (1992) 31–36.
[35] E. Rodríguez, M.A. Pickard, R. Vazquez-Duhalt, Industrial dye decolorization by laccases from ligninolytic fungi, Curr. Microbiol. 38 (1999) 27–32.
[36] S. Erdal, M. Taskin, Uptake of textile dye Reactive Black-5 by Penicillium chrysogenum MT-6 isolated from cement-contaminated Soil, Afr. J. Microbiol. Res. 4 (2010) 618–625.
[37] M. Taskin, S. Erdal, Isolation of a reactive black-5-decolourizing fungus, *Absidia californica* MT-1, from cement-contained soil and the optimization of culture conditions for dye removal, Asian J. Chem. 22 (2010) 7123–7134.