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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

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The present study focused on the removal of textile dye Reactive Black-5 by actively growing mycelia of fungus *Absidia californica* MT-1, which was isolated from the cement-contamined soil. The temperature of 28 ºC, shaking speed of 180 rpm, pH 5, inoculum amount of 0.2 g/L, 15 g/L sucrose and 1 g/L ammonium chloride were the most efficient conditions to achieve optimum removal of dye. Nutrient-poor medium was found to be more beneficial for dye removal. Biosorption/ bioadsorption was likely the dominant mechanism for dye removal by the fungus and found to be associated with mycelial morpholgy. The mycelial morphology in small uniform pellet form was found to be better for dye removal. The maximum dye removal by *A. californica* MT-1 was 92 % (0.276 g/L bioadsorbed-dye) with 3.95 g/L of biomass production at an initial dye concentration of 0.3 g/L in 108 h. In the present study, both the ability of this fungus species to remove a textile dye and the usability of cement-contamined soil as a source of microorganisms, which are capable of removing snythetic textile dye, were investigated for the first time.

Key Words: *Absidia californica* MT-1, Bioadsorption, Optimization, Reactive Black-5.

INTRODUCTION

Synthetic dyes find use in a wide range of industries such as textile dyeing, paper printing, cosmetics and pharmaceuticals[1]. Approximately 10,000 different dyes and pigments are used in industries and over 7×10^5 tons of these dyes are annualy produced world-wide. Due to inefficiencies of the industrial dyeing process, 10-15 % of the dyes are lost in the effluents of textile units, rendering them highly coloured. Among the various classes of dyes, reactive dyes are more difficult to remove. They contain chromophoric groups such as azo, anthraquinone, triarylmethane, *etc*. and reactive groups *e.g*., vinyl sulphone, chlorotriazine, trichloropyrimidine *etc*., that form covalent bonds with the fiber. Azo reactive dyes are the largest class of water-soluble synthetic dyes with the greatest variety of colours and structure and are generally resistant to aerobic biodegradation processes[2-4].

There are some reports about the negative effects of textile dyes, especially azo dyes, towards aquatic life and humans. For example, the discharge of these coloured wastewaters into rivers and lakes leads to a reduction of sunlight penetration in natural water bodies which in turn decrease both photosynthetic activity and dissolved oxygen concentration. This create anaerobic conditions thereby killing aerobic marine organism. Furthmore, textile dyes pose serious health threats to human due to their carcinogenicity and lead to mutagenic and toxic effects on organism[5-7]. Currently, various treatment methods exist for the removal of colour from industrial effluents, including physico-chemical and biological processes. Important physico-chemical processes include ozonization, adsorption, chemical precipitation and flocculation[8], but these are usually inefficient, costly and not adaptable to a wide range of dye wastewaters[9]. Conversely, biological processes have received increasing interest due to their cost, effectiveness, ability to produce loss sludge and environmental benignity[10,11].

Up to now, several reports have been published on the microbial decolourization of synthetic dyes[12-16]. However, the best of our knowledge, there has been no report on the usage of fungus *Absidia californica* for dye removal processes.

Reactive black-5 is one of the most important synthetic dyes used in the textile industry. Hence, the basic objective of present study is to obtain optimum physico-chemical conditions for the decolourization of reactive black-5 at laboratory scale by actively growing biomass of fungus *Absidia californica* MT-1, which was isolated from the cement-contamined soil.

EXPERIMENTAL

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

Isolation of dye-decolourizing microorganisms: The fungus *A. californica* MT-1 used in this study for the decolourization of dye was isolated from soil samples collected from the near of the cement fabric of Askale, Erzurum (Turkey). In brief, *ca.* 1 g of the contamined soil was suspended in 10 mL sterile saline water. Two mL of the mixture for the selection of fungal isolates being resistant against the toxic effect of dye was inoculated in 200 mL of the dye decolourization medium consisted of (g/L) glucose 5, ammonium sulphate 1, KH_2PO_4 1, $MgSO_4 \cdot 7H_2O$ 0.5, NaCl 0.5, $CaCl_2$ 0.01, dye 0.1 and pH 6. The inoculated medium was incubated aerobically at 28 ºC and 150 rpm in a shaker for 3 days. One mL of this culture was then plated on the agar plate of same medium. The dye decolourization capacities of isolates growing on agar medium were assessed by the formation of clear zone around the colonies. Among 6 fungal isolates, MT-1 displaying the widest diameter was subcultured, prufied and used for the following experiments. This fungal isolate was identified as *Absidia californica*. Its identification processes was performed using mature cultures on standard potato dextrose agar (PDA) in order to ensure a good development

of taxonomically relevant features and following the identification keys[17,18]. Observations for identification were made by staining the isolated fungus using lactophenol-cotton blue and examination under low power microscope.

Preparation of inoculum: *Absidia californica* MT-1 was first grown on petridish containing 20 mL of PDA. One disc with 4 mm × 4 mm size, cut from the growing edge of the mycelia on PDA, was then inoculated in 250 mL flask containing 100 mL of dye-deficient decolourization medium. The flask was incubated at 28 ºC and 150 rpm. After 48 h of incubation, the mycelia was seperated from the culture broth and then homogenized by using a homogenizer. The homogenized mycelia was used as inoculant for the following decolourization experiments.

Decolourization experiments with *A. californica* MT-1 in liquid medium: The decolourization experiments with *A. californica* MT-1 were performed in 250 mL Erlenmeyer flasks containing 100 mL of the decolourization medium as described before. The flasks were stopped with cotton plug, sterilized at 121 ºC for 15 min, cooled at the room temperature, suplemented with 0.1 g/L sterilized-dye and then inoculated with 0.1 g/L (dry weight) of the homogenized mycelia. To determine the most favourable initial pH for decolourization and biomass production, a pH range of 2-10 was screened out. The influence of carbon sources for decolourization and growth was studied in media containing various carbon sources, *i.e.*, glucose, sucrose, maltose, fructose and lactose, where each carbon source was added to the medium at 5 g/L. To investigate the effect of nitrogen sources on mycelium growth and dye decolourization, organic nitrogen sources (peptone and yeast extract) and inorganic nitrogen sources (ammonium sulphate, ammonium chloride, ammonium nitrate, sodium nitrate and ammonium tartarate) were tested at a concentration level of 1 g/L. The concentrations of the best carbon source were studied from 5-15 g/L and best nitrogen source were from 0.5-1.5 g/L. The optimization of temperature was studied at 16, 19, 22, 25, 28, 31, 34, 37 and 40 ºC and the optimization of shaking speed 100, 150, 180 and 200 rpm. The concentration of inoculum was studied from 0.1-0.3 g/L (dry weight). To determine the maximum dye amount the mould was able to remove, experiments were carried out at different initial concentration of dye from 0.1-1.0 g/L under the optimized culture conditions.

Decolourization and biomass assays: One mL of sample taken from culture broth was initially centrifuged at 5000 rpm for 10 min to separate the supernatant and mycelium from each other and the obtained supernatant was then used for the determination of residual dye concentration in liquid medium. The degree of decolourization in liquid culture was measured spectrophotometrically at a wavelength corresponding to the maximum absorbance, k_{max} (595 nm) by means of a UV-vis spectrophotometer (Shimadzu UV-160A).

Control experiments were performed using the same medium but without microorganism (control flask). Percentage (%) removal of dye in liquid medium was determined by using the following formula.

Percentage removal = (initial concentration of dye - final concentration of dye)/ initial concentration of dye × 100

The concentration of fungal biomass concentration produced at the end of each experiment were estimated according to dry weight method.

The obtained pellets or mycelium was washed three times with distilled water. The washed biomass was dried at 105 ºC untill weight was stable.

Statistical analysis: Each experiment was repeated at least three times two replicates. Statistical analysis was performed using one way analysis of variance (ANOVA). $p \leq 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Effect of pH on mycelial biomass and dye removal: As it was shown in Table-1, even if dye decolourization occured in the range of pH 3-9, it was maximum (76 %) at pH 5. In the case of dye decolourization, biomass production was maxium (2.92 g/L) at pH 5. On the other hand, no fungal growth and colour removal were observed at pH 2 and 10. The experiments elucidated that initial pH affected the fungal morphology in decolourization medium. Uniform mycelial pellets with 5 mm size were observed at pH 5. Conversely, non-uniform mycelial pelllets occured at the other pH values. Hence, lower decolourization efficiencies at pH conditions below or above 5 might be attributed to a decrease in pellet formation and biomass concentration. This situation may be also due to decreased metabolic activity of fungus mycelia.

Effect of different carbon sources on mycelial biomass and dye removal: The experiments elucidated that although biomass production and dye decolourization occurred on all the carbon sources tested, glucose and sucrose resulting in mycelial pellets with 5 mm size were more suitable carbon sources. Uniform pellet formation was not achieved with the other carbon sources. Accordingly, high decolourization efficiencies with glucose and sucrose could be attributed to high biomass yield and dense pellet formation. The fungus was able to decolourize 76 % of the dye in glucose medium in 60 h. Following glucose, sucrose gave relatively high dye decolourization (74 %) with 2.85 g/L biomass production. It is known that the most readily usable carbon source for most of the fungi is glucose. However, glucose is a costly carbon source and is generally not used in wastewater treatment. In this context, sucrose being a cheaper carbon source than glucose is more advantageous for dye decolourization processes and therefore it was chosen as carbon source for the following decolourization experiments. In decolourization processes, various inexpensive carbon sources such as starch and molasses have also been utilized. For example, Donmez[19] indicated that the yeast species *Candida tropicalis* showed relatively high dye decolourization capacity in the molasses medium. Similarly, molasses being sucrose source may be used as a cheap carbon source in the culture medium of this fungal isolate for dye removal processes; however, a further study is needed to prove this assumption.

TABLE-1
EFFECTS OF pH, CARBON AND NITROGEN SOURCES ON DYE REMOVAL, MYCELIAL BIOMASS AND MYCELIAL MORPHOLOGY

Culture parameters	Dye removal (%)	Mycelial biomass (g/L)	Mycelial morphology
Initial pH			
2.0	No removal	No growth	–
3.0	30 ± 1.12	1.61 ± 0.16	Non-uniform pellet
4.0	49 ± 0.99	2.45 ± 0.08	Non-uniform pellet
5.0	76 ± 1.86	2.92 ± 0.10	Uniform pellet (5 mm size)
6.0	70 ± 1.33	2.74 ± 0.03	Non-uniform pellet
7.0	61 ± 0.86	2.46 ± 0.12	Non-uniform pellet
8.0	50 ± 1.21	2.31 ± 0.02	Non-uniform pellet
9.0	33 ± 0.95	1.41 ± 0.15	Non-uniform pellet
10.0	No removal	No growth	–
Carbon sources (5 g/L)			
Glucose	76 ± 1.86	2.92 ± 0.10	Uniform pellet (5 mm size)
Sucrose	74 ± 0.98	2.85 ± 0.13	Uniform pellet (5 mm size)
Fructose	56 ± 1.11	2.52 ± 0.18	Non-uniform pellet
Maltose	36 ± 1.54	1.98 ± 0.06	Non-uniform pellet
Lactose	31 ± 1.22	1.74 ± 0.08	Non-uniform pellet
Sucrose concentration (g/L)			
5.0	74 ± 0.98	2.85 ± 0.13	Uniform pellet (5 mm size)
10.0	83 ± 2.07	3.61 ± 0.04	Uniform pellet (4 mm size)
15.0	64 ± 1.64	3.78 ± 0.07	Clump
Nitrogen sources (1 g/L)			
Ammonium sulphate	83 ± 2.07	3.61 ± 0.04	Uniform pellet (4 mm size)
Ammonium chloride	88 ± 1.66	3.49 ± 0.02	Uniform pellet (4 mm size)
Ammonium nitrate	56 ± 2.33	2.31 ± 0.10	Uniform pellet (4 mm size)
Sodium nitrate	46 ± 0.67	2.87 ± 0.05	Uniform pellet (4 mm size)
Yeast extract	17 ± 0.13	3.96 ± 0.03	Uniform pellet (6 mm size)
Peptone	15 ± 0.06	3.85 ± 0.04	Uniform pellet (6 mm size)
Ammonium chloride (g/L)			
0.5	72 ± 1.49	3.11 ± 0.19	Non-uniform pellet
1.0	88 ± 1.66	3.49 ± 0.02	Uniform pellet (4 mm size)
1.5	61 ± 0.87	3.81 ± 0.07	Clump

Optimization of initial pH: carbon source (5 g/L) = glucose, nitrogen source (1 g/L) = ammonium sulphate, Shaking speed = 150 rpm, temperature = 28 ºC, inoculum amount (dry weight) = 0.1 g/L, incubation time = 60 h. Optimization of carbon source (5 g/L): initial pH = 5.0, nitrogen source (1 g/L) = ammonium sulphate, shaking speed = 150 rpm, temperature = 28 ºC, inoculum amount (dry weight) = 0.1 g/L, incubation time = 60 h. Optimization of sucrose concentration (g/L): initial pH = 5.0, nitrogen source (1 g/L) = ammonium sulphate, Shaking speed = 150 rpm, temperature = 28 ºC, inoculum amount (dry weight) = 0.1 g/L, incubation time = 60 h. Optimization of nitrogen source (1 g/L): initial pH = 5.0, carbon source = 10 g/L sucrose, shaking speed = 150 rpm, temperature = 28 ºC, inoculum amount (dry weight) = 0.1 g/L and incubation time= 60 h. Optimization of ammonium chloride concentration (g/L): initial pH= 5.0, carbon source = 10 g/L sucrose, shaking speed = 150 rpm, temperature = 28 ºC, inoculum amount (dry weight) = 0.1 g/L and incubation time = 60 h. All values are mean of three times 2 replicates ± SD.

When sucrose concentration was increased from 5-10 g/L, the size of uniform pellets decreased from 5-4 mm but the dye removal increased and reached to the maximum percentage (83 %) with 3.61 g/L biomass production (Table-1). Although maximum mycelial biomass production (3.78 g/L) was achieved at 15 g/L sucrose concentration, the lowest dye removal percentage (64 %) was recorded at the same concentration of sucrose. This might be due to mycelial morphology. Because, mycelial growth in clump form occured at this concentration of sucrose. It is also possible to say that high sucrose concentration might negatively affect dye removal by creating highly acidic condition. Because, the final pHs of culture were 4.8, 4.7 and 4.5 at the 5, 10 and 15 g/L sucrose concentrations, respectively. On the other hand, a similar result for optimal concentration of carbon source was also reported by Kapdan and Kargi[20]. They observed that minimum glucose concentration for highest decolourization efficiency was 5 g/L, although, best fungal growth was obtained at 10 g/L glucose.

Effect of different nitrogen sources on mycelial biomass and dye removal: The data presented in Table-1 clearly show that inorganic nitrogen sources were more effective in dye removal, while organic nitrogen sources were more effective in biomass production. Amongst all the nitrogen sources tested, yeast extract resulted in the highest mycelium biomass (3.96 g/L). However, only 17 % dye removal was achieved with the same nitrogen source. Maximum dye removal (88 %) was achieved in the ammonium chloride medium with 3.49 g/L of biomass production. Differences between dye removal percentages in the mediums with organic and inorganic nitrogen source might be attributed to the size of mycelial pellets. Because, inorganic nitrogen sources gave rise to uniform pellets with 4 mm size, while organic ones gave rise to uniform pellets with 6 mm size. High decolourization performances with inorganic nitrogen sources can make this fungal isolate more attractive in dye removal processes since inorganic nitrogen sources are cheaper than organic ones.

It was noted that the maximum dye decolourization (88 %) was achieved with 3.49 g/L biomass production at 1 g/L ammonium chloride concentration, where uniform pellets with 4 mm size formed (Table-1). On the other hand, the lowest dye removal percentage (61 %) was recorded at 1.5 g/L ammonium chloride concentration, where mycelial biomass production was maximum (3.8 g/L) and the mycelial morphology was clump. These results meant that ammonium chloride concentrations affected the mycelial morphology during growth, thereby increasing dye removal. It was also clear from these results that the optimum concentrations of ammonium chloride for biomass production and decolourization were different from each other. This finding was similar to the result reported by Ali *et al.*[21], who demonstrated that optimum concentration of nitrogen source (urea) for the highest dye bioadsorption efficiency was 0.1 g/L, but the optimum concentration of the same nitrogen source for the maximum production of fungal biomass was 0.5 g/L.

Effect of temperature on mycelial biomass and dye removal: As seen from data presented in Table-2, even if dye removal occured in the range of temperature

19-37 ºC, it was maximum (88 %) at 28 ºC. In the case of dye decolourization, biomass production reached to maximum (3.49 g/L) at 28 ºC and gradually decreased with the increase in the temperature from 28-37 ºC. On the other hand, no dye decolourization and fungal growth were observed at 16 and 40 ºC. Uniform pellets with 4 mm size formed at 28 ºC, while non-uniform pellets formed at the other temperatures. Hence, lower decolourization percentages above and below 28 ºC might be attributed to the mycelial morphology and the decrease in biomass concentration. This situation might be also due to decreased surface activity of the fungus mycelia.

TABLE-2
EFFECTS OF TEMPERATURE, SHAKING SPEED AND INOCULUMS AMOUNT ON DYE REMOVAL, MYCELIAL BIOMASS AND MYCELIAL MORPHOLOGY

Culture parameters	Dye removal (%)	Mycelial biomass (g/L)	Mycelial morphology
Temperatures (ºC)			
16	No removal	No growth	Non-uniform pellet
19	78 ± 2.12	2.65 ± 0.21	Non-uniform pellet
22	51 ± 1.08	2.10 ± 0.12	Non-uniform pellet
25	23 ± 0.66	1.65 ± 0.09	Non-uniform pellet
28	88 ± 1.25	3.49 ± 0.22	Uniform pellet (4 mm size)
31	72 ± 1.24	3.11 ± 0.13	Non-uniform pellet
34	53 ± 1.06	2.56 ± 0.16	Non-uniform pellet
37	21 ± 0.54	1.84 ± 0.10	Non-uniform pellet
40	No removal	No growth	Non-uniform pellet
Shaking speed (rpm)			
100	78 ± 2.12	2.65 ± 0.21	Uniform pellet (7 mm size)
150	88 ± 1.63	3.49 ± 0.06	Uniform pellet (4 mm size)
180	96 ± 1.13	3.63 ± 0.09	Uniform pellet (3 mm size)
200	57 ± 0.69	3.87 ± 0.12	Clump
Inoculum amount (1g/L)			
0.1	96 ± 1.13	3.63 ± 0.09	Uniform pellet (3 mm size)
0.2	100 ± 0.00	3.79 ± 0.04	Uniform pellet (2 mm size)
0.3	71 ± 1.86	3.96 ± 0.08	Clump

Optimization of temperature (ºC): initial pH = 5.0, carbon source = 10 g/L sucrose, nitrogen source = 1 g/L ammonium chloride, shaking speed = 150 rpm, inoculum amount (dry weight) = 0.1 g/L and incubation time = 60 h. Optimization of shaking speed (rpm): initial pH = 5.0, carbon source = 10 g/L sucrose, nitrogen source = 1 g/L ammonium chloride, temperature = 28 ºC, inoculum amount (dry weight) = 0.1 g/L and incubation time = 60 h. Optimization of inoculum amount on dry basis (g/L) = initial pH = 5.0, carbon source = 10 g/L sucrose, nitrogen source = 1 g/L ammonium chloride, temperature = 28 °C, shaking speed = 180 rpm and incubation time = 60 h. All values are mean of three times 2 replicates ± SD.

Effect of shaking speed on mycelial biomass and dye removal: The uniform pellets with the largest size (7 mm) were attained at a shaking speed of 100 rpm, but the number of these pellets was much less. The increase in shaking speed from

100-180 rpm decreased the size of uniform pellets but increased the number of uniform pellets per unit volume. The average size of uniform pellets was determined as 4 and 3 mm at 150 and 180 rpm, respectively. When shaking speed was increased from 180-200 rpm, the mycelial growth in clump form was observed. Besides, the lowest dye removal percentage (57 %) was obtained at 200 rpm, where mycelial biomass production was the maximum (3.87 g/L). Table-2 shows that the maximum dye revomal (96 %) was achieved with 3.63 g/L of biomass production at 180 rpm, where the density of uniform pellets in the medium was the maximum. Taking into the account of these results, it is concluded that the shaking speed increased dye removal by affecting mycelial morphology rather than mycelial biomass yield.

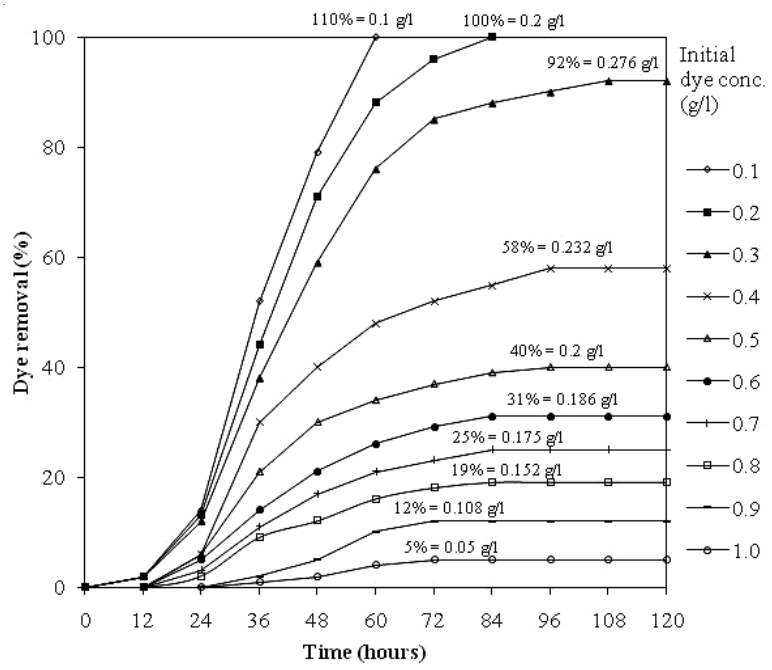
Effect of inoculum amount on mycelial biomass and dye removal: Although maximum biomass production (3.96 g/L) was achieved at an inoculum amount of 0.3 g/L (dry weight), the lowest dye removal percentage (71 %) was recorded at the same inoculum amount. The data presented in Table-2 indicate that dye removal percentage reached to maximum (100 %) at an inoculum amount of 0.2 g/L (dry weight) with 3.79 g/L biomass production (dry weight). The size of uniform pellets was 3 and 2 mm at 0.1 and 0.2 g/L inoculum amounts, respectively. On the other hand, the mycelial morphology was clump at the inoculum amount of 0.3 g/L. These results also demonstrated that mycelial morphology for dye removal was very important.

Mechanism of dye removal: Biological methods such as biodegradation and biosorption/bioadsorption possess good potential for the removal of synthetic dyes. Biodegradation is an energy dependent process and involves the breakdown of dye into various by products through the action of various enzymes. If the growth medium does not contain additional carbon and nitrogen sources, microorganisms can use dyes as carbon and nitrogen sources through their biodegradative abilities. The biodegradative abilities of fungi is due to extracellular non-specific and non-stereoselective enzyme systems composed of laccases, lignin peroxidases and manganese peroxidases[20,22-25]. In the present study, growth of the fungus was initiated in the decolourization medium containing 10 g/L glucose and other components in the absence of nitrogen source. However, no microbial growth and dye removal were observed in this medium. Similarly, no microbial growth and dye removal was recorded in the decolourization medium containing 1 g/L ammonium chloride and other components in the absence of carbon source (data not shown). These results meant that the fungus could not utilize the dye as carbon or nitrogen source in the absence of glucose or ammonium chloride and thus the chemical structure of dye was not biodegraded by the actively growing mycelia of *A. californica* MT-1.

Brahimi-Horn *et al.*[26] have reported that azo dyes from fungal biomass can be effectively recovered only through extraction with methanol. After the dye-containing culture medium was completely decolourized, we extracted the dye-uptaking mycelial pellets with methanol. At the end of this, 96 % of the total dye was recovered. The other part (4 %) of the total dye might be converted to byproducts

through biodegradation mechanism. However, this value (4 %) was too low relative to 96 %. Therefore, in the present study we did not need to investigate the activities of liglynoliytic enzymes (laccase, lignin peroxidase and manganese peroxidase), which are capable of degrading textile dyes. Considering the above results, it is assumed that dye removal was probably associated with fungal growth and hyphal uptake mechanism (biosorption/bioadsorption).

Effect of initial dye concentration on dye bioacumulation and fungal growth: Fig. 1 depicts the dye removal efficiencies with contact time at initial dye concentrations in the range of 0.1-1.0 g/L. At the initial dye concentrations of ≤ 0.2 g/L, there was no inhibitory effect on dye uptake and mycelial growth. The removal of the dye by bioadsorption was complete at the initial dye concentrations of ≤ 0.2 g/L but incomplete at the initial dye concentrations of ≤ 0.3 g/L. At 0.1 and 0.2 g/L initial dye concentrations, the complete removal (100 %) of dye took place in 60 and 84 h, respectively. Dye removal and mycelial growth were slightly inhibited at an initial dye concentration of 0.3 g/L. In spite of this, dye removal reached up to 92 % (0.276 g removed-dye) with 3.95 g/L of biomass production in 108 h and no further removal was detected. The calculation of removed dye as gram was performed by using the following formulation. Removed-dye amount (g/L) = initial dye



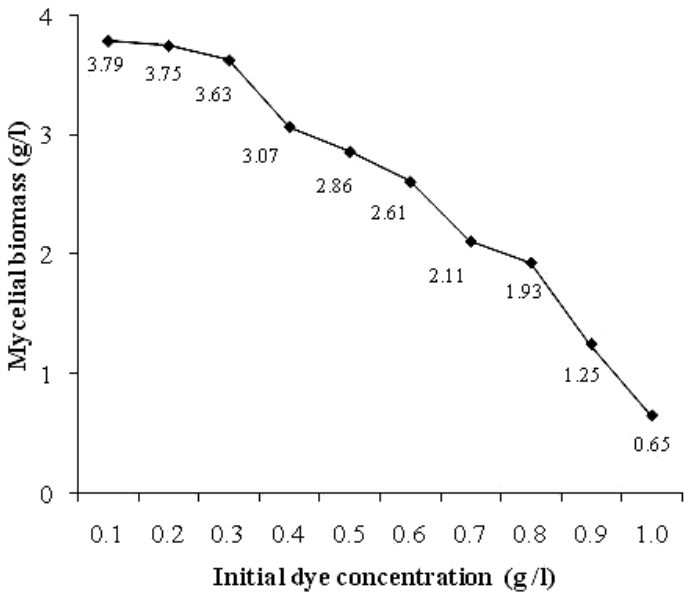
Optimal culture conditions: Sucrose 10 g/L; ammonium chloride 1 g/L; KH_2PO_4 1 g/L; $MgSO_4 \cdot 7H_2O$ 0.5 g/L; NaCl 0.5 g/L; $CaCl_2$ 0.01 g/L; pH 5; tempreture 28 ºC; inoculum amount of 0.2 g/L and shaking speed of 180 rpm

Fig. 1. Time profile of dye uptake in *Absidia californica* MT-1 at the different initial dye concentrations under the optimized culture conditions

due to the toxic effect of the dye. Mycelial growth in uniform pellet form was observed at the initial dye concentrations of ≥ 0.3 g/L, while mycelial growth in concentration (g/L) × (%) dye removal at any time/100. At the initial dye concentrations of ≥ 0.4 g/L, dye removal and fungal growth were strongly inhibited non-uniform pellet form was observed at the initial dye concentrations of ≥ 0.4 g/L. At the initial dye concentrations from 0.4-0.8 g/L, dye removal started after an initial lag period of 2 h. At 0.4, 0.5 and 0.6 g/L initial dye concentrations, no further dye removals were detected after 96 h. On the other hand, dye removal by mycelia ended at the 0.7 and 0.8 g/L initial dye concentrations after 84 h.

With 0.9 and 1 g/L reactive black-5, dye removal starded after an initial lag period of 24 h and slowly progressed and no further dye uptakes were detected after 70 h. Hence, only 12 and 7 % dye removals were able to reach at the initial dye concentration of 0.9 and 1.0 g/L, respectively. As it was shown in present studies, dye removal by fungus at all the initial dye concentrations tested was faster within the first 60 h of the cultivation and then gradually slowed down. Hence, it is concluded that young fungal biomass for dye removal was more effective than old fungal biomass. This is not unexpected, since the dye removal must be dependent on metabolic activity of actively growing biomass. The experiment shows the toxicity of reactive black-5 on the concentration of net dry mass of mycelia cultured in dye-containing culture media at different initial dye concentrations within the first 60 h, where dye uptake and growth were faster. From Figs. 1 and 2, it is clear that mycelial biomass and dye removal were correlation with each other at high dye concentrations. Namely with an increase in dye concentration the biomass production decreased due to the inhibitory effect of the dye on the growth and led to a reduction in the percentage colour removal. This result could be attributed to the fact that azo dyes generally contain one or more sulfonic acid groups in the aromatic rings, which may act as detergents, thereby inhibiting the growth of the microorganisms. Such dyes may affect DNA synthesis, as it has also been reported that dyes are inhibitors of nucleic acid synthesis or cell growth[27,28].

The results obtained from the optimization of environmental and nutritional parameters elucidated that initial pH, initial dye concentration, shaking speed, temperature, initial inoculum amount, nitrogen and carbon sources significantly affected the mycelial morphology and the dye removal in the decolourization medium during the growth of *A. californica* MT-1. Highest dye removal efficiencies were achieved when the fungus grew in uniform pellet form. Small uniform pellets with 2 mm size were the most favourable mycelial morphology. The worst mycelial morphology for dye removal was found to be clump form. Dye removal was inversely proportional to the size of mycelial pellets. Namely, dye uptake and the density of the pellets per unit volume increased as the size of the mycelial pellets decreased. This is not suprising, since the total surface size of the pellets in the decolourization medium increases when pellet density increases. As a result of this, the increasing surface contacts with more amount of dye.



Optimal culture conditions: Sucrose 10 g/L; ammonium chloride 1 g/L; KH_2PO_4 1 g/L; $MgSO_4 \cdot 7H_2O$ 0.5 g/L; NaCl 0.5 g/L; $CaCl_2$ 0.01 g/L; pH 5; tempreture 28 ºC; inoculum amount of 0.2 g/L and shaking speed of 180 rpm

Fig. 2. Toxic effect of the different initial dye concentraions on the mycelial growth in *Absidia californica* MT-1 under the optimized culture conditions for 60 h

In selection of a microorganism for decolourization of synthetich dyes, it is very important that living biomass of microorganism is capable of decolourizing dyes at a wide range of temperature, shaking speed and pH values on cheap medium components. The other two essential characteristics of a microorganism used in dye removal process are tolerance and decolourization potential. The present study elucidated that the fungi isolated from the cement-contamined soil had these potential properties.

Conclusion

It was shown for the first time in the present study that the soil polluted by cement industry was a good source of fungi, which are capable of decolourizing the synthetic textile dye reactive black-5. Optimization of nutritional and environmental conditions could significantly increase the dye removal potential of fungus *Absidia californica* by affecting mycelial morphology and growth in decolourization medium. The fungus showed decolourizing activity through adsorption mechanism rather than degradation and it could tolerate high concentrations of dye. The cheap medium components and the small uniform pellets with 2 mm size were more suitable for bioadsorption of dye. From the above results, it is believed that the bioaccumulation potential of *Absidia californica* MT-1 make significant contributions to the solution of the decolourization problem of aquous solutions containing dye.

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