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Uptake of textile dye Reactive Black-5 by *Penicillium chrysogenum* MT-6 isolated from cement-contaminated soil

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The present study focused on the decolorization of textile dye Reactive Black- 5 by actively growing mycelium of fungus *Penicillium chrysogenum* MT-6, which was isolated from the cement-contaminated soil. Dye decolorization was probably associated with fungal growth and hyphal uptake mechanism (Biosorption/Bioadsorption) . Dye uptake was strongly depended on mycelial morphology. Small uniform pellets with 2 mm size and nutrient-poor medium were found to be better for dye uptake. Optimal conditions for dye uptake by the fungus were determined as initial pH of 5.0, shaking speed of 150 rpm, temperature of 28°C, spore concentration of 10^7/ml, 10 g/l sucrose and 1 g/l ammonium chloride. The maximum removal/uptake of dye by fungus was 89% (0.267 g removed-dye) with 3.83 g/l of biomass production at an initial dye concentration of 0.3 g/l in 100 h. The fungus was found to be a good bio -system for the decolorization of the medium containing Reactive Black-5. It was shown for the first time in the present study that the cement-contaminated soil was a good source of microorganisms, being capable of removing snythetic textile dye.

Key words: *Penicillium chrysogenum* MT-6, dye removal, optimization, reactive black-5.

INTRODUCTION

Synthetic dyes find use in a wide range of industries such as textile dyeing, paper printing, cosmetics and pharmaceuticals (Marmion, 1991). 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. vinylsulphone, 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 colors and structure and are generally resistant to aerobic biodegradation processes (Lin and Peng, 1994; Sanghi et al., 2006; Daneshvar et al., 2007).

There are some reports about the negative effects of textile dyes, especially azo dyes, towards aquatic life and humans. For example, the discharge of those colored 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 will 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 (Brown and De Uito, 1993; Yesilada et al., 2003; Kalyani et al., 2007).

Currently, various treatment methods exist for the

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removal of color from industrial effluents, including physico-chemical and biological processes. Important physico-chemical processes include ozonation, adsorption, chemical precipitation and flocculation (Slokar and Majcen-Le Marechal, 1998), but these are usually inefficient, costly and not adaptable to a wide range of dye wastewaters (Banat et al., 1996). Conversely, biological processes, such as biodegradation, bioaccumulation and biosorption, have received increasing interest due to their cost, effectiveness, ability to produce less sludge and environmental benignity (Fu and Viraraghavan, 2001; Aksu, 2005).

Up till now, several reports have been published on the microbial decolorization/removal of synthetic dyes (Demir et al., 2003; Ramya et al., 2007; Dave and Dave, 2009; Lyra et al., 2009). On the other hand, it is known that Reactive Black -5 is one of the most important synthetic dyes used in the textile industry. Hence, the major aims of the present study were to obtain optimum physicochemical conditions for the decolorization/removal of Reactive Black-5 at laboratory scale by actively growing biomass of an isolated fungus *Penicillium chrysogenum* and to investigate the effect of mycelial morphology on dye decolorization.

MATERIALS AND METHODS

Dye

Reactive Black-5 dye which is commonly used in cotton textile industry in Turkey was obtained from AYTEM Z Textile Co., Turkey, in pure form and used without further purification.

Isolation of dye-decolorizing microorganisms

The fungus *P. chrysogenum* MT-6 used in this study for the decolorization of dye was isolated from soil samples collected from near the cement fabric of Askale, Erzurum (Turkey). In brief, approximately 1 g of the cement -contaminated soil was suspended in 10 ml sterile-saline water. Two ml of the supernatant for the selection of fungal isolates being resistant against the toxic effect of dye was inoculated in 200 ml of the liquid medium: glucose 5 g, KH_2PO_4 1 g, Ammonium sulphate 1 g, $MgSO_4$ 0.5 g, yeast extract 0.2 g, streptomycin 0.03 g, dye 0.1 g, distilled water 1000 ml, pH 5 – 6 (Yang et al., 2003). The inoculated medium was incubated aerobically at 28°C and 180 rpm in a shaker for 4 days. One ml of this culture was plated on the agar plate. After 4 days of incubation, fungal colonies surrounded by decolorized zones were picked up, subcultured and prufied. The obtained fungal isolates were termed as MT-1, 2, 3, 4, 5, 6 and 7.

Preparation of inoculum

The isolates were initially grown at 28°C on patato dextrose agar (PDA) slants. At the end of 6-days incubation period, ten milliliters of sterile saline water (0.9% NaCl) was added to culture slant of each isolate and these slants were vortexed. 1 ml of the homogenized spore suspension of each isolate was directly used for the inoculation. The final concentration of each spore suspension was adjusted to 10^6 /ml spores with sterile-saline water.

Selection and identification of the best isolate for dye removal

The capacities of the seven fungal isolates to decolorize/remove Reactive Black-5 were screened in 250 ml flasks containing 100 ml of the decolorization medium: Glucose 5 g, Ammonium sulphate 1 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.5 g, $CaCl_2$ 0.01 g, $FeSO_4$ 0.003 g and distilled water 1000 ml. The pH value of mediums was adjusted to 6 by using 0.1M HCl and NaOH. The flasks were stopped with cotton plug, sterilized at 121°C for 15 min, cooled at the room temperature and then inoculated with 1 ml of the homogenized spore suspension. No inoculum was added in the control flasks and the sterilized dye was incorporated in each flask before inoculation of the fungus mycelium. The inoculated flasks were incubated at 28°C and 100 rpm for 60 h. Although all of the fungi isolated from the cement-contaminated soil had high capacity to decolorize the dye, the isolate MT-6 showed slightly higher decolorization capacity (53%) than the other isolates. Therefore, only fungal isolate MT-6 was selected for the following decolorization experiments. It was identified as *P. chrysogenum*. The identification processes of the fungus were 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 (Von Arx, 1981; Domsch et al., 1983). Observations for identification were made by staining the isolated fungus using lactophenol-cotton blue and examination under low power microscope.

Dye decolorization/removal experiments with *P. chrysogenum* MT-6

The experiments were performed in 250 ml erlenmeyer flasks containing 100 ml of the decolorization medium. The optimization studies of different physicochemical conditions were carried out at an initial dye concentration of 0.1 g/l for 60 h. To determine the most favourable initial pH for the decolorization of dye-containing medium and the production of mycelial biomass, a pH range of 2 – 9 was screened out at a temperature of 28°C and shaking speed of 100 rpm. The optimization of temperature was studied at 20, 24, 28, 32, 36 and 40°C, and the optimization of shaking speed 100, 150 and 200 rpm. The optimization of spore concentration was studied at 10^5, 10^6, 10^7 and 10^8/ml spore concentrations. The influence of carbon sources was studied in media containing various carbon sources, that is, 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, organic nitrogen sources (peptone and yeast extract) and inorganic nitrogen sources (ammonium sulphate, ammonium chloride, ammonium nitrate and sodium nitrate) were tested at a concentration level of 1 g/l. The concentrations of the best carbon source were studied from 5 to 20 g/l, and best nitrogen source were from 0.5 to 1.5 g/l. To determine the maximum dye concentration, which the mould was able to decolorize/remove, experiments were carried out at different initial concentration of dye from 0.1 to 1 g/l under the optimized culture conditions. All the experiments were performed in triplicate and the average values were used in calculations.

Dye decolorization/removal and biomass assays

1 ml 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 dye removal in liquid culture was measured spectrophotometrically at a wavelength corresponding to the maximum absorbance (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. % Removal (Removal efficiency) = (Initial concentration of dye − Final concentration of dye) / Initial concentration of dye × 100. The concentration of fungal biomass was estimated according to dry weight method. The obtained pellets or mycelium was washed three times with distilled water. The washed biomass was dried at 60°C untill weight was stable.

Statistical analysis

Each experiment was repeated at least three times 2 replicates. Statistical analysis was performed using one way analysis of variance (ANOVA). P 0.05 was considered as significant.

RESULTS AND DISCUSSION

Mechanism of dye decolorization

Biological methods such as biodegradation and biosorption/bioadsorption possess good potential for the decolorization/removal of synthetic dyes. Biodegradation is an energy dependent process and involves the breakdown of dye into various byproducts 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 (Heinzkill et al., 1998; Kapdan and Kargi, 2002; Wang and Hu, 2008). In the present study, when growth of the fungus was initiated in the decolorization medium containing 5 g/l glucose and other components in the absence of nitrogen source, no mycelial biomass production and dye decolorization /removal were observed in this medium. Similarly, no microbial growth and dye decolorization/removal were recorded in the decolorization medium containing 1 g/l ammonium sulphate 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 *P. chrysogenum*.

Brahimi–Horn et al (1992) 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 decolorized, it was seen that the fungus mycelia were colored. When we extracted the dye-uptaking mycelia with methanol, 90% of the total dye was recovered from the mycelia. The other part (10%) of the total dye might be converted to byproducts through biodegradation mechanism; however, this value (10%) was too low relative to 90%. 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, we assumed that dye removal was probably associated with fungal growth and hyphal uptake mechanism (Biosorption/Bioadsorption).

In the present study, we aimed to determine the optimum culture conditions for dye decolorization rather than biomass production. Hence, the most suitable parameters of different physicochemical conditions were chosen for the subsequent decolorization experiments. Uninoculated controls except for pH 2 and 9 showed no color removal. Hence, control results were not shown in tables.

Effects of pH, shaking speed, temperature and spore concentration on fungal growth and dye removal

The results summarized in Table 1 shows that even if dye removal occured in the range of pH 3 - 8, it was maximum (53%) at pH value of 5.0 that caused mycelial pellets with 4 mm size. In contrast to dye removal, biomass production was maxium (2.92 g/l) at pH 6.0, where mycelial pellets with larger size (5 mm) were obtained. Besides, although fungal growth did not occur at pH 2 and 9, somewhat dye removal was observed at these pH values. This could be attributed to fact that very low and high pH may cause a modifiying effect on chemical sutructure of dye.

The uniform pellets with the largest size (4 mm) were attained at a shaking speed of 100 rpm, but the number of these pellets was less. The increase in shaking speed from 100 to 150 rpm decreased the size of uniform pellets (pellets with 3 mm size) but increased the number of uniform pellets per unit volume. With further increase in shaking speed from 150 to 200 rpm, biomass

Table 1. Effects of pH, shaking speed, temperature and spore concentration on dye decolorization, mycelial biomass and mycelial morphology.

Culture parameters	Dye removal (%)	Mycelial biomass (g/l)	Mycelial morphology
Initial pH			
2.0	4 ± 0.66	No growth	-
3.0	24 ± 1.01	1.28 ± 0.06	Non-uniform pellet
4.0	33 ± 1.73	1.68 ± 0.31	Non-uniform pellet
5.0	53 ± 2.08	2.44 ± 0.22	Uniform pellet (4 mm size)
6.0	43 ± 1.05	2.92 ± 0.17	Uniform pellet (5 mm size)
7.0	38 ± 1.15	1.78 ± 0.09	Non-uniform pellet
8.0	22 ± 0.59	1.42 ± 0.11	Non-uniform pellet
9.0	3 ± 0.85	No growth	
Shaking speed (rpm)			
100	53 ± 2.08	2.44 ± 0.22	Uniform pellet (4 mm size)
150	63 ± 2.66	2.61 ± 0.04	Uniform pellet (3 mm size)
200	47 ± 1.86	2.97 ± 0.19	Clump
Temperature (^{0}C)			
20	38 ± 2.41	1.86 ± 0.19	Non-uniform pellet
24	54 ± 3.32	2.32 ± 0.14	Non-uniform pellet
28	63 ± 2.66	2.61 ± 0.04	Uniform pellet (3 mm size)
32	33 ± 2.45	2.88 ± 0.12	Clump
36	24 ± 2.87	1.56 ± 0.13	Non-uniform pellet
40	No removal	No growth	-
Spore number/ml			
10^5	47 ± 1.67	1.74 ± 0.25	Uniform pellet (5 mm size)
10^6	63 ± 2.66	2.61 ± 0.04	Uniform pellet (3 mm size)
10^7	73 ± 3.12	2.78 ± 0.08	Uniform pellet (2 mm size)
10^8	44 ± 1.16	2.92 ± 0.02	Clump

Optimization of initial pH: shaking speed = 100 rpm, temperature = 28°C, spore number = 10^6, carbon source (5 g/l) = glucose, nitrogen source (1 g/l) = ammonium sulphate and Incubation time = 60 h. Optimization of shaking speed: Initial pH = 5.0, temperature = 28°C, spore number = 10^6, Carbon source (5 g/l) = glucose, nitrogen source (1 g/l) = ammonium sulphate and Incubation time = 60 h. Optimization of temperature: Initial pH = 5.0, shaking speed = 150 rpm, spore number = 10^6, carbon source (5 g/l) = glucose, nitrogen source (1 g/l) = ammonium sulphate and incubation time = 60 h. Optimization of spore number: Initial pH = 5.0, shaking speed = 150 rpm, temperature =28°C, carbon source (5 g/l) = glucose, nitrogen source (1 g/l) = ammonium sulphate and incubation time = 60 h. All values are mean of three times 2 replicates ± SD.

production increased but pellet formation decreased. Accordingly, the fungal growth in clump form was observed at a shaking speed of 200 rpm that resulted in maximum biomass production (2.97 g/l). Table 1 shows that high dye removal (63%) was achieved with 2.61 g/l of biomass production at 150 rpm that resulted in more uniform pellet per unit volume. On the basis of these observations, lower dye removal efficiencies at pH conditions below or above 5 might be attributed to the mycelial growth in non-uniform pellet form or the size of uniform pellets per unit volume. This might be also due to decreased metabolic activity of fungus mycelium.

Table 1 indicates that although fungus was capable of decolorizing the dye in the range of temperature 20 - 36°C, dye removal was maximum (63%) at 28°C. On the other hand, no dye removal and fungal growth were observed at 40°C. Fungal growth in uniform pellet form (3 mm size) was observed at only 28°C. In contrast to dye

removal, biomass production reached to maximum (2.88 g/l) at 32°C, where mycelium growth in clump form took place. Hence, lower dye removal efficiencies below and above 28°C could be attributed to mycelial morphology. This situation might be also due to decreased surface activity of the mycelium.

Among the spore concentrations tested, 10^8/ml spore concentration gave rise to maximum biomass production (2.92 g/l), whereas 10^7/ml spore concentration gave rise to maximum dye removal (73%). Mycelial growth in pellet form was achieved at 10^5, 10^6 and 10^7 /ml spore concentrations; however, mycelial pellet size was quite distinct. Small pellets formed at high spore concentrations but large pellets at low spore concentrations. On the other hand, a spore concentration of 10^8 caused fungal growth in clump form. The optimal pellet size for maximum dye removal was found to be 2 mm. The applications below and above this optimal spore concentration caused a decrease in dye removal (Table 1).

Effect of different carbon and nitrogen sources and their various concentrations on fungal growth and dye removal

Although biomass production and dye removal occurred on all the carbon sources tested, glucose and sucrose resulting in uniform pellet form (2 mm size) seemed to be more suitable carbon sources (Table 2). Uniform pellet formation was not achieved with the other carbon sources. The fungus was able to decolorize 73% of the dye in glucose medium in 60 h. Following glucose, sucrose gave relatively high dye removal (71%) with 2.65 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 decolorization processes, and therefore it was chosen as carbon source for the following experiments. In decolourization processes, various inexpensive carbon sources such as starch and molasses have been utilized.

When sucrose concentration was increased from 5 to 10 g/l, dye decolorization progressed more rapidly and 89% removal of the total dye was achieved with 3.12 g/l biomass. A further increase in sucrose concentration from 10 to 20 g/l exerted a negative effect on dye decolorization. This may stem from C/N rate of culture medium. It is also possible to say that high sucrose concentrations may negatively affect dye decolorization by creating highly acidic condition. In contrast to dye removal, biomass production was maximum (4.08 g/l) at a sucrose concentration of 20 g/l. A similar result for optimal concentration of carbon source was also reported by Kapdan and Kargi (2002). They observed that minimum glucose concentration for highest decolorization efficiency was 5 g/l, although, best fungal growth was obtained at 10 g/l glucose. Mycelial pellet formation in 2 mm size was achevied at all sucrose concentrations tested.

Large uniform pellets with 5 mm size were achieved with organic nitrogen sources (peptone and yeast extract), while smaller uniform pellets with 2 mm size were achieved with inorganic ones. Amongst all the nitrogen sources tested, although the highest biomass productions were achieved with organic nitrogen sources, the lowest dye removal percentages were recorded with them. Maximum dye removal (96%) was achieved in the ammonium chloride medium with 3.27 g/l of biomass production. High removal 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.

The experiments also concluded that the optimum concentrations of ammonium chloride for biomass production and dye removal were different from each other. This finding was similar to the result reported by Ali et al. (2007), who demonstrated that optimum concentration of nitrogen source (urea) for the highest dye removal 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. In the present study, optimum concentration of ammonium chloride was found to be 1 g/l for maximum dye removal (96%) but 1.5 g/l for maximum biomass production (3.73 g/l). The uniform pellets were observed at the ammonium chloride concentrations of 0.5 and 1.0 g/l, whereas mycelial growth in clump was observed at an ammonium chloride concentration of 1.5 g/l.

Effect of initial dye concentration on dye uptake and fungal growth

At the initial dye concentrations of 0.2 g/l, there was no inhibitory effect on dye removal and fungal growth. The decolorization of the dye- containing culture media was complete at the initial dye concentrations of 0.2 g/l but incomplete at the initial dye concentrations of 0.3 g/l. At0.1 and 0.2 g/l initial dye concentrations, the complete removal (100%) of dye took place in 70 and 80 h, respectively. At an initial dye concentration of 0.3 g/l, dye

Table 2. Effects of different carbon and nitrogen sources and their various concentrations on dye decolorization, mycelial biomass and mycelial morphology.

Culture parameterS	Dye removal (%)	Mycelial biomass (g/l)	Mycelial morphology
Carbon sources (5 g/l)			
Glucose	73 ± 3.12	2.78 ± 0.08	Uniform pellet (2 mm size)
Sucrose	71 ± 1.26	2.65 ± 0.11	Uniform pellet (2 mm size)
Fructose	56 ± 2.35	2.20 ± 0.21	Non-uniform pellet
Maltose	33 ± 3.28	1.96 ± 0.13	Non-uniform pellet
Lactose	24 ± 2.96	1.75 ± 0.05	Non-uniform pellet
Sucrose concentration (g/l)			
5	71 ± 1.26	2.65 ± 0.11	Uniform pellet (2 mm size)
10	89 ± 2.35	3.12 ± 0.24	Uniform pellet (2 mm size)
15	64 ± 3.46	3.78 ± 0.16	Uniform pellet (2 mm size)
20	53 ± 2.14	4.08 ± 0.14	Clump
Nitrogen sources (1 g/l)			
Ammonium sulphate	89± 2.35	3.12 ± 0.08	Uniform pellet (2 mm size)
Ammonium chloride	96 ± 1.68	3.27 ± 0.06	Uniform pellet (2 mm size)
Ammonium nitrate	58 ± 3.43	2.36 ± 0.12	Uniform pellet (2 mm size)
Sodium nitrate	47 ± 4.11	2.98 ± 0.07	Uniform pellet (2 mm size)
Yeast extract	27 ± 1.65	3.96 ± 0.04	Uniform pellet (5 mm size)
Peptone	24 ± 1.23	3.82 ± 0.09	Uniform pellet (5 mm size)
Ammonium chloride (g/l)			
0.5	81 ± 1.11	3.11 ± 0.09	Uniform pellet (2 mm size)
1	96 ± 1.68	3.27 ± 0.06	Uniform pellet (2 mm size)
1.5	78 ± 0.78	3.73 ± 0.11	Clump

Optimization of carbon source (5 g/l): Initial pH = 5.0, shaking speed = 150 rpm, temperature =28°C, spore number = 10^7, nitrogen source (1 g/l) = ammonium sulphate and incubation time = 60 h. Optimization of sucrose concentration (g/l): Initial pH = 5.0, shaking speed = 150 rpm, temperature = 28°C, spore number = 10^7, nitrogen source (1 g/l) = ammonium sulphate and incubation time = 60 h. Optimization of nitrogen source (1 g/l): Initial pH = 5.0, Shaking speed = 150 rpm, temperature = 28°C, spore number = 10^7, carbon source = 10 g/l, sucrose and incubation time = 60 h. Optimization of ammonium chloride concentration (g/l): Initial pH = 5.0, shaking speed = 150 rpm, temperature =28°C, spore number = 10^7, carbon source = 10 g/l sucrose and Incubation time = 60 h. All values are mean of three times 2 replicates ± SD.

removal and fungal growth were slightly inhibited; however, dye removal reached up to 89% (0.267 g removed- dye) with 3.83 g/l of biomass production in 100 h and no further uptake was detected. The calculation of removed-dye as gram was performed by using the following formulation. Removed-dye amount (g/l) = Initial dye concentration (g/l) × dye removal (%) at any time / 100 (%). Since dye removal and fungal growth were strongly inhibited at the initial dye concentrations of 0.4 g/l, dye removal started after an initial lag period of 20 h (data not shown). 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 (Chen et al., 2003; Asad et al., 2007). No futher dye removals were observed at the 0.4, 0.5 and 0.6 g/l initial dye con-centrations after 90 h. On the other hand, dye removal at the 0.7 and 0.8 g/l initial dye concentrations ended after 80 h. With 0.9 and 1 g/l Reactive Black-5, dye removal

Table 3. Effects of initial dye concentration and cultivation time on dye uptake and mycelial biomass in *P. chrysogenum* MT-6.

Initial dye con. (g/l)	Incubation time									
	60 h		70 h		80 h		90 h		100 h	
	MB (g/l)	DR (g/l)	MB (g/l)	DR (g/l)	MB (g/l)	DR (g/l)	MB (g/l)	DR (g/l)	MB (g/l)	DR (g/l)
0.1	3.27 ± 0.06	0.096 ± 0.002	3.54 ± 0.02	0.100 ± 0.019	ND	ND	ND	ND	ND	ND
0.2	3.22 ± 0.04	0.140 ± 0.008	3.51 ± 0.03	0.178 ± 0.011	3.68 ± 0.11	0.200 ± 0.002	ND	ND	ND	ND
0.3	3.11 ± 0.05	0.222 ± 0.024	3.41 ± 0.05	0.247 ± 0.022	3.58 ± 0.07	0.255 ± 0.014	3.73 ± 0.11	0.261 ± 0.009	3.83 ± 0.02	0.267 ± 0.009
0.4	2.88 ± 0.03	0.168 ± 0.004	3.18 ± 0.09	0.196 ± 0.008	3.26 ± 0.08	0.208 ± 0.003	3.34 ± 0.09	0.224 ± 0.011	3.41 ± 0.09	0.224 ± 0.016
0.5	2.78 ± 0.02	0.155 ± 0.001	2.98 ± 0.07	0.175 ± 0.010	3.12 ± 0.02	0.190 ± 0.006	3.20 ± 0.04	0.202 ± 0.006	3.24 ± 0.05	0.202 ± 0.008
0.6	2.52 ± 0.09	0.138 ± 0.006	2.68 ± 0.13	0.156 ± 0.005	2.86 ± 0.16	0.168 ± 0.011	2.96 ± 0.16	0.176 ± 0.019	3.02 ± 0.14	0.176 ± 0.012
0.7	2.23 ± 0.12	0.121 ± 0.010	2.39 ± 0.12	0.140 ± 0.011	2.52 ± 0.13	0.151 ± 0.004	2.62 ± 0.13	0.151 ± 0.001	2.68 ± 0.11	0.151 ± 0.011
0.8	1.98 ± 0.14	0.108 ± 0.002	2.11 ± 0.09	0.120 ± 0.003	2.20 ± 0.09	0.128 ± 0.017	2.26 ± 0.20	0.128 ± 0.004	2.29 ± 0.20	0.128 ± 0.010
0.9	1.38 ± 0.11	0.072 ± 0.013	1.48 ± 0.22	0.081 ± 0.001	1.57 ± 0.21	0.081 ± 0.019	1.62 ± 0.10	0.081 ± 0.002	1.65 ± 0.16	0.081 ± 0.017
1.0	1.06 ± 0.12	0.050 ± 0.005	1.16 ± 0.06	0.060 ± 0.010	1.21 ± 0.04	0.060 ± 0.009	1.24 ± 0.21	0.060 ± 0.007	1.26 ± 0.08	0.060 ± 0.006

Culture conditions: Initial pH = 5.0, shaking speed = 150 rpm, temperature =28°C, spore number = 10^7, carbon source = 10 g/l sucrose, nitrogen source = 1 g/l ammonium chloride, KH_2PO_4 = 1 g, $MgSO_4$ = 0.5 g, NaCl = 0.5 g, $CaCl_2$ = 0.01 g, $FeSO_4$ = 0.003 g and distilled water = 1000 ml. MB (Mycelial biomass), DR (Dye removal), ND (Not determined). All values are mean of three times 2 replicates ± SD.

started after an initial lag period of 30 h (data not shown). It was slowly progressed and no further dye removal was detected at these dye concentrations after 70 h. As it was shown in Table 3, dye removal was faster at all the initial dye concentrations within the first 60 h of the cultivation and then gradually slowed down. Hence, we concluded that young fungal biomass in dye uptake was more effective than old fungal biomass. This is not unexpected, since the dye uptake must be dependent on metabolic activity of actively growing mycelium. Besides, it was noted that at all the initial dye concentrations of 0.1 g/l even if fungal growth continued after 60 h, a disruption in structures of uniform or non-uniform pellets occured after 60 h (data not shown). This situation may be also an answer to the decrease in dye uptake performance of fungus after 60 h. In selection of a microorganism for decolorization of synthetich dyes, it is very important that living biomass of microorganism is capable of decolorizing dyes at a wide range of temperature, shaking speed and pH values on cheap medium components. In the recent years, there has been a growing interest in isolation of microorganisms from various sources for dye removal. On the other hand, the screening of isolated microorganisms is known to be very critically important to obtain microorganisms with high ability. In the present study, isolation and screening methods could be effectively used for the selection of the best fungal isolate. Besides, high dye removal efficiencies were achieved with cheap medium components. The other two essential characteristics of a microorganism used in dye removal process are tolerance and decolorization potential. The fungus could tolerate high concentrations of dye. As a result, the present study eluciated that the *P. chrysogenum* MT- 6 isolated from the cement-contaminated soil had these potential properties.

The results elucidated that the optimization of initial pH, initial dye concentration, shaking speed, temperature, spore concentration, nitrogen and carbon sources could increase the dye removal potential of the fungus by affecting significantly mycelial morphology and growth in the medium containing Reactive Black-5 (Tables 1, 2 and 3).

Dye removal percentages were relatively high when the fungus grew in uniform pellet form. The worst mycelial morphology for dye removal was

found to be clump form. Small uniform pellets with 2 mm size were the most favorable mycelial morphology. Dye removal was inversely proportional to the size of mycelial pellets. 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 increases when pellet density increases in the medium. As a result of this, there is increasing surface contacts with more amount of dye. From the above results, we belive that the dye removal potential of *P. chrysogenum* MT-6 make significant contributions to the solution of the decolorization problem of aquous solutions containing dye. Especially, the usage of this fungal isolate as a bio- system benefits to solve the problem of decolorization by biodegradation, which may produce new toxic byproducts owing to incomplete mineralization.

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