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Decolorization of Remazol Black-B azo dye in soil by fungi

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Abstract

Textile industry is known to release huge amount of dyes in the water and soil environments during the dyeing process. The present study was planned with the aim to remove azo dye toxicants from the soil using fungal strains. The fungi were isolated by using Remazol Black-B azo dye as the sole source of C and N. Ten isolates were initially selected for testing their decolorization potential in the liquid medium. Three most effective strains were used to study the decolorization of Remazol Black-B in soil. The strain S4 was found to be very effective in removing the dye Remazol Black-B from liquid medium as well as in soil suspension. More than 95% decolorization by the strain S4 was observed in soil under optimal incubation conditions. Overall, the dye decolorization was maximum at 100 mg dye kg^{-1} soil at pH 7-8 under static conditions. Glucose, moisture and aeration also affected the decolorization efficacy of the fungal strain in soil. This study implies that fungi could be used for bioremediation of dye-contaminated sites.

Keywords: *biodegradation, dyes, fungi, soil, factors*

Introduction

Azo dyes are commonly used as a coloring agent in textile, food, paper, leather, cosmetics, pharmaceutics and other industries (Erkurt, 2010). The largest amount of azo dyes is used for the dyeing of textile products (Bafana *et al*., 2008). It has been estimated that about 2-50% of the dye-stuff used during these dyeing processes does not bind to the fibers and is therefore released into the environment via wastewater facilities (Reisch, 1996). In particular, the soluble reactive dyes which are being used in increasing quantities are hydrolyzed during application without a complete fixation. Consequently, a large proportion of these dyes are released into the soil and water environment (Carliell *et al*., 1994; Jeckel, 1997). These dye compounds retain their color and structural integrity under exposure to sunlight, chemical attack (Guelli *et al*., 2008) and also exhibit high resistance to microbial degradation in wastewater treatment systems. This results in severe contamination of the rivers and ground water in those areas having a big number of textile industrial units (Gong *et al*., 2005).

Azo dyes contain at least one nitrogen-nitrogen double bond (–N=N–); however, many different structures are possible (Zollinger, 1991). Azo dyes are of great concern because dye precursors or their biotransformation products such as aromatic amines show carcinogenic and mutagenic effects (Ozturk and Abdullah, 2006; Alves de Lima *et al*., 2007; Daneshvar *et al*., 2007). Contrary to physico-chemical methods, biodegradation is an environment-friendly and cost competitive approach that could be used for the detoxification of azo dyes contaminants (Maier *et al.,* 2004; Hernández *et al*., 2008). Fungi have been found capable of mineralizing a diverse range of persistent organic pollutants (Ramya *et al.,* 2007; Casieri *et al*., 2008).

Although several reports provide evidence that microorganisms including fungi degrade azo compounds but very little is known about the biodegradation of these compounds under soil conditions. Further, screening efficient fungal strains that can cleave azo bonds of complex azo dye structures could be useful for the effective treatment of textile effluents. The treated industrial wastewater can be used for irrigation purpose, particularly for growing crops in degraded soils (Iqbal *et al*., 2010). It is even more imperative when quality of the underground water is not good in the most areas of Pakistan (Waheed *et al*., 2010). Here, fungal isolates were evaluated for their potential to decolorize Reactive Black B azo dye and some of the strains were capable of decolorizing the azo dye efficiently in a suspension of soil under aerobic conditions.

Materials and Methods

A laboratory study was carried out to assess the potential of fungal isolates in degrading Remazol Black-B azo dye in the soil.

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Isolation of dye degrading fungi

Soil samples were collected from the surroundings of Sandal Bar Dyestuff Industry, Faisalabad. Fungal strains native to that area were isolated on modified peptone agar medium by dilution plate technique. The following composition of medium (pH7) was used containing Remazol Black-B azo dye as a source of carbon and nitrogen: K_2HPO_4, 0.5 g L^{-1}; $MgSO_4.7H_2O$, 0.2 g L^{-1}; $FeSO_4.7H_2O$, traces; Remazol Black-B, 0.1 g $L^{-1,}$

The medium was autoclaved for 20 min at 120°C. Petri plates containing solidified medium were inoculated by adding 1 mL soil suspension and then thoroughly spreading on the agar medium containing 1.5% agar. Petri plates were incubated at 28°C for six days. Ten fungal strains differing in growth pattern and morphology were isolated and further streaked 3 times to get purified strains.

Preserved strains were transferred to liquid medium lacking agar. Instead of dye, glucose (1.5 g L^{-1}) was used as a carbon source. This culture was placed in shaker for 48 h at 30° C. Liquid culture was prepared to ensure the equal distribution of fungal cells.

Screening was done to find out fungal strains capable of degrading Remazol Black-B azo dye efficiently in liquid medium. The fungal strains that showed the highest decolorization of Remazol Black-B in liquid medium in 96 h were selected. Out of ten strains, three potential isolates were selected and examined again for their potential to decolorize the Remazol Black-B in soil.

The soil collected from upper 15 cm layer was air-dried, ground, and passed through a 2 mm sieve. It was analyzed for physico-chemical properties. The soil was clay loam having pHs of 7.4 and ECe of 2.45 dS m^{-1}.

Biodegradation of Remazol Black-B in soil

The soil was sterilized and spiked by adding 2 mL azo dye solution (200 mg L^{-1}). One mililiter inoculum was added to soil and 60% water holding capacity, WHC (field capacity level) was maintained. The fungal inocula were prepared in liquid medium. Instead of dye, glucose (1.5 g L^{-1}) was used as a carbon source. This culture was placed in a shaker (100 rpm) for 96 h at 30 ^{o}C. Liquid culture was prepared to ensure the equal distribution of fungal cells.

In control, azo dye solution was added to the soil without inoculum. The flasks were incubated for 96 h at 25 ^{o}C. All the treatments were replicated three times.

The contaminated soil was analyzed for Remazol Black-B recovery after 96 h. The dye was extracted from the soil using a multi-solvent system (chloroform, methanol, distilled water: 1:1:1, v/v, 3 mL each). Each solvent was added separately in above order following mixing and vigorous shaking of the soil samples. Then the flasks contents were sonicated for 15 min and filtered through filter paper. Chloroform was separated from the filtrate with the help of micro-pipette. Then filtrate was centrifuged for 10 min at 10,000 rpm. The absorbance was measured at 578 nm (UV- Spectrophotometer) as described by Novotny *et al*. (2001). Change in color was observed which gave the rate of biodegradation of dye (Greenberg *et al*., 1992).

One of the efficient fungal strains was selected for optimization of environmental/ incubation conditions. Using above described method, the following factors were studied to optimize dye degradation process in soil.

Five levels of Remazol Black-B (50, 100, 150, 200 and 250 mg L^{-1}) were used while different concentrations of glucose such as 2, 4, 6, 8 and 10 g kg^{-1} were used as a co-substrate to find the best glucose level for maximum degradation.

Four kinds of carbon sources namely glucose, lactose, mannitol and sucrose at the rate of 10 g kg^{-1} were studied. Similarly, four levels of pH (5, 6, 7, 8 and 9) were optimized having 100 mg L^{-1} azo dye concentration, 60% water level and 10 g kg^{-1} glucose concentration, and incubated for 96 h at 30 ^{o}C. Other factors include temperature (25, 30, 35 and 40 ^{o}C), moisture (40, 60, 80 and 100% WHC) and aeration (shaking vs. static).

All the above factors were optimized under the following conditions except the factor under investigation:- soil, 10 g; inoculum, 1 mL; dye concentration, 100 mg kg^{-1} soil; glucose concentration, 10 g kg^{-1} soil; water, 60% WHC; pH 7.4; incubation temperature, 30 ^{o}C and incubation time 96 h, under shaking conditions.

Results

The results revealed that different fungal isolates had variable potential to degrade Remazol Black-B and decolorization varied from 21 to 68% (Table 1). The most efficient fungal strain in degrading the Remazol Black-B was S4. It was followed in descending order by S7 and S3. The strain S9 was the least efficient degrader of Remazol Black-B.

Three selected strains of fungi (S3, S4 and S7) were further evaluated for their ability to degrade Remazol Black-B under soil conditions. The results reveled that maximum degradation (57%) was found where the soil was inoculated with S4 fungal strain (Figure 1). Next to it, the strain S7 showed better performance with 47% decolorization of the tested azo dye in soil. The strain S3

was the least efficient degrader of azo dye among the three tested fungal strains.

Based upon the preliminary studies, one of the most efficient strains namely, S4 was used for optimizing incubation conditions for maximum biodegradation of Remazol Black-B in the soil environment. Effects of various levels of substrate (Remazol Black-B), glucose concentration and type of carbon sources (sucrose, glucose, lactose, mannitol), pH, temperature, incubation time, moisture and aeration on Remazol Black-B degradation in soil by fungi were investigated under standard conditions.

Table 1: Biodegradation of Remazol Black-B in liquid medium by fungi

Fungal isolate	Percent Degradation (± SD)
S1	42 ±4
S2	44 ±6
S3	52 ±6
S4	68 ±7
S5	25 ±3
S6	34 ±2
S7	55 ±5
S8	29 ±3
S9	22 ±2
S10	40 ±3

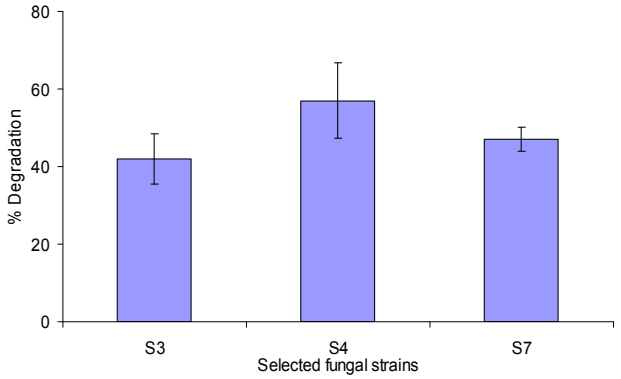

Figure 1: Biodegradation of Remazol Black-B in soil by three strains of fungi. Ten gram soil containing 200 mg kg^{-1} Remazol Black-B was inoculated with one mL inoculum and incubated at 25°C for 96 h

It is evident from Figure 2 that the decolorization of Remazol Black-B azo dye was increased up to 100 mg kg $^{-1}$ of substrate concentration and maximum degradation (75%). Then, there was a gradual decrease in the azo dye degradation up to substrate concentration of 200 mg kg^{-1} soil and then it almost leveled off.

Effect of different levels (0, 2, 4, 6, 8 and 10 g kg^{-1} soil) of glucose was assessed on substrate Remazol Black-B by fungal strain S4 and a stimulatory effect of increasing concentration of glucose on Remazol Black-B degradation was observed (Figure 3). There was a rapid increase in degradation of Remazol Black-B up to glucose concentration of 6 g kg^{-1} soil. Afterwards, a slower increase in Remazol Black-B degradation was observed up to 8 g kg^{-1} soil, and then the reaction was leveled off and no further increase in degradation rate was observed.

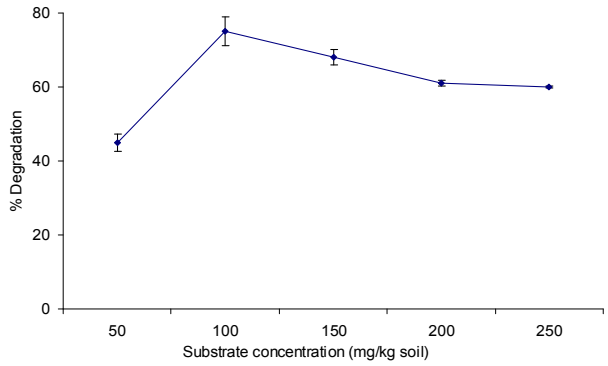

Figure 2: Effect of substrate (Remazol Black-B) concentration on fungal biodegradation

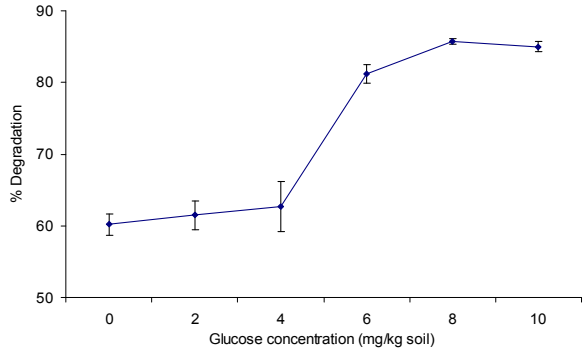

Figure 3: Effect of different levels of glucose on biodegradation of Remazol Black-B by fungal strain S4

Among different carbon sources such as lactose, mannitol, glucose and sucrose, glucose applied at the rate of 8 g kg^{-1} soil caused maximum degradation (79.5%) (Figure 4). Lactose applied had almost similar effect on degradation as glucose. Two other sources of carbon i.e. mannitol and lactose, although stimulated the azo dye degradation than control but the effect was non-significant.

The maximum degradation (93%) of azo dye was obtained between soil pH 7-8 by fungus S4 (Table 2). As the pH decreased from 7 to 5 (towards high acidity), degradation rate decreased. The highest biodegradation was recorded between 30 to 35 ^{o}C temperature (Table 2). Lowering of temperature from 30 ^{o}C inhibited the biodegradation of Remazol Black-B azo dye in soil by

fungi S4. The degradation of Remazol Black-B by fungus strain S4 was increased as the moisture level increased from 40 to 60% WHC (Table 2). The maximum biodegradation percentage (75%) was obtained at 60% moisture level and then it sharply decreased as moisture level increased up to 80%. There was no effect between degradation rate at 80 and 100% moisture level.

for the treatment of Remazol Black-B contaminated water and soil environments. Fungi are considered efficient degraders because of their ability to produce large variety of extra cellular enzymes, organic acids and other metabolites (Lilly and Barnett, 1951). Glenn and Gold (1983) reported that *Phanerochate chrysosporium* degrade polymeric dyes by means of secondary metabolic process

Table 2: Effect of pH, temperature (T) and moisture (M) on degradation of Remazol Black-B in soil by fungal strain S4

	Percent degradation													
Factor	**pH5**	**pH6**	**pH7**	**pH8**	**pH9**	**T25**	**T30**	**T35**	**T40**	**M20**	**M40**	**M60**	**M80**	**M100**
pH	61 (±5)	78 (±8)	93 (±7)	92 (±6)	71 (±5)									
Temp						82 (±6)	93 (±7)	94 (±8)	91 (±7)					
Moist										36 (±4)	67 (±5)	75 (±5)	30 (±3)	28 (±3)

T: ^{o}C
M: % WHC

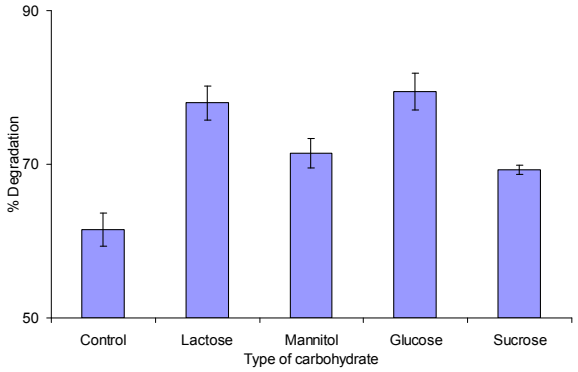

Figure 4: Effect of different sources of carbon on biodegradation of Remazol Black-B by fungi

Under static conditions, fungus S4 decolorized 84% of the dye in soil suspension; however, degradation rate was decreased to 67% when the dye suspension was incubated under shaking conditions (Figure 5).

Discussion

This study demonstrated the biodegradation potential of fungi isolated from azo dye contaminated soil using Remazol Black-B as the sole source of C and N. The results showed that all the ten strains of fungi were capable of degrading Remazol Black-B azo dye in the liquid medium but with different degrees of efficacy. However, the strain (S4) capable of degrading azo dye in liquid medium was also the most effective in soil environment and degraded Remazol Black-B up to 95% under optimized conditions. These findings imply that the fungi can be effectively used (lignindegration). Similarly, Enayatzamir *et al*. (2010) reported the ability of the white-rot fungus *Phanerochaete chrysosporium* immobilized into Ca-alginate beads to decolorize different recalcitrant azo dyes such as Direct Violet 51, Reactive Black 5, Ponceau Xylidine and Bismark Brown R in successive batch cultures.

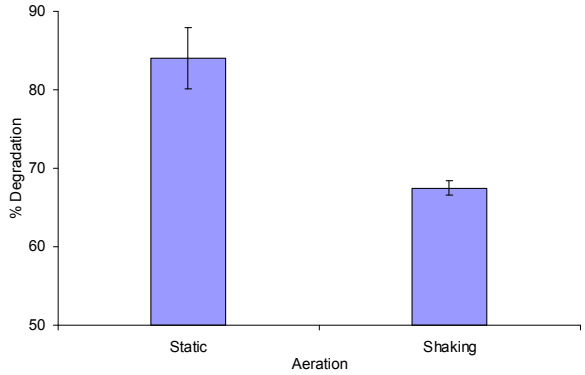

Figure 5: Effect of aeration on biodegradation of Remazol Black-B by fungal strain S4 in soil

This study showed that Remazol Black-B degradation was concentration dependent and maximum biodegradation was observed at 100 mg dye kg^{-1} soil. Biodegradation by fungus reduced as dye concentration increased beyond 100 mg kg^{-1} soil. Azo dyes are considered recalcitrant xenobiotic compounds due to the presence of N═N bond (Adosinda *et al*., 2001). Presence of azo dyes in environment reduces the gas solubility, so increased Remazol Black-B concentration can decrease the fungus

growth (Carliell *et al*., 1994). The higher concentration of the dye might have inhibited fungal growth.

This study also suggested that fungus worked efficiently at field capacity (60% moisture level) in soil. Less than 60% moisture was not enough for fungus growth, and greater than 60% moisture reduced the bio-availability of Remazol Black-B in soil to fungus. These results support the previous findings also (Adosinda *et. al*., 2001).

Glucose had stimulatory effect on Remazol Black-B degradation because glucose acted as a source of C and energy and thus promoted fungal growth. Since absence of co-substrate decreases mycelial weight (Jeffries *et al*., 1981), so its addition stimulated degradation of Remazol Black-B due to increased fungal activity. However, after certain level of glucose, its addition had negative effect on degradation. This negative effect may be due to the shift of fungal population to glucose rather to utilize Remazol Black-B as a C and N source. This study also suggested that among different co-substrates, glucose and lactose were the best source of carbon for the degradation of Remazol Black-B. Nigam *et al*. (1996) had also observed that the medium supplemented with glucose and lactose had given better degradation of dye.

At pH 7, 93% degradation was achieved. This study indicated that neutral pH supported fungal activity to degrade Remazol Black-B in soil. Similarly, increasing temperature had positive effect on fungus activity and maximum biodegradation was observed at 35°C. The results also showed that the degradation percentage was greater under static condition than shaking/agitating condition. Spadaro *et al*. (1992) studied that treatment of azo dyes contaminated environment with fungus is mainly due to the expression of some extracellular enzyme, responsible for the degradation and in some fungus agitation had been reported to suppress the expression of lignolytic system.

This study suggested that toxic pollutants like azo dyes concentrated in industrial waste and contaminated sites can potentially be eliminated by low cost bioremediation systems using microbial cultures. The knowledge about the optimum environmental factors/ conditions could help to employ biological approaches efficiently to clean up the water and soil environment polluted by azo dyes discharged from textile and dyeing industry.

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