African Journal of Biotechnology Vol. 6 (12), pp. 1441-1445, 18 June 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Biodecolorization and biodegradation of Reactive Blue by *Aspergillus* sp.

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Accepted 15 June, 2007

A*spergillus* sp. effectively decolorized Reactive Blue and other structurally different synthetic dyes. Agitation was found to be an important parameter, while glucose (99%), sucrose (97%) and mannitol (98%) were the best carbon sources for the decolorization. Decolorization was effective in an acidic environment (pH 3). Few chemically different dyes such as Reactive Black (75%), Reactive Yellow (70%), Reactive Red (33%) and Coloron Violet (66%) were decolorized moderately.The dye Coloron Black (9%) was highly resistant for decolorization by *Aspergillus* sp. Both spectral analysis and HPLC analysis were confirmatory to degradation.

Key words: Decolorization, degradation, HPLC, Reactive Blue, *Aspergillus* sp.

INTRODUCTION

A major environmental problem facing the textile dyeing and finishing industry is that the industry produces large volumes of high strength aqueous waste continuously. The discharge of wastewaters containing recalcitrant resiues into river and lakes lead to higher biological oxygen demand (BOD) causing serious threat to native aquatic life (McMullan et al., 2001).

As synthetic dyes are relatively resistant to biodegradation, the elimination of coloured effluents in wastewater is based mainly on physical and chemical methods (Banat, et al., 1996). Although these methods are effective, they suffer from short-comings such as high cost, formation of hazardous by-products and intensive energy requirements. The majority of color removal techniques are based on coagulation /adsorption of dyes by physical methods or the complete destruction of dye molecules by chemical methods such as electrolysis, ozonation, etc. However, these procedures have inherent drawbacks as they generate a significant amount of sludge or cause secondary pollution due to formation of sludge or hazardous by-products (Verma et al., 2003; Zhang et al., 2004).

Although the decolorization is a challenge for textile industry as well as for waste water treatment systems, the literature suggest that there is a great potential for developing microbiological decolorization systems with total color removal in some cases within few hours (Balan and Monterio, 2000). Development of efficient dye degradation requires a suitable strain and its use under favorable conditions to realize the degradation potential. In recent years there has been an intensive research on fungal decolorization of dye wastewater. It is turning into a promising alternative to replace or supplement present treatment processes. The most studied fungus is the white rot fungus *Phanerochate chrysosporium*, which is able to decolorize various dyes (Swamy et al., 1992). The use of species of the genera *Pleurotus, Bjerkanera, Tremetes, Poyporus and Phelinus* and the species *Iprex Lacteus, Fungalia trogii, Ganoderma* sp. *and Thelephora* sp. have been also investigated (Selvam et al., 2003; Wesemberg et al., 2002; Yesilada et al., 2003; Revankar et al., 2006; Fu and Virarragavan et al., 2002). Knapp et al. (1995) reported that adsorption of dyes to the microbial cell surface is the primary mechanism of decolorization.

The present study aimed at using *Aspergillus* sp for decolourization of Reactive Blue and other synthetic dyes. Various conditions required for decolorized have

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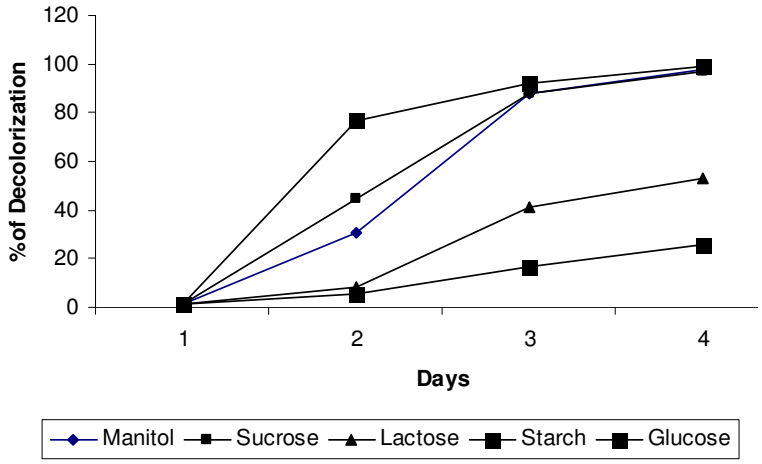


Figure 1. Effect of various carbon sources Reactive Blue (100 mg/l) by *Aspergillus* sp.

been optimized and dye degradation has been analyzed and confirmed by high performance liquid chromatography.

MATERIALS AND METHODS

Dyes and chemicals

Various textile dyes such as, Reactive Black, Reactive Yellow, Reactive Red, Reactive Blue were purchased from Evergreen Industries, Ahmedabad, India. Coloron Violet and Coloron Black were purchased from Coloron India pvt Limited, Mumbai. λ max of textile dyes were determined by using UV-Visible Spectrophotometer (labomed, USA). Dyes were selected based on the basis of their structural diversity and frequency of use in the local textile industries. The chemicals and medium components were purchased from Hi-Media labs, Bombay, India. All chemicals used were of analytical grade.

Screening and selection of decolorizes

Soil sample were collected from a textile industry near Kumbakonam, Tamilnadu, India and it was spread over minimal medium of the following compositions (g/l) $C_6H_{12}O_6$ (1.00), K_2HPO_4 (7.00), KH_2PO_4 (2.00), $Na_3C_6H_5O_7$ (0.50), $MnSO_4$ (0.10), $(NH_4)_2SO_4$ (1.00) with Reactive blue 100 mg/l. The pH of the medium adjusted to 6.0. The fungal colony with highest decolorization zone was selected and stored in potato dextrose agar plate at 15˚C. Disc of 0.7 mm containing mycelium and spores (0.6 mg dry weight) were removed from the borders of colonies, and then added to Erlenmeyer flasks (250 ml) with 100 ml of minimal medium with the dye as the unique source of carbon, in 100 mg/l concentration. They were subsequently grown in the same liquid medium containing the selected dyes for decolorization studies. Filter sterilized dyes were used for decolourization studies and they were added after fungi was inoculated.

Decolourization assay

Decolourization studies were carried out in minimal medium with Reactive Blue (100 mg/l) under orbital shaking condition (150 rpm/min) at room temperature. At intervals of every 24 h, 2 ml of culture media was taken from the flask, centrifuged using refrigerated centrifuge (Eppendorf 5804 R) and was read at 585 nm using UV-VIS-Spectrophotometer (Labomed USA). Medium without dye and inoculum was used as blank. Medium with dye but without inoculum was used as control. All experiments were carried out in triplicates and the mean value was taken. The decolourization efficiency was expressed as per the following equation; Decolourization (%) = [(Initial Absorbance – Final Absorbance) / Initial Absorbance] × 100.

Effects of physico–chemical factors on decolorization

Decolorization ability of *Aspergilus* sp at various pH between 3 to 8 was studied. pH 3 was found to be the optimal pH for the decolorization activity. Based on that acidic pH 3 was selected to study the effects of various physico chemical factors such as different carbon sources (Mannitol, Sucrose, Lactose, Starch, and Glucose), static and non-static conditions, dye concentration (25, 50,100 mg/l) in the medium containing Reactive Blue (100 mg/l). Ability of this isolated fungi to decolorize various dyes (100 mg/l) based on changes in their respective λ max.

HPLC analysis of degraded products

The dye degradation was monitored by HPLC (Shimadzu) as the decolorization continued. Ten milliliters of samples were taken at 0 and 24 h, centrifuged, filtered through 0.4 5µm membrane filters (Millipore). The filterate was extracted three times with methylene chloride and evaporated in rotary vaccum vaporator with 45 – 50^{o}C waterbath, after which the residue was dissolved in 2 ml methanol. Extracted samples were analyzed using the mobile phase consisting of 60% of acetonitryl and 40% water (HPLC grade). The sample was eluted isocratically using a C18 reversed phase column (RPC -18 phenomenex). The flow rate of mobile phase was 0.5 ml/min, and the UV-VIS detector was set at 285 nm.

RESULT AND DISCUSSION

About seven different morphologically distinct fungi were isolated from the soil. Among them one fungal isolate

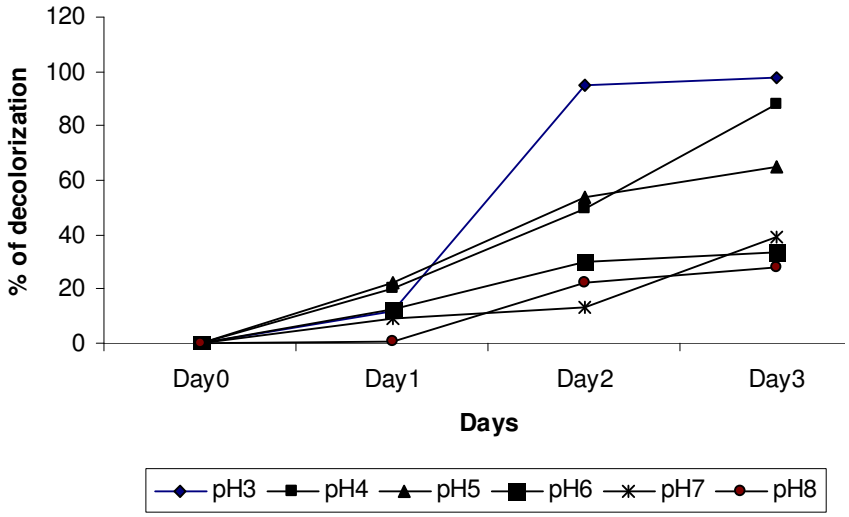


Figure 2. Effect of various pH on decolorization Reactive Blue (100 mg/l) by *Aspergillus* sp.

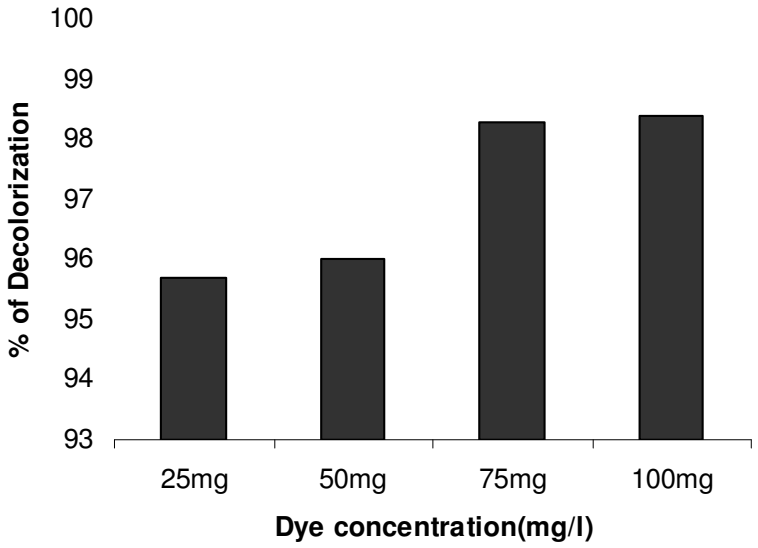


Figure 3. Effect of Reactive Blue dye concentration on decolorization by *Aspergillus* sp.

showed higher decolorization and it was identified as *Aspergillus sp* based on staining using lacto phenol cotton blue and colony morphology and used for our study.

The effects of carbohydrates such as sucrose, mannitol and glucose on the decolorization performance was found best with more than 95% at end of 3rd day of incubation. Figure 1 shows that starch was found to be the strong inhibitor of dye decolorization (26% at the end of 3rd day). When compared to all other carbohydrate sources lactose gave a moderate effect (53%) of decolorization. High percentage of decolorization was due to dye adsorption by mycelium of fungi as well as reduction of dye intensity in solution because of changes caused by them (Balan et al., 2001). No dye decolorization was observed in control flask with out inoculum.

pH variation in comparison had a significant effect on the decolorization of Reactive Blue by *Aspergillus* sp. Figure 2 shows that the highest color removal was detected in acidic pH 3 (98%) when compared to neutral and basic pH. At the pH of 8, the decolorization was strongly inhibited. These results provides an information that acidic pH is required for growth and decolorization. UV visible spectra did not show any change at this pH range (data not shown) in the control sample.

Generally the concentration of dye compounds found in the effluent or rivers ranged as low as 12 to 16 mg/l. As shown in Figure 3, the decolorization efficiency was found to be more than 95% when the dye concentration used was 100 mg/l. In addition the growth of fungi was strongly inhibited at dye concentration above 200 mg/l

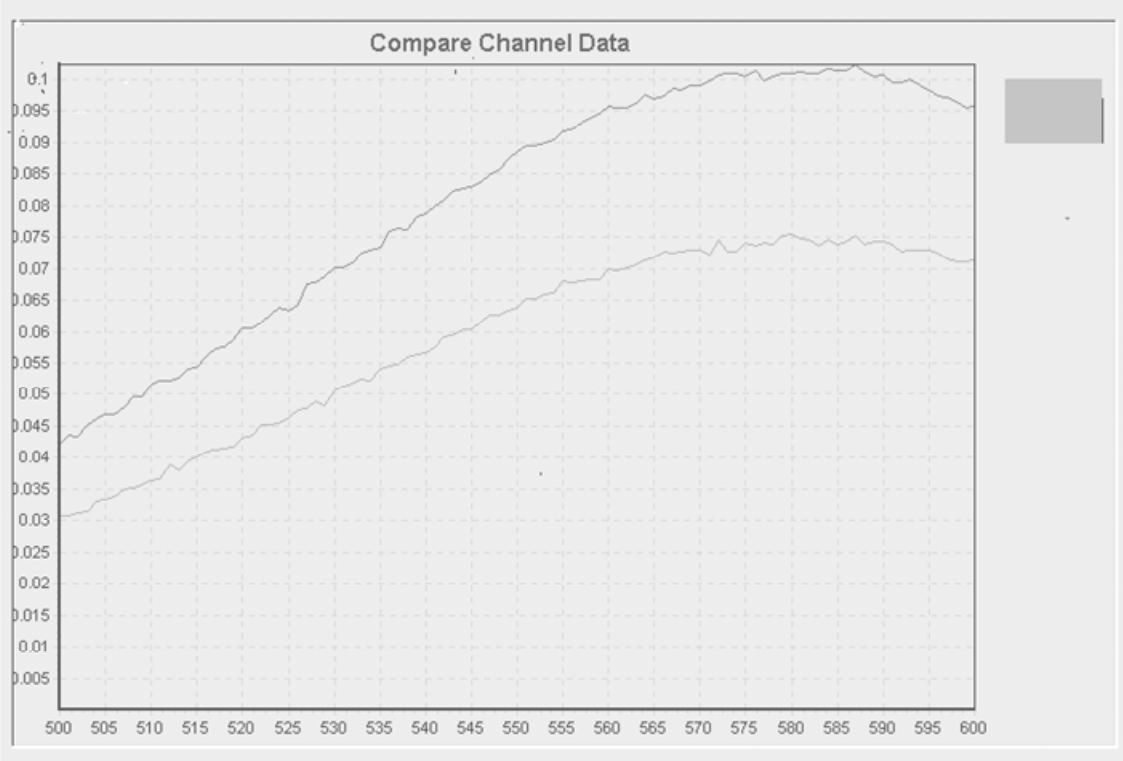


Figure 4. Spectral changes (500 – 600 nm) during decolorization Reactive Blue by *Aspergillus* sp.

Table 1. Decolorization of various dyes by *Aspergillus* sp. At pH 4.5, temperature of30°C and incubation duration of 24 h.

Dye used	λ max	% of Decolorization
Reactive blue	585	99
Reactive Black	594	75
Reactive yellow	425	70
Reactive Red	528	33
Coloron Violet	567	66
Coloron Black	594	9

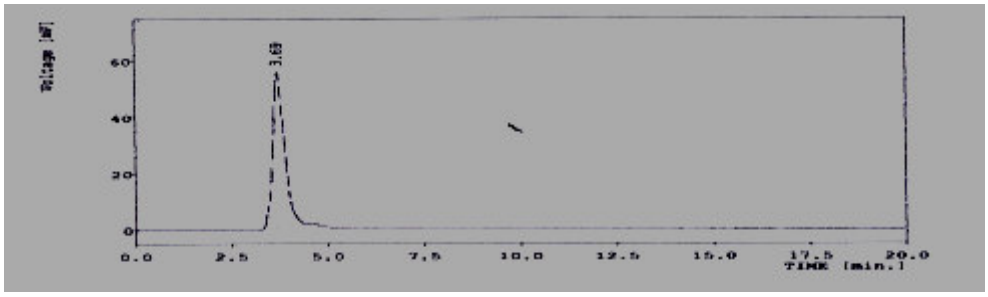
(data not shown). Higher concentration dye may be toxic to metabolic activities.

Dyes of different structures are often used in the textile processing industry, and therefore, the effluents from the industry are markedly variable in composition A nonspecific biological process may be vital for treatment of textile effluents. As shown in Table 1, the fungal isolate decolorizes all the dyes such as Reactive Black (62.6%), Reactive Yellow (74.8%), Reactive Red (69.8%) and Coloron Violet (66.8%) moderately at the end of 24 h. This organism shows very low decolorization with Coloron Black (8.7%). This may be due to complexity in chemical structures. A slower rate of decolorization was attributed to higher molecular weight, structural complexicity and the presence of inhibitory groups like $–NO_2$ and $–SO_3Na$ in the dyes (Hu and Wu, 2001).

The supernatant sample obtained at 0 and 24 h were subjected to spectral scan between 400-800 nm. A spectrophotometric scan of the dye Reactive Blue showed a narrow peak between 500 to 600 nm with a slight maximum at 585 nm (Figure 4). After decoloration, the absorbance at wavelength 585 nm decreased with the largest decrease at the peak. A general decrease of this kind is usually attributed to dye degradation rather than adsorbtion (Wesenberg et al., 2002). These data show that decolorization was caused by degradation of dye. As reported by Glenn and Gold (1983) decolorization of several polymeric dyes might be due to the process of secondary metabolism and the degradation system of lignin.

The *Aspergilus* sp cells were mixed with 200 mg/l of Reactive Blue (200 mg/l) in minimal broth for decolorization. The HPLC analysis (Figure 5) for the sample taken at the beginning of static incubation shows that a major peak appears at a retention time of 3.69 min, which represents the retention time of pure Reactive Blue. The corresponding peaks were absent in both, in control as well as in the treated sample. In the treated sample two new peaks were emerged with the retention time of 4.32 and 4.61min, which clearly indicate that dye, has degraded. The present study confirms the ability of *Aspergilus* sp. to decolorize and degrade Reactive blue and other

Standard (100 mg/ml)



Treated sample (100 mg/ml)

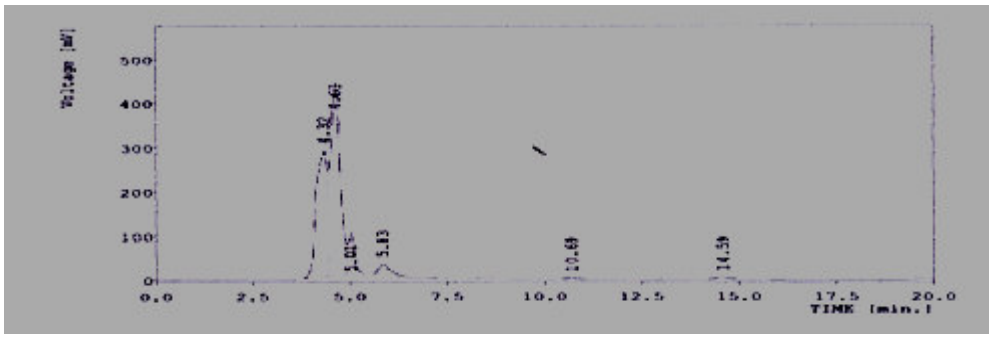


Figure 5. HPLC analysis of Reactive Blue and its degradation products by *Aspergillu*s sp.

structurally different dyes. Further studies are needed to identify the biochemical machinery of decolorization.

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