

## Studies on the Biodegradation of Azo Dyes by White rot Fungi *Daedalea Flavida* in the Absence of External Carbon Source

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**Abstract**—Dye degradations by white rot fungi have been studied in the presence of an easily metabolizable external carbon source that is unlikely to be present in waste water. For wastewater treatment, it is important to study the potential of white rot fungi to decolorize the azo dyes in the absence of external carbon source. The potential of *D. flavida* to decolorize the azo dyes in the absence of external carbon source has been studied. Decolorization of Amaranth, Metanil yellow, Trypan blue and Chlorazole black was studied with *D. flavida*. Decolorization was studied in 250 ml Erlenmeyer flasks at 36°C, stationary, 50 ml N-limited medium with dye 50 mg/l in the absence and presence of glucose. Decolorization and enzyme activity were measured. It was observed that decolorization for Amaranth was 99% after five days, Metanil yellow 82.5% after 10 days, Trypan blue 99% and Chlorazole black E 72% after 10 days of treatment both in the absence and presence of glucose. UV – VIS spectra of dyes before and after the treatment of dyes with *D. flavida* showed a decrease in the absorbance at their maximum absorption wavelength in visible region and a shift towards shorter wavelength indicating the degradation of dyes. It showed that *D. flavida* could be used for the treatment of azo dyes in waste water without an external carbon source for reduction of cost.

**Keywords**—azo dyes; carbon source; decolorization; *D. flavida*; white rot fungi

### I. INTRODUCTION

Synthetic dyes are produced annually in large quantities. The textile industry is a major consumer of these dyes and accounts for two-thirds of the total dyestuff market. Azo dye is the largest class of dyes with 60-70% of total dyes. During dyeing processes, entire dye is not fixed to the fiber and a certain amount of the dye remains in dye bath is released with effluents. Azo dyes are potential health hazards as they may be converted to toxic and/or carcinogenic products under anaerobic conditions [1]. Biological methods are considered environmental friendly for the treatment of waste water. White rot fungi have attracted a lot of attention due to their ability to attack a wide variety of recalcitrant compounds including dyes [2, 3, 4]. *Phanerochaete chrysosporium* and *Coriolus versicolor* / *Trametes versicolor* are the most studied species [4]. *Trametes hirsuta* [5], *Bjerkandera adusta* [6], *Irpex lacteus* [7], *Dichomitus squalens* [8], *Funalia trogii* [9], *Pycnoporus sanguineus* [10], *Phlebia radiata* [11], *Daedalea flavida* [12] and *Flavodon*

*flavus* [13] have also been studied for their ability to decolorize various dyes.

Dye decolorization/degradations by above-mentioned strains have been studied in the presence of an easily metabolizable external carbon source such as glucose etc. that is unlikely to be present in wastewater. Textile industry generates a large volume of wastewater. From the point of view of wastewater treatment, it becomes important to study the potential of white rot fungi to decolorize the azo dyes in the absence of glucose. Dye decolorization ability of white rot fungi is often associated with the ligninolytic enzymes produced by them and production of ligninolytic enzymes depends upon nutrient limiting conditions [14]. *T. versicolor* has been reported to exhibit the decrease in the laccase activity with increased glucose concentration and a maximum activity in the carbon limited conditions (in the absence of glucose) [15]. So, there is possibility that some white rot fungi are able to produce ligninolytic enzymes in the absence of glucose and would be able to decolorize the dyes in the absence of glucose. Earlier, we observed that *D. flavida* was able to decolorize the azo dyes in the absence of external carbon source glucose on agar plates. The objective of the present work was to study the potential of *D. flavida* to decolorize the azo dyes in the absence of glucose in suspension culture to test its applicability in aquatic environment.

### II. MATERIALS AND METHODS

#### A. Microorganism

*Daedalea flavida* (MTCC 145) was obtained from the Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India and was maintained in the medium (g/l): glucose 10, yeast extract 5, agar 15 and pH 5.8. Culture was stored at 4°C and sub cultured every four weeks.

#### B. Chemicals

The Amaranth ( $\lambda_{\max}$  520 nm), Metanil yellow ( $\lambda_{\max}$  430 nm), Trypan blue ( $\lambda_{\max}$  599 nm) and Chlorazole black E ( $\lambda_{\max}$  487 nm) were purchased from Hi-Media Chemicals, Mumbai, India. ABTS (2, 2'-azinobis (3 ethylbenzthiazoline-6-sulphonate)) was purchased from M P Biomedicals USA. All other reagent grade chemicals were purchased from Hi-Media Chemicals, Mumbai, India.

### C. Decolorization study

Decolorization of dyes was studied in 250 ml Erlenmeyer flasks containing 50 ml N-limited medium [16] in the absence of glucose. Decolorization studies were also carried out in the presence of glucose for comparison. The medium had the following composition (g/l): ammonium tartrate, 0.22;  $\text{KH}_2\text{PO}_4$ , 0.2;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.05;  $\text{CaCl}_2$ , 0.01; thiamine-HCl, 0.001, trace element solution, 10 ml/l. Trace element solution contained (g/l):  $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.08;  $\text{H}_2\text{MoO}_4$ , 0.05;  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 0.07;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.043 and  $\text{Fe}_2(\text{SO}_4)_3$ , 0.05. Dye was added at a final concentration of 50 mg/l. The pH was adjusted at 5.5 by using sodium acetate (20 mM) buffer. The inoculum was prepared by transferring the fungal cultures from 10 days old slants to the flasks containing 100 ml medium. The flasks were incubated under stationary condition at 28°C in complete darkness. After the six days of incubation, the culture broths were homogenized. 10% homogenate (5 ml homogenate equivalent to 0.0081 g dry biomass) was employed to inoculate the experimental flasks. Uninoculated controls were maintained in parallel. The experimental flasks were incubated under stationary condition at 36°C in complete darkness.

Samples were withdrawn and centrifuged at 10,000 rpm for 5 min to remove fungal mycelia. Supernatants were diluted 10 times and absorbance of supernatants was measured spectrophotometrically at maximum absorption wavelength in visible region for respective dye. The decolorization % was calculated from absorbance values as follows:

$$\text{Decolorization \%} = 100 * (A_0 - A) / A_0,$$

where,  $A_0$  = Initial absorbance, A = Absorbance at time t

### D. Enzyme assay

Laccase activity was assayed by oxidation of ABTS (2, 2'-azinobis (3 ethylbenzthiazoline-6- sulphonate)) to ABTS radicals ( $\epsilon_{420} = 36,000 \text{ M}^{-1} \text{ cm}^{-1}$ ). The assay mixture contained 1.0 ml of supernatant, 2 mM ABTS and 0.1 M sodium acetate buffer (pH 4.5) in 2 ml of reaction mixture [17]. One unit (U) of laccase activity was defined as 1.0  $\mu\text{mol}$  of product formed per minute.

## III. RESULTS AND DISCUSSION

The potential of *D. flavida* to decolorize Amaranth, Metanil yellow, Trypan blue and Chlorazole black E in the absence of external carbon source was studied. Decolorization studies were also carried out in the presence

of glucose for comparison and to observe the difference, if

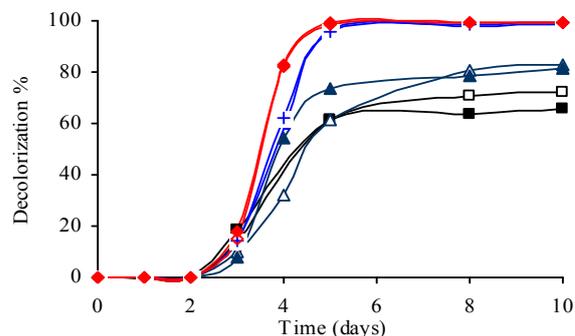


Figure 1. Decolorization of different dyes: Decolorization of Amaranth in the absence ( $\circ$ ) and presence ( $\bullet$ ) of glucose; decolorization of Metanil yellow in the absence ( $\Delta$ ) and presence ( $\blacktriangle$ ) of glucose; decolorization of Trypan blue in the absence ( $-$ ) and presence ( $+$ ) of glucose; decolorization of Chlorazole black E in the absence ( $\square$ ) and presence ( $\blacksquare$ ) of glucose.

any, on the extent of dye decolorization. Fig. 1 showed the results of decolorization in the absence and presence of glucose. Only 15-18% decolorization of Amaranth was observed after three days, which reached upto 99% after five days both in the absence and presence of glucose.

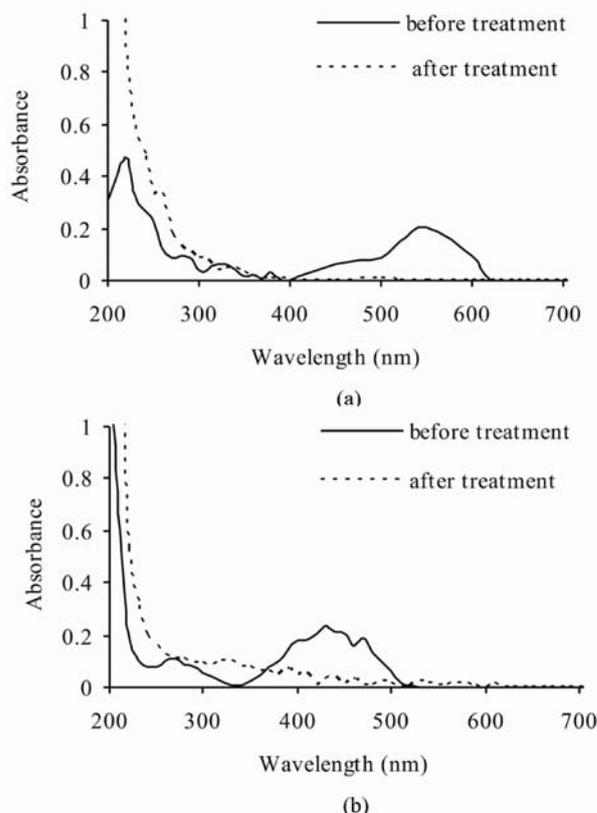


Figure 2. UV-VIS spectra of dyes before and after the treatment: (a) Amaranth, (b) Metanil yellow

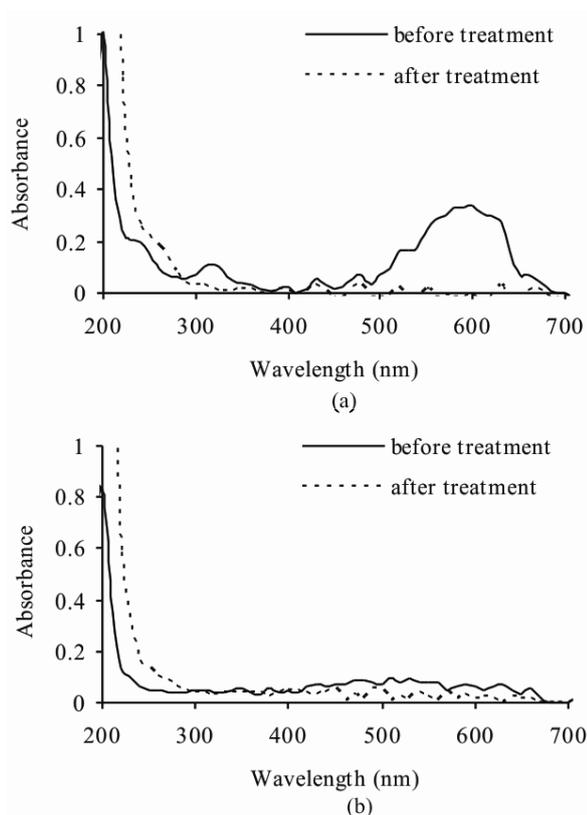


Figure 3. UV-VIS spectra of dyes before and after the treatment: (a) Trypan blue, (b) Chlorazole black E

Decolorization was 61% for Metanil yellow after five days and reached upto 82.5 after 10 days in the absence of glucose. In the presence of glucose, 73% decolorization of Metanil yellow was observed after five days which reached upto 81.5 after 10 days. Decolorization was 96 and 61% for Trypan blue and Chlorazole black E respectively after five days which slightly increased for Trypan blue (99%) and Chlorazole black E (72%) after 10 days both in the absence and presence of glucose. UV-VIS spectra of dyes before and after the treatment with *D. flavida* showed a decrease in absorbance at their maximum absorption wavelength in visible region and a shift towards shorter wavelength indicating the degradation of dyes (Fig. 2 and 3).

Recently, Anastasi et al., 2010 reported the decolorization of dye baths in liquid system in the absence of any additional nutritive source [18]. However, they added the dyes to the biomass already generated in the presence of 1% glucose and other nutrients. Other studies have observed the effect of carbon source on dye decolorization in order to optimize the yields of decolorization [19, 20]. Amaral et al., 2004 reported that decolorization is more effective in the presence of glucose; reaching 97% for 50 mg/l of dye [21]. Table I summarizes the results of these studies. However, the present study is the first one having the objective to investigate the potential of a white rot fungus to decolorize azo dyes in the absence of an external carbon source.

Table II shows that laccase production was more in the presence of glucose but the decolorization efficiency was not higher in the presence of glucose.

TABLE I. COMPARISON WITH SOME RELEVANT STUDIES

| Authors                | Fungus strain                      | Dye   | Study   | Remark/ Finding   |
|------------------------|------------------------------------|---|---|---|
| This study             | <i>D. flavida</i>                  | Amaranth<br>Metanil yellow<br>Trypan blue<br>Chlorazole black E | Decolorization study in the absence and presence of glucose   | 99% decolorization of Amaranth both in the absence and presence of glucose; 61 and 73% decolorization of Metanil yellow in the absence and presence of glucose respectively; 96 and 61% for Trypan blue and Chlorazole black E respectively both in the absence and presence of glucose after 5 days. 82, 99 and 72% decolorization of Metanil yellow, Trypan blue and Chlorazole black E respectively both in the absence and presence of glucose after 10 days. |
| Anastasi et al. (2010) | <i>Bjerkandera adusta</i> 2295     | Acid, direct and reactive dye bath                              | Biomass generated in the presence of 1% glucose and other nutrients was added to dye bath in the absence of glucose | 90% decolorization of acid dye bath in 3 days, 16% decolorization of reactive dye bath in 1 days, 57% decolorization of direct dye bath in 5 days.  |
| Faraco et al. (2009)   | <i>Pleurotus ostreatus</i>         | Acid, direct and reactive dye bath                              | Decolorization in the presence and absence of malt extract  | 40% decolorization of acid dye bath in the absence of malt extract in 1 day; 60 and 66% decolorization of acid dye bath in the presence of malt extract in 7 and 14 days respectively, Other dyes were not decolorized.   |
| Pant et al. (2008)     | <i>Phanerochaete chrysosporium</i> | Congo red<br>Malachite green<br>Crystal violet                  | Effect of the presence and absence of glucose   | A readily available carbon source was imperative to enhance the bioremediation and it was 76.1, 78.9 and 71.5% for Congo red, Malachite green and Crystal violet respectively in the presence of glucose and urea in 15 days.   |
| Amaral et al. (2004)   | <i>Trametes versicolor</i>         | Synthetic waste water   | Effect of the presence and absence of glucose   | 65% decolorization in the absence of glucose and 97% in the presence of glucose in 13 days.   |

TABLE II. MAXIMUM LACCASE DURING THE DECOLORIZATION OF DYES

| Dye                | Maximum Laccase activity (U/l) |                            |
|--------------------|--------------------------------|----------------------------|
|                    | In the absence of glucose      | In the presence of glucose |
| Amaranth           | 8.5                            | 18.5                       |
| Metanil yellow     | 12.7                           | 20                         |
| Trypan blue        | 18.7                           | 20.5                       |
| Chlorazole black E | 16                             | 19                         |

Metanil yellow was decolorized upto 82% both in the absence and presence of glucose although more laccase was produced during its decolorization in the presence of glucose. It has been reported that the different isoforms of enzyme were produced in different culture conditions and these isoforms had different affinity for dyes and reaction substrate during measurement [3]. This could be the reason behind the apparent lack of correlation between dye decolorization and laccase activity observed in the present study.

#### IV. CONCLUSION

*D. flavida* was able to decolorize Amaranth, Metanil yellow, Trypan blue and Chlorazole black E upto same extent both in the absence and presence of glucose. Dye decolorization ability of *D. flavida* indicated that this strain could be used for the treatment of azo dyes in textile wastewater without using an external carbon source in order to reduce the cost of treatment.

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