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## STUDIES ON FUNGAL DECOLOURIZATION OF SYNTHETIC DYES

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### ABSTRACT

Decolourization of synthetic dyes like direct greenish blue, direct brilliant violet using *Aspergillus niger* and *Phanerochaete Chrysosporium* was carried out at a dye concentration of about 10mg/l. Asthana and Hawker's broth was used for decolourization study 10mg/l of two dyes were added and mixed well. After that, the flasks were inoculated with pre-grown fungal mycelial cultures. During the incubation, samples were drawn at 1, 3, 7 and 14 days and analyzed for decolourization, after centrifuging the samples at 8000-9000rpm for 15 minutes. The Supernatant was collected and absorbance was measured using Spectronic 20 at 490nm. The maximum decolourization of direct brilliant violet of about 65%,60% were achieved by *Aspergillus niger* and *Phanerochaete Chrysosporium* respectively during 14th day of incubation and the maximum decolourization of direct greenish blue about 76%,70% were achieved by *Aspergillus niger* and *Phanerochaete Chrysosporium* respectively during 14th day of incubation. The maximum percentage of degradation of direct brilliant violet was 65% and direct greenish blue was 76% obtained from *Aspergillus niger*. In order to treat the dyes more effectively, microorganism capable of degrading the toxic compound present in the textile dyes will be used on a large scale and will be introduced into effluent treatment plants for Bioremediation.

**Keywords:** Fungal isolates, Synthetic dyes, Decolourization, *Aspergillus niger* and *Phanerochaete Chrysosporium*.

### INTRODUCTION

Azo dyes are the largest group of dyes used in industry (Ramalho, et al., 2002; Mane, et al., 2008) representing more than half of the annual production (Stolz, 2001). Technological advance has seen an increase in diversity and complexity of synthesized textile dyes with the objective of product improvement through enhancement of dye properties such as resistance to fading, improved delivery of dyes to fabrics and increased variety of shades. This increase in diversity and complexity of dyes is coupled with higher resistance to

environmental degradation leading to pollution problems by textile effluent. A larger proportion of these azo dyes which can pass through normal water treatment procedures (Stolz, 2001; Pearce, et al., 2003; Pandey, et al., 2007; Chamunorwa Aloius Togo, 2008) resulting in aesthetically unappealing water.

Due to the fungal oxidative mechanisms, it is possible to avoid the formation of hazardous anilines, formed by reductive cleavage of the azo dyes. Compared to other fungal oxidative enzymes, laccases can act oxidatively, non-

specifically at the aromatic rings and has the potential to degrade a wide range of compounds. Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are multicopper containing enzymes that catalyze the oxidation of a variety of phenolic and inorganic compounds, with the concomitant reduction of oxygen to water. Due to their wide substrate specificity, laccases have gained much attention over the last number of years in many industrial and environmental fields (Fernandes et al., 2008; Moldes et al., 2008; Sadhasivam et al., 2009). Enzymatic processes have various advantages over conventional, biological, physical, and chemical treatment processes including selective removal of particular pollutants, application to xenobiotic recalcitrant compounds, high reaction rate and reduction in sludge volume (Sadhasivam, 2007). In recent years, dye decolorization by laccases has received a significant attraction by various researchers (Domi'nguez et al., 2005; Moldes and Sanroman, 2006).

Adequate treatment of textile effluent requires more than one stage as there is need for both colour removal and degradation of aromatic compounds from the decolourization process. Physico-chemical treatment methods are the least desirable owing to their high costs and generation of secondary pollutants. On the other hand, biological treatment methods are attractive due to their cost effectiveness, diverse metabolic pathways and versatility of microorganisms (Singh, et al., 2004; Van de Zee and Villaverde, 2005).

## **MATERIALS AND METHODS**

### **Dyes and soil sample collection**

Dyes used in the experiments were purchased from Infra-Tex dye industry, Perunthurai, in Tirupur and were of the highest purity. The soil sample was collected from dye contaminated area nearby textile industry situated in Perunthurai (Erode District), Tamilnadu, India.

### **Decolourization media**

Asthana Hawker's broth (Laxminarayana, et al., 2010) was composed of (5 gm glucose, 3.5 g KNO<sub>3</sub> 1.75 g KH<sub>2</sub>PO<sub>4</sub>, 0.75 g MgSO<sub>4</sub> 7H<sub>2</sub>O and 1 L distilled water) was prepared and added with 10mg dye the medium was sterilized at 121°C for 20 minutes and used for the cultivation of mycelia and for decolorization experiments.

### **Isolation techniques**

The soil sample was serially diluted in sterile physiological saline and was transformed to sabouraud's dextrose agar. The plates were incubated at room temperature for 3 days. After incubation the plates were noted for the presence of fungal colonies (Cappucino Sherman, 2007).

### **Identification of fungal isolates- lacto phenol cotton blue staining- tease mount preparation**

A drop of lacto phenol cotton blue was placed on a clean glass microscopic slide. With a straight wire slightly bended at the tip, a small portion of the colony was removed and placed it in the drop of LPCB. With the help of another straight wire (or) needle the fungal culture tint was teased into small bits and spread in LPCB. Cover slip was placed and pressure was gently applied over the agar bits to spread evenly. After giving sufficient time for the structure to take up the stain, the slide was examined using microscope under 10x, 45x objectives to examine the fungal morphology. Based on morphological and cultural characteristics, the isolated fungal cultures were identified as *Aspergillus niger*, *Phanerochaete chrysosporium* (Cappucino Sherman, 2007).

### **Decolourization**

Asthana and Hawker's broth was used for decolourization study 20ml broth was taken in 100ml Erlenmeyer flask and sterilized. Then 10mg/l (Dilek Asma, et al., 2006) of direct brilliant violet and direct greenish blue were added and mixed well. After that, the flasks were inoculated with pre-grown fungal mycelial cultures.

During the incubation, samples were drawn at 1, 3, 7 and 14 days and analyzed for decolourization, after centrifuging the samples at 8000-9000rpm for 15 minutes. The supernatant was collected and absorbance was measured using spectronic 20 at

490nm. The percentage of decolourization was calculated using the formula given below. Decolourizing activity was expressed in terms of percent decolourization (Yatome et al., 1993).

$$\text{Percentage of decolourization (D)} = \frac{\text{Initial Absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

### Scanning of dyes for its absorbance maximum

The maximum absorbance of the dyes was scanned using spectronic 20 from 490nm. The dyes direct brilliant violet and direct greenish blue, gave a maximum absorbance at 490nm (Soeprijanto et al., 2002) respectively and the samples were analyzed at their respective absorbance throughout the study.

### Biomass Estimation

The fungal biomass (pellets) produced was filtered out from the liquid medium by using Whatman filter paper No. 1. It was washed twice with deionized water before application in the experiments. The fungal biomass dried at 110°C for 5 hrs in hot air oven and the dry weight was measured (Ashu – Augustine, et al., 2006).

p<sup>H</sup> of the medium

p<sup>H</sup> of the supernatant was measured using the p<sup>H</sup> meter during initial stage and 1, 3, 7 and 14 days of incubation in the case of commercial dyes.

## RESULTS

The decolourization studies carried out with fungal isolates from the dye effluent. Based on morphological and cultural characteristics, the isolated fungal cultures were identified as *Aspergillus niger*, *Phanerochaete chrysosporium* (table-1). The results shows that *Aspergillus niger* performed best when compared to *Phanerochaete chrysosporium* (tables 2, 3 and figures 1, 2).

The synthetic dyes removal was tested using two approaches. The first one was based on the biomass formation the second one was based on p<sup>H</sup>. Samples were withdrawn at different intervals

after dye amendment until 14<sup>th</sup> day of incubation to determine the dye bio removal and growth media p<sup>H</sup> changes. The results showed that *Aspergillus niger* strain gave good efficiency in the removal of direct brilliant violet in 14<sup>th</sup> day of incubation, where the decolourization reached to 76% (figure). The same fungal strain gave 65% percentage of decolourization in direct greenish blue at 14<sup>th</sup> day of incubation. Another fungal isolate *Phanerochaete chrysosporium* gave 70% in direct greenish blue than the direct brilliant violet (60%), on 14<sup>th</sup> day of incubation. In, the second approach the dye removal in the p<sup>H</sup> 3.0 – 6.0 showed biomass production of maximum 0.9g / 20ml by *Aspergillus niger* at p<sup>H</sup> 3.0, and 0.8 g / 20 ml by *Phanerochaete chrysosporium* at p<sup>H</sup> 5.0. Fungal growth did not occur at p<sup>H</sup> 2 and 9.

## DISCUSSION

Direct greenish blue resulted with maximum decolourization of about 76% in *Aspergillus niger* 70% in *Phaenerochaete chrysosporium*. As the decolourization was proceeded, the concentration of the dye decreased in terms of its absorbance value at the initial stage, dye decolourization was probably due to the absorption of dye to the mycelium (Cripps, et al., 1990). Likewise, few other studies have also clearly mentioned biosorption/ bioadsorption of certain brown rot Fungi *Aspergillus niger*, *Aspergillus foetidus* (Ali, et al., 2007). The primary dyes removal phenomenon coupled with electrostatic pull between the positively charged cell wall and negatively charge dyes (Aksu et al., 1999; Aksu

and Tezer, 2000). In this work, the use of *Aspergillus niger* to remove direct dyes commonly used in textile industry revealed that this fungal culture was capable to remove dyes in short time from the media. This interaction could be based on a biosorption of dyes on the intact fungal biomass; this is in harmony with Juliana and Thuy, 2002.

Brilliant green is much more decolorized by *Phanerochaete chrysosporium* than the structurally similar crystal violet (Paszczyński, A and Crawford, R.L, 1991). Presumably, these differences are due partly to electron distribution and charge density, although steric factors may also contribute. There appears to have been only one systematic study relating dye structure to degradation by a white rot fungus (*Phanerochaete chrysosporium*). Pasti-Grigsby, et al (1992) showed that the nature and position of substituents on one of the aromatic rings of azo dyes can markedly influence decolorization, although a simple, clear pattern was not established. In the present study *Phanerochaete chrysosporium* showed lesser activity when compared *Aspergillus niger*. Among the two fungal strains tested we found strain, *Aspergillus niger* which had better potential for the dye decolorization than strain *Phanerochaete chrysosporium*.

White-rot fungi were the fastest decolorizers, but additionally we found very effective decolorizers among brown-rot fungi. The decrease of  $p^H$  at the end of these experiments may be referred to the excretion of the organic acid by the fungus itself (Abdel – Aal et al., 2001 and Naima et al., 2007). In the present study we also found that the decrease in  $p^H$  at the end of experiment. Considering the above result we assumed that dye was probably associated with fungal growth and hyphal uptake mechanism (biosorption / bioadsorption). Textile industries discharge colored dyes and toxic compound in to the environment. Physicochemical methods that are have operation problem and do not provide satisfactory results. But biological treatment

methods are cheap and offer the best alternative with proper analysis and environmental control.

In order to treat the dyes more effectively, Fungal isolates capable of specifically degrading the toxic compound present in the textile dyes are cultured on a large scale and introduced into effluent treatment plants.

## CONCLUSION

The present study reveals that the fungal culture can be used successfully for decolorizing direct brilliant violet and direct greenish blue. 80% of the decolorization of the above dyes were achieved by *Aspergillus niger*. On the basis of the result of the present study suitable strategy can be developed for the treatment of waste water contaminated with dye. These biological methods can be promoted to degrade the variety of dyes from the textile industries.

The treated textile dyes when disposed to the land it has several applications.

\*improves soil fertility.

\*very little quantity of bioearth, compost is sufficient for crops and thus input and transport cost could be reduced.

\*Humus rich, very slow release of nutrient which is essential for growth.

\*Increases water holding capacity of the soil.

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**Table: 1 Morphology of fungal isolates**

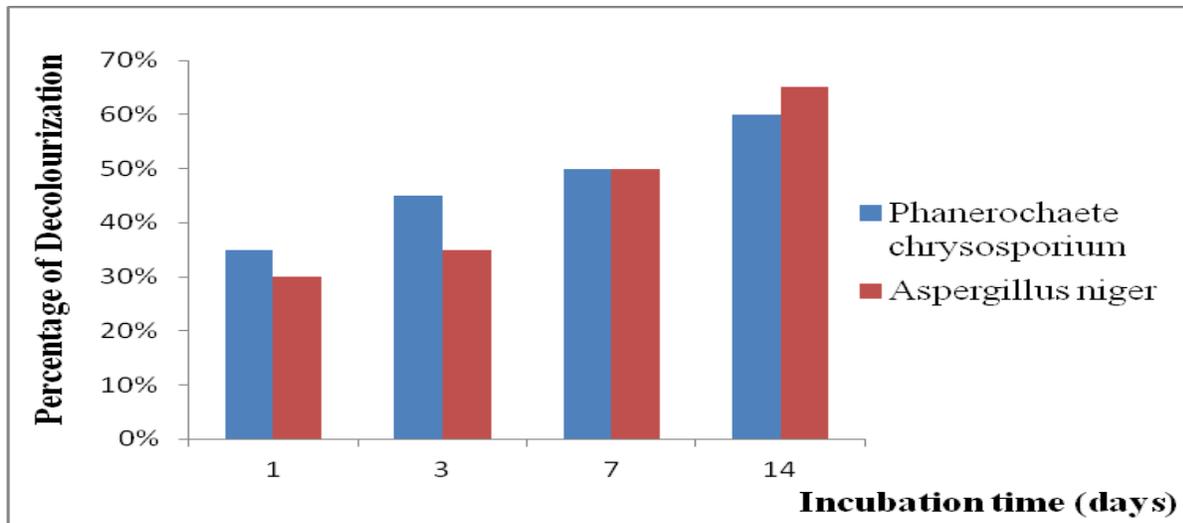
| Fungal isolates                    | Colonial morphology                                    | Microscopic appearance   |
|------------------------------------|--|--|
| <i>Aspergillus niger</i>           | White colonies become greenish- blue, black, or brown. | Single - celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle; long conidiophores arise from a septate mycelium. |
| <i>Phanerochaete chrysosporium</i> | White colour puffy colonies.                           | Multicelled conidia.   |

**Table: 2 Growth of Phanerochaete chrysosporium and decolourization**

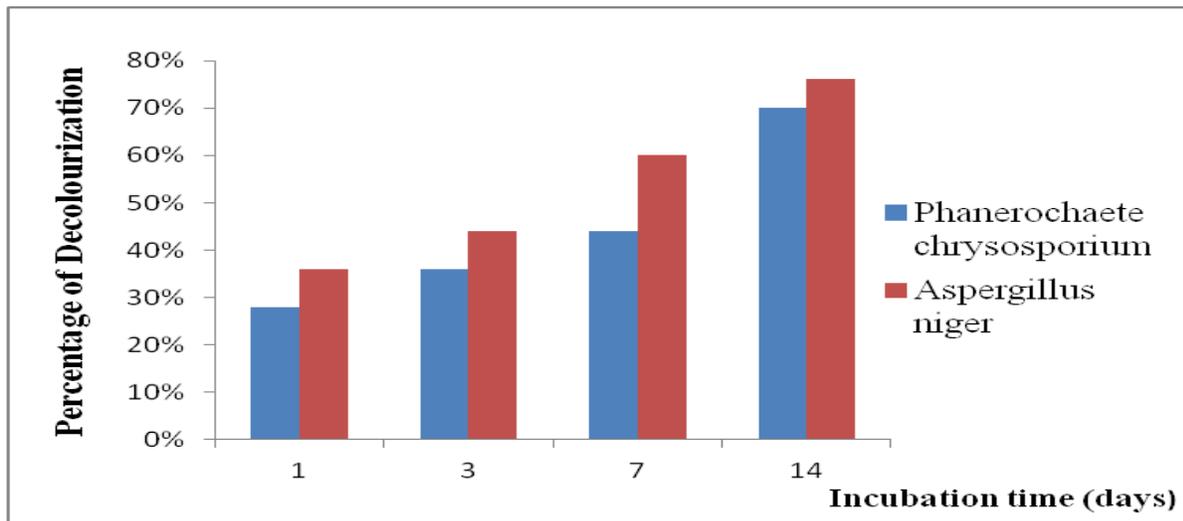
| Name of the dyes        | Incubation Time (days) | p <sup>H</sup> of the medium |       | Biomass g/20ml | Absorbance value OD at 355nm |       | Decolourization (%) |
|-------------------------|------------------------|------------------------------|-------|----------------|------------------------------|-------|---------------------|
|                         |                        | Initial                      | Final |                | Initial                      | Final |                     |
| Direct brilliant violet | 1                      | 6.01                         | 5.5   | 0.2            | 0.2                          | 0.13  | 35%                 |
|                         | 3                      | 6.01                         | 4.8   | 0.4            | 0.2                          | 0.11  | 45%                 |
|                         | 7                      | 6.01                         | 4.6   | 0.6            | 0.2                          | 0.10  | 50%                 |
|                         | 14                     | 6.01                         | 4.4   | 0.7            | 0.2                          | 0.08  | 60%                 |
| Direct greenish blue    | 1                      | 6.01                         | 5.57  | 0.3            | 0.25                         | 0.18  | 28%                 |
|                         | 3                      | 6.01                         | 5.2   | 0.4            | 0.25                         | 0.16  | 36%                 |
|                         | 7                      | 6.01                         | 5.15  | 0.5            | 0.25                         | 0.14  | 44%                 |
|                         | 14                     | 6.01                         | 5.00  | 0.8            | 0.25                         | 0.07  | 70%                 |

**Table: 3 Growth of Aspergillus niger and decolourization**

| Name of the dyes        | Incubation Time (days) | p <sup>H</sup> of the medium |       | Biomass g/20ml | Absorbance value OD at 355nm |       | Decolourization (%) |
|-------------------------|------------------------|------------------------------|-------|----------------|------------------------------|-------|---------------------|
|                         |                        | Initial                      | Final |                | Initial                      | Final |                     |
| Direct brilliant violet | 1                      | 6.03                         | 4.0   | 0.3            | 0.2                          | 0.14  | 30%                 |
|                         | 3                      | 6.03                         | 3.8   | 0.4            | 0.2                          | 0.13  | 35%                 |
|                         | 7                      | 6.03                         | 3.5   | 0.45           | 0.2                          | 0.10  | 50%                 |
|                         | 14                     | 6.03                         | 3.2   | 0.53           | 0.2                          | 0.07  | 65%                 |
| Direct greenish blue    | 1                      | 6.0                          | 4.57  | 0.3            | 0.25                         | 0.16  | 36%                 |
|                         | 3                      | 6.0                          | 4.20  | 0.4            | 0.25                         | 0.14  | 44%                 |
|                         | 7                      | 6.0                          | 3.25  | 0.5            | 0.25                         | 0.10  | 60%                 |
|                         | 14                     | 6.0                          | 3.21  | 0.9            | 0.25                         | 0.06  | 76%                 |



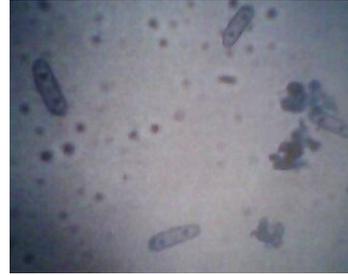
**Figure 1 Percentage of fungal decolourization in direct brilliant violet**



**Figure 2 Percentage of fungal decolourization in direct greenish blue**



**Figure 1. Cultural morphology of *Aspergillus niger*, *Phanerochaete chrysosporium***



**Figure 2. Microscopic observation of *Aspergillus niger*, *Phanerochaete chrysosporium***



**Figure 3. Decolourization of direct greenish blue by *Aspergillus niger*, *Phanerochaete chrysosporium*.**