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EFFECTIVITY OF THE DECOLORIZATION OF CRYSTAL VIOLET BY VARIOUS FUNGI, WITH SPECIAL REFERENCE TO THE WHITE ROT FUNGUS, *Trametes hirsuta*

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ABSTRACT:
Decolorizations of the dye, Crystal violet was investigated using the white rot fungus, Trametes hirsuta and other fungi like Aspergillus flavus, Aspergillus fumigatus, Penicillium sp and Fusarium oxysporium. Among the fungi used, Trametes hirsuta gave maximum decolorization for the dye, and among the other fungi Aspergillus niger and Aspergillus fumigatus gave maximum decolorization than the other fungi. The medium and growth conditions were optimized for the effective decolorization of the fungus, Trametes hirsuta. Decolorization of Crystal violet was optimum when maltose (89.3%) was used as the carbon source, yeast extract (81.6%) as the nitrogen source, at 30°C (88.5%) and at pH 4 (80.1%). The immobilized cells of Trametes hirsuta also showed decolorization for the dye, Crystal violet.

Key words: *Fungi, Decolorization, Crystal violet, Immobilized cells.*

INTRODUCTION

Industrialization is vital to a nation's economy because it serves as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment. Many of these products are problematic because of persistence (low biodegradability) and/ or toxicity. The textile industries produce effluents that contain several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes (Cooper, 1995). Dye wastes and other effluents from textile processing can cause problems like foaming, color persistence, have abnormally high pH, temperature and heavy metal concentrations and variations in the hydraulic flow rates (Buckley, 1992). This alters the biochemical oxygen demand (BOD) and gives rivers intense colorations (Ajayi and Osibanjo, 1980). The use of these water resources is limited and ecosystem is affected.

Dyes are stable to light and oxidizing agents and are sometimes resistant to biooxidation and they contain chromophores. Many chemical dyes have been used increasingly in the textile and dyeing industries because of their ease and cost effectiveness in synthesis, firmness and variety in color compared to the natural dyes.

The release of dyes may therefore present an ecotoxic hazard and introduces the potential danger of bioaccumulation that may eventually affect man by transport through the food chain. Moreover, various dyes are also one of the major sources of heavy metals (Wagner, 1993) in water and soil and at times, they can be phototoxic (Raskin *et al.*, 1994) and due to their persistent nature cause a misbalancing in the ecosystem.

Currently various physical, chemical and biological treatment methods are used to remove color (Zhang *et al.*, 2003). Because of the high cost and disposal problems, most of the chemical and physical methods for treating dye wastewater have not been widely applied in the textile industries (Robinson *et al.*, 2001). Biological dye removal techniques are based on microbial biotransformation of dyes. In the present study an attempt was made to degrade Crystal Violet using white rot fungus, *Trametes hirsuta* and the ability of other fungi like *Aspergillus niger, Aspergillus fumigatus, Penicillium sp* and *Fusarium oxysporum* to degrade the dye were also studied.

MATERIALS AND METHODS

Organisms used for the study:

The white rot fungi *Trametes hirsuta* (MTCC 1171) was procured from Institute of Microbial Type Culture Collection, Chandigarh, India to test for the dye degradation abilities. Other fungi used for the dye degradation include *Aspergillus flavus, Aspergillus*

fumigatus, Penicillium sp and Fusarium oxysporium.

Dye used:

The dye used for this study was Crystal Violet. Stock solutions of the dye was prepared with 100mg of dye in 100ml of sterile distilled water and filter sterilized and used for decolorization studies.

Studies on the decolorization of dye by the fungi on solid medium:

Malt Extract Agar (MEA) medium was prepared and sterilized. To each 15ml of the medium 5ml of sterile dye, Crystal Violet solution was added and poured into Petri dish separately. For each plate, a disc (5mm) of fungal mycelium was placed at the centre. Uninoculated plates served as a control. The plates were then incubated at 30°C and observed for visual disappearance of color.

Studies on the decolorization of dye by the fungi in liquid medium:

Decolorization Assay:

Decolorization studies were carried out in Malt Extract broth medium. Media was prepared, sterilized and supplemented with the dye, Crystal Violet solution. To this medium 5mm disc of fungal mycelium was inoculated. Following third day of inoculation, 2ml of culture media was taken, centrifuged and read at 580 nm using UV- Visible spectrophotometer. Medium without dye and inoculum was used as blank. Medium with dye but without inoculum was used as control. The rate of decolorization was estimated by the following formula:

$$\text{Rate of decolorization (\%)} = 100 - \frac{\text{Absorbance of treated dye solution}}{\text{Absorbance of control dye solution}} \times 100$$

Optimization of the medium and growth conditions for the effective decolorization of the dye by *Trametes hirsuta*:

Different parameters like temperature, pH, carbon source and nitrogen source of the medium were optimized for the decolorization assay. 350ml of Malt extract broth medium was prepared and sterilized. 25ml of the above said sterilized medium was dispensed into 100ml Erlenmeyer flasks. To this 25ml of stock dye, Crystal Violet solution was added separately. Three discs (5mm) of actively growing fungal mycelium were inoculated into each flask. To check for the optimum temperature for the effective decolorization the above said sterilized medium were incubated at temperature ranging from 25°C-40°C, for testing the optimum pH for the decolorization assay the pH of the medium was adjusted using 0.1N HCl and 0.1N NaOH and the pH ranging from 3-7 was used. To check for the carbon source for the effective decolorization, the medium was prepared with carbon sources like Glucose, Sucrose, Galactose, Maltose and Mannitol and for the effective decolorization of the dye the nitrogen source of the medium was also optimized, for which the medium was prepared using nitrogen components like Yeast extract, Ammonium chloride, Urea, and Peptone.

Efficacy of immobilized cells on the decolorization of dye:

Immobilized cells were prepared using sodium alginate. The fungal mycelium was immobilized for the decolorization studies. 150ml of Malt Extract broth medium was prepared and sterilized. 25ml of the sterilized broth was dispensed into 100 ml Erlenmeyer flasks. To this 25ml of stock dye solution, Crystal violet was added separately. Then using sterile spatula 10 beads (fungal beads) were added to the media and incubated at 30°C and rate of decolorization was studied using UV-Visible Spectrophotometer as said above. Medium with dye and beads alone served as control.

RESULTS AND DISCUSSION

Decolorization of dye on solid medium by *Trametes hirsuta* and other fungi:

The decolorization of the dye, Crystal Violet by the fungus *Trametes hirsuta* and the other fungi was performed on dye-agar plate. The decolorization ability of *Trametes hirsuta* was supported by many studies as reported by Abadulla *et al.* (2000).

The mycelial growth of the fungus is started from first day but decolorization started from third day. The decolorization rate was differing among the fungi used. *Trametes hirsuta* could effectively degrade the dye compared to the other fungi.

The solid-state decolorization assay was carried out among the other fungi like *Aspergillus niger, Aspergillus fumigatus, Penicillium sp*, and *Fusarium oxysporum. Aspergillus niger* and *Aspergillus fumigatus* showed maximum efficiency to decolorize the dye, Crystal violet. *Fusarium oxysporum and Penicillium sp.* also showed decolorization but was relatively low when compared to other fungi. Similar studies regarding Congo red and Crystal violet degradation by *Aspergillus niger, Aspergillus fumigatus, Penicillium sp, Fusarium oxysporum, Rhizopus and Cladosporidium sp.* had been observed by Poonkothai *et al.* (2009).

The solid-state decolorization assay of the present study revealed that high percentage of decolorization was observed with the white rot fungus. The decolorization started after the sufficient growth of mycelium. The results were similar to the reports of Sathiyamoorthi *et al.* (2006) in which the solid state decolorization of Blue CA, Black B133 and Corazol violet SR dyes were observed.

Decolorization of different dye in liquid medium by *Trametes hirsuta* and other fungi:

The efficiency of the fungi to decolorize the dye, crystal violet was studied. Maximum decolorization was observed with *Trametes hirsuta*. *Aspergillus niger* and *Aspergillus fumigatus* also showed moderate decolorization. The decolorization was very low in the case of *Penicillium sp* and *Fusarium oxysporium* (Fig: 1)

Optimization of medium and Growth Conditions for the Effective Decolorization by *Trametes hirsuta:*

As the decolorization was found to be maximum for the white rot fungus, *Trametes hirsuta* the medium and growth conditions were optimized for their effective decolorization. Decolorization rate was observed at different temperatures ranging from 25°C - 40°C at an interval of 5°C. Decolorization was optimum at 30°C (88.5%) (Fig: 2). Sharma *et al.*, (2009) reported that the optimum decolorization of Orange II dye by *Phanerochaete chrysosporium* on 5th day at 30°C.

The decolorization efficiency was also checked at various pH ranging from 3 - 7. The decolorization rate was maximum at pH 4 (80.1%) (Fig: 3). This was similar to the results obtained by Mohandass *et al.*, (2007) who observed maximum decolorization at pH 3 on *Aspergillus* sp. Decolorization study at different pH was also carried out by Rani *et al.*, (2005) who observed 96.4% decolorization after 8 days of incubation at a pH of 6.

The decolorization was maximum (89.3%) with maltose compared to other carbohydrates tested (Fig: 4). Mohandass *et al.*, (2007) reported glucose and mannitol as the best carbon source for the decolorization in which the decolorization was more than 95%. Sathiyamoorthi *et al.*, (2007) reported that effective color removal was observed with 2% glucose.

The effect of nitrogen sources such as Yeast extract, Peptone, Ammonium chloride and urea on decolorization showed maximum (81.6%) with Yeast extract, followed by Peptone (79.8%), Urea (75.1%) and Ammonium chloride (70%) (Fig: 5).

Decolorization of dye using immobilized cells of *Trametes hirsuta*:

The efficacy of the fungus, *Trametes hirsuta* for the decolorization of the dye was also observed by immobilizing the cells in sodium alginate to form beads. The decolorization was 44% for Crystal Violet (Table: 1). Similarly Pandey *et al.*, (2009) studied the biodegradation of texile dyes using the immobilized cells of microorganisms like *Phanerochaete chrysosporium* and *Pseudomonas fluorescens*. The results showed good decolorization of textile dyes.

The present study reveals that the White rot fungus *Trametes hirsuta* could effectively decolorize Crystal violet. The extent of decolorization differed from dye to dye. This may be due to the differerences in the components of the dye. Decolorization of dye using *Aspergillus niger, Aspergillus fumigatus, Penicillium sp, and Fusarium oxysporum* was also checked but the rate of decolorization was low than *Trametes hirsuta*. Thus the results prove that the test fungus, *Trametes hirsuta* is more efficient in the decolorization of the dye

and can be used for the treatment of dye industry effluents reaching the environment.

Fig: 1 Effect of decolorization of Crystal Violet by all the fungi used

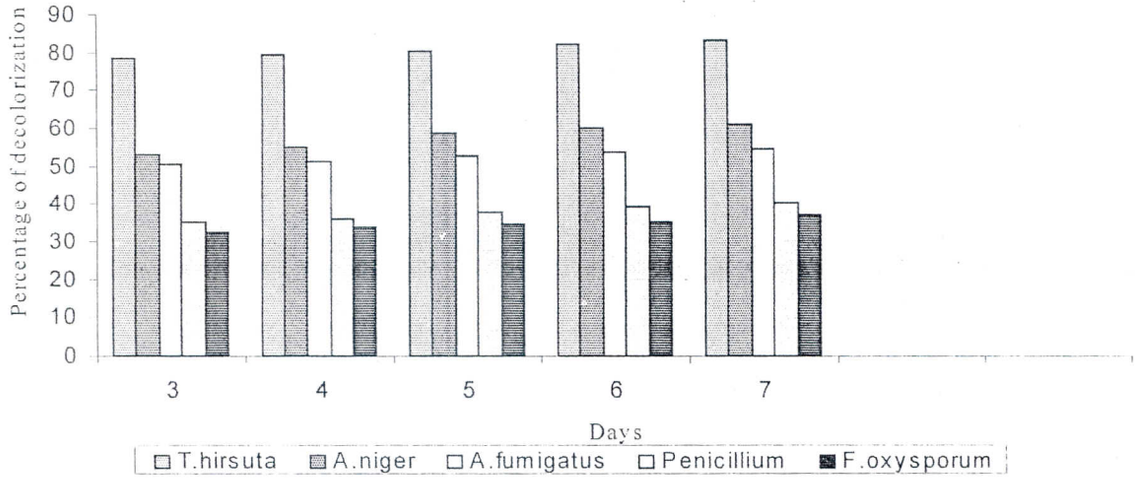

Fig: 2 Effect of temperature on the decolorization of Crystal Violet by *T. hirsuta*

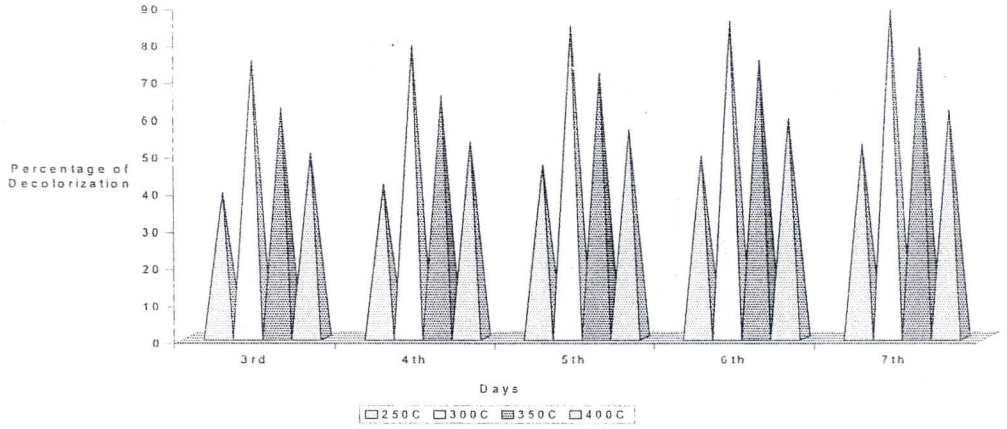

Fig: 3 Effect of pH on the decolorization of Crystal violet by *T.hirsuta*

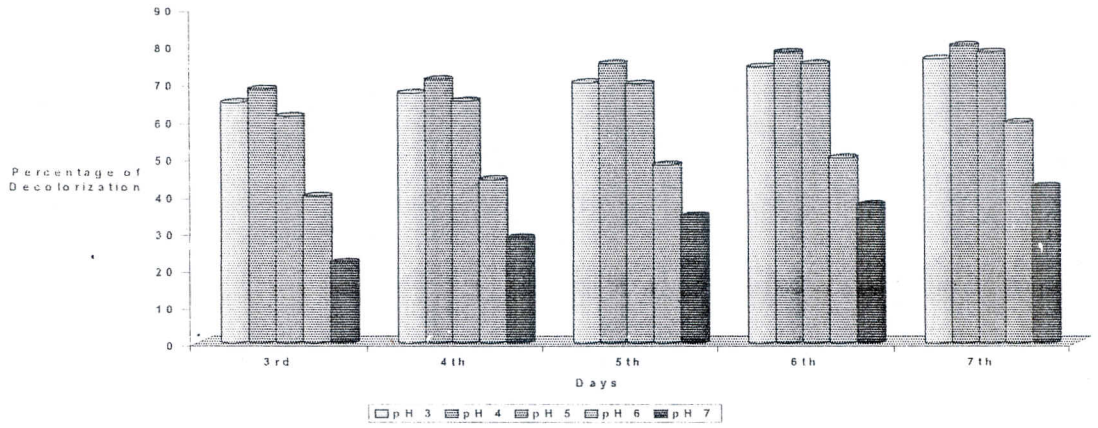

Fig: 4 Effect of carbon source on the decolorization of Crystal violet by *T.hirsuta*

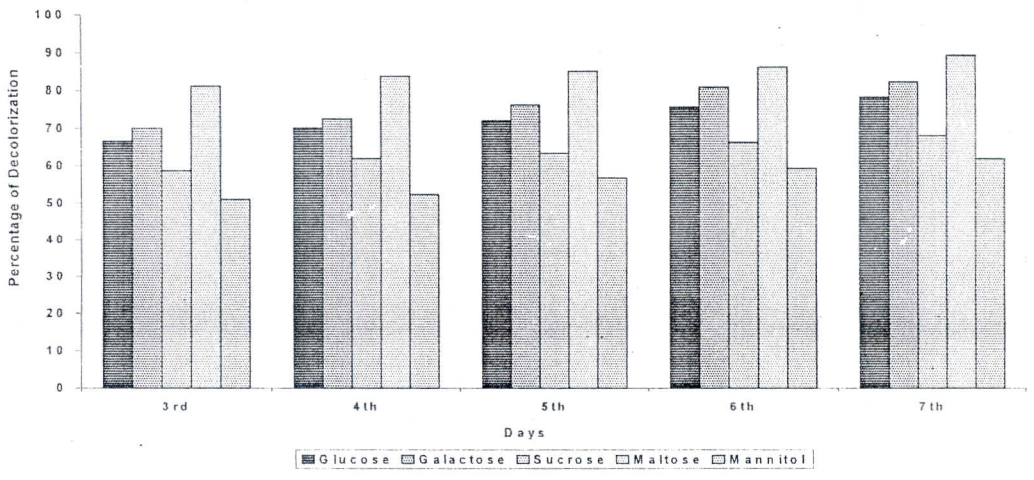

Fig: 5 Effect of nitrogen source on the decolorization of Crystal violet by *T. hirsuta*

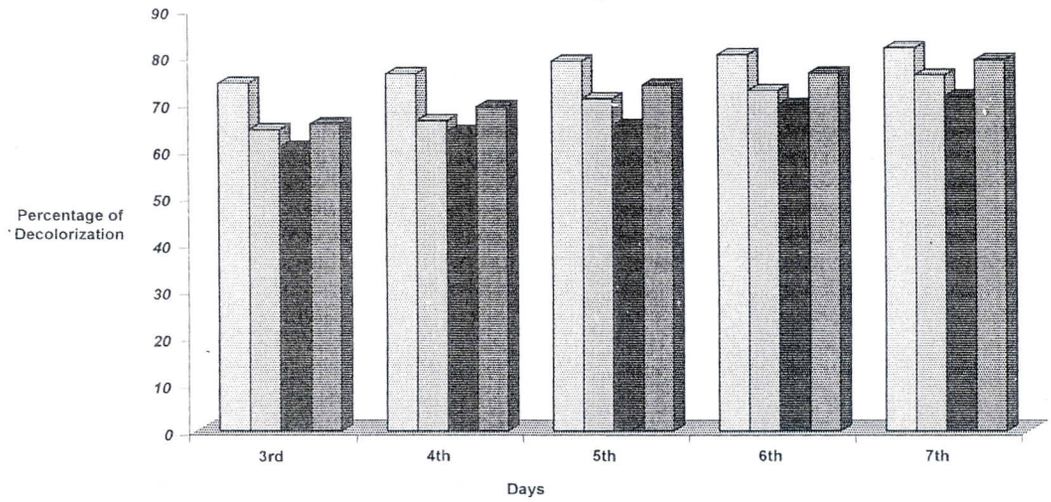

Table: 1 Decolorization of Crystal violet using immobilized cells of *Trametes hirsuta*

Days	Percentage of Decolorization (%)
3rd	36.4
4th	37.9
5th	40.8
6th	42.1
7th	44

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