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ORIGINAL RESEARCH ARTICLE

Decolourization of Azo Dyes and Dye Industry Effluents by Lignin Degrading Fungus *Trametes versicolor*

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ABSTRACT

Lignin degrading white rot fungus, *Trametes versicolor* isolated from the logs of *Acacia nilotica L.Del.sp. indica* in the Western Ghats region of Tamil Nadu, India. The fungus was used for the decolourization of azo dyes such as orange G, congo red and amido Black 10 B and also for colour removal from the dye industry effluents. Batch studies were conducted in a defined medium with pH-4.5. The results revealed that the fungus could remove only 34.60% of orange G in the synthetic solution whereas 98.27% and 78.05% removed congo red and amido black 10B respectively. An enzymatic dye decolourization study showed that a maximum of 21% orange G was removed by lignin Peroxidase (LiP) at 15 U/ml whereas manganese dependant peroxidase (MnP) and laccase at the same concentration decolorized at 13.5% and 8.10%. A maximum decolourization of 13.5% and and 12.5% for Congo red and amide Black 10B, respectively, was recorded by laccase. Maximum decolourization of the effluent by this fungus was evidenced on fouth day treatment .On the fourth day the colour removal was up to 98.43%.

From the results, it was interpreted that the colour removal by the basidiomycetes fungi was mainly due to adsorption of the dyes to the mycelial surface and also due to metabolic breakdown. The results suggested that the batch mode treatment of *Trametes versicolor* is one of the most efficient ways for colour removal in dye industry effluents.

Key words: Azo Dyes, Decolourization, Effluents, *Trametes versicolor.*

INTRODUCTION

Synthetic dyes are extensively used for textile dyeing, paper printing, colour photography, as an additive in petroleum products and food industries [36]. Azo dyes (-N = N group) form the largest class of synthetic dyes with a variety of colour and structure [13]. The worldwide production of these dyes is currently estimated at 4, 50,000 tons/year with almost 50,000 tons/year lost in effluent during application and manufacture. At the time of production and application about 2-50% of these dyes are lost as waste effluents [31]. Waste removal inadequately eliminates many azo dyes from effluent waters of textile mills and dye stuff factories [25]. All dyes used in the textile industry are designed to resist fading upon exposure to sweat, light, water, many [42] chemicals including oxidizing agents, and microbial attack..The azo dyes exposure can reduce fertility .When male and female mice were exposed to Congo red (II), uterogonadal development in both sexes were adversely affected [17]. There are a variety of treatment methods for dye effluent that are broadly classified as chemical, physical and biological [40]. The physiochemical methods include flocculation combined with floatation. Electrofloatation, membrane filtration, electrokinetic coagulation, electrochemical destruction, ion-exchange, irradiation, precipitation, ozonation and katox treatment method involving the use of activated carbon, Howerever these technologies are usually inefficient in the removal of color, little adaptable to a wide range of dye waste waters, generally lead to production of secondary pollution [2,45]. Adsorption has been observed to be an effective process for color removal from dye waste waters. Adsorption on activated carbon is an effective

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method for the removal of color, but it is too expensive. Many studies have been undertaken to find low cost adsorbents which include peat, china clay, fly ash, wood shavings and silica, but these adsorbents have low adsorption capacities and require large amount of adsorbents [34]. In recent years, a number of studies have focused on microorganisms which are able to biodegrade and biosorb dyes in waste waters. Biological treatment methods are more desirable as they are environmentally friendly, do not produce secondary pollutants and have a higher possibility of wider application [39]. Various bacteria and fungi have decolourizing abilities and an extensive review of microbiological decolourization is available [14] Treatment with basidiomycetous fungi or their lignin-degrading enzymes, lignin peroxidase, manganese-dependent peroxidase and laccases has been widely reported [3]. [43] reported the adsorption of acid green 27, acid violet 7 and indigo carmine dyes on living and dead mycelia of Trametes versicolor. Adsorption of dyes to the microbial cell surface is the primary mechanism of decolourization [24]. (1999)[39] reported that White rot fungi secrete extracellular ligninases that could degrade the azo dyes. **[11]** reported Synthetic dye decolorization capacity of white rot fungus *Dichomitus squalens. Daedalea flavida Dichomitus squalens*, , *Irpex flavus* and *Polyporus sanguineus* were tested for their potential to decolourise various chromophoric groups of dyes, employed in different industries **[4]**. [20] reported the decolorization of congo red dye**.** [6] reported the decolorization of synthetic dyes by *Trametes hirsuta* in expanded-bed reactors. Decolorization of two azo dyes namely Direct Red–80 (DR–80) and Mordant Blue–9 (MB–9), by *Phanerochaete chrysosporium* was investigated both individually and in mixtures in batch shake flasks **[37]**. **[11]** reported the decolorization of high concentration (1.2mM) of Congo red. [8] compared the degradation of a polymeric dye (PolyR-478) by 127 strains to estimate the peroxidative activity: the correlation between the polymeric dye decolourization and the peroxidase activity was pointed out. [33] also studied the enzymatic properties of 90 *white-rot fungi* strains. Pleurotus ostreatus produces laccases which is responsible for the biodegradation of lignin and textile dyes [35]. [31]Reported that 115 fungi from different physio-ecological groups were compared for their capability to decolorize anthroquinone and azo dyes. **S**ix fungal isolates, *Aspergillus niger, Penicillium* spp., and *Pleurotus osstreatus* were used for decolourization activities of the dyes, Basic Blue 9, Acid Blue 29, Congo Red and Disperse Red 1 [12]. Decolorization of reactive orange 16, reactive black 5, direct violet 51, Acid red 88, Acid red 114, Basic orange II, Bismark brownR by *Phanerochaete chrysosporium* RP78 under optimal conditions [15]**.** [47] reported high decolorization efficiency of orange II using white rot fungus. [5] demonstrated the degradation of azo and heterocyclic dyes of orange II, tropeolin O, congo red and azure B by *Phanerochaete chrysosporium.* [22] observed 98% decolorization of Orange II by wood rotting fungs F29. [28] reported that in liquid culture, D. squalens decolorized both Orange G and RBBR efficiently, The early step in azo dyes decolorization is the breaking of the azo bond—and further degradation involving aromatic cleavage, depends on the identity, number and position of functional groups in the aromatic region and the resulting interaction with the azo bonds In the present study *Trametes versicolor* was isolated from Western Ghats area of South India and studied for dye decolourization and dye industry effluent treatment.

MATERIALS AND METHODS

Microorganism and Media

The fungus, *Trametes versicolor* was isolated from the *Acacia nilotica L Del sp. Indica* logs tree in Western Ghats region in Tamil Nadu, India. The fungus was identified based on the key provided previously [1,16]. Fungal growth was cut, sterilized with 1% mercuric chloride solution, repeatedly washed with sterile distilled waster as described previously [45]and inoculated on 2% Malt agar medium. The fungal growth was subcultured and incubated for 6 days at 37^0 C and maintained on Malt agar slants. Then, the spores were harvested without disturbing the mycelial growth using a camel hairbrush and filter sterilized. The spore concentration was adjusted to 10^5 spores/ml and used as inoculum for futher studies. Growth kinetics, the ligninolytic enzyme production, and dye deccolourisation studies were carried out in C-limited medium (M14) of Janshekar and Fiechter [19], to which spores in the one-tenth volume of the medium were inoculated.

Ligninolytic enzyme production

The enzyme, Lignin Peroxidase (Lip), Manganese Dependentant Peroxidase (MnP) and Laccase were purified from the culture filtrates by acetone precipitation (66% v/v), dialysis and Sephadex G100 column chromatography (15x13 cm; NaCl

gradient of 0-0.14M).Lip activity was assayed by the method of Linko [27].One unit of enzyme activity was defined as the amount of enzyme oxidizing one μ mole of veratryl alcohol in 1 min. MnP was assayed using a spectrophotometer by the method of Kuwahara [26]. One unit of enzyme activity was expressed as the amount of enzyme capable of increasing one Optical density (OD) at 610nm in a minute. The Laccase activity was determined spectrophotometrically [9] with guaiacol as a substrate .In this case, one unit of the enzyme activity was expressed as the amount of enzyme able to increase one OD at 440nm.

Decolourization of Azo dyes

To study the ability of the fungus to remove azo dyes from aqueous solutions, C-limited medium containing orange G (50μM), Congo red (50μM) and amido black 10B (25 μM) were inoculated with spore suspension of *Trametes versicolor* and incubated in rotary shaker (120rpm) at 39°C for 6 days. After 6 days, the samples were withdrawn at regular time intervals and filtered through a G3 sintered glass filter. The Optical density of the clear filtrate was measured at 479, 497 and 618nm respectively for Orange G, Congo red and amido black 10B in a spectrophotometer (Shimadzu, TCC 240). For enzyme treatment, the reaction mixture containing 90ml of the dye at various concentrations (5, 10 and 15U/ml) of LiP, MnP and Laccase were incubated with dyes in specific buffers. For LiP and MnP enzyme treatment, sodium succinate buffer -20mM; pH 4.5 was used, For Laccase, Phosphate buffer -0.1M; pH 7.0 was used .At regular time intervals, samples were withdrawn and the OD was measured.

Decolourization of textile industry effluent

To analyze the efficiency of treatment of a dye industry effluent, two modes of treatment were adopted, since different modes can show different efficiencies in the treatment. The ability of the fungus to remove colour from a dyeing industry effluent was assayed in the modified C-Limited medium [19].The medium contained the textile dye effluent instead of distilled water in equal volume. The pH of the solution was adjusted to 4.5 .To the effluent amended medium (950 ml), 50 ml of spore suspension (10^5 spore/ml) was inoculated and maintained at 39°C. Samples were withdrawn at regular time intervals and analyzed for colour removal .The intensity of the effluent colour was measured at 490nm.For enzyme treatment, the reaction mixture containing 90ml of the effluent, various concentrations (5, 10 and 15 U/ml as final concentration) of LiP, MnP (in sodium succinate buffer, 20 mM, pH 4.5) and Laccase (in Phosphate buffer 0.1ml, pH 7.0) were added. The reaction mixture was incubated at 37°C for 1 hour. After 1 hour, the color intensity of the effluent was measured. Boiled enzyme was used as a control.

RESULTS AND DISCUSSION

Decolourization of Azo dyes by mycelia

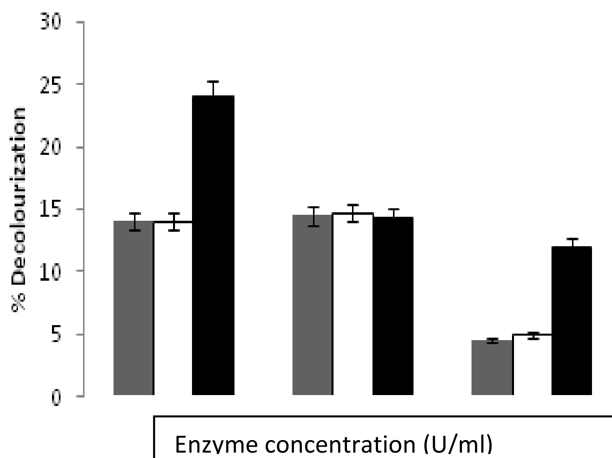
The dyes are aromatic compounds and the ligninolytic fungi degrade these dyes during secondary metabolism. The growth and decolourization efficiency of the test fungus is shown in table 1.It was observed that *Trametes versicolor* was able to decolourise only 34.60% Orange G within 9 days, Whereas Congo red was removed by 98.27% within 20 hours and amido black 10B by 78.05% by 8h. *Phanerochaete chrysosporium* could remove 80-97% of tropeolin O and congo red within 5 days [7]. [18] reported that *Bjerkendra aedusta* and *T.versicolor* removed 95% of HRB38 dye within 4 days . [37] established that *Fomes lividus* was able to decolourise 30.8% of Orange G within 9 days where as Congo red was removed upto 74% within 8 hours and amido black 10B upto 98.9% in 6 hours. [41] reported complete decolorization of Amido black, congo red, trypan blue, methyl green, remazol brilliant blue R (RBB), methyl violet, within six days. [46] reported the decolorization of azo dyes Congo red, Fast Blue RR salt orange G, Acid red, and amidoblack 10B by Trametes versicolor. More than 325 white rot fungi strains belonging to 76 fungal genera were compared with regards to their capability to decolorize five azo and two antroquinine dyes as well as dye mixtures [29]**.** [21] reported that a newly isaolated fungus *Geotrichum candidum* Decl, decolorized 21 types of dyes

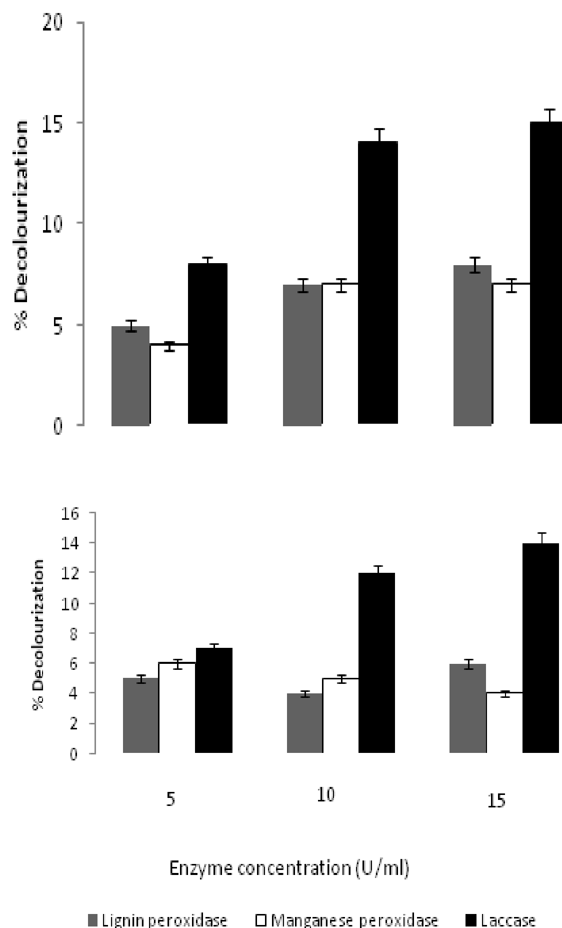
Treatment of Azo dyes by enzymes

In the present study (**Figure 1**) through present removal was low in Orange G, dye degradation was comparatively high of about 21.62% and the enzyme Lignin Peroxidase was found to play a major role in Orange G degradation. In Congo red and amido Black 10B, the degradation was in the range of 12.50% to 13.43% and the enzyme lactase was the effective degrader. The results revealed that *Trametes versicolor* could effectively remove Congo red and Amido Black 10B and degrade Orange G.The enzyme Lignin Peroxidase is involved in degradation of Orange G and laccase degraded Congo red and amdio Black 10B.Enzymatic degradation of adsorbed dye was the major mechanism in the regeneration of the adsorption capacity of the fungal mycelium. [30]

showed that 54% decolourizations of Congo red occur in the presence of a crude preparation of Lignin peroxidase and these enzymes utilize Congo red as the substrate. [43] reported that enzymes such as Lignin Peroxidase, Manganses Dependant Peroxidase (MnP) and Lactase all of which are involved in lignin degradation, participate in the decolourization of the dyes. [38] reported that in white rot fungi *Trametes versicolor*, the enzymes laccase and Manganses Dependant Peroxidase (MnP) were detected in decolourising cultures of amaranth. [22] purified and characterized a novel Peroxidase (DyP) that is responsible for the dye concentration on decolourization activity of *Geotrichum candidum* Dec1. The effect of dye concentration on decolonization rate was rarely reported .A general tendency is that high concentration will cause slower decolourization rate. Orange G and amido black were good substrates for laccase and was degraded within 12 hours. [10] reported that the fungi *Dichomitus squalens*, *Ischnoderma resinosum* and *Pleurotus calyptratus*, efficiently decolorized both Orange G and RBBR but they differed in decolorization capacity depending on cultivation conditions and ligninolytic enzyme production. Two different decolorization patterns were found in these strains: Orange G decolorization in *Ischnoderma resinosum* and *Pleurotus calyptratus* was caused mainly by laccase, while RBBR decolorization was effected by manganese peroxidase (MnP); in D. squalens laccase and MnP cooperated in the decolorization processes. [38] reported that the enzyme peroxidase play a strong role in dye decolorization. Biodegradation of azo-dyes using *Phanerochaete chrysosporium* is one the most environmentally friendly methods available. The main enzymes responsible for mycodecolorization process are lignin and manganese peroxidases [15].

Figure 1: Decolourization of Azo dyes by purified ligninolytic enzymes of *T.versicolor*





Treatment of dye industry effluents

In the present study, when the textile industry effluent was treated with *Trametes versicolor* in batch mode and continuous mode, in batch mode 98.43% of colour was removed on the fourth day of incubation, later there was a decrease in colour removal in continuous mode, a maximum of only 40.63% colour removal was observed on 5th day (**Figure 2**).When the enzyme were used for effluent treatment, Manganses Dependant Peroxidase (MnP) at 15 U/ml concentrations removed 23.52% of color (**Figure 3**). Hence of textile industry effluent treatment, *Trametes versicolor* fungal mycelial treatment, in batch mode with an incubation period of four days will be preferred. [23] reported that *Trametes versicolor* removed 70% to 80% colour of chemistry industry effluent; *Phanerochaete chrysosporium* removed 70% colour from the oil mill waste water. A continous decolourization of industrial effluent by *T.versicolor* would be difficult because of the cycles of mycelia growth and enzyme production [[44]. [36] reported that removal of colour in dye industry effluent by white rot fungi *Fomes lividus* and *Thelephora sp.* in batch mode were 84.4% and 61% and in continuous mode were 37.5% and 50%. [7]

compared dye decolorization in both batch and continuous mode and reported that Batch cultivation led to high decolorization percentages in a short time (100% for Indigo Carmine in 3 h and 85% for Bromophenol Blue in 7 h) .

Figure 2: Decolourization of dye industry effluent by culture of *Trametes versicolor*

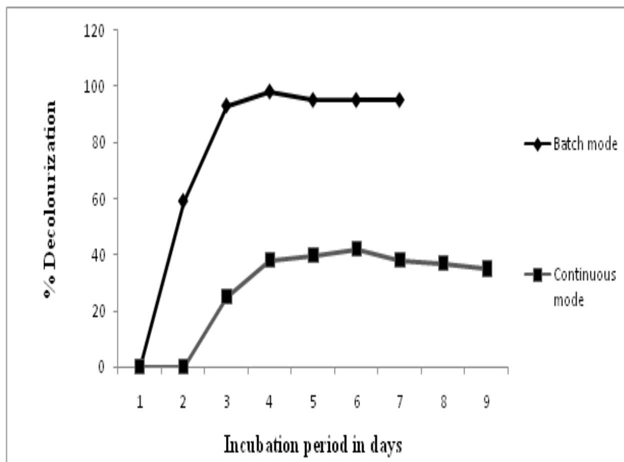
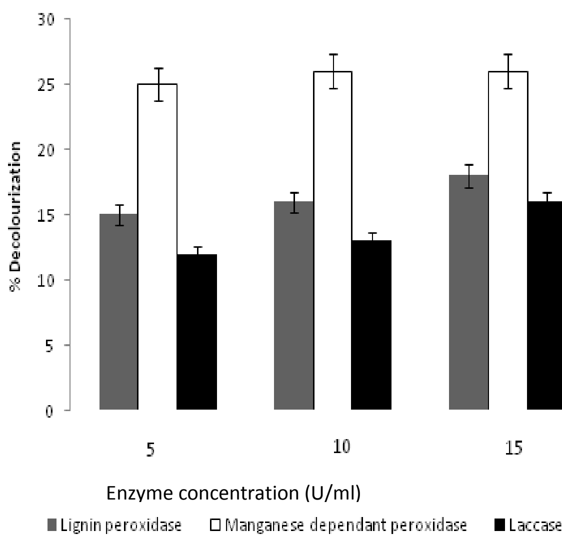


Figure 3: Decolourization of dye industry effluent by purified ligninolytic enzymes of *T. versicolor*



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