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Biological treatment of AZO dyes and textile industry effluent by newly isolated White ROT fungi *Schizophyllum commune and Lenzites eximia*

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ABSTRACT

Lignin degrading white rot fungi, *Schizophyllum commune and Lenzites eximia* were collected from the living tree of *Tramarindus indica* and burnt tree respectively from the Western Ghats region of Tamil Nadu, India. The fungi were used for the decolourization of azo dyes such as congored, methylorange, erichrome black- T and also for decolourization of the dye industry effluents. Removal of azo dyes from aqueous solution by *Schizophyllum commune* (50 µm) concentration showed 96.86% of colour removal in congored, 97.57% in methylorange and 97.40% in erichrome black-T on fourth day respectively. *Lenzites eximia* decolourised congo red by 95.50%, methylorange by 94.79% and erichrome black-T by 95.36% at (50 µm) concentration respectively on fourth day. Removal of textile dye effluent in batch mode showed 76.15% decolourisation and in continuous mode 55.92% on 5th day by *Schizophyllum commune.* In *Lenzites eximia* 75.23% and 54.60% of decolourization was observed in batch and continuous mode respectively on 5th day. From the results, it was interpreted that the colour removal by the basidiomycetes fungi were mainly due to adsorption of the dyes to the mycelial surface and also due to metabolic breakdown. The results suggested that *Schizophyllum commune* is more efficient than *Lenzites eximia* for the treatment of azo dyes and textile dye industry effluent in both batch mode and continuous mode.

Keywords: Azo dyes, Decolourization, Effluents, *Schizophyllum commune* and *Lenzites eximia*

1. Introduction

The dyestuff usage has been increased day by day because of tremendous increase of industrialization and man's urge for color (Mohan *et al.,* 2002) The chemical structure of dyes is comprised of a conjugated system of double bonds and aromatic rings. Azo dyes (N= N group) form the largest group of synthetic dyes with a variety of colour and structure (Gharbani *et al.,* 2008) More than 1,00,000 commerical dyes are available to textile industries worldwide and at the time of production and application about 2-15 per cent of these dyes are lost as waste effluents (Park and Lee, 2007). The presence of these dyes in the aqueous ecosystem are the cause of serious environmental and health concerns (Asad *et al.,* 2007). Dyes are mutagenic and carcinogenic and also cannot be completely removed by conventional wastewater treatment systems (Lopez *et al*., 2002). Textile industry effluents differ widely in their chemical characteristics and pH (Hai *et al*., 2007). There are a variety of treatment methods for dye effluent treatment that are broadly classified as chemical, physical and biological (Ferrero, 2007). The physicochemical methods include filtration, coagulation, carbon activated and chemical flocculation (Gogate and Pandit, 2004a, b). These methods are effective but they are expensive and involve the formation of a concentrated sludge that

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creates a secondary disposal problem (Maier *et al*., 2004). Green technologies to deal with this problem include adsorption of dyestuffs on bacterial and fungal biomass or low-cost non-conventional adsorbents (Prigoine *et al.*, 2008, a,b). Biological processes, such as biodegradation, bioaccumulation and biosorption, have received increasing interest due to their cost, effectiveness, ability to produce less sludge and environmental friendly (Aksu, 2005). Though bacteria could utilize dye stuff under anoxic conditions, the disadvantages of this method is the production of aromatic amines by these organisms. These amines may be toxic and carcinogenic (Meyer, 1981). Aerobic bacteria usually tend to be specific towards a particular dye (Reddy, 1995). Therefore there is a necessity to develop a new method, the single class of microorganisms most efficient in breaking down synthetic dyes are fungi (Saratale, 2006). Treatment with basidiomycetous fungi or their lignin degrading enzymes, lignin peroxidase, manganese dependent peroxidase and laccases has been widely reported (Blanquez, 2008). Ligninolytic fungi are the most widely used fungi involved in dye decolourization (Bumpus, 2004). Microbial decolourization and degradation has appeared as an environmental friendly and cost competitive alternative to chemical decomposition processes (Dhanve, 2008). Biological processes have the potential to convert or degrade the pollutant into water, carbon dioxide and various salts of inorganic nature. These treatment methods are more desirable as they are environmental friendly, do not produce secondary pollutants and have a higher possibility of wider application (Togo *et al.,* 2008). The isolation of potent species and their degradative mechanisms is one of the major interests in biological aspects of effluent treatment (Mohan *et al.*, 2002. (Glenn and Gold, 1987) reported that the decolorization of synthetic dyes by *Trametes hirsuta* in expanded-bed reactors. Decolourization 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 (Singh and Pakshirajan). In the present study newly isolated white rot fungi *Schizophyllum commune and Lenzites eximia* from Western Ghats area of South India, India and studied for dye decolourization and textile industry effluent treatment in both batch mode and continuous mode.

2. Materials and method

2.1 Microorganism and Media

The fungi, *Schizophyllum commune* and *Lenzites eximia* were isolated from Western Ghats region in Tamil Nadu, India. The fungi were collected from living tree of *Tamarindus indica* and burnt tree and used for dye decolourization studies. The fungi were identified based on the key provided previously (Bakshi, 1971; Gilbertson and Ryvarden, 1986). The fungal growth was cut and then sterilized with 1per cent mercuric chloride solution, repeatedly washed with sterile distilled water as described previously and inoculated on 2 per cent Malt agar medium (Watling R, 1971). The fungal growth were subcultured and incubated for 6 days at 37^0C 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 10^5spores/ml and used as inoculum for further studies. Dye decolourisation studies were carried out in C-limited medium (M14) to which spores in the one-tenth volume of the medium were inoculated (Janshekar and Fiechter, 1988).

2.2 Decolourization of Azo dyes

The ability of the fungi to decolourize azo dyes from aqueous solutions were studied in C-limited medium containing congo red (50μM), methylorange (50μM) and erichrome black-T(50μM) and this was inoculated with spore suspension of *Schizophyllum commune* and

Lenzites eximia 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 497, 465 and 503 nm respectively for congo red, methylorange, and erichrome black T in a spectrophotometer (Shimadzu, TCC 240).

2.3 Decolourization of textile industry effluent

To analyze the efficiency of effluent from of a dye industry, two modes of treatment were carried out in the present study, since different modes can show different efficiencies in the treatment. The ability of the fungi to remove colour from a dyeing industry effluent was assayed in the modified C-Limited medium (Janshekar and Fiechter, 1988). The C-limited medium contained the textile dye effluent instead of distilled water. 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 488nm.

3. Conclusion / Suggestions/ Findings

3.1 Decolourization of Azo dyes by fungi

In the present study two newly isolated white rot fungi *Schizophyllum commune* and *Lenzites eximia* were used for dye decolourization studies. The maximum mycelial dry weight were obtained on the fourth day as 60.1mg/day, 62.5mg/day, 58.0mg/day for congo red, methylorange, erichrome balck-T respectively when *Schizophyllum commune* was used for dye decolourization studies. The decolourization of congo red, methylorange and erichrome black- T at 50µm concentration were observed as 96.86%, 97.57% and 97.40% respectively on fourth day when treated with same fungi (Fig.1). In *Lenzites eximia* maximum mycelial dry weight was observed on fourth day as 56.4% for congo red, 58.8% for methyl orange, 54.0% for erichrome black-T. The decolourization of congo red, methylorange and erichrome black T at 50µm concentration were observed as 95.50%, 94.79%, 95.36% respectively on fourth day (Fig. 2).Cripps, (1990) reported that *Phanerochaete chrysosporium* could remove 80-97 per cent of tropeolin O and congo red within 5 days. Heinfling *et al.* (1997) showed that *Bjerkendra aedusta* and *Trametes versicolor* removed 95 per cent of HRB38 dye within 4 days. Selvam *et al.* (2005) reported that *Fomes lividus* was able to decolourize 30.8 per cent of orange G within 9 days where as congo red was removed upto 74 per cent within 8 hours and amidobalck 10B upto 98.9per cent in 6 hours. Tychanowicz, *et al*. (2004) reported complete decolourization of amido black, congo red, trypan blue, methyl green, remazol brilliant blue R (RBB), methyl violet within six days. The decolourization of azo dyes congo red, fast blue RR salt, methylorange, acid red and amidoblack 10B by *Trametes versicolor* was reported by Yang *et al*. (2009). *Pleurotus ostreatus* was used for decolourization activities of the dyes, basic blue 9, acid blue 29, congo red and disperse red1(Fu and Viraraghavan, 2002). Kim *et al.* (1995) reported that a newly isolated fungus *Geotrichum candidum* Decl, decolourized 21 types of dyes. Wang, (1998) 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 (Knapp and Newby, 1995). Eichlerova *et al*. (2006) reported that *pleurotus sp* were able to decolourize orange G and and remazol brilliant blue R on agar plates and decolourization process was complete after an incubation period of 12-18 days. (Liu, 2004) reported that in liquid culture, *Dichomitus squalens* decolourized both methylorange and

RBBR efficiently. Diwaniyan *et al*. (2010) reported that 69 per cent decolourization of congo red by the white rot fungal isolate RCK-3. The results suggested that decolourization of azo dyes (congo red, methyl orange and erichrome black T) at 50µm concentration by *Schizophyllum commune* proved to be efficient.

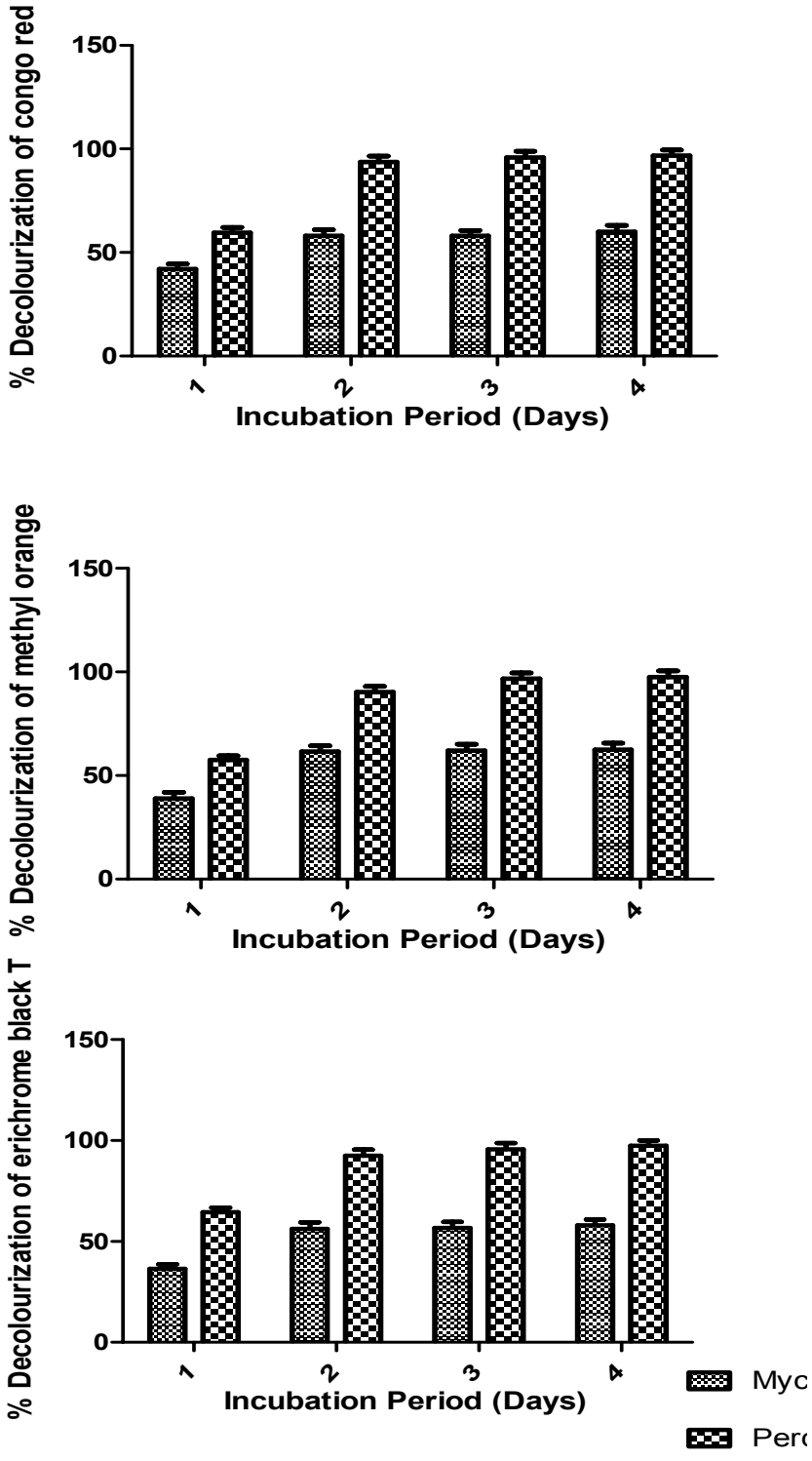

Figure 1: Removal of Azo dyes from aqueous solution by *Schizophyllum commune*

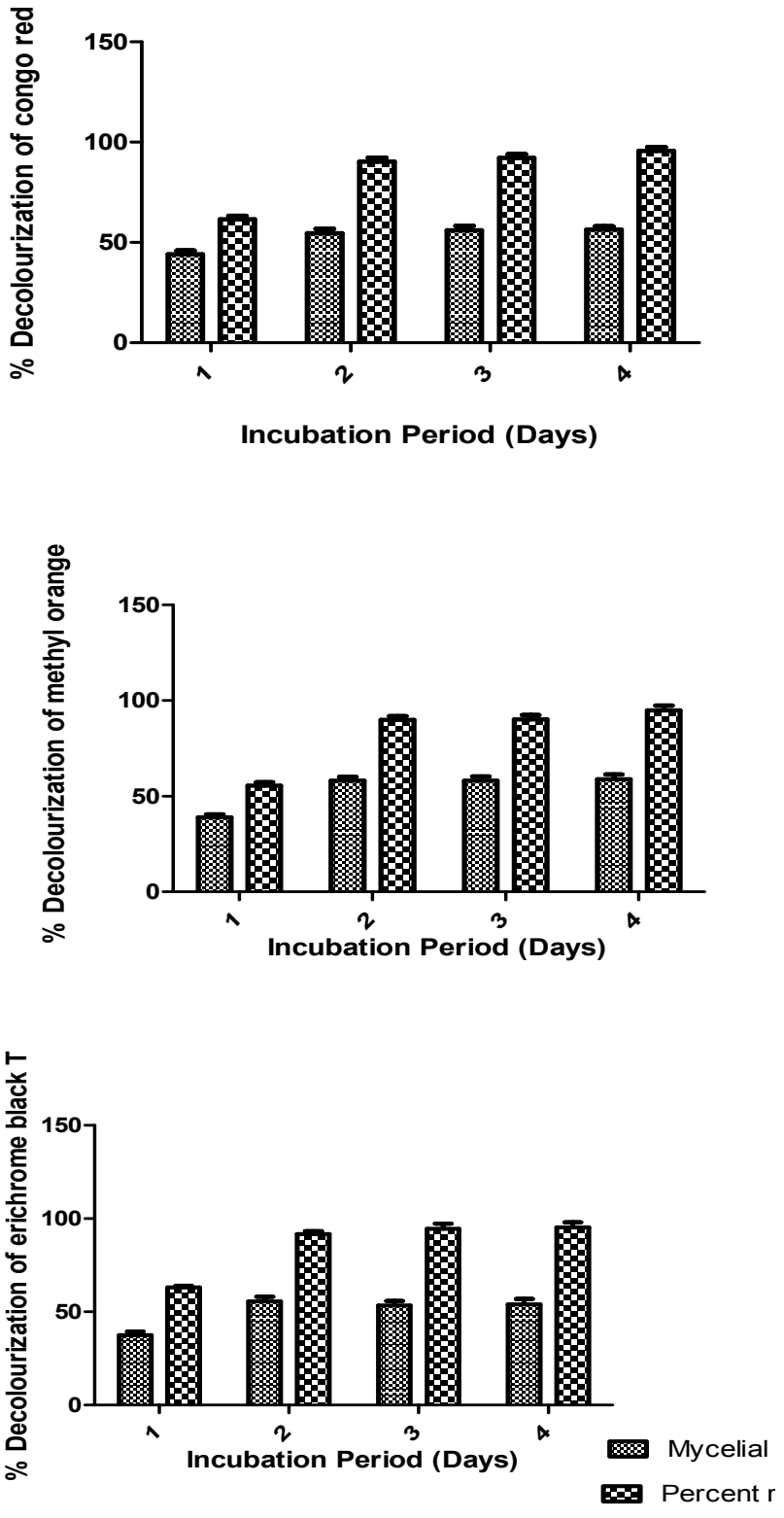

Figure 2: Removal of Azo dyes from aqueous solution by *Lenzites eximia*

3.2 Treatment of dye industry effluents in two modes

In the present study, when the textile industry effluent was treated with *Schizophyluum commune* in batch mode and continuous mode, in batch mode 76.15 per cent of colour was removed on the 5th day of incubation, in continuous mode, a maximum of only 55.92 per cent colour removal was observed on 5th day (Fig. 3). When the textile industry effluent was treated with *Lenzites eximia* in batch mode and continuous mode, in batch mode 75.23 per cent of colour was removed on the 5th day of incubation, and in continuous mode a maximum of 54.60 per cent was observed on 5th day (Fig. 4). Knapp and Newby (1995) reported that *Trametes versicolor* removed 70 to 80 per cent colour of dye industry effluent. Selvam *et al*. (2003) 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 per cent and in continuous mode were 37.5 per cent and 50 per cent respectively.

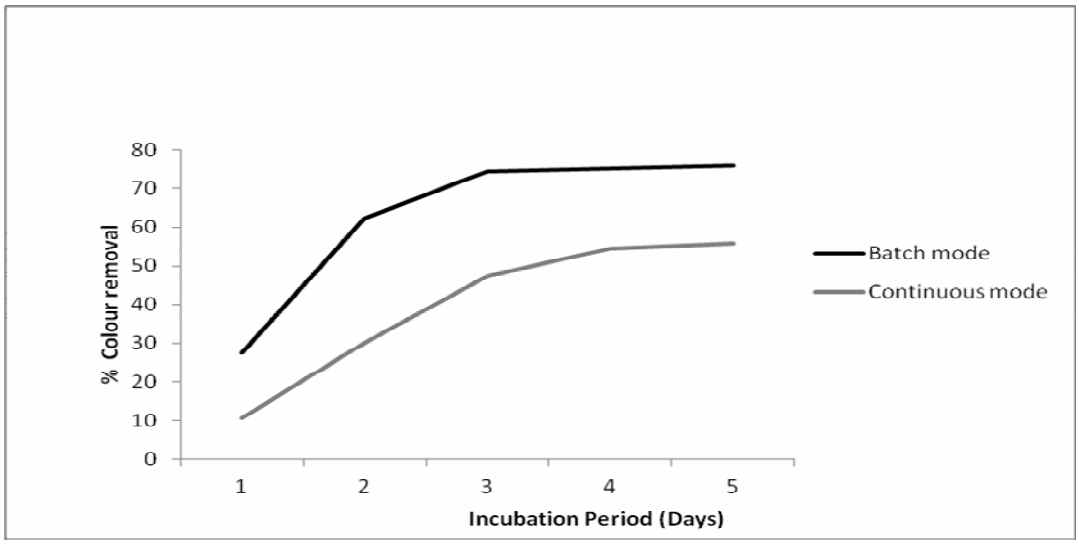

Figure 3: Colour removal of textile industry effluent by *Schizophyllum commune*

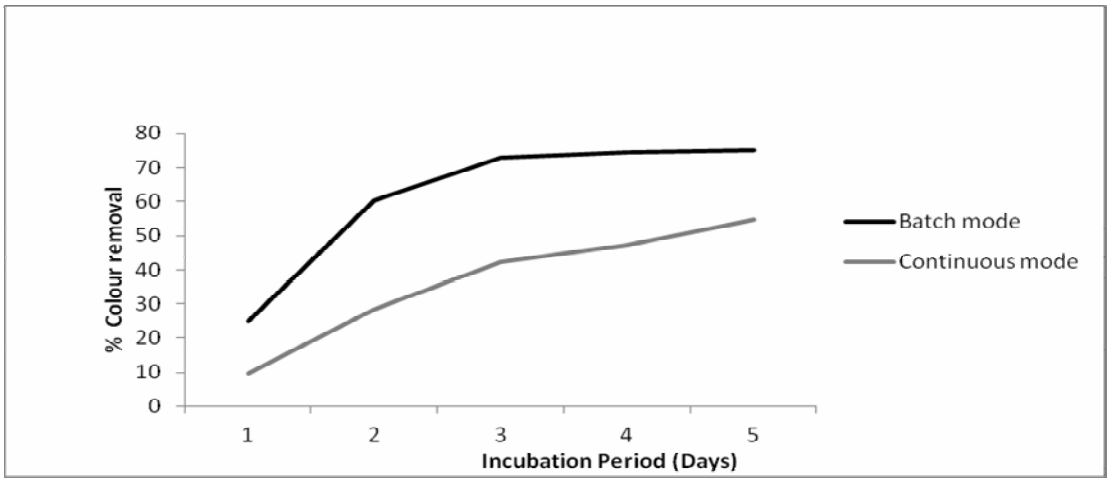

Figure 4: Colour removal of textile industry effluent by *Lenzites eximia*

Couto *et al.* (2006) compared dye decolourization in both batch and continuous mode and reported that batch cultivation led to high decolourization percentages in a short time. Olukanni *et al*. (2009) reported the decolourization of textile waste water effluent by white rot fungus *Pleurotus flabellatus* growing on sponge packed in a continuous reactor. Asgher, (2009) studied 36.3 per cent decolourisation of textile industry effluents **c**ollected from five different textile industries of Faisalabad, Pakistan. Diwaniyan *et al.* (2010) reported that 54 per cent decolourization of textile effluent by the white rot fungal isolate RCK-3. In the present study the batch mode treatment of textile industry effluent by *Schizophyllum commune* is more efficient when compared to continuous mode.

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