DECOLOURIZATION OF AZO DYES AND DYE INDUSTRY EFFLUENT BY *Ganoderma applanatum*, *Inonotus dryadeus*AND *Trametes hirsuta*

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ABSTRACT

In the present study the selected wood rot fungi were isolated from forest area of Chitteri hills, Eastern Ghats of Tamilnadu, India. From the study area three different wood rot fungi namely, *Ganoderma applanatum*, *Inonotus dryadeus* and *Trametes hirsuta* were isolated from *Albizia lebbek*, decayed wood logs and *Prunus avium* respectively. The fungi were used to decolourize the azo dyes such as Orange G, Methyl orange and Congo red. The results were obtained for dye removal. The dye industry effluents were decolourized by wood rot fungi in batch mode and continuous flow mode. The results exhibits the colour removal by the fungi was mainly due to adsorption of the dyes to the mycelial surface and due to metabolic breakdown. Microbial decolourization processes for the textile industry wastewater have the benefit of being cost-effective, eco-friendly and producing lesser sludge.

Keywords: Azo dyes, Decolourization,*Ganoderma applanatum*, *Inonotus dryadeus*, *Trametes hirsuta*

INTRODUCTION

Dyes usually have a synthetic origin and complex aromatic molecular structures which create them more stable and more difficult to degrade. Textile dyes are crucial, widespread contaminants of industrial wastewaters. They are discharged in the effluents as part of the manufacturing process in textile industries where they represent 10-15% (Trella *et al*., 2006; Cristovao *et al*.,2009; Gopinath *et al*.,2009) and even 90% of the used dyestuffs (Abadulla *et al*., 2000). Once discharged, the dyes can confer toxic and carcinogenic properties to the water and can contribute to the total organic loading (Dos Santos *et al.*, 2004). Structurally, they contain azo groups (-N=N-) mostly linked to benzene or naphthalene rings and occasionally to aromatic heterocyclic or to analyzable aliphatic groups (Zollinger, 2003).

Colour removal of these dyes by chemical, physical, and electrochemical method demonstrated some good results, but these methods require high cost. Recently, many researchers utilize microorganisms like fungi which have ability to decolourize and degrade dyes (Singh, 2004). The fungal possess the extracellular oxidative enzymes with the presence of Lignin Peroxidase (LiP), Manganese Peroxidase (MnP) and Laccase these enzymes were playing a vital role in colour removal of synthetic dyes (Hamedani *et al*., 2007).

The white rot fungi *Phanerochaete chrysosporium* has lignin peroxidase (LiP) and manganese peroxidase (MnP) that decolourise the textile effluent upto 99% in 7 days. *Hirschioporus larincinus*, *Inonotus hispidus*, *Phlebia tremellosa* and *Coriolus versicolor* have also decolourization effect on dye containing effluent (Rana, 2010). Biological processes, such as biodegradation, bioaccumulation and biosorption have received increasing interest due to their cost effectiveness, ability to produce less sludge and ecological friendly.

MATERIALS AND METHODS

Study area

Chitteri Hills was located in Dharmapuri District of Tamilnadu over 654.52 km^2 and was considered as one of the segments of Eastern Ghats of Tamil Nadu. The geographical limit was 78°15'−78°45' E longitude and 11°44'−12°08' N latitude. The hill endowed with different vegetation types. The minimum and maximum temperature of the area is 19 °C (in winter) and 40 °C (in summer) respectively. The annual rainfall varies from 620 to 900 mm and it received both northeast and southwest monsoons.

Collection and identification of fungi

The collected fungi were isolated from the study area. Collection numbers were given and preserved using proper techniques. The samples were deposited in Department of Botany, Periyar University, Salem. Morphologically the samples identified based on the key provided previously (Bakshi, 1971, Gilbertson & Ryvarden, 1986) and DNA sequencing also performed for species level identification. The collected fungi were identified and screened for further studies.

Preparation of Inoculum

The wood rot fungi were isolated and maintained on 2% malt agar medium. Fungal growth was cut out, sterilized with 1% mercuric chloride solution and continuously washed by sterile distilled water then incubated on 2% malt agar plates (2 g of malt extract, 2 g of agar in 100 ml distilled water) (Walting, 1971). The spore suspension obtained from the malt agar plates were used as inoculums for further studies.

Preparation of spore suspension

The fungal growth on a plate was sub cultured for 6 days at 37°C and maintained in malt agar slants. The pH was kept as 6.5 then the spores in plates were harvested without disturbing the mycelia growth flooded in sterile distilled water and brushed with camel hair brush smoothly and filtered through a sterile filter. The concentration of the filtrate was adjusted to 10^5spores/ml and inoculum was used for further studies. Dye decolourization studies was carried out in C-limited medium (M 14) the spores were in the one-tenth volume of the medium were inoculated (Janshekar & Fiechter, 1988).

Decolourization of azo dyes

The ability of the fungi to decolourize the azo dyes namely orange G (50 µM), methyl orange (50 µM) and congo red (50 µM) from aqueous solution were studied in C-limited medium. The medium was inoculated with fungal spore suspension (10^5spores/ml) and incubated at 39°C for 6 days in rotary shaker about 120rpm. After 6 days, the dyes orange G (50 µM), methyl orange (50 µM) and congo red (50 µM) were added. 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, 465 and 497 nm respectively for orange G (50 µM), methyl orange (50 µM) and Congo red (50 µM) in a spectrophotometer (Shimadzu, TCC 240).A control was also maintained.

Decolourization of textile industry effluent

To assess the efficiency of wood rot fungi for the treatment of a textile industry effluent, two modes of treatment were adopted. The ability of the wood rot fungi to remove colour from dyeing industry effluent was assayed in the modified C- limited medium (Janshekar & Fiechter, 1988), where instead of distilled water, the effluent amended medium (950 ml) was taken the pH of the solution was adjusted to 4.5 in the rotating biological contractor and inoculated with 50 ml of spore suspension (10^5spore /ml) and maintained at 39° C. In batch mode the treatment was given in a specific period. The samples were withdrawn at regular time intervals and analysed for colour removal. In continuous mode the treatment process continued for a week but the samples were withdrawn at regular time intervals. The intensity of effluent colour was measured at 490 nm the adsorption maximum of the dye industry effluent (Shimadzu, TCC 240). The untreated raw effluent served as control.

RESULTS AND DISCUSSION

Decolourization of azo dyes by wood degrading fungi

In the present study, the three newly isolated wood rot fungi namely *Ganoderma applanatum*, *Inonotus dryadeus* and *Trametes hirsuta* were taken for dye decolourization studies (Plate 1).

Ganoderma applanatum

The results show the effect of fungal treatment on orange G removal from aqueous solution. In *Ganoderma applanatum* the mycelial growth was 57 mg dry weight with 40.1% removal after eighth day incubation period. In decolourization of Methyl orange, *Ganoderma applanatum* shows maximum of 74.0 % dye was removal after eight days of incubation period. The initial dry weight of the mycelial was 48.0 mg, at eighth day of dry weight was 55.0 mg whereas the mycelial dry weight was increased along with increased the dye removal. The removal of congo red (50µM) from aqueous solution by *Ganoderma applanatum* treatment eighth day of incubation showed 81.5% dye removal and the mycelial growth was increased along with incubation period. At eighth day the mycelial dry weight was found to be 56.0 mg

(Figure 1 a,b,c).

Inonotus dryadeus

In Orange G dye removal *Inonotus dryadeus* resembles 60.0 mg dry weight after eight days of fungal treatment, the dye removal was gradually increased and results 45.6% respectively. While treating with methyl orange from aqueous solution showed maximum removal of dye upto 87.8% at eighth day incubation, at the same day maximum mycelial growth was reached at 61.0 mg. In Congo red dye *Inonotus dryadeus* shows the removal of 97.9% dye was observed at eighth day of incubation period and increased mycelial growth was observed at 57.0 mg (Figure 2 a,b,c).

Trametes hirsuta

Trametes hirsuta on Orange G decolourization exhibits 54.0 mg dry weight after eight days of fungal treatment, the dye removal was gradually increased to 44.6% respectively. *Trametes hirsuta* with Methyl orange treatment shows 74.8% of colour removed from the aqueous solution within eight days of incubation; the dry weight of the mycelial was 54.0 mg at eighth day. In Congo red decolourization *Trametes hirsuta* results 88.7% of dye removal at eighth day along with mycelial dry weight was increased upto 57.0 mg/day (Figure 3 a,b,c).

Treatment of textile industry effluent in two modes

The textile industry effluents were decolourized by wood degrading fungi in batch mode and continuous flow mode were studied. In batch mode, the fungi had taken seven days for maximum removal of colour from the effluent. After seventh day *Ganoderma applanatum* removed 81.5 % of the effluent colour was shown in figure 4. The wood rot fungi *Inonotus dryadeus* and *Trametes hirsuta* shows the maximum colour reduction achieved at 83.9 and 89.2 % respectively on seventh day (Figure 5). According to the results, batch mode was most effective than continuous mode. In continuous mode 65.9% of colour removal was resulted in *Ganoderma applanatum* on seventh day. *Inonotus dryadeus* can decolourize the effluent 60.5% on seventh day. The colour removal of *Trametes hirsuta* was observed to be 75.3 % on 7th day (Figure 6). These industrial dyes were decolourized by extra cellular enzymes.

Decolourization of Remazol Brilliant Blue R was investigated using a new isolated white rot fungus, strain KRUS-G decolourized RBBR efectively compared to *Phanerochaete crysosporium* and *Ceriporiopsis subvermispora*. The highest decolourization was obtained at pH 4 with 89% loss of RBBR used (Tito sumandono *et al*., 2015). Lignin degrading white rot fungi, *Poria* sp. *Ganoderma* sp. and *Trametes* sp. were collected from the decayed wood of *Tectona grandis* from the Western Ghats of Tamilnadu, India. The fungi were used for the decolourization of azo dyes such as congored, rhodamine 6G, malachite green (Selvam *et al*., 2013).

Da Paz *et al*. (2012) reported that decolourization of dyes orange II and Black V by the fungi *Pycnoporus sanguineus* and *Trametes membranacea* was assessed at 6, 12 and 18 days, through fractional design, with a total of 16 trials. Microbial decolourization of azodyes by *Fomes lividus* was considered most efficient for decolourization of dye industry effluents (Selvam *et al*., 2003). *Pleurotus ostreatus* was used for decolourization activities of the dyes, basic blue 9, acid blue 29, congo red and dispersered1 (Fu and Viraraghavan, 2001). Selvam *et al*., (2003) reported the decolourization of azo dyes and dye industry effluent by the white rot fungi *Thelephora sp.* in liquid medium which effectively decolourize the dyes like Orange G, Congo red and Amido black 10 B and in both lab scale and pilotscale.

Trametes versicolor serves as a potential source for decolourization of industrial effluents by enzymatic degradation. The maximum decolourization upto 98.43% was studied in *Trametes versicolor* in fourth day treatment (Selvam *et al*., 2012). *Lenzites eximia* decolourize congo red by 95.50%, methyl orange and erichrome black-T by 94.79%, 95.36% respectively (Selvam and Shanmuga Priya, 2012). The methylene blue dye was highly adsorbed by *Inonotus dryadeus*, the wood rot fungi can separate engine oil, used engine oil and has high adsorption on certain effluents (Balaprasad Ankamwar, 2016).

Alternaria alternata CMERI F6 can decolourize 99.99% of 600 mg/L and 78% of 800 mg/L CR in 48 h, at maximal decolourization rate of18.01 mg/L h and 20.21 mg/L h, respectively (Samayita *et al*., 2013). A variety of chemical, physical and biological treatment methods are used to remove colour (Zhang *et al*., 2003). Man-made artificial dyes are lethal, mutagenic and carcinogenic (Chung & Stevens, 1993). White-rot organisms possess enhanced biodegradation. (Rivela *et al*., 2000). *Bjerkandera* sp. BOS55 and *Trametes versicolor* exhibit the utmost level of dye decolouration in agar plates. (Swamy and Ramsay, 1999)

CONCLUSION

From the present study it could be concluded, that the three wood degrading fungi namely *Ganoderma applanatum, Inonotus dryadeus* and *Trametes hirsuta* possess decolourization activity. Among these wood rot fungi *Inonotus dryadeus* could be employed for treating the azo dyes. *Trametes hirsuta* was found to be most effective fungus for decolourization of azodyes and to treat textile industry effluent could be recommended with batch mode. The dye decolourization depends on structure and complexity of dyes and the compounds were sensitive to enzymatic decolourization.

The wood rot fungi symbolize an eco-friendly and fewer pricey and being unconventional for the treatment of such effluents. The result justifies the applicability of the wood rot fungi in removal of azodyes from textile industry wastewaters and ensures the safe disposal. The textile industry is a significant user of water and produces enormous volumes of impure water. The vital contaminants of textile industry were resulted the noxious wastes. Microbial decolourization processes for the waste water management of textile wastewater have the benefit of being economic and eco-friendly especially producing lesser sludge.

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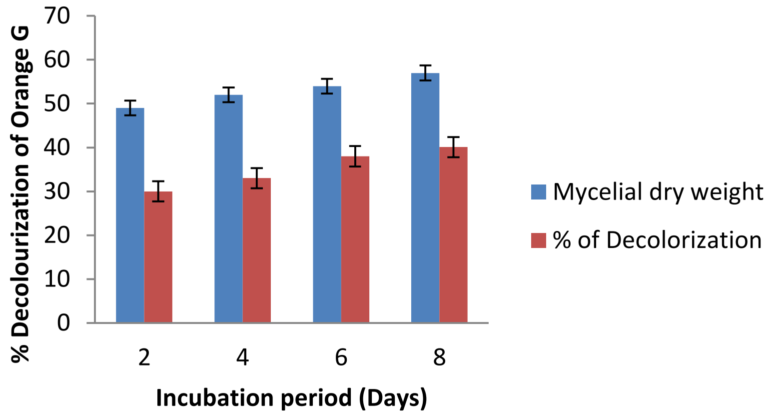
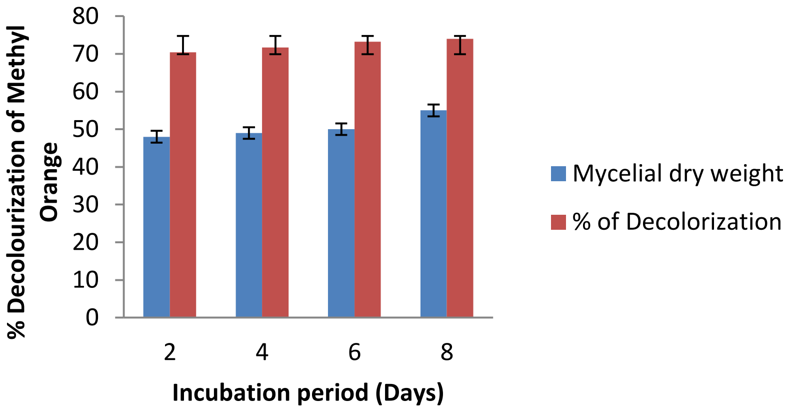
Figure 1: Removal of azo dyes from aqueous solution by *Ganoderma applanatum*

a



b



c

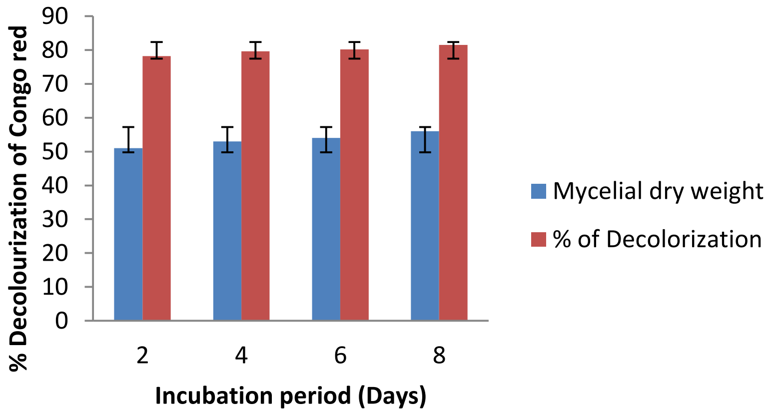
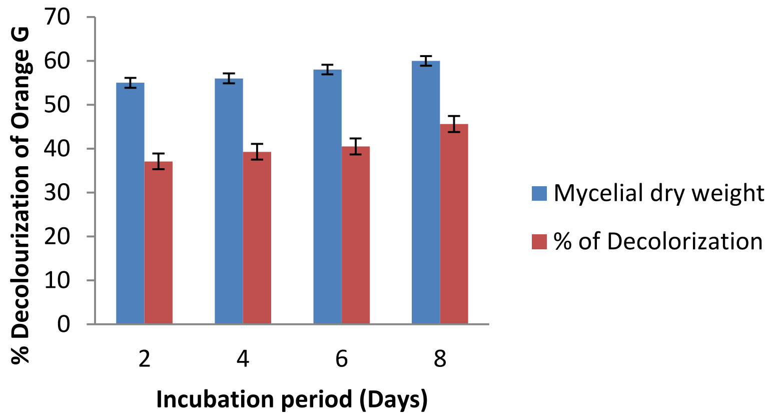


Figure 2: Removal of azo dyes from aqueous solution by *Inonotus dryadeus*

a



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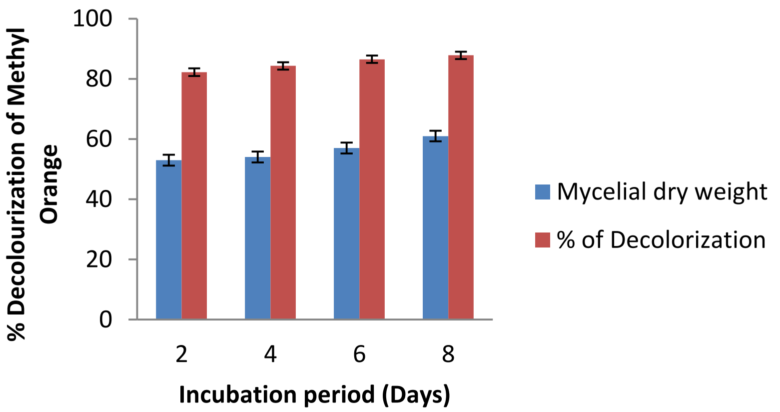

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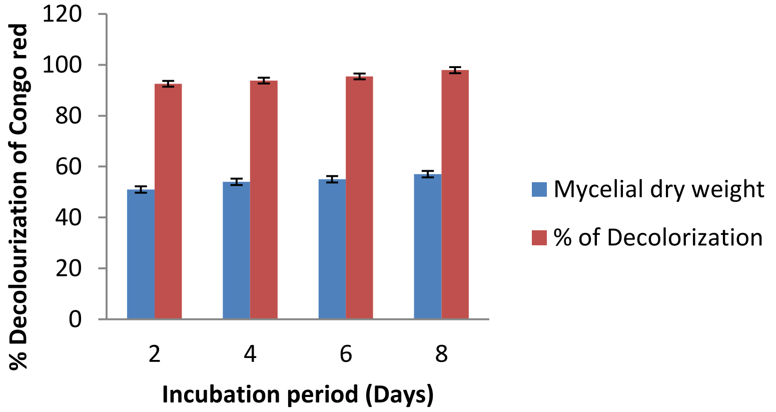
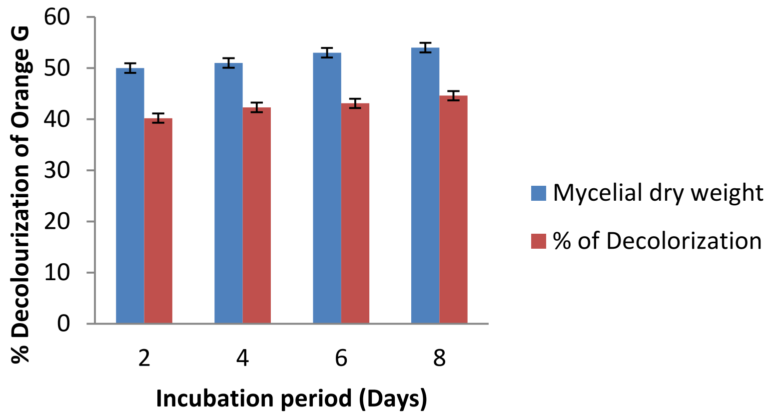


Figure 3: Removal of azo dyes from aqueous solution by *Trametes hirsuta*

a



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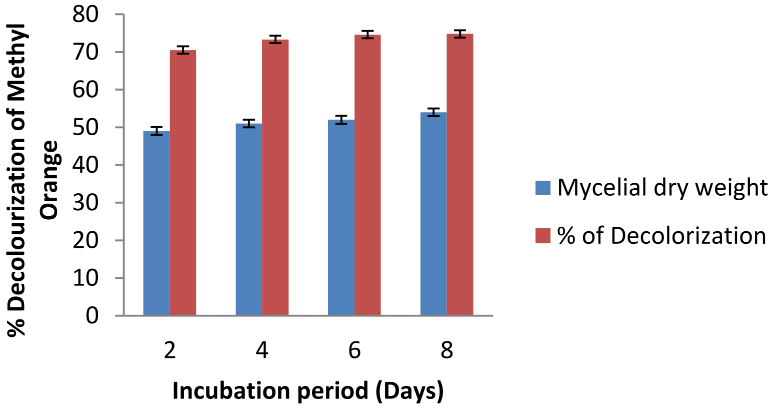

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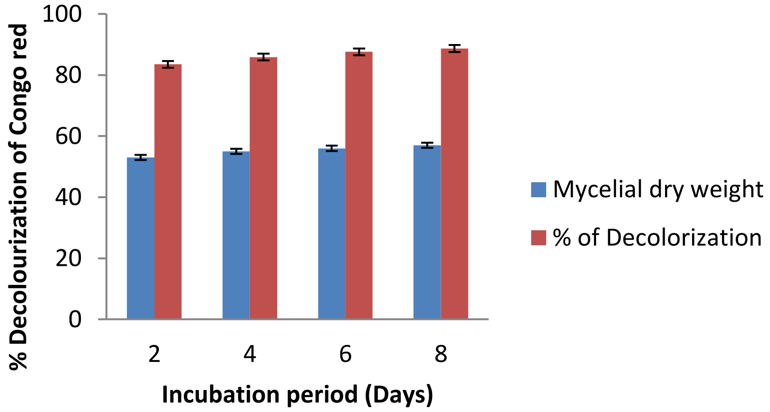

Figure 4: Decolourization of dye industry effluent by *Ganoderma applanatum*

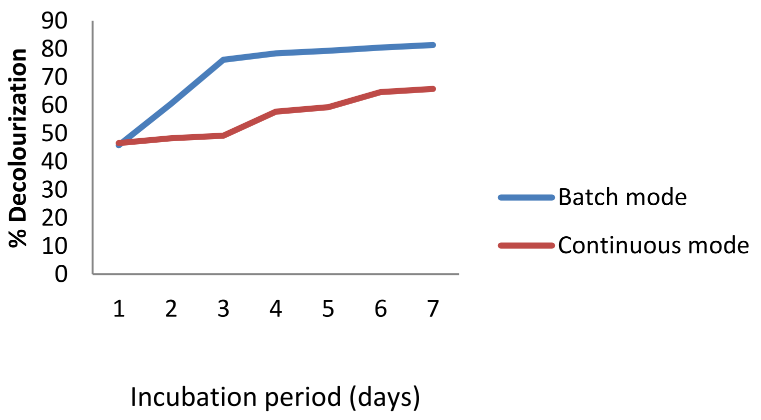

Figure 5: Decolourization of dye industry effluent by *Inonotus dryadeus*

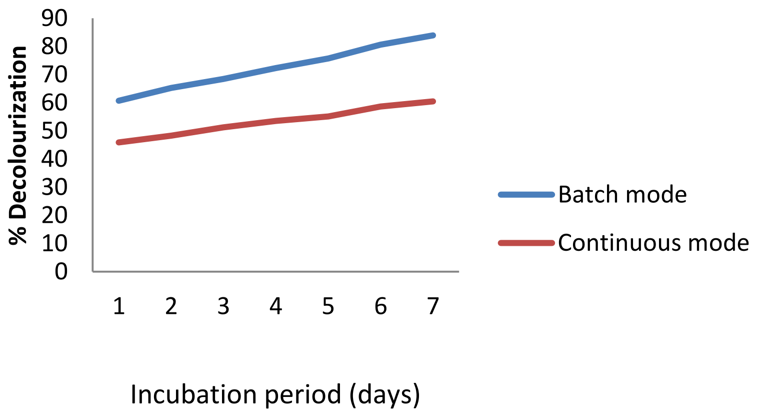

Figure 6: Decolourization of dye industry effluent by *Trametes hirsuta*

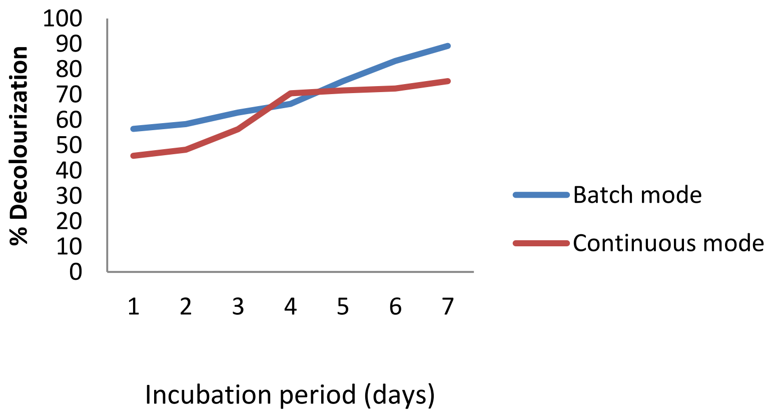

Plate 1: Wood rot fungi isolated from the study area

Ganoderma applanatum

Inonotus dryadeus

Trametes hirsuta

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