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Decolorization of AZO Dyes and Dye Industry Effluent by the Screening of Novel Wood Rot Fungi

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Abstract : The textile industries were producing a massive quantity of highly contaminated effluents. The emancipation of those effluents without a suitable treatment is an issue of severe concern due to their lethal impacts on receiving waters. The conventional technologies used for the wastewater treatment are inept or pricey in the treatment of textile effluents. The present research paper helps to identify new technologies to replace or to complement the accessible ones by the microbial decolorization of dye industry effluent by three wood-rot fungi. Three different wood rot fungi, *Daldenia concentrica, Lepiota sp.* And *Trametes serialis* were collected from the Western Ghats region of Tamil Nadu, India. The fungi were used to degrade the azo dyes such as orange G, methyl orange and congo red from aqueous solutions was determined by estimating the per cent of dye removal and the effect of dye concentration. The dye industry effluents were decolorized by ligninolytic fungi in batch mode and continuous flow mode. The results exhibit the colour removal by the basidiomycetes fungi were mainly due to adsorption of the dyes to the mycelial surface and due to metabolic breakdown. These results showed that *Lepiota sp.* was found to be the most effective fungus in decolourization of azo dyes especially orange G; *Daldenia concentrica* could be used for decolourization of methyl orange and *Trametes serialis* could be used for congo red for dye removal.*Trametes serialis*for batch mode and *Lepiota sp.*for continuous mode could be recommended.

Keywords : Azo dyes, Decolourization, *Daldenia concentrica, Lepiota sp., Trametes serialis.*

Introduction

Wood rot fungi can degradelignin and can arrange environmental pollutants by many of their extracellular ligninolytic enzymes[1].Dye is an integral part which is used to impart colour to materials. The waste generated during the process and operation of the dyes, contains the inorganic and organic contaminant leading to the hazard to ecosystem and biodiversity causing impact on the environment. The physico-chemical treatment does not remove the colour and dye compound concentration. The decolourization of the dye takes place either by adsorption on the microbial biomass and enzymatic degradation.Bioremediation takes place by anaerobic and aerobic process[2].

Dyes are chemical which bind to material and impart colour to that material. The colour of a dye is due to the presence of chromophore group. They are widely used to colour the substrate like textile fiber, paper, leather and hair, for plastic material, wax a cosmetic base and food stuff [3.]. Based on chemical structure of chromophore there are 20 - 30 different groups of dyes. Azo (Monoazo, diazo, triazo, polyazo) anthraquinone, phthalocyanine and triarylmethane dyes are the most important groups [4]. The majority of industrial important azo dyes belong to the following classes like acid dyes, basic dyes, direct dyes, mordant dyes, reactive dyes and

solvent dyes. The acid dyes, direct and reactive azo dyes are ionic dyes [5].

Azo dyes are the largest groups of synthetic aromatic dyes composed with one or more (N=N) groups and sulfonic (-SO_3-) groups with lots of commericial interest. Azo dyes contain one, two or three azo linkages, linking phenyl, naphthyl rings that are usually substituted with some functional groups including triazine amine, chloro, hydroxyl, methyl, nitro and sulphonate[6]. There are more than 10000 azo dyes which include Astrazon Red GTLN; Maxilon Blue GRL and sandolan yellow are widely used by the textiles, leather, cosmetics, food colouring and paper production industries[7-8]. About 80% of azo dyes are used in the dyeing process of textile industries and has been estimated approximately 10% of the dyes used in dying process do not bind to the fiber and are released into the environment [9].

The textile industry effluents are the great threat of the environment.Textile waste waters include various dyes which are hardly decolourized by conventional treatment system.Generally, these effluents are highly coloured with high biological oxygen demand (BOD) and chemical oxygen demand (COD). Dye decolourization through biological means has gained force of the cost effective and can be applied to wide range of dyes[10].

Materials and Methods

Collection of fungi

Thefungi *Daldenia concentrica*, *Lepiota sp.* and *Trametes serialis* were collected from decayed wood of Western Ghats area of Tamilnadu and Karnataka, south India. The collection site was situated in the latitude of 11.58°S and longitude of 76.93°E at 420 ± 50M MSL. It receives rain fall of about 300 cm per year with high humidity and even temperature.The collected fungi were isolated and identified based on the key providedpreviously[11-12]. 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)[13]. The spore suspension obtained from the malt agar plates were used as inoculums for further studies.

Preparation of spore suspension

The fungi were grown in malt agar medium. The pH was maintained as 6.5 at 37°C then the plates were flooded with sterile distilled water and brushed with camel hair brush smoothly without disturbing the mycelial growth 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 decolorization studies was carried out in C-limited medium (M 14) the spores were in the one-tenth volume of the medium were inoculated [14].

Screening of wood rot fungi for ligninolytic activity

The wood rot fungi were screened for their ligninolytic activity based on their ability to oxidize dyes, Poly R [15] and remazol brilliant blue. The fungi were also tested for their ability to degrade native lignin [16]and confirmation was done by the liberation of ethylene from 2-keto-4- thiomethylbutyric acid (KTBA).

Decolourization of azo dyes

The ability of the fungi to decolourize the azo dyes, orange G (50 µM), methyl orange (50 µM) and congo red (50 µM) were studied in C-limited medium. The medium was inoculated with fungal spore suspension (10^5spores/ml) and incubated at 30°C for 6 days in an orbital shaker. After 6 days, the dyesorange 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, 497 and 620 nm respectively fororange 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 azo dyes by wood rot fungi

The enzymes were used to degrade the azo dyes such as orange G, methyl orange and congo red from aqueous solution were determined by estimating the per cent of dye removal and the effect of dye concentration.

Decolourization of textile industry effluent

The ability of the wood rot fungi to remove colour from dyeing industry effluent was assayed in the modified C- limited medium, where instead of distilled water, the effluent amended medium (950 ml) was taken in the rotating biological contractor and inoculated with 50 ml of spore suspension (10^5spore /ml) and maintained at 39^o C. The samples were withdrawn at regular time intervals and analysed for colour removal. The intensity of effluent colour was measured at 490 nm.

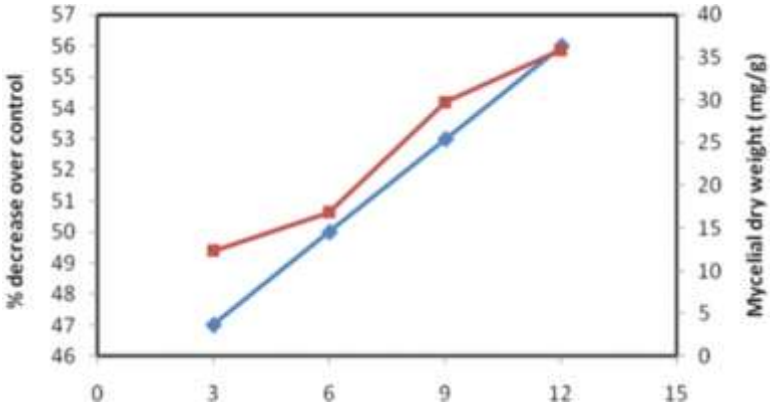
Results

Decolourization of azo dyes by ligninolytic fungi

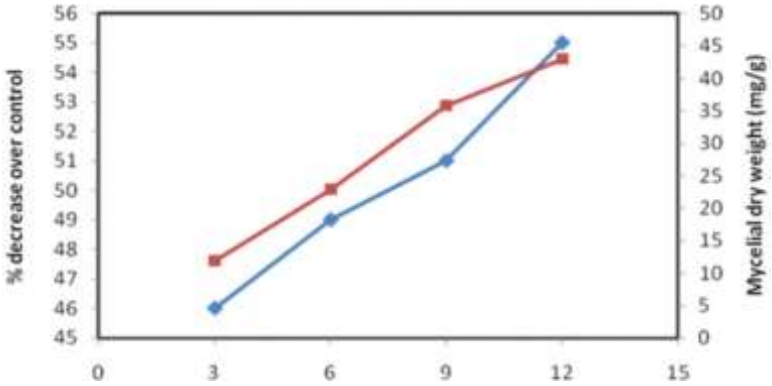
Orange G

The results presented in figure 1 revealed the effect of fungal treatment on orange G removal from aqueous solution. In all the three fungi shows the maximum dye removal was observed on twelth day of incubation and increase in mycelial growth was observed along with increase in incubation period. In *Daldenia concentrica* the mycelial growth was 56 mg dry weight in 35.88 % removal of twelth day incubation peroid, where as in *Lepiota sp*. and *Trametes serialis* it was 55 and 51 mg dry weight. After twelve days of fungal treatment, the dye removal was gradually increased from 43.00 % and 32.00% in *Lepiota sp*.and *Trametes serialis.*

a. Daldenia concentrica



b. Lepiotasp.



c. Trametes serialis

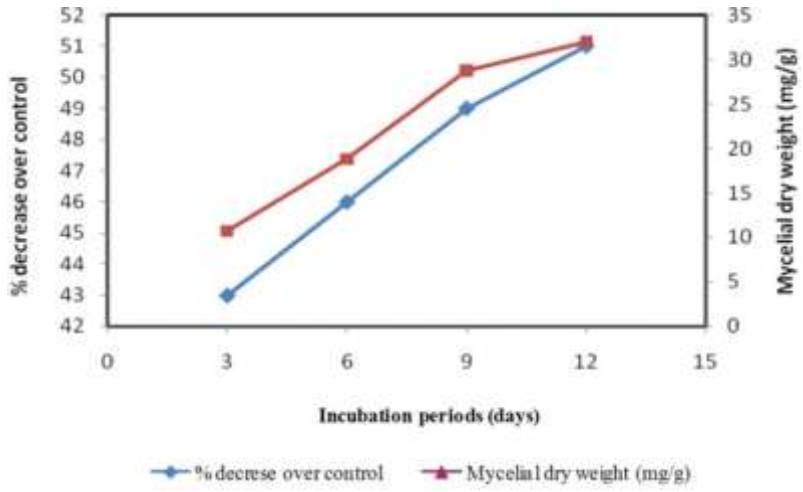
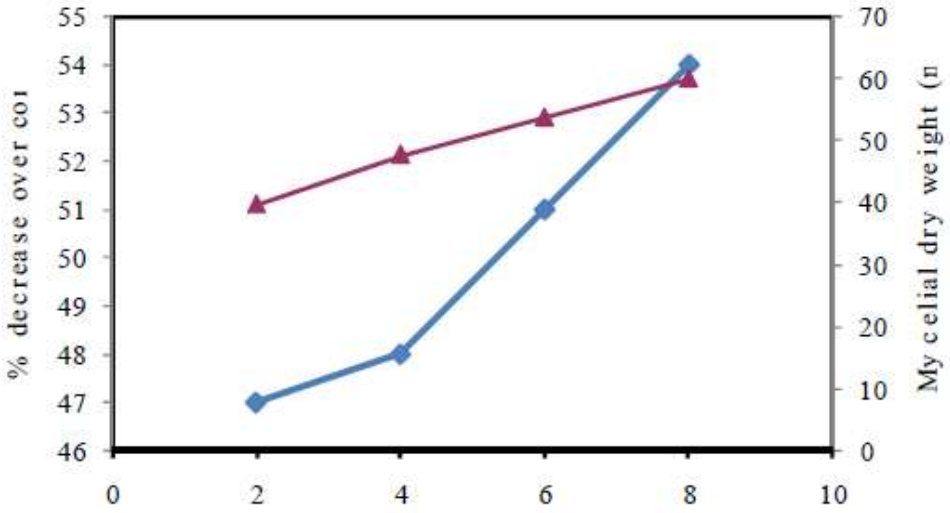
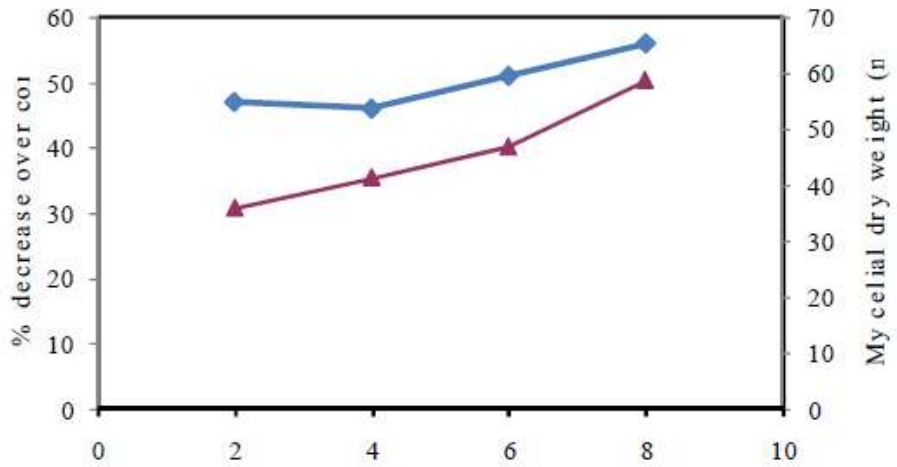


Figure 1: Effect of dye concentration Orange G (50µm) from aqueous solution by White rot fungi

a. *Daldenia concentrica*



b. *Lepiota sp.*



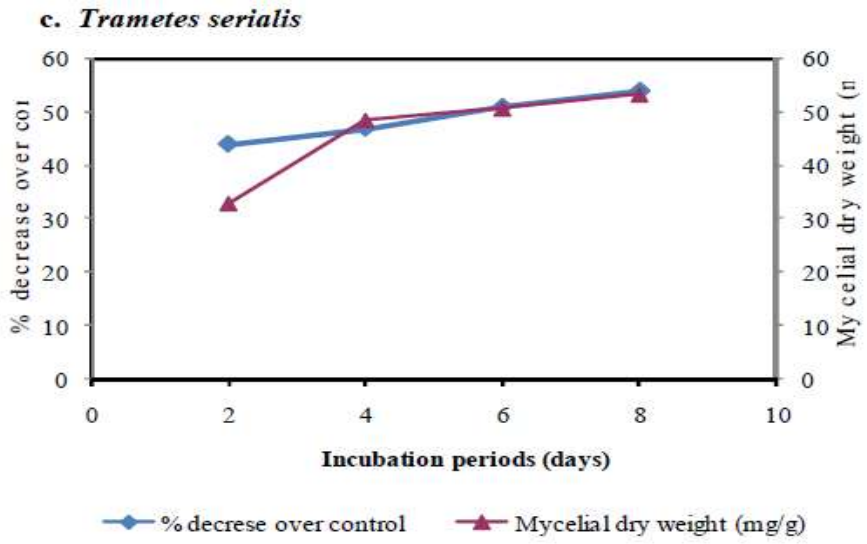


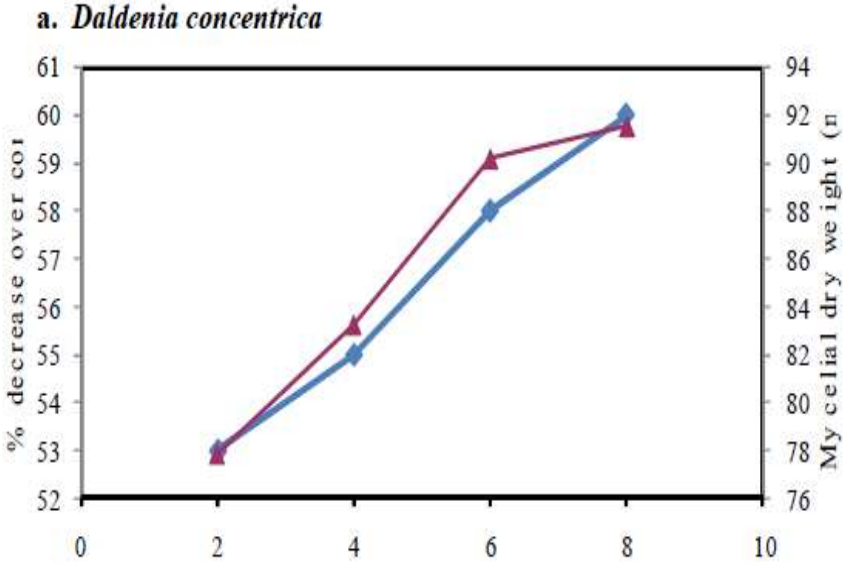
Figure 2: Effect of dye concentration Methyl Orange (50µm) from aqueous solution by White Rot fungi

Methyl orange

The removal of Methyl orange(50 µM) from the aqueous solution was represented in figure 2.In *Daldenia concentrica*, maximum of 60.00 % dye was removed after eight days of incubation period. The initial dry weight of the mycelial was 47.0 mg, at eigth day of dry weight was 54 mg whereas the mycelial dry weight was increased along with increased the dye removal. In *Lepiota sp*. was treated with methyl orange from aqueous solution showed maximum removal of dye upto 58.65 % at sixteenth day incubation, at the same day maximum mycelial growth was reached at 56 mg. In *Trametes serialis* treatment 53.28 % of methyl orange was removed from the aqueous solution within eight days of incubation; the dry weight of the mycelial was 54 mg at eighth day.

Congo red

The removal of congo red (50µM) from aqueous solution showed in figure. In *Daldenia concentrica* treatment eighth days of incubation showed 91.54 % dye removal and the mycelium growth was increased along with incubation period. At eighth day the mycelial dry weight was found to be 60 mg. In *Lepiota sp.* removal of 90.07 % dye was observed at eighth day of incubation period and increased mycelial growth was observed at 56 mg. In *Trametes serialis* the per cent of dye removal was observed to be 95.48 at eighth day along with mycelial dry weight was increased upto 54 mg.



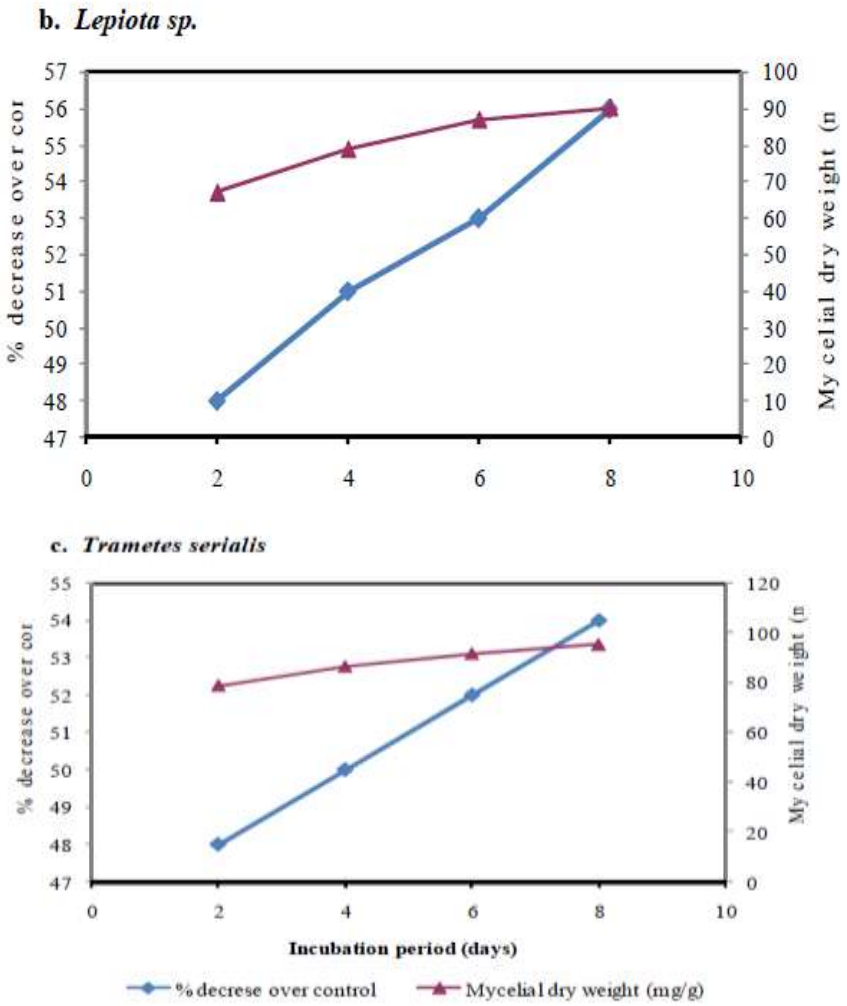
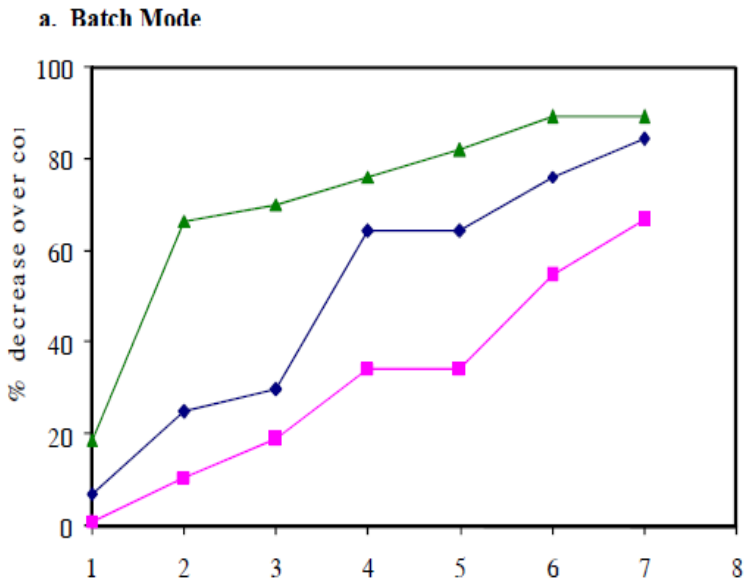


Figure 3: Effect of dye concentration Congo red (50µm) from aqueous solution by White Rot fungi



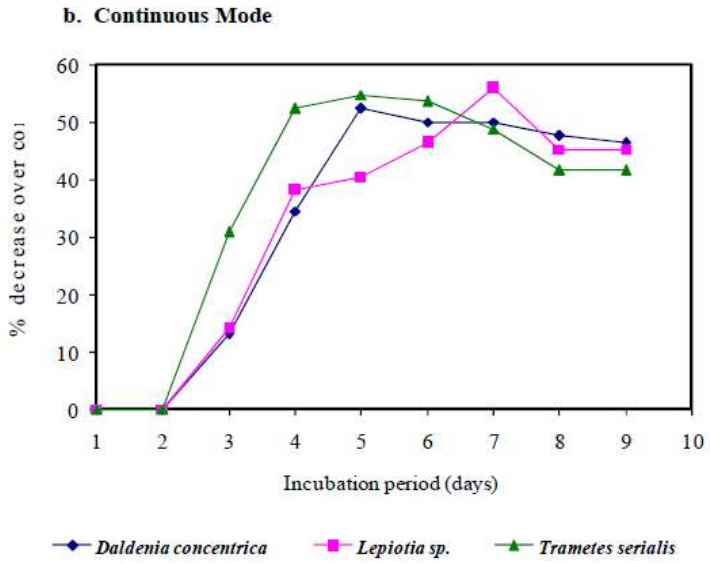


Figure 4: Effect of fungal treatment on colour removal in dye industry effluent by ligninolytic fungi

Treatment of textile industry effluent in two modes

The dye industry effluents were decolourised by ligninolytic fungi in batch mode and continuous flow mode and the results were presented in figure 4.In batch mode, the fungi had taken seven days for maximum removal of colour from the effluent. After seventh day *Daldenia concentrica* removed 84.52 % of the effluent colour in *Lepiota sp.* and *Trametes serialis* the maximum colour reduction were 66.67 and 89.28 % respectively on seventh day. According to the results, batch mode was most effective than continuous mode. In continuous mode colour removal was showed maximum in *Lepiota sp.*on seventh day 55.95 %. *Daldenia concentrica* decolourize the effluent 52.38 % on fifth day. The removal of *Trametes serialis* was observed to be 54.76 % on 5^{th} day.

Discussion

Daldenia concentrica results 35.88 % removal on the fungal treatment, the dyeremoval was gradually increased from 43.00 % in *Lepiota sp*. and *Trametes serialis* was observed at 32.00 % removal in orange G. The present study showed that removal of Methyl orange dye concentration of *Daldenia concentrica* was maximum of 60.04 % after eight days of incubation period. In *Lepiota sp*. was treated with methyl orange showed maximum removal of dye upto 58.65 % at sixteenth day of incubation. In *Trametes serialis* treatment 53.28 % of methyl orange was removed from the aqueous solution within eight days of incubation. *Daldenia concentrica*, treatment eight days of incubation showed 91.54 % dye removal and the mycelium growth was increased along with incubation period. In *Lepiota sp.* removal of 90.07 % dye and in *Trametes serialis*, the per cent of dye removal was observed to be 95.48 at eight day incubation while treated with congo red. Among these three fungi maximum decolourization of azo dyes and mycelial growth of all the three test fungi were favouredat twelfth day of incubation and *Lepiota sp* efficiently removed orange G (43.00 %). *Daldenia concentrica* had efficiently removed methyl orange at eighth day of incubation (60.00 %)and *Trametes serialis* (95.48%)removed congo red most effectively.

In thepresent study, the dye industry effluent was treated with ligninolytic fungi for colour removal in batch mode and continuous mode. The results showed (Fig 4) that in batch mode treatment; *Daldenia concentrica* removed 84.52 % of the effluent colour, in *Lepiota sp.* and *Trametes serialis* showed the maximum colour reduction upto 66.67 and 89. 28%. In continuous mode colour removal was showed maximum by *Lepiota sp.*on seventh day 55.95 %. *Daldenia concentrica* decolourize the effluent 52.38 % on fifth day. The removal of *Trametes serialis* was observed to be 54.76 % on 5th day.According to this results, batch mode was

most effective than continuous mode.

The maximum decolourization of azo dyes and mycelial growth of all the three test fungi were favoured at twelth day of incubation and *Lepiota sp*. efficiently removed orange G. In methyl orange, all the three wood rot fungi were favoured at 8thday of incubation. *Daldeniac oncentrica* had efficiently removed methyl orange. *Trametes serialis* (95.48%)removed congo red efficiently.

The dye industry effluents were treated for colour removal in batch mode and continuous mode. In batch mode, the fungi take seven days for maximum removal of colour from the effluent. *Trametes serialis* removed the maximum colour reduction upto89.28 % on seventh day. In continuous mode colour removal was showed maximum by *Lepiota sp.*on seventh day of 55.95 % respectively.

Lignin degrading wood rot fungi, *Poria* sp. *Ganoderma* sp. and *Trametes* sp. were collected from the decayed white of *Tectona grandis* from the Western Ghats of Tamilnadu, India. The fungi were used for the decolourization of azo dyes such as congo red, rhodamine 6G, malachite green[17].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[18].

Aspergillus orhraceus could remove 100% of reactive blue-25 within 7 days [19]. *Ganoderma austral* and *Pleurotus ostreatus sp.*3 removed 93.4% and 66.25% of poly R-478 dye in 18 days respectively, where as *Polyporus sp*.2 removed the dye upto 86.53% in 18 days [20]. *Cunnighamella elegans* was used for the decolourization of orange II dye and it removed 88% of azobased reactive group in 96hours [21]. *Fomes lividus* could remove 30.8% of Orange G, Congo red and Amino black 10B removed 74.0 and 98.9% respectively. In Batch mode maximum decolorization was achieved of 84.4% on 4th day whereas in continuous mode the decolorization was 37.5% on 5th day [22]. The decolourization of congo red, fast blue RR salt, methyl violet in six days [23]. *Pleurotus sp* were decolourize orange G and remazol brilliant blue R after 12-18 days [24]. *Armillaria sp*. removes 65% of reactive black 5 and 70% Remazol Brilliant blue in 96 hours [25]. *Fusarium oxysporum* remove 100% of yellow GAD within 144hours[26]. *Coriolus versicolor* degraded 85% of Acid orange II [27]. Thus the microbial decolorization of these novel fungi showed potential control over industrial effluents and other compounds.

Conclusion

The wood rot fungi symbolize an eco-friendly and fewer pricey and being unconventional for the treatment of such effluents. The results justify the applicability of the wood rot fungi in removal of azodyes from textile wastewaters and their safe disposal.The textile industry is a significant user of water and produces enormous volumes of impurewater the vital contaminants in noxious wastes are azo dyes. Microbial decolorizationprocesses for the waste water management oftextile wastewater have the benefit of being cost-effective and eco-friendly and producinglesser sludge.

From the present study it could be concluded, that the three wood rot fungi namely *Daldenia concentrica, Lepiota sp.* And *Trametes serialis* possess ligninolytic activity. These wood rot fungi could be employed for treating the textile industry waste water. *Lepiota sp.*could be used for treating dye like orange G;*Daldenia concentrica* could be used for methyl orange and *Trametes serialis* could be used for congo red.*Trametes serialis* for batch mode and *Lepiota sp*. for continuous mode could be recommended.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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