Bioremediation of Textile Dyes by White Rot Fungi Isolated From Western Ghats Area

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Abstract— ***Lignin degrading white rot fungi, Phanerochaete chrysosporium, Schizophyllum commune and Lenzites eximia were isolated from the logs of Acacia nilotica, Tarmarindus indica, Eucalyptus grandis from the Western Ghats region of Karnataka, India. The fungi were used for the decolourization of azo dyes such as Acid orange 7, Methly red, and Evans blue. . P. chrysosporium 787 removed 94.8 per cent of acid orange 7 50 µM concentration. In S .commune removed 96.7 per cent of Acid orange 7 from aqueous solution. When the dye was treated with L. eximia maximum (95.5 %) dye removal was observed. For methyl red, P. chrysosporium 787 treatment maximum of 92.2 per cent dye was removed. In S. commune treatment, 98.5 per cent of methyl red was removed. The per cent of methyl red removal from aqueous solution by L. eximia was observed up to 96.0 per cent. In Evans blue, P. chrysosporium 787 treatment, resulted in 90.2 per cent dye was removed. When the dye was treated with S. commune, 97.4 per cent dye was removed. In L. eximia treatment, the per cent of dye removal was observed 95.5 per cent. All the above results were obtained on fourth day of incubation. Maximum decolourization of azo dyes and mycelium growth of all the three test fungi were favoured on fourth day of incubation. The fungus S. commune efficiently removed acid orange 7 (96.7), methyl red (98.50 %) and evans blue (97.4 %) from the effluent when compared with the other two fungi.***

Keywords— ***Acid orange 7, Methyl red, Evans blue and Effluent***

I. INTRODUCTION

Synthetic dyes are widely used in the textile, paper, cosmetics, leather, dyeing, colour photography, pharmaceutical and food industries. In textile industries, during dyeing process, about 10 to 30 per cent or more of the dyes are released into water bodies causing serious environmental problem in many parts of the world. The main common property of dyes is to absorb light due to the chromophore, a part of the molecule responsible for its colour. The colour arises when a molecule absorbs certain wavelengths of visible light and transmits or reflects the others.

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However, the variation in the structure is enormous and many thousand different dyes are produced for commercial use. In general, dyes can be classified according to their chemical structure, particularly chromophore, and the method of application

Azo pigments are colourless particles, which have been coloured using an azo compound. Depending upon the number of azo groups present they are called as monazo, diazo, triazo, tetraazo and polyazo dyes [1].Commercial dyes have a great variety of colours, high stability to light, temperature, detergent and microbial attack. The colours in industrial effluents cause environmental concern, but they are not toxic, however many azo dyes constituting the largest dye group is decomposed into potential carcinogenic amides under anaerobic conditions after discharge into effluent [2]. Several amino-substituted azo dyes including 4-phenylazoaniline and N-methyl and N, N-dimethyl 4-phenylazo anilines are mutagenic as well as carcinogenic. In mammals azo dyes are reduced to aryl amines by cytochrome p 450 and a flavin-dependent cytosolic reductase [3]. Most of the dyes currently used could be degraded and removed by physical and chemical processes and sometimes the degradation products are more toxic. The azo dyes exposure can reduce fertility. When male and female mice were exposed to Acid orange 7 (II), uterogonadal developments in both sexes were adversely affected [4]. Azo dyes generally contain one or more sulphonic groups on aromatic rings which might act as detergents and inhibit the growth of microorganisms. But it has been reported that microorganisms are capable of degrading azo dyes and could be used in effluent treatment plants for removal of these dyes. Microbial decolourisation has been proposed as a less expensive and less environmentally intrusive alternative. Various bacteria and fungi have decolourising abilities and an extensive review of microbial decolourization is available [5] [6].

Many species of white rot fungi have been reported to decolourize textile dyes containing azo dyes. The sequential adsorption and degradation of dye molecules on living fungal hypae may provide a mechanism for feasible application of white rot fungi in a continuous treatment of industrial effluent. [7] first attempted that ligninolytic cultures of *P. chrysosporium* was able to decolourize several polymeric dyes. The biodegradation of dyes by white rot fungi and their ligninolytic enzyme system offers an advantage over other processes because of their ability to completely mineralize various dyes to CO_2 and H_2O. Enzymes such as lignin peroxidase (LiP), manganese dependent peroxidase (MnP) and laccase have been reported to decolourize dyes [8]. Lignin peroxidase, laccase and polyphenol oxidase (PPO) of white rot fungi degrade many aromatic compounds due to their non specific activity [9]. Laccase are mutlicopper enzymes, which catalyse the oxidation of wide range of

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inorganic and organic substances, using oxygen as electron acceptor [10]. Swamy and Ramsay (1999) reported that white rot fungi secrete extracellular ligninases that could degrade the azo dyes.[11] reported the ability of white rot fungus, *P. chrysosporium* and its enzymes to decolourise Orange II in liquid systems, besides role of enzymatic activity on colour removal. [12] reported the decolourization of azo dyes and dye industry effluent by a white rot fungus *Thelephora sp*. [13] suggested the binding of dyes to fungal hypae, physical adsorption and enzymatic degradation by extracellular and intracellular enzymes as major mechanisms for the colour removal. As a result of the studies on the physical adsorption and the enzymatic degradation of the adsorbed dye molecules, the enzymatic degradation of adsorbed dyes has been regarded as a major mechanism in the regeneration of dye adsorption capacity of the mycelium. [13] reported the microbial decolourization of azo dyes and dye industry effluent by *Fomes lividus*. [14] reported decolourisation of Orange II by immobilized thermotolerant white rot fungus *Coriolus versicolor* RC3 in packed-bed bioreactor. The decolourisation of Remazol brilliant blue R by laccase from the spent compost of *Lentinus polychrous* has also been reported [15]. The rate of decolourisation for crystal violet, tryphan blue, amido black, acid orange 7 and RBBR dyes by enzymes from spent mushroom compost (SMC) has been reported [16]. [17] reported the decolourization of synthetic dyes by the white rot fungi *Coriolus versicolor* and *Funalia trogii*. [18] reported the biodegradation of textile azo dyes by the white rot fungus *Phanerochaete chrysosporium*. [19] reported the decolourization of textile dyes by the white rot fungi *T.versicolor*. Sathiyamoorthi et al. (2006) reported that the two species of white rot fungi were evaluated for their ability to decolourize the dyes Blue CA, Black B133, Corazol violet SR by the white rot fungi *T. hirusta* and *Pleurotus florida*. [20] reported the bioreactor designs for industial effluent decolourization from white rot fungi in submerged and immobilized liquid cultures. [21] screened eighteen fungal isolates for their potential to decolourize the commercial used reactive textile dyes, reactive orange 96, reactive blue 15, reactive blue 38 and reported that only few organisms of white rot fungi such as *T. versicolor, Bjerkandera adusta and P. chrysosporium* were able to decolourize all the dyes.[22] reported the decolourization of synthetic dyes by *T. hirsuta* in expanded-bed reactors. Decolourization of two azo dyes namely Direct Red–80 and Mordant Blue–9 by *P. chrysosporium* was investigated both individually and in mixtures in batch shake flasks**.** [23] compared the degradation of a polymeric dye (polyR-478) by 127 strains to estimate the peroxidase activity, the correlation between the polymeric dye decolourization and the peroxidase activity was pointed out.[24] also studied the enzymatic properties of 90 white-rot fungi strains. [25] reported that 115 fungi from different physio-ecological groups were compared for their capability to decolorize anthroquinone and azo dyes. [26] reported that in liquid culture, *Dichomitus squalens* decolourized both methylorange and RBBR efficiently. The early step in azo dyes decolourization 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.

II. MATERIALS AND METHODS

White rot fungi were collected from various sources like decaying wood logs, tree trunks and tree stumps. Mostly fruit bodies were collected from the forest of Western Ghats area of Karnataka, India. The collection sight was situated in the latitude of -11.58,°S and longitude of 76.93°E at 400 ± 50M MSL. It receives rain fall of about 600 mm per year with high humidity and temperature. Mostly fresh fruit body was collected from the woody substrate, when the fruit bodies were not available the part of the host plant showing the fungal growth were collected. The exterior surface of the fruit body was sterilized with 1 per cent mercuric chloride solution repeatedly washed with sterile distilled water and inoculated on 2 per cent malt agar medium plates. The plates were incubated at 37°C. Further subculturing were done and pure cultures were obtained .

Malt agar medium

Malt extract	- 20 g
Agar	- 20 g
Distilled water	- 1000 ml
pH	- 6.5

A. *Preparation of spore suspension*

The fungi were grown in malt agar plates for 6 days at 37°C. Then the plates were flooded with sterile distilled water and brushed with camel hair brush smoothly without disturbing the mycelial growth. The suspension was filtered over a sterile filter system to remove the mycelia fragments and the concentration of the filtered spore suspension was adjusted to 10^5 spores/ml and inoculum is used for dye decolourization studies.

B. *Decolourization of azo dyes*

The ability of the fungi to degrade the azo dyes, acid orange 7, methyl red and evans blue, were studied in C-limited medium. C-limited medium was inoculated with fungal spore suspensions (10^5 spores/ml) and incubated at 30 °C for 6 days in an orbital shaker. After six days, the dyes were added. The samples were withdrawn at regular times intervals, filtered and the optical density of the clear filtrate was measured in a spectrophotometer at 497 nm, 465 nm and 479 nm respectively for Acid orange 7, Methyl red and Evans blue. Samples were withdrawn at regular time intervals and analysed for total colour removal.

III. RESULTS AND DISCUSSION

A. *Acid orange 7*

In the present study three fungi were studied for the removal of acid orange 7 from aqueous solution. The results were tabulated in Table 1.

The control value was observed to be 0.214 at 497 nm. The fungus *P. chrysosporium* 787 removed 94.8 per cent of 50 µM dye from the aqueous solution within fourth day of incubation period. Mycelial growth also increased up to 54.0 mg on fourth day. Data in figure 1 showed that at 25 µM concentration 94.5 per cent of the dye was removed. Dye above 100 µM concentration became toxic to the mycelium growth.

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In *S. commune* fourth day of incubation period, increased the mycelial dry weight (60 mg) removed 96.7 per cent of acid orange 7 from aqueous solution. In Data in figure 1 showed that at 25 µM concentration 97.9 per cent of the dye was removed.

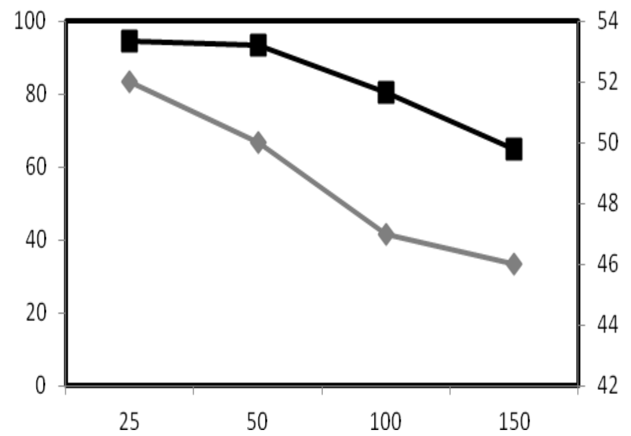
When the dye was treated with *L. eximia* maximum (95.5%) dye removal was observed at four days of incubation period. The mycelial dry weight was observed to be 56.0 mg on fourth day of incubation. The Data in figure 1 showed that at 25 µM concentration 95.8 per cent of the dye was removed.

Table 1: Removal of Acid orange 7 (50µM) from aqueous solution by white rot fungi
Control value = OD 0.214 at 497nm

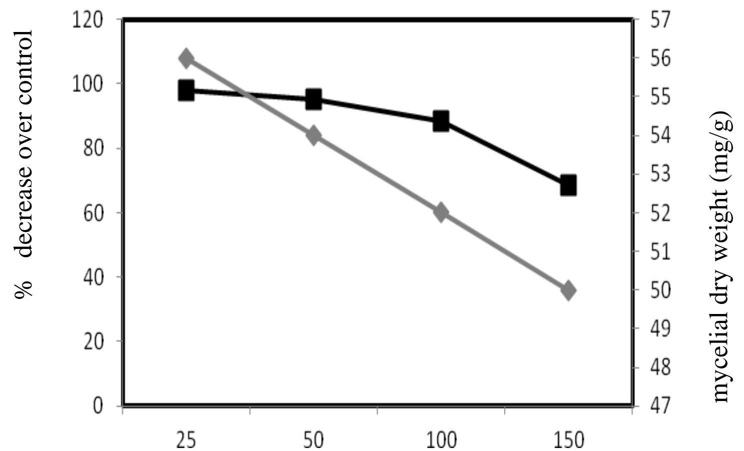
Incubation period (days)	Mycelial growth (mg dry weight)	Optical densit
P. chrysosporium 787		
1	40	0.095 (55.1)
2	45	0.018 (90.6)
3	50	0.010 (93.9)
4	54	0.010 (94.8)
S. commune		
1	42	0.086 (60.0)
2	58	0.012 (93.9)
3	58	0.010 (95.3)
4	60	0.007 (96.7)
L. eximia		
1	44	0.085 (60.0)
2	54	0.019 (90.1)
3	56	0.010 (92.5)
4	56	0.009 (95.5)

Fig. 1: Effect of dye concentration (Acid orange 7) on decolourization by white rot fungi

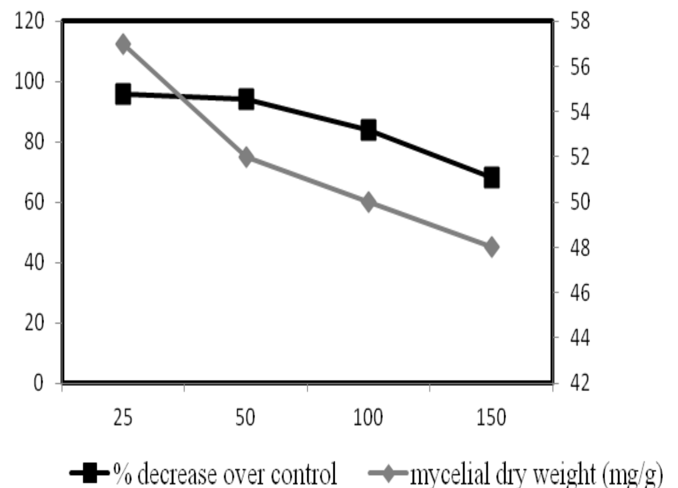
a. *P. chrysosporium* 787



b. *S. commune*



c. *L. eximia*



B. *Methyl red*

The removal of methyl (96.5)(50 µM) from the aqueous solution was presented in Table 2.

In *P. chrysosporium* 787 treatment maximum of 92.2 per cent dye was removed after fourth day of incubation period. The initial dry weight of the mycelium was 35.0 mg, at fourth day the dry weight was 56.0 mg. Data in figure 2 showed that at 25 µM concentration 93.8 per cent of the dye was removed, the maximum mycelium growth was 56.0 mg. Dye above 100 µM concentration became toxic to the mycelium growth.

In *S. commune* treatment, 98.5 per cent of methyl red was removed from the aqueous solution within fourth day of

incubation; the dry weight of the mycelium was 62.0 mg at fourth day. Data in figure 2 showed that at 25 µM concentration, 96 per cent of dye was removed maximum mycelium growth was 52.0 mg. Dye concentration maximum at 100 µM become toxic to the mycelium growth.

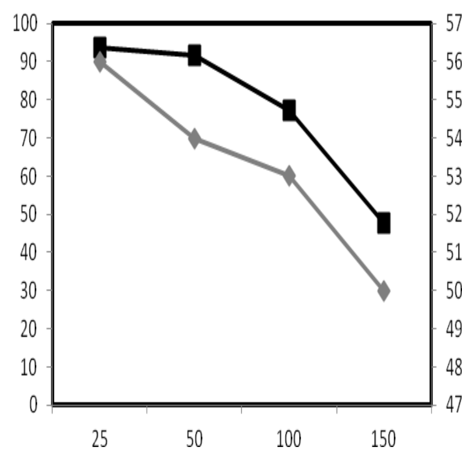
The per cent of methyl red removal from aqueous solution by *L. eximia* was observed up to 96.0 per cent at fourth day of incubation. The growth of mycelium was increased up to 58 mg at fourth day. Data in figure 2 showed, among 95.2 per cent of dye was removed at 25 µM concentration, the mycelial growth was maximum at 57.0 mg. Dye above 100.0 µM concentration become toxic to the mycelial growth.

Table 2: Removal of Methyl red (50µM) from aqueous solution by white rot fungi
Control value: OD 0.220 at 465nm

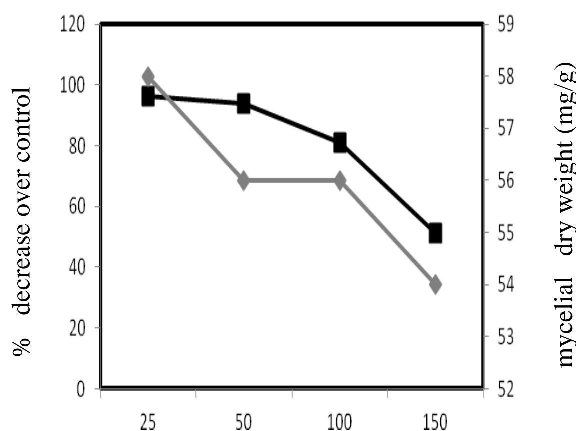
Incubation period (days)	Mycelial growth (mg dry weight)	Optical density
P. chrysosporium 787		
1	35	0.104 (53.1)
2	50	0.035 (83.6)
3	56	0.020 (88.6)
4	56	0.017 (92.2)
S. commune		
1	38	0.091 (59.0)
2	61	0.014 (93.6)
3	62	0.011 (95.0)
4	62	0.006 (98.5)
L. eximia		
1	38	0.096 (55.9)
2	56	0.020 (90.9)
3	58	0.014 (93.1)
4	58	0.012 (96.0)

Fig. 2: Effect of dye concentration (methyl red) on decolourization by white rot fungi

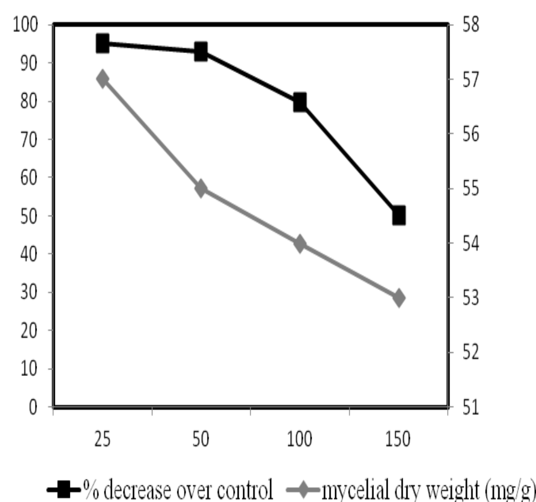
a. *P. chrysosporium 787*



S. commune



b. *L. eximia*



C. Evans blue

The results tabulated in Table 3 and figure 3 showed that the removal of Evans blue (50 µM) from aqueous solution. (94.7)

The control value was found to be 0.225 at 503 nm. In *P. chrysosporium* 787 treatment, four days incubation resulted in 90.2 per cent dye removal and the mycelial growth was increased along with incubation period. At fourth day the mycelial dry weight was found to the 53.0 mg. At 25 µM dye concentration, per cent removal was 90.1 and mycelia growth was observed 56.0 mg dry weight.

When the dye was treated with *S. commune*, 97.4 per cent dye was removed at fourth day of incubation period and increased

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mycelial growth was observed (58 mg). At 25 µM dye concentration, per cent removal was 97.0 and mycelia growth was observed 58.0 mg dry weight.

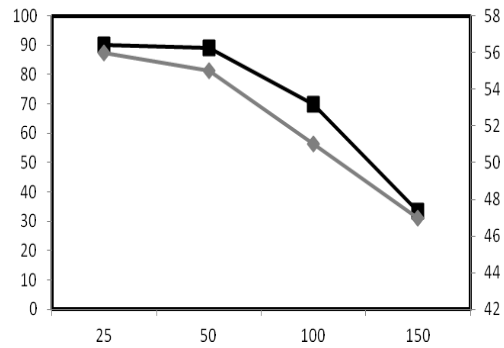
In *L. eximia* treatment, the dye removal was observed 95.5 per cent at fourth day along with mycelial dry weight was increased up to 54 mg. At 25 µM dye concentration, per cent removal was 95.3 and mycelial growth was observed 56.0 mg dry weight.

Table 3: Removal of Evans blue (50µM) from aqueous solution by white rot fungi
Control value = OD 0.225 at 503 nm

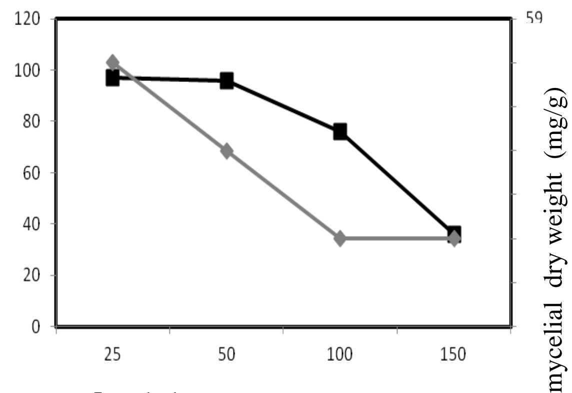
Incubation period (days)	*Mycelial growth (mg dry weight)*	*Optical dens*
P. chrysosporium **787**		
1	33	0.089 (60.4)
2	51	0.036 (84.0)
3	52	0.030 (86.6)
4	53	0.026 (90.2)
S. commune		
1	36	0.080 (64.4)
2	56	0.015 (93.3)
3	56	0.008 (96.4)
4	58	0.006 (97.4)
L. eximia		
1	37	0.083 (63.1)
2	50	0.018 (92.0)
3	53	0.012 (94.6)
4	54	0.010 (95.5)

Fig. 3: Effect of dye concentration (Evans blue) on decolourization by white rot fungi

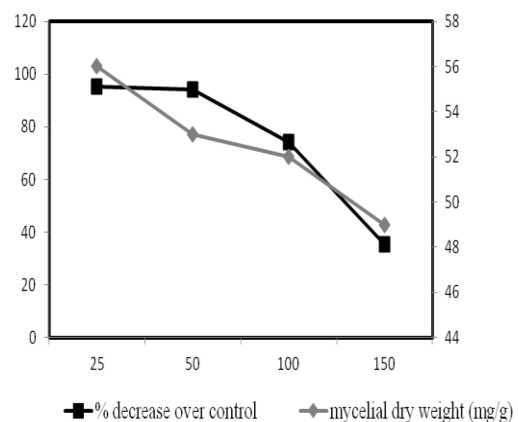
a. P. chrysosporium 787



b. *S. commune*



c. L. eximia



White-rot basidiomycetes are a group of fungi capable of depolymerizing and mineralizing otherwise not easily degradable lignin with their extracellular and non-specific ligninolytic enzymes. In the 1980s, this fact stimulated research on the ability of ligninolytic fungi to degrade organic pollutants [27], [28]. It was established that Phanerochaete chrysosporium is capable of biodegrading various pollutants and it soon became a model white-rot fungus with most of the research done up to now. The enzymes produced with this fungus are lignin peroxidase (LiP) and manganese peroxidise (MnP) [29], [30]. In the next decade, a few new species of white-rot fungi like *Pleurotus ostreatus* and *Trametes versicolor* [31], [33] were characterized for the dye degradation. A more intense research with *Irpex lacteus* [34] and *Bjerkandera adusta* [35], [36] started in the last decade, while the interest in the decolourization capability of *Ceriporiopsis subvermispora* [37], [38].

In the present study three fungi were studied for the removal of Acid orange 7, methyl red and Evans blue from aqueous solution. The results were tabulated in Table 1, 2 and 3

respectively. *P. chrysosporium* 787 removed 94.8 per cent of acid orange 7 50 µM concentration. In *S .commune* removed 96.7 per cent of Acid orange 7 from aqueous solution. When the dye was treated with *L. eximia* maximum (95.5 %) dye removal was observed. For methyl red, P. *chrysosporium* 787 treatment maximum of 92.2 per cent dye was removed. In *S. commune* treatment, 98.5 per cent of methyl red was removed. The per cent of methyl red removal from aqueous solution by *L. eximia* was observed up to 96.0 per cent. In Evans blue, *P. chrysosporium* 787 treatment, resulted in 90.2 per cent dye was removed. When the dye was treated with *S. commune*, 97.4 per cent dye was removed. In *L. eximia* treatment, the per cent of dye removal was observed 95.5 per cent. All the above results were obtained on fourth day of incubation. Maximum decolourization of azo dyes and mycelium growth of all the three test fungi were favoured on fourth day of incubation. The fungus *S. commune* efficiently removed Acid orange 7 (96.7), Methyl red (98.50 %) and Evans blue (97.4 %) from the effluent when compared with the other two fungi.

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