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Decolourisation of synthetic textile dyes by using *Calocybe indica*

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Abstract
The present study reveals the potentials of macrofungi namely *Calocybe indica* for decolourization of dye. Azo dyes are released in large quantities into the environment from textile industries. These dyes are recalcitrant to microbial degradation, causing problems in the usual biological treatment of the industrial effluents. Pollution is particularly associated with the reactive azo dyes, because of their strong colour which leads to aesthetic problems and obstructs light penetration and oxygen transfer into water bodies. Biomass production varied with different fungal isolates in the decolurization of dyes. The decolorization of viscose orange-A was found to be 78.54 and 94.72%, with *Calocybe indica* -1 and *Calocybe indica* -2 respectively after 15 days of incubation. The decolorization of direct green PLS, was found to be 81.56% and 66.41 with *Calocybe indica* -1 and *Calocybe indica* -2 respectively after 15 days of incubation. Among the two fungi, *Calocybe indica* -1 produced the maximum fungal biomass. Fungal isolates showed a highly correlation between the dry weight of the fungi and colour removal percentage.

Keywords: *Calocybe indica*, decolorization, azo dyes, effluent

Introduction
Synthetic dyes are colouring agents mainly used in textile industries which generate a huge amount of wastewater in the process of dyeing. A very small amount of dye in water (10-50 mg L) affects the aesthetic value, transparency of water and gas solubility of water bodies. The effluents from these industries are complex, contain a wide variety of dyes and the other products such as dispersants, acids, bases, salts, detergents, humectants, etc. The toxicity of effluent is because of the presence of a dye or its degraded products which are mutagenic or carcinogenic (Kalyuzhnyi and Sklyar, 2000) [5]. Various kinds of physicochemical methods are in use for the treatment of wastewater contaminated with dye. Various methods were employed but they are not ecofriendly, cost effective and hence become commercially unattractive. Over the past decades many microorganisms have been found to be capable of degrading dyes, these include bacteria, fungi, yeasts, actinomycetes and algae.

Fungal lignolytic enzymes system, manganese perooxidase may also involved in the bio oxidation of dyes. However the requirement of low ph for enzyme activity and the long hydraulic retention time for complete degradation are major disadvantages in using fungi. Thus large scale applications using filamentous fungi for decolourization have been limited.

Compared to bacteria and filamentous fungi, yeasts have many advantages. They not only grow rapidly like bacteria but like filamentous fungi, they also have the ability to resist unfavourable environments. Furthermore, some yeasts have been found to be efficient in treating high-strength organic wastewaters, such as food industry effluents. They can decolorize and even completely mineralize many Azo dyes under certain environmental conditions including medium composition (Ramya *et al*., 2010) [9].

Medium optimization using statistical designs was recently used for the decolorization of dyes (Pavan *et al*., 2005) [6]. Response surface methodology (RSM) is an efficient experimental strategy to determine optimal conditions for a multivariable system rather than optimization by the conventional method which involves changing one independent variable while keeping the other factors constant. These conventional methods are time-consuming and incapable of detecting the true optimum, especially the absence of interactions among factors.

Optimization of conditions for maximum removal of textile dye by statistical approach has been planned to determine the exact conditions for removal by edible mushroom which would be useful for industrial applications. Hence, the present study was aimed to examine the most influential variables for maximum decolorization of textile dye through Plackett - Burman design using an edible mushroom.

Materials and methods
The present investigation was carried out at Poultech Agro Research Centre, Namakkal during December 2016-march-2018.Direct azo dyes were used in this study. The dye samples were commercially graded and kindly supplied by the dealers of Global Dyes, Salem. Direct azo dyes used in this research are, Viscose Orange – A (λm = 480 nm), Direct Green PLS (λm = 580 nm) and Reactive blue R (λm = 580 nm).

Sample collection
Spent mushroom waste was collected from milky mushroom (*Calocybe indica*) production unit Namakkal in sterilized polypropylene bags maintained around 4°C and

processed within 12 hr of collection during the month of January 2018. Each sample were collected in sterile polyethylene bags and brought to the laboratory.

Isolation of mushroom fungi

A suitable portion of spent mushroom was cut with sterile scalpel surface sterilized with 0.1% solution of mercuric chloride and finally washed with sterile distilled water to remove the antiseptic. After soaking dry using sterile filter paper, the small portion of waste was cut and a surface layer peeled off under aseptic conditions. Subsequently, a small bit of the inner tissue was removed using a sharp forceps and the mycelial plugs were aseptically transferred to Sabouraud's Dextrose Agar SDA plates. The plates were incubated around 25°C for 6-8 days to get the pure culture of the isolate.

Maintenance of culture

The fungal isolates were maintained on Sabouraud's Dextrose Agar (SDA) slants.

Screening of fungal isolates

The suspension of 4 days old cultures of fungi were used to investigate their abilities to decolourize dyes. They were prepared in saline solution (0.85% Sodium chloride). The fungal cultures were inoculated into 50 ml of saline and incubated at room temperature for 5 hours (Benson, 1994).

Dye decolourization experiments (Saranraj *et al*., 2010) [10]**.**

The three textile dyes namely Viscose orange – A, Direct green PLS and Reactive blue R were used for the dye decolourization studies. Dye decolourization experiments were carried out in 250 ml flask containing 500 ppm of azo dyes in Czapek-Dox broth. The pH was adjusted to 7.3 ± 0.2 using Sodium hydroxide and Hydrochloric acid solution. The autoclaved flasks were inoculated with 5 ml of fungal inoculums of each isolates. The flasks were kept in mechanical shaker at room temperature for 15 days.

Samples were drawn at 5 days intervals for observation. 10 ml of the dye solution was filtered and centrifuged at 5000 rpm for 20 minutes. Decolourization was assessed by measuring absorbance of the supernatant with the help of UV Spectrophotometer at wavelength maxima (λm) of respective dye.

Decolourization assay

Decolourization assay was measured in the terms of percentage decolourization using UV Spectrophotometer. The percentage decolourization was calculated from the following equation,

$$\% \text{ Decolourization} = \frac{\text{InitialOD} - \text{FinalOD}}{\text{InitialOD}} \times 100$$

Measurement of fungal my celial dry weight (El-Rahim and Moawad, 2010) [3]**.**

The inoculated flask for dye decolourization was removed after specific incubation periods and the contents were filtered through pre weighed Whatmann No-1 filter papers which were dried in an oven at 105°C for 48 h. The dried filter papers along with mycelium were re-weighed. The fungal biomass were calculated according to the following formula

Weight of mycelium= Weight of filter paper with mycelium – weight of filter paper.

Toxicity study

Phytotoxicity tests were carried out in order to assess the toxicity of azo dyes and metabolites formed after decolourization of azo dyes by efficient fungal isolates. Phytotoxicity tests were carried out at a final concentration of 500ppm on two kinds of seeds. One from grains *Sorghum vulgare* (monocot) and second from pulses *Vigna mungo* (dicot), commonly cultivated. Phytotoxicity was conducted at room temperature (10 seeds of each) by watering separately 5ml sample of control dyes and its degradation products per day. Control set was carried out using distilled water at the same time. Germination % was recorded after 10 days (Kalyani *et al*., 2008) [4].

18s rRNA based identification and phylogenetic relationship

The multi drug resistant *E. coli* AS-13 was selected based on antibiotic resistant pattern. *E. coli* AS-13 genomic DNA was isolated and PCR amplified with 16S rDNA. Electrophoretical analysis of PCR products obtained from the amplification of 16S rDNA genes confirmed that full length (1207bp) genes were amplified for *E. coli* AS-13 (Plate; Figure 1). The Molecular Weight was 366348.00 Daltons in single stranded and 734901.00 Daltons in double stranded. The G+C content was 55.51% and A+T content was 44.49% while 306 number of adenine with 25.35 mol %, 284 number of cytosine with 23.53 mol %, 386 number of guanine with 31.98 mol % and 231 number of thymine with 19.14 mol % were found (Figure 2).

The amplified product was sequenced and sequence of DNA fragment was compared to the sequences available in Gen Bank, NCBI. Sequence analysis of these isolates was also performed using BLAST (blastn) search tool (http: //www.ncbi.nlm. nih.gov) available on the NCBI homepage. The MDR *E. coi* AS-13 strains used in the study exhibited 96 to 98% sequence similarity to the *E. coi* available in NCBI database. Theses sequence data has been deposited in the Gen Bank (Submission number: SUB2484782) as the detailed below in figure.

The phylogenetic tree generated by a weighted neighbor-joining (Figure) method clearly revealed the evolutionary relationship of the strain AS-13 to a group of *E. coi*. Thus, this strain was designated as *E. coi* AS-13.

Results

Spent mushroom waste was collected from milky mushroom (*Calocybe indica*) production unit Namakkal during the month of January 2017.

Isolation and identification of fungi

Calocybe indica was in creamy white even growth on SDA plate.

Decolourization of textile dyes

The three textile dyes namely Viscose orange – A, Direct

green PLS and Reactive blue R were used for the dye decolourization studies. Table-1 revealed that different fungal species showed 78.54 to 94.72 % decolourization of viscose orange –A with the maximum on 15 days after incubation. The range of decolourization activity was found to be 78.54 and 94.72%, with *Calocybe indica* -1 and *Calocybe indica* -2 respectively after 15 days of incubation. The dry matter production was the maximum in *Calocybe indica* -1 (294 mg) followed by *Calocybe indica* -2 (271 mg) after 15 days of incubation.

Similarly in Direct green PLS, the range of decolourization activity was found to be 81.56% and 66.41 with *Calocybe indica* -1 and *Calocybe indica* -2 respectively after 15 days of incubation Table-2. In biomass production, the maximum was obtained in *Calocybe indica* -1 (336mg) followed by *Calocybe indica* -2 (202mg) after 15 days of incubation. In Reactive blue R, the maximum decolourization was given by the fungi *Calocybe indica* -1 (82.58%) followed by *Calocybe indica* -2 (74.62) after 15 days of incubation Table-3. The range of biomass production was found to be 306 and 268 mg with *Calocybe indica* -1 followed by *Calocybe indica* -2 respectively after 15 days of incubation.

Phytotoxicity study of dye

The degraded metabolites of dyes obtained by *Calocybe indica* -1was used for toxicity study (Table 4). The phytotoxicity study was carried out at room temperature of *Sorghum vulgare and Phasealus mungo* has revealed considerable decrease in growth in presence of dye when compared with dye degraded metabolites and control. Similarly results were observed in germination (%) of both *Sorghum vulgare and Phasealus mungo* seed was found to be less.

In *Sorghum vulgare*, Viscose Orange – A affected both germination and growth. 30% seeds only germinated with dye alone and exhibited 70% with decolourized metabolities. 42 and 44 % of germination observed with direct green PLS and Reactive blue R and its degraded metabolites. In the aspect of growth, the maximum growth was noticed in control followed by degraded product of dye and dye (500ppm).

Phasealus mungo was highly sensitive to dye and its degraded products. When compared with control (90%) germination, 20% germination was noticed with orange – A, 25% in direct green PLS and 22% in reactive blue R (500ppm). The maximum germination (68%) was exhibited in direct green PLS degraded product followed by orange – A (60%) and reactive blue (55%). In the aspect of growth, the maximum growth was noticed in control followed by degraded product of dye and dye (500ppm).

Table 1: Decolourization of viscose orange-a and biomass production by fungi

S. No	Name of the fungi	5days		10 days		15days	
		% of DC*	MDW**	% of DC*	MDW**	% of DC*	MDW**
1	*Calocybe indica* -1	34.82	192	79.94	228	94.72	294
2	*Calocybe indica*-2	28.68	183	55.07	215	78.54	271

* Decolourization; ** Mycelial Dry Weight (mg)

Table 2: Decolourization of direct green pls and biomass production by fungi

S. No	Name of the fungi	5days		10 days		15days	
		% of DC*	MDW**	% of DC*	MDW**	% of DC*	MDW**
1	*Calocybe indica* -1	32.09	192	74.65	239	81.56	336
2	*Calocybe indica*-2	24.18	168	54.82	184	66.41	202

* Decolourization; ** Mycelial Dry Weight (mg)

Table 3: Decolourization of reactive blue r and biomass production by fungi

S. No	Name of the fungi	5 days		10 days		15 days	
		% of DC*	MDW**	% of DC*	MDW**	% of DC*	MDW**
1	*Calocybe indica* -1	28.56	182	64.53	228	82.58	306
2	*Calocybe indica*-2	24.35	148	59.64	205	74.62	268

* Decolourization; ** Mycelial Dry Weight (mg)

***Calocybe indica* 18S rRNA, partial sequence**

10 20 30 40 50 60 70 80 90
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

AAGAGTTTGAGTCGAGAGTGATCTTGGCGCTTACACATCCGAGTCTATGTCTCTTCATATCATTTTACTC
TGTGTATAAGAATGTCTTCT

100 110 120 130 140 150 160 170 180
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

AAGGCATTATTAATGCCTTTAAATCATATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGA
AGAACGCAGCGAAATGCGAT

190 200 210 220 230 240 250 260 270
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AGGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCC
GAGGAGCATGCCTGTTTGAG

280 290 300 310 320 330 340 350 360
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TGTCATGAAATTCTCAACCTTTGTTACTTTCTTGTTTCAAAGAGTCTGGAAGTGGAGGTTGCTGGCTATT
GTTGAAAGAGTCGGCTCCTC

370 380 390 400 410 420 430 440 450
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TGAAATACATTAGTGGGACCCATCGTTGATTAGCTCCCTGGTGTGATAGTTATCTACGCCGTGGCTCATC
ACGATATTGTGTGGTTCAGC

460 470
....|....|....|....|....|
TCTCTAACGAGACACAACAACTGTCs

Fig 1: Partial sequencing of *Calocybe indica* -1 18S rRNA, partial sequence

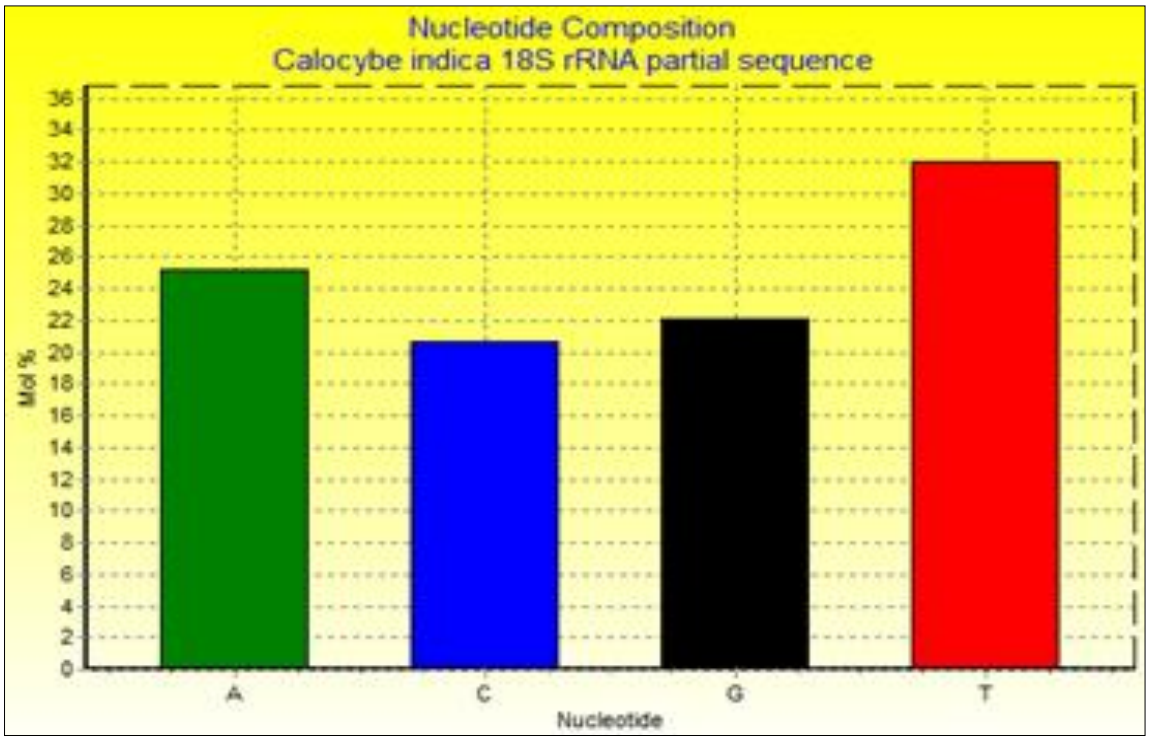

Fig 2: Nucleotide molecular percentage of *Calocybe indica* -1 18S rRNA, partial sequence

Discussion

Many physical and chemical treatment techniques are effective for colour removal but use more energy and chemicals than biological processes. In recent years, a number of studies have focused on some microorganism which is to biodegrade and bio sorb dye in wastewaters. A wide variety of micro -organisms capable of decolourizing a wide range of dyes include some bacteria, fungi, yeast. Among micro organisms, fungi represent an inexpensive and promising material for removal of the azo dye- reactive blue from textile dye effluents. Fungal systems appear to be a most appropriate biological agent in the treatment of colored and metallic effluents. In recent years, several adsorbents have been identified as possessing good dye-binding capabilities (Pavan *et al.,* 2008) [7]. Therefore *Pleurotus* sp. is one of the promising eco-friendly tools for the dye containing an effluent treatment of textile industries. Against these backdrops, this study was aimed to evaluate the efficiency of mushroom fungal isolates on the degradation of various azo dyes. *Calocybe indica* was isolated from spent mushroom waste which were collected from a mushroom retailer of Namakkal during the month of January 2018. *Calocybe indica* was in creamy white even growth on SDA plate and designated as *Calocybe indica* -1 and *Calocybe indica* -2. The three textile dyes namely Viscose Orange – A, Direct green PLS, and Reactive blue R were used for the dye decolourization studies.

Decolourization ability of the selected white-rot fungi such as *P. florida* LCJ 65, *P. ostreatus* LCJ 183 and *P. sajorcaju* LCJ 184 were compared to investigate their potential to decolourize the three textile dyes such as Bromophenol blue, Brilliant green and Methyl red. The selected *Pleurotus* sp. was able to completely decolourize all three dyes within 10-14 days of incubation (Radhika *et al*., 2014) [8]. In the present investigation, the decolourization of viscose orange –A was found to be 78.54 and 94.72%, with *Calocybe indica* -1 and *Calocybe indica* -2 respectively after 15 days of incubation. The decolorization of Direct green PLS, was found to be 81.56% and 66.41 with *Calocybe indica* -1 and *Calocybe indica* -2 respectively after 15 days of incubation. In Reactive blue R, the maximum decolourization was given by the fungi *Calocybe indica* -1 (82.58%) followed by *Calocybe indica* -2 (74.62) after 15 days of incubation. In the present study, *Calocybe indica* -1was found to efficient fungal isolate which was used for further study.

Biomass production varied with different fungal isolates in the decolurization of dyes. The dry matter production was the maximum in *Calocybe indica* -1 (294mg in viscose orange – A, 336mg in direct green PLS mg; 306 mg in reactive blue R) followed by *Calocybe indica* -2 (271 mg in viscose orange – A, 202 mg in direct green PLS mg; 268mg in reactive blue R) after 15 days of incubation. Among the two fungi, *Calocybe indica* -1 produced the maximum fungal biomass. Fungal isolates showed a highly correlation between the dry weight of the fungi and colour removal percentage.

Sorghum vulgare and Phasealus mungo have revealed a considerable decrease in growth in the presence of dye when compared with dye degraded metabolites and control. Similarly, results were observed in germination (%) of both *Sorghum vulgare and Phasealus mungo* seed was found to be less. In *Sorghum vulgare*, Viscose Orange – A affected both germination and growth. 30% seeds only germinated with dye alone and exhibited 70% with decolourized metabolites, 42 and 44 % of germination observed with direct green PLS and Reactive blue R and its degraded metabolites. The maximum germination (68%) was exhibited in direct green PLS degraded product followed by orange – A (60%) and reactive blue (55%) in *Phasealus mungo.* Similarly, results were observed in germination (%) and growth of both *Triticum aestivum* and *Phaseolus mungo* by the degraded metabolites of Direct Violet 51 and Tatrazine (Chaube *et al.*, 2010) [2].

The present study reveals the potentials of macrofungi namely *Calocybe indica* for decolourization of dye. The results would stimulate interest and investigations into the development and adoption of ecofriendly, biological treatment of colored dye from effluents and some other sources.

Conclusion

In conclusion, the decolorization of textile dyes using *Calocybe indica* were successfully studied. Between the two isolates *Calocybe indica* -1 were found to be more effective against decolorization of azo dyes. *Calocybe indica* -1 was found to efficient fungal isolate for decolorization of dye and biomass production this studies concluded that the bioremediation process is ideal to reduce dyes toxicity with low-cost and environmentally friendly. Mushroom fungal decolorization is a promising alternative to present treatment processes. However, using *Calocybe indica* -1 strains to remove colour in dye is still in the research stage. Further research is needed to establish the relationships between dye molecule structure and fungal decolorization.

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