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Decolourization of Textile Dyes by Ligninolytic Fungi Isolated from Spent Mushroom Substrate

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ABSTRACT
The aim of study was to executive the efficiency of white rot fungi, Schizophyllum commune (DMRF-7) and Pezizomycotina sp.(DMRF-8) for higher decolourization of Rhodamine B and Methyl violet 2B (MV2B). Both the potential fungi isolated from spent mushroom substrates of Pleurotus spp. were identified by using 5.8S rRNA gene sequencing followed by BLASTn technique. The decolourization potential of both fungi was evaluated at different temperatures, pH values, ligninolytic enzyme activities and biomass production during decolourization process. The activities of laccase, manganese peroxidase and lignin peroxidase enzymes were estimated to reveal the roles of enzymes in dyes decolourization. The dye decolourization process was confirmed by UV-visible spectroscopy and functional group analysis was performed by Fourier transform infrared spectroscopy (FTIR). The most significance of this work to efficiently first time utilize Pezizomycotina sp. for decolourization of Rhodamine B and Methyl Violet 2B. Both fungi is useful for bioremediation of textile dyes.
Keywords: *Decolourization , Azo dyes , Fungi , Ligninolytic enzymes, Pezizomycotina sp.*

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INTRODUCTION

Waste effluents from the textile industry can be considered as the most polluting wastewater and their treatment is greatly challenging for their safe discard. Approximately, around 280,000 tons of dyes are discharged worldwide annually by these textile and allied industry into the environment [1]. During the dyeing process, about 10–15% of the dyes used are released into the wastewater. The presence of these dyes in the aqueous ecosystem causes serious environmental and health concerns [2]. The coloured wastewater is aesthetically objectionable, it deteriorate the water quality of receiving water bodies. It also reduces light penetration into the water body and consequently photosynthetic activities, lower gas solubility and all of which causes acute toxic effect on aquatic fauna and flora. Increasing use of different dyes led to enhance the pollution in the form of textile wastewater. Therefore, the discharge of highly coloured dyes effluents from those industries can result in serious environmental pollution problems [3]. Among dyes, Azo class contains aromatic compounds and are the most demandable class of synthetic dyes used in textile industry applications . Azo dyes are also considered as xenobiotic compounds that are very recalcitrant to biodegradation [4]. Several methods are used to treat textile effluents to achieve decolourization of textile dyes and their wastewater. Various physicochemical methods such as coagulation, filtration, carbon activated and chemical flocculation of synthetic dyes and wastewater. Physiochemical methods are effective but they are expensive and involve the formation of a secondary sludge problem [5].

Bioremediation is a popular and attractive technology that utilizes the metabolic potential of microorganisms to clean up the environment from the pollutants [6]. Recently, biological processes are getting more attention since it is environment friendly, cost effective, and produce less secondary sludge [5]. The removal of dyes and other organo-pollutants by bacterial communities often results from adsorption [7] which leads to concentration of recalcitrant aromatic toxicants in the sludge. The

degradation levels of these compounds in the sludge can be increased by filamentous fungi especially white-rot fungi and with the help of their active mycelia [8]. White rot fungi are efficient in the biodegradation of recalcitrant compounds like dyestuffs, xenobiotics and lignin by their extracellular ligninolytic enzyme system [9]. Their extracellular enzymes including manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase being nonspecific and attacks a wide variety of complex aromatic dyestuffs [10]. Most of white rot fungal strains seem to produce laccase as main enzyme during the dye decolourization process.

This study presents the role of *Schizophyllum commune* and *Pezizomycotina* sp. for decolourization of textile dyes. These fungal isolates were also studied for ligninolytic enzyme activities, biomass production, temperature and pH values of medium for decolourization of dyes.

MATERIALS AND METHODS

Dyes and Chemicals

Rhodamine B (C_{28} H_{31} N_2O_3Cl) and Methyl violet 2B ($C_{25}H_{30}ClN_3$) were procured from Sigma-Aldrich (Fig.1). All other chemicals were of analytical grade and obtained from Merck and Hi-media labs, Mumbai, India.

Fungal Strains

Spent substrate of *Pleurotus* spp. was collected from Directorate of Mushroom Research, Solan (HP), India and used for isolation of fungal isolates (DMRF-7 & DMRF-8). Fungi were isolated using the serial dilution and plating method on Potato dextrose agar (potato dextrose - 24 g, agar agar - 20 g, distilled H_2O - 1000 ml, pH - 7.2) and incubating at 30±2 ˚C for 6 days. Well isolated colonies were further purified by growing on respective media and stored thereafter at 4 ˚C for further use.

Screening of Potential Fungal Isolates

Initially, both strains were screened for laccase activity using guaiacol as indicator compound in agar plate. Intense reddish brown colour in the presence of guaiacol was appeared in the medium around the fungal colonies and was taken as the positive reaction for the presence of laccase. Indentification and screening of laccase producing fungus was done on plates using Potato dextrose agar supplemented with 0.02% guaiacol and was incubated at 30 ˚C for a period of seven days [11]. Both fungal strains showed definite colour changes and considered laccase producing strains which further selected for synthetic dyes decolourization.

Dye Decolourization on Agar Plate

Fungi were inoculated as an agar plug (4 mm of diameter), taken from the edge of an actively growing colony, in the middle of Petri dishes containing 25 ml of potato dextrose agar media, supplemented with each dyes at a final concentration of 100 ppm. The experiment was performed in triplicates and plates were incubated at 30±2 ˚C and after 1, 4, 8, 12 and 16 days, mycelia growth and decolourization was measured. Since the fungal colonies determined a widespread loss of dye over the whole surface of the plate, it was not possible to measure the extent of a halo of discolouration.

Phylogenetic Determination by 5.8S rRNA Gene Sequence

Both fungal cultures were grown separately on Malt extract agar plates at 30 ± 2 ˚C for 7 days. The mycelia from respective fungus plates was scrapped and placed at -85 ˚C at least for 2 h, followed by freeze drying for 16-18 h. The genomic DNA was extracted from approximately 100 mg of freeze dried fungal mycelia by crushing in 1.5 ml micro-centrifuge tubes. QIAGEN DNeasy Plant Mini Kit (QIAGEN GmbH, D-40724 Hilden) was used for DNA extraction as per the protocol supplied by the manufacturer. The polymerase chain reaction (PCR) primer ITS-1 and ITS-4 developed by White et al.,1990 [12] were used to amplify the ITS region of 5.8S rRNA gene. PCR amplification was performed in a reaction mixture of 50 µl, containing 5 µl 10X PCR buffer (250 mM KCl, 100 mM Tris-HCl, pH-8.3, 15 mM $MgCl_2$), 0.2 µl *Taq* DNA polymerase (5 U μl^{-1}), 1 µl each of ITS-1 and ITS-4 primers (0.01 mM), 5 µl dNTP mix (2.0 mM each), 1 µl glycerol (5%), 2 µl of genomic DNA (50 ng) and 2 µl $MgCl_2$ (25 mM). PCR reaction was performed in PCR Master Cycler Gradient in 36 cycles each of 95˚C for 1 min, 50˚C for 30 sec, 72˚C for 1 min 20 sec and final elongation at 72˚C for 10 min with lid heating option at 104˚C. PCR amplified amplicons was ascertained on 2.0% agarose gel (w/v) prepared in 1% TBE. Gel electrophoresis was carried out at 90 V for 1.35 h in 0.5X TBE buffer. Finally, staining was done with ethidium bromide and the gel was visualized using Bio Imaging System (Gene Genius, Syngene).

PCR Products Sequencing and Blasting

PCR amplified amplicons of 5.8S rDNA were cleaned up by using RCB kit (Banqiao City, Taipei County 220, Taiwan) for removing undesired DNA fragments, if any. Cleaned PCR products were got sequenced using 3730Xl (96 capillary) electrophoresis instruments from Bioserve Biotechnologies, Hyderabad, India. Cleaned and improved consensus sequences were blasted using BLASTn of NCBI

(http://blast.ncbi.nlm.nih.gov/Blast) and the species considered as identified against which highest total score was exhibited.

Ligninocellulosic Substrates

For extracellular ligninolytic enzyme, the growing medium was prepared by overnight soaking of wheat straw in water to achieve moisture content of 65-75%. After filling of wet substrate in flasks (@ 50g/250 ml flask), it was autoclaved at 121 ˚C for 1 h, followed by inoculation with respective fungus separately (2 bits of 4 mm dia./flask) in triplicate. Inoculated flasks were incubated at 30 ± 2 ˚C for 12 days to achieve complete colonization of substrate by the respective fungus. The crude enzymes extract was obtained by mixing 0.1 M sodium acetate buffer (pH 4.5) with mycelial colonized substrate in the ratio of 1:5 (w/v) under magnetic stirring ambient temperature conditions for 30 minutes. Resultant supernatant was filtered through muslin cloth sheet and the crude filtrate was centrifuged at 3,000 rpm for 10 min at 4 ˚C and stored at -20 ˚C for its further use.

Enzymes activity was determined spectrophotometrically at 25˚C in triplicate and data obtained was subjected to statistical analysis using AGRES software. Lignin peroxidase (EC: 1.11.1.14) activity was measured by the oxidation of veratryl alcohol to veratryl aldehyde and corrected for veratryl alcohol oxidase activity [13]. One unit of activity is represented as 1 µmol of veratryl alcohol oxidized to veratrylaldehyde per min. Manganese peroxidase (EC: 1.11.1.13) activity was measured by the method of Harazono et al. 2003 [14] using Mn^{2+} as the substrate to record enzyme activity. Amount of enzyme that oxidizes 1 µmol of substrate per minute considered as one unit of enzymes. Laccase (EC: 1.10.3.2) was assayed according to the method [15] by measuring oxidation of 2,2-azino bis (3-ethylbenzthiazoline-6-sulfonic acid). Oxidation of ABTS was determined by an increase in A_{420} (ϵ420= 36 Mm/cm). One unit is defined as the amount of enzyme that oxidizes 1 µmol of substrate per min and the activity is expressed in UL^{-1}.

Decolourization at Different pH

Seven different pH (4.0 to 10.0, with gap of one each) were tested for studying the effect of pH on decolourization of two dyes with *S. commune* and *Pezizomycotina* sp. Potato dextrose broth was prepared by adjusting pH with 0.1N solution of HCl or NaOH. Prepared broth was distributed in 250 ml Erlenmeyer flasks @ 100 ml each and sterilized at 15 psi for 20 min. The 0.1 ml stock solution (1g/10 ml) of each dye was added in 100 ml sterilized broth in flasks to give a final concentrations of 100 ppm and bit of mycelia (3 mm) of each fungus was added separately and incubated at 30±2 ˚C for next 18 days. Flasks devoid of culture but with different dyes were kept as control treatments. Before recording optical density, 3 ml sample from each flask was withdrawn and centrifuged (Sigma) at 10,000 rpm for 10 min and clear supernatant was used for recording optical density. Colour removal if any, was recorded by decrease in optical density at λ_{max} using UV-Visible Spectrophotometer (Unico-3802) starting from 0 day to 18 days of incubation.

Incubation Temperature

Five different temperatures (15, 20, 25, 30 and 35˚C) were used for studying effect of different temperatures on decolourization of two dyes using *S. commune* and *Pezizomycotina* sp. Protocol for media preparation and setting up of experiment was same as was for pH studies, excepting medium pH, which was kept 7.2. All the treatments was carried out in triplicate and all flasks including control were incubated at 5 different temperatures (15, 20, 25, 30 and 35˚C) for next 18 days.

Sampling and Analytical Techniques

The controls and samples were dried and mixed with potassium bromide (1:20; 0.02 g of sample with KBr at a final weight of 0.4 g). The collected samples grounded, desorbed at 60 ˚C for 24 h and dried to obtain IR-transparent pellets. Before analysis, FT-IR spectra of the samples were recorded using an FT-IR Spectrum 2000 Perkin–Elmer spectrometer. FT-IR spectra were collected within a scanning range of 400–4000 cm^{-1}. The FT-IR was first calibrated for signal scanning with a control sample of pure potassium bromide, and then the experimental sample was scanned and analyzed. The FT-IR spectrum of the control was finally subtracted from the spectra of the non-degraded and degraded dyes.

Measurement of Decolourization Extent

Sample (3 ml) collected each time from each replication and centrifuged at 10000 rpm for 10 min was used for measuring decolourization extent by measuring absorbance of supernatant at specific λ_{max} for each dye by using UV-Visible double beam Spectrophotometer (Unico-3802). Decolourization extent was calculated as:

Decolourization extent (%) = $[100 \times (OD_1 - OD_t)]/OD_1$

Where OD_1 is initial absorbance at 0 day, OD_t is absorbance after different periods of decolourization under different experimental conditions, t is incubation time [16].

RESULTS AND DISCUSSION

Identification of Fungi

The blasting of the sequences of Internal transcribed spacer (ITS) region of 5.8S rRNA gene from two fungal isolates, DMRF-7 and DMRF-8 using NCBI BLAST(n) tool helped them to be identified as *Schizophyllum commune* and *Pezizomycotina* sp., respectively (Fig. 2). The ITS sequences of these two fungi were further aligned against other closely matching ITS sequences of *Schizophyllum commune* (AM493689.1, FJ426395.1, JX848644.1 and HQ331059.1) and *Pezizomycotina* sp. (GU212422.1, GU212416.1, HQ607902.1 and EU003047.1) available in NCBI genebank using Mega 5.05 software. The resultant phylogenetic tree was analysed for evolutionary relationship between different isolates. The isolate DMRF-7 (*S. commune*) showed a bootstrap value of 99 along with other four ITS sequences of *S. commune* isolate obtained from NCBI genebank, proving the closeness of the present isolate to the earlier reported isolates of this fungus. The other isolate DMRF-8 (*Pezizomycotina* sp.) again showed a bootstrap value of 98 with the ITS sequences of *Pezizomycotina* sp. or other related fungi obtained from NCBI genebank, elucidating the close relationship of present isolate with other isolates of same or related species, sequences of which are already available in public domain.

Dyes Decolourization on Agar Plate

The screening on solid agar made it possible to identify the potential fungi with promising degradation capacity aganist Rhodamine B and MV2B for further exploitation in sub-merged conditions. The best dye removal and mycelial growth rate were recorded for fungi, *Schizophyllum commune* followed by *Pezizomycotina* sp., and able to completely decolourize dyes in solid agar plate. Differences have been observed in the ability of the two fungal strains to decolorize both azo dyes due to their structural differences. Out of two fungi,, *S. commune* exhibited whole plate decolourization (100%) against Rhodamine B and Methyl violet 2B in agar plate assay (Fig. 3). It has also been stressed upon that different fungi have varied decolourization potential against chemically different dyes, hence the screening of fungi for ligninolytic enzymes activity and dye decolourization must be conducted under similar conditions to screen the potential strain for industrial applications [17].

Ligninolytic Enzymes Production

Both the fungi were analyzed for the extracellular ligninolytic enzymes production potential in the growing medium containing their natural substrates. Amongst the two potential fungi, highest activity of laccase was recorded in *S. commune* (11.8 U mL^{-1}), followed by *Pezizomycotina* sp. (8.32 U mL^{-1}) (Fig. 4b). Higher activity of manganese peroxidase (MnP) was recorded in *Pezizomycotina* sp. (417.5 U L^{-1}) followed by *S. commune (*395 U L^{-1}). *Schizophyllum commune* was reported highest for Lignin peroxidase (LiP) activity (339 U L^{-1}) followed by *Pezizomycotina* (318 U L^{-1}) (Fig. 4a). Among ligninolytic enzymes, laccase, lignin peroxides, manganese peroxidase and H_2O_2 dependent peroxidases are functional extracellular enzymes secreted by fungi in biodegradation of lignin and dyes [18]. Some white rot fungi produce all these enzymes while others produce only one or two of them. Fungal ligninolytic enzymes are commercially in use in textile industry and have potential for more industrial applications [5]. Moreover, the present results showed that out of three enzymes, activity of laccase and LiP was higher in both fungi. The data on ligninolytic enzymes activity produced by fungi and bacteria and their role in dye decolourization are also reported previously [3],[5]. Similarly, Bhatti et al. 2008, have specifically demonstrated the major role of manganese peroxidase in dye decolourization, while minor role of lignin peroxidase and laccase while optimizing the cultural conditions for decolourization of Cibacron Red FN-2BL with *S. commune* [19]. Fungus has proved to be a suitable organism for the treatment of textile effluent and dye removal. The fungal mycelia have an advantage over single cell organisms by solubilizing the insoluble substrates by producing ligninolytic enzymes.

Dye Decolourization on Different Temperature

For the decolourization of MV2B & Rhodamine B at varied temperature optima (15-35 ˚C) were studied using *S. commune* and *Pezizomycotina* sp. Maximum decolourization (100%) of Methyl violet 2B was recorded after 15 days of incubation and Rhodamine B was after 18 days at 35^{o}C by *Pezizomycotina* sp. and *S. commune*. Lower temperature range (15˚C) supported less decolourization as compared to medium temperature range (25-35˚C). The decolourization temperature for both the isolates was almost same but *S. commune* was most suitable in wide temperature range. Temperature optima between 25 to 35˚C recorded for best decolourization of MV2B & Rhodamine B (Fig. 5 & 6). Whereas, *S. commune* started slightly higher decolourization of MV2B from 6 days onwards and *Pezizamycotina* sp. showed little higher decolourization from 9th day onwards against both dyes. Although both fungi has shown greater dye degradation ability and there is scarce information available with *S. commune* and no available literature on azo dye decolourization with *Pezizomycotina* sp. Similarly the role of temperature on dye decolourization potential of a fungus has also been studied and temperature optima of 30 to 37 ^{o}C has been recorded for highest decolourization of chemically diverse dyestuffs by using white-rot fungi [5],

while 25 to 35 ˚C specifically for decolourization of Cibacron Red FN- 2BL by *Schizophyllum commune* [20].

Effect of pH on Dye Decolourization

Out of various pH tested for decolourization, 5.0 to 10 showed almost higher decolourization after 8 days of incubation(Fig. 7 & 8). But pH 7.0 to 10 was recorded for complete decolourization of Rhodamine B using *S. Commune*. Whereas *Pezizamycotina* sp. was recorded in the pH range 7.0-10 for the best decolourization but highest (100%) was recorded in pH 10.0 for decolourization of Rhodamine B. Similarly, in case of Methyl violet 2B, highest decolourization rate were recorded in pH 7.0 to 10 on the 15th day onward by *S. commune*. The *S. communes* have wide pH ranges (4.0 to 10) for the decolourization of MV2B and almost same pattern was also followed by the *Pezizomycotina* sp. for the decolourization of MV2B. Both fungi were recorded for highest decolourization of Rhodamine B and MV2B in pH ranging from 7.0 to 10.0 and incubation temperature at 30 ˚C. In present study, highest fungal biomass was recorded almost in the same pH range where highest decolourization of two azo dyes was recorded with the two potential fungi. It proves the adaptability of the two fungi to grow and decolourize the dyes simultaneously at same pH and temperature optima, which is crucial for their industrial scale application. Fungi growth conditions had a great influence on effective decolourization of textile wastewater. In batch scale experiment, it was recorded that decolourization of Acid orange 10 was a function of both the substrate (dye) and biomass concentrations. The main property of white rot fungi is to withstand in a wide range of pH which further enhances their pollutant degradation capabilities. Most previous reports indicated pH optima in the range 3.5 to 5.0 in case of *Trametes versicolor, Trichderma* sp. and *Phanerochate chrysosporium* [21].

Mycelial Biomass Production and Dyes Decolourization

Effect of varied pH values of medium on biomass production of *Pezizomycotina* sp. and *S. commune* along with decolourization of Rhodamine B and MV2B was studied at a pH range of 4.0 to 10.0. Highest decolourization of Rhodamine B was recorded at pH from 8.0 to 10.0, while highest biomass (6.5g/l) was recorded at pH of 9.0 (Fig. 9). The pH from 4.0 to 6.0 was not suitable for higher growth of the fungus as well as decolourization of the dye. Five different temperatures (15, 20, 25, 30 and 35 ˚C) were employed for studying the effect of temperature on biomass production as well as decolourization of Rhodamine B (Fig.9). The highest decolourization of Rhodamine B was recorded at temperature ranging from 30 to 35 ºC, while highest biomass was recorded at temperature of 30 ˚C. The pH optima for biomass production of *S. commune* along with Methyl violet 2B decolourization was studied at pH ranging from 4.0 to 10.0. The pH ranging from 7.0 to 10.0 was recorded to be optimum for achieving nearly 100% decolourization of MV2B. However, the pH optima for biomass production was narrower and it was 9.0 to 10.0, where highest biomass (7.2 g/l) production was recorded (Fig. 10). Reports on dyes decolourization with fungi isolated from spent mushroom substrates are quite scanty. However, work carried out with other fungi has proved the synthetic dye decolourization capability of white rot fungus, *Phanerochaete chrysosporium* [1]. In one study conducted with *Lentinula edodes*, it has been noted that the fungal mycelial growth became visible after 24 hrs of inoculation, and the medium became completely decolorized within 6 days [10]. *Schizophyllum commune* and *Pezizomycotina* spp. are basidiomycetes and ascomycetes fungi isolated from spent substrate of *Pleurotus* spp. showed similar trend of biomass production during azo dyes decolourization. In present study, highest fungal biomass was recorded almost in the same pH range where highest decolourization of two dyes was recorded with the two fungi. It proves the adaptability of the two fungi to grow and decolourize the dyes simultaneously at same pH and temperature optima, which is crucial for their industrial scale application.

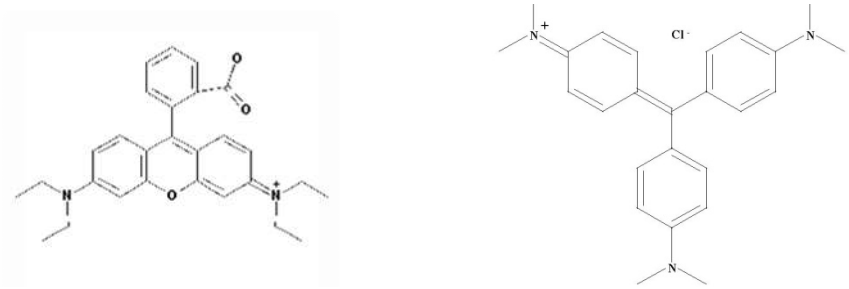
Fourier Transformed Infra Red Spectrum Analysis

The FTIR spectrum of Rhodamine B and MV2B was studied before degradation and after degradation with *Schizophyllum commune*. The FTIR spectrum of the control showed the presence of different sharp and strong absorption peaks around 3400.97 cm^{-1} corresponding with $-NH_3$ stretches (Fig 10). The peaks at 2926.15 cm^{-1} indicated the stretching band of –CH. The sharp peaks observed at 1642.20 cm^{-1} could be assigned to the stretching band of C=O from amide. The peaks at 1406.66 cm^{-1} were attributed to the stretching vibration indicative of benzene ring. Other peaks observed at 1077.02 cm^{-1} indicates –OH stretching of aromatics ring and peaks at 619.72 cm^{-1} demonstrated the primary alcohols and sulphoric acid (-S=O). The FTIR spectrum after degradation of Rhodamine B with *Schizophyllum commune* displayed peaks at 3369.33, 2920 and 2851.16 cm^{-1} correspond to –C-H deformation of alkanes, -C-H symmetric stretch of the methylene group ($-CH_2$) and deformation vibration of methyl group ($-CH_3$), alkane ($-CH_2$) stretching and vibration (Fig. 11). The other peaks at 1627.92 cm^{-1} represent –C=O from amide stretching vibration, whereas 1573.31 cm^{-1} peak shows –N=N stretching of azo compound and breaking of azo bond.

The FTIR spectrum of the undegraded MV2B showed presence of different peaks at 3399 cm^{-1} for – NH_3 stretching of amino acid, 2923 cm^{-1} for C–H stretch of alkenyl group, 1647 and 1418 cm^{-1} for aromatic –C=C bending, 1196 cm^{-1} for –SO stretching of sulfites, 1047 cm^{-1} for –SO stretching of sulfonic acids (Fig. 12). The FTIR spectrum of sample after treatment with *S. commune* showed major peaks at 2920 cm^{-1} for –CH stretching of alkene, 1644 and 1404 cm^{-1} for stretching of aromatic –C=C, 1323 cm^{-1} for – C=O stretching of carboxylic acids, 1047 cm^{-1} for –C=O stretching of secondary alcohols, 838 cm^{-1} for –C–H stretching of monosubstituted alkenes and 522.83 for sulphonic acid (S=O). The presence of additional group, due to the conjugation of C=C and C=O groups, suggested that this peak could belong to a carbonyl group in a carboxylic acid, ketone, ester or conjugated aldehyde group attached to an aromatic ring [22]. Remarkably, in contrast to the expectation that bio-recalcitrant aromatic amines would tend to autoxidise forming coloured products, in the present experiment no increase in colour in the visible region was observed in the aerobic stage, suggesting that the aromatic amines were effectively biodegraded. Moreover, the azo compounds with a hydroxyl or amino groups were more likely to be degraded than those with methyl, methoxy, sulfo or nitro groups. However, although in some cases biodegradation of the dye's cleavage products was demonstrated [23], it is difficult to predict the fate of the aromatic amines during the decolourization of azo dyes, because it is not clear whether their removal was due to biodegradation, adsorption or chemical reactions [24].

Dyes with simple structures and low molecular weights usually exhibit higher rates of colour removal, whereas colour removal is more difficult with highly substituted, high molecular weight dyes [25],[26]. Due to this reason, Rhodamine B and MV2B are both high molecular weight dyes, showed a longer decolourization time (14 and 18 days, respectively) but completely decolourize with both the fungi.

Ability of the potential microorganisms to degrade azo dyes depends on the various factors like structural characteristics of the dye, temperature and the pH of the medium [27], presence of intermediates and the difference between the redox potentials of the biocatalyst and the dye. Another important aspect is that our system did not require any addition of nutrients during decolourization of the azo dyes. Addition of additives, immobilized and consortium form of fungal mycelium and increase in initial inoculations needs more attention to make it more applicable in field level and ultimately led to reduce decolourization time.



A **B**

Figure 1 : Chemical structure a) Rhodamine B; b) Methyl Violet 2B

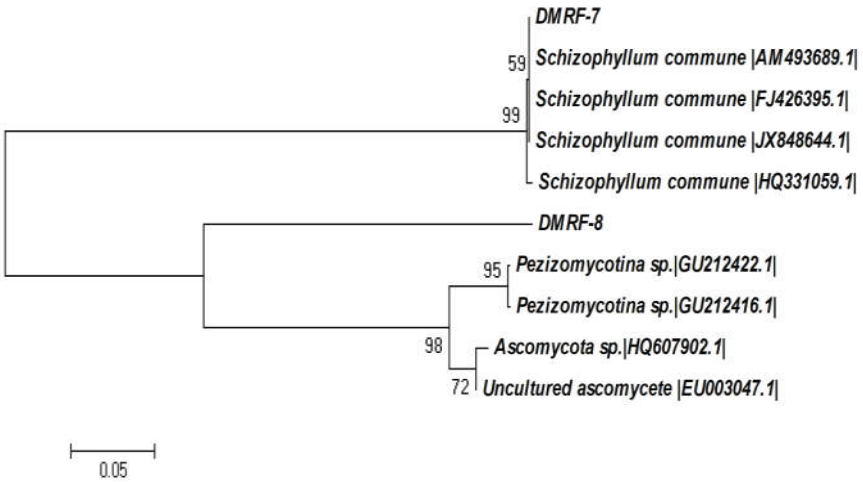


Figure 2: Maximum parsimony phylogenetic tree derived from ITS sequences of 5.8S rRNA gene of potential fungal isolates from spent mushroom substrate of *Pleurotus* spp. and related sequences obtained from NCBI genebank. (DMRF-7-*Schizophyllum commune* (This work); DMRF-8 *Pezizomycotina* sp. (This work).

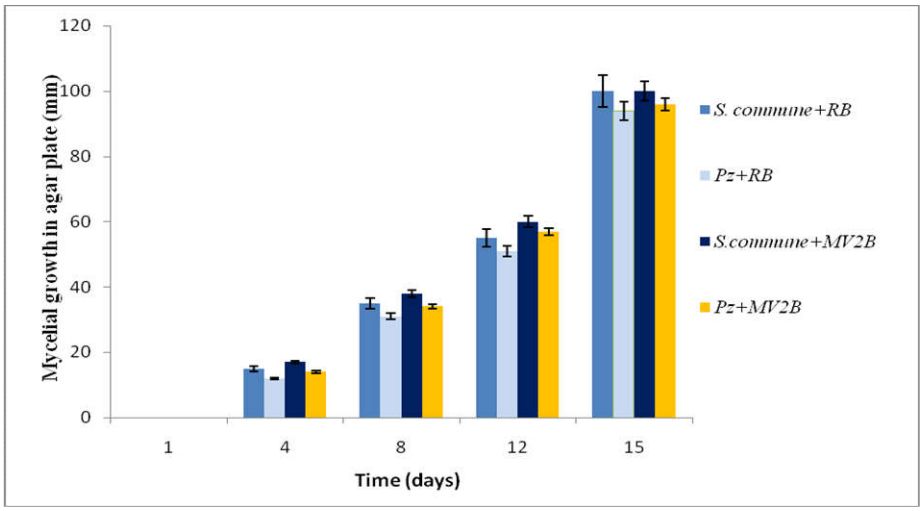


Figure 3: Mycelial growth of *Schizophyllum commune* and *Pezizomycotina* sp. in agar plate supplemented with each dye. [RB= Rhodamine B; Pz= *Pezizomycotina*; MV2B= Methyl violet 2B]

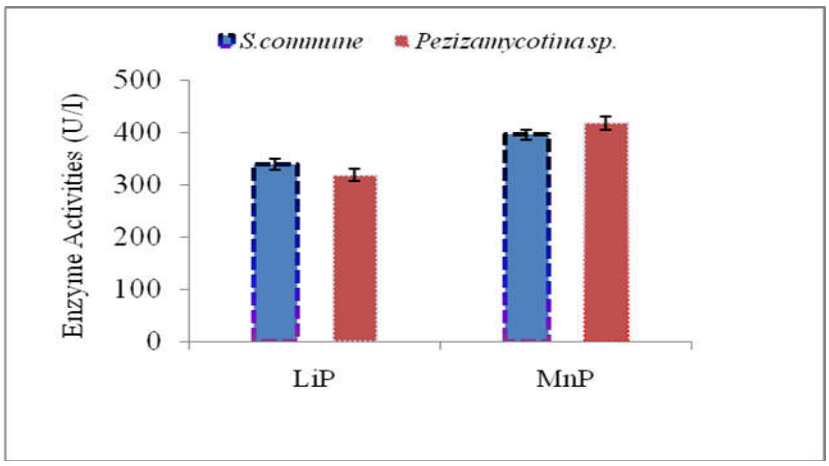


Figure 4a: Lignin peroxidase and Manganese peroxidase activity of potential fungi isolated from spent substrate of *Pleurotus* spp.

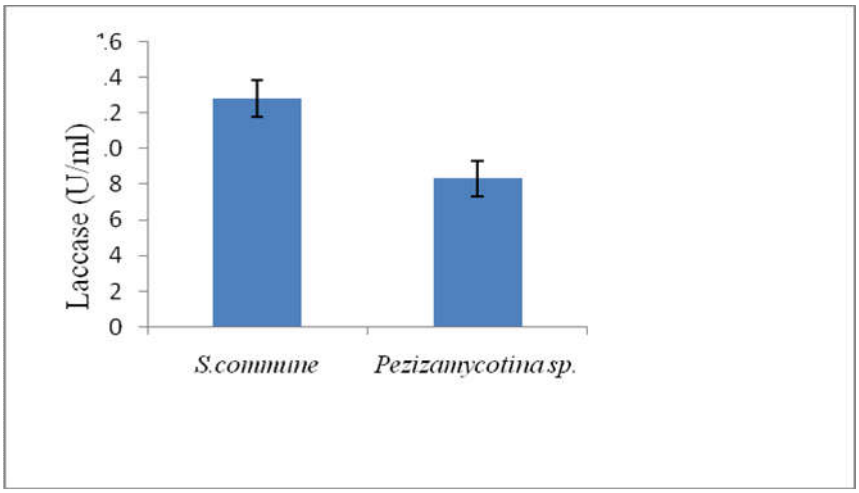


Figure 4b: Laccase activity of potential fungi isolated from spent substrate of *Pleurotus* spp.

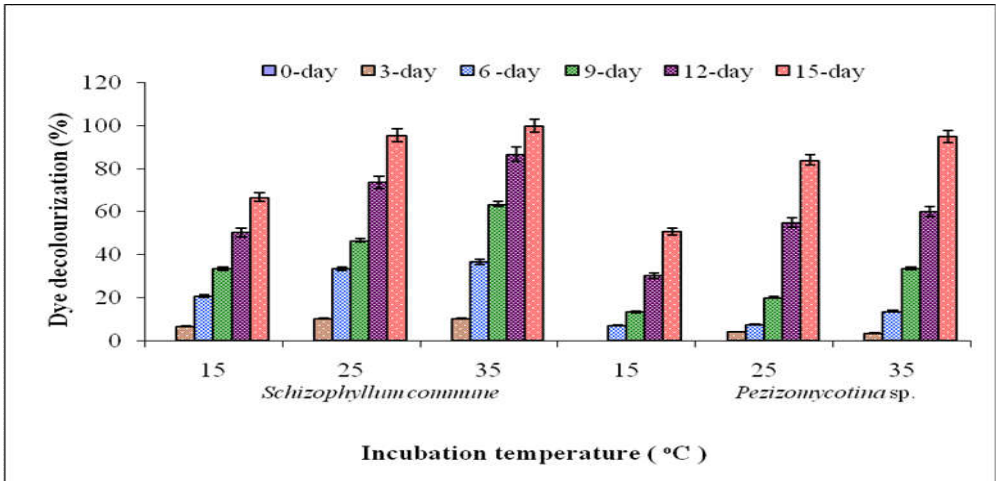


Figure 5: Decolourization of Methyl violet 2B using *Schizophyllum commune* and *Pezizomycotina* sp. at different temperature.

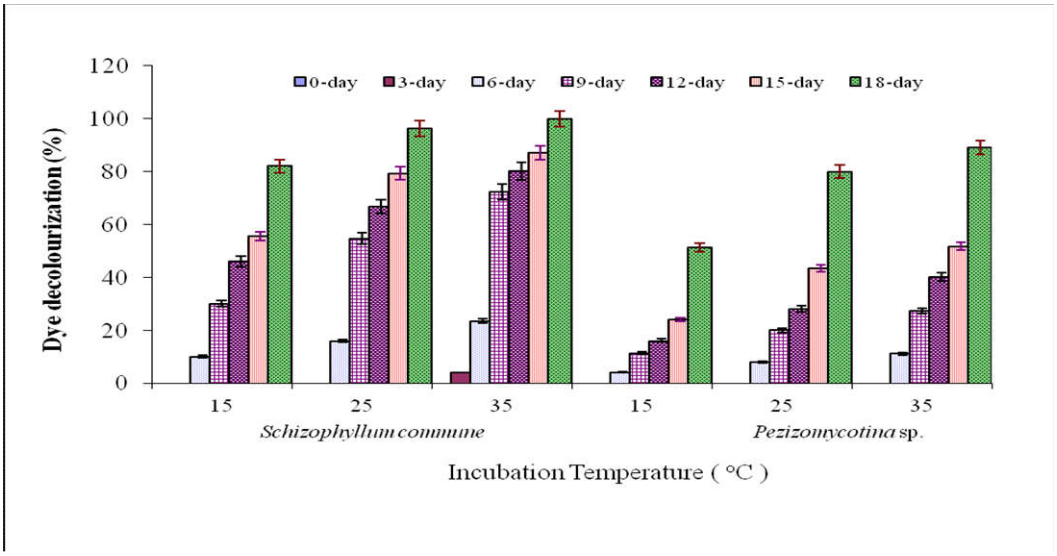


Figure 6: Decolourization of Rhodamine B using *Schizophyllum commune* and *Pezizomycotina* sp. at different incubation temperature.

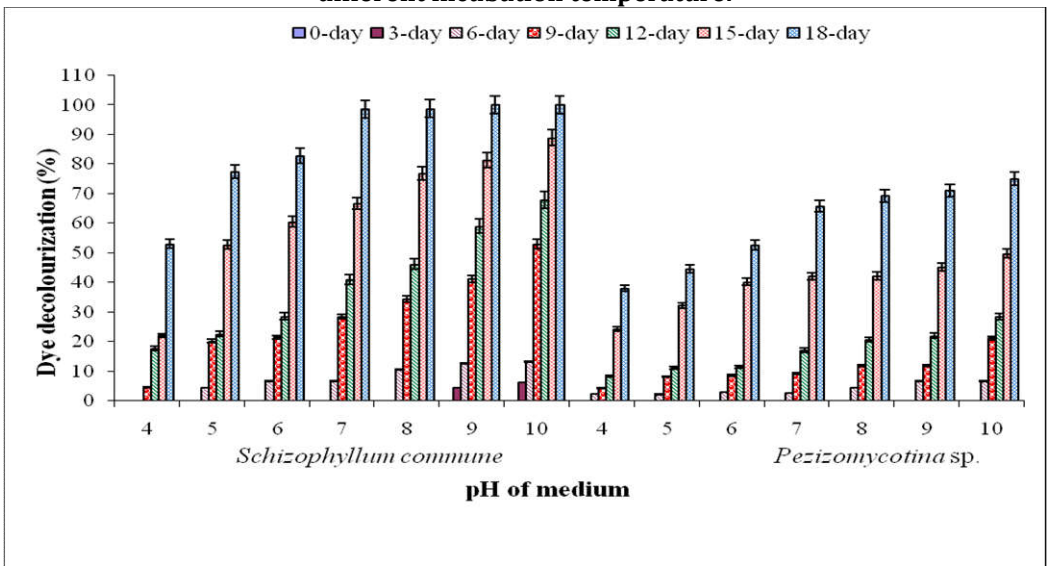


Figure 7: Decolourization of Rhodamine B using *Schizophyllum commune* and *Pezizomycotina* sp. at different pH of medium.

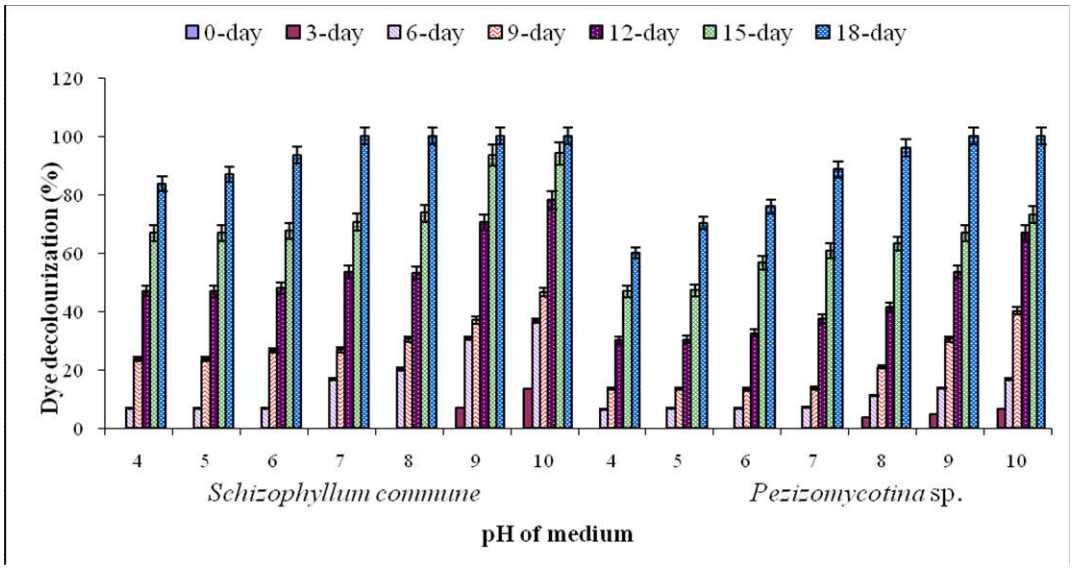
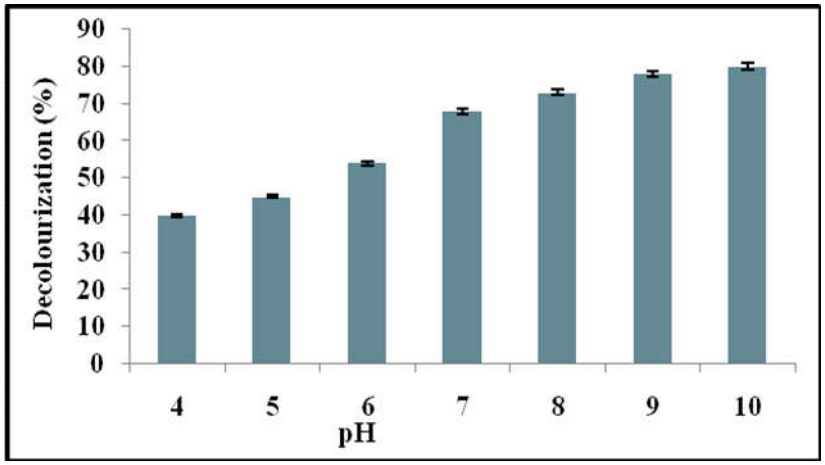
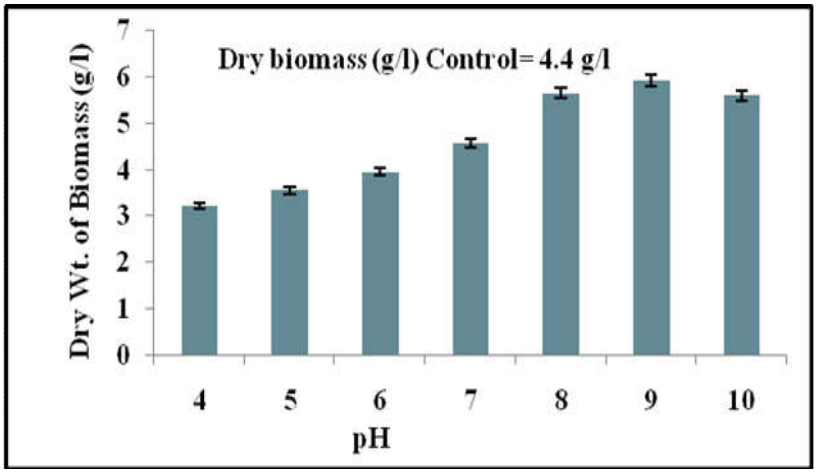


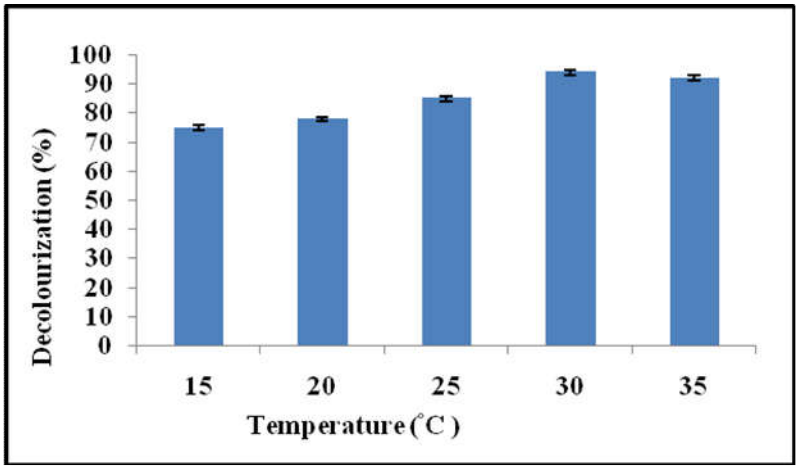
Figure 8: Decolourization of Methyl violet 2B using *Schizophyllum commune* and *Pezizomycotina* sp. at different pH of medium.



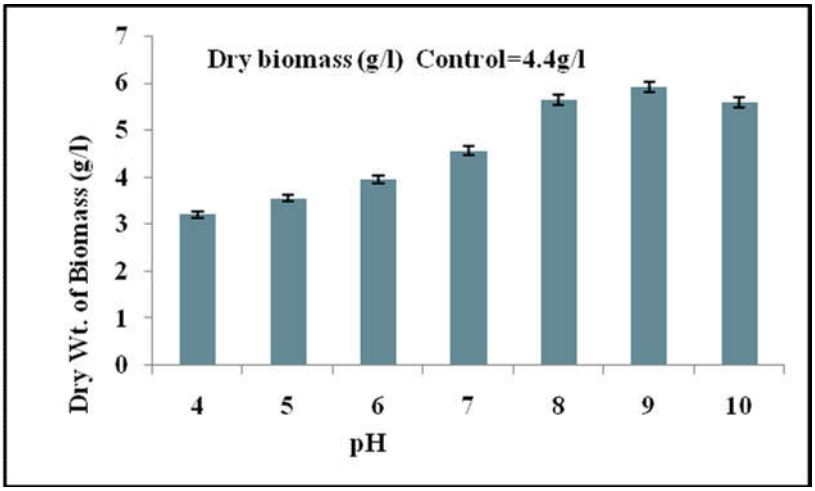
a)



b)

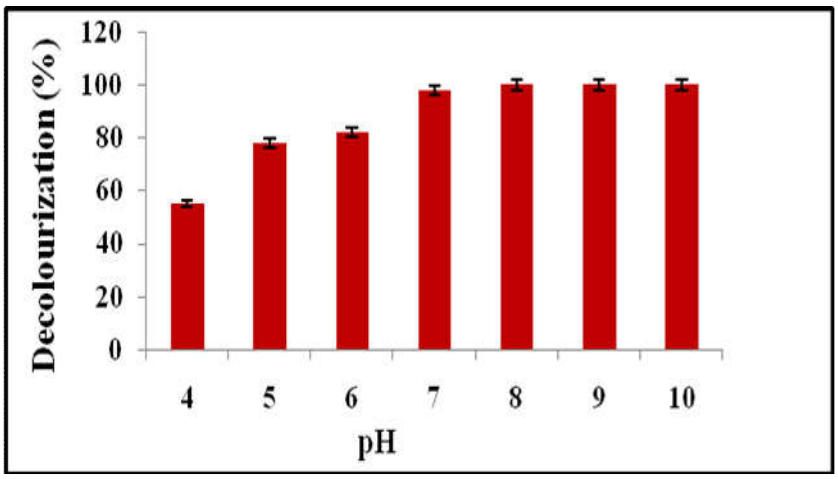


c



d)

Figure 9: Decolourization of Rhodamine B and mycelial biomass production potential of *Pezizomycotina* sp. a) Rhodamine B decolourization at different pH values; b) Dry weight of mycelial biomass at different pH values; c) Rhodamine B decolourization at different temperature; d) Dry weight of mycelial biomass at different temperature, where the control was the amount of biomass of *Pezizomycotina* sp. growing on medium without dye; control = 4.4 gl^{-1})



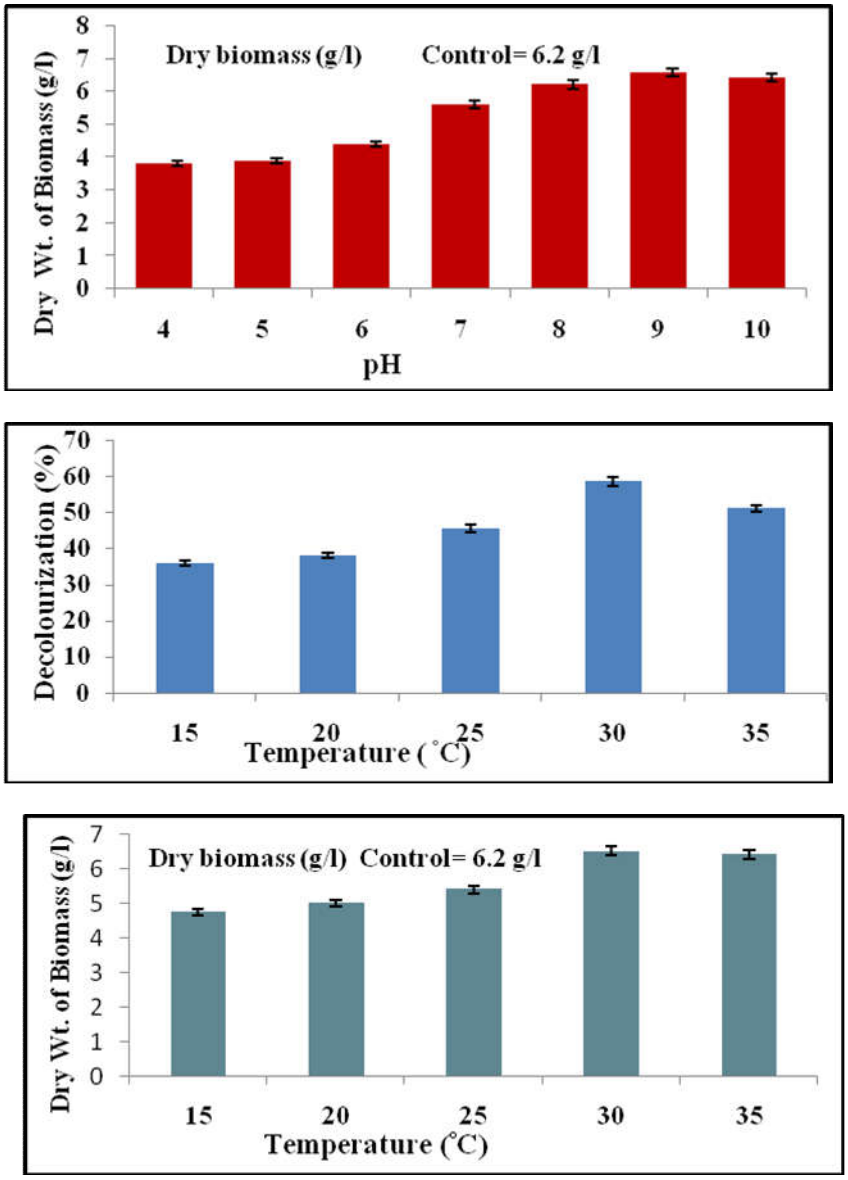
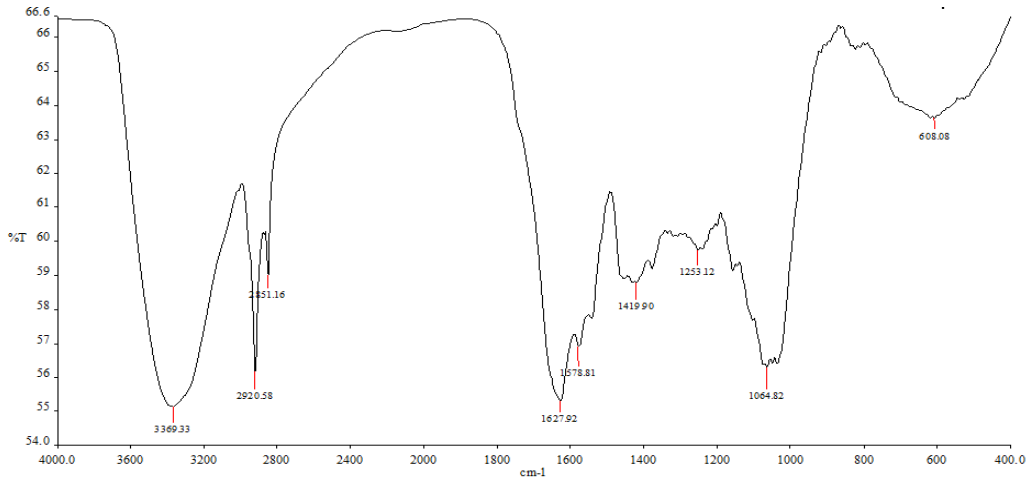


Figure 10: Decolourization of Methyl violet 2B and mycelial biomass production potential of *S. commune;* a) MV2B decolourization at different pH; b) Dry weight of mycelial biomass at different pH; c) MV2B decolourization at different temperature ; d) Dry weight of mycelial biomass at different temperature, where the control was the amount of biomass of *S. commune* growing on medium without dye; control=6.2gl^{-1}



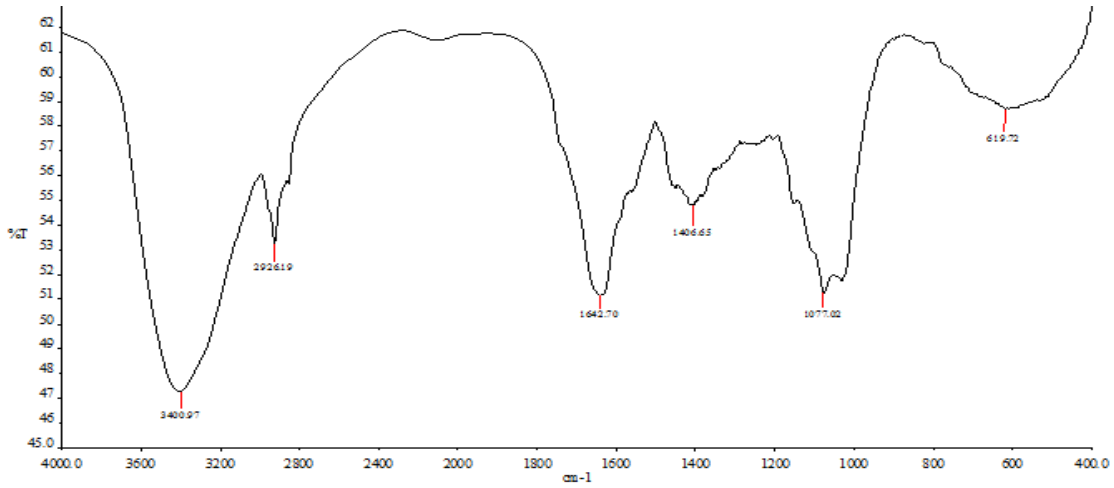


Figure 11: FTIR analysis of the Rhodamine B a) Control and b) After biodegradation by *S. commune*

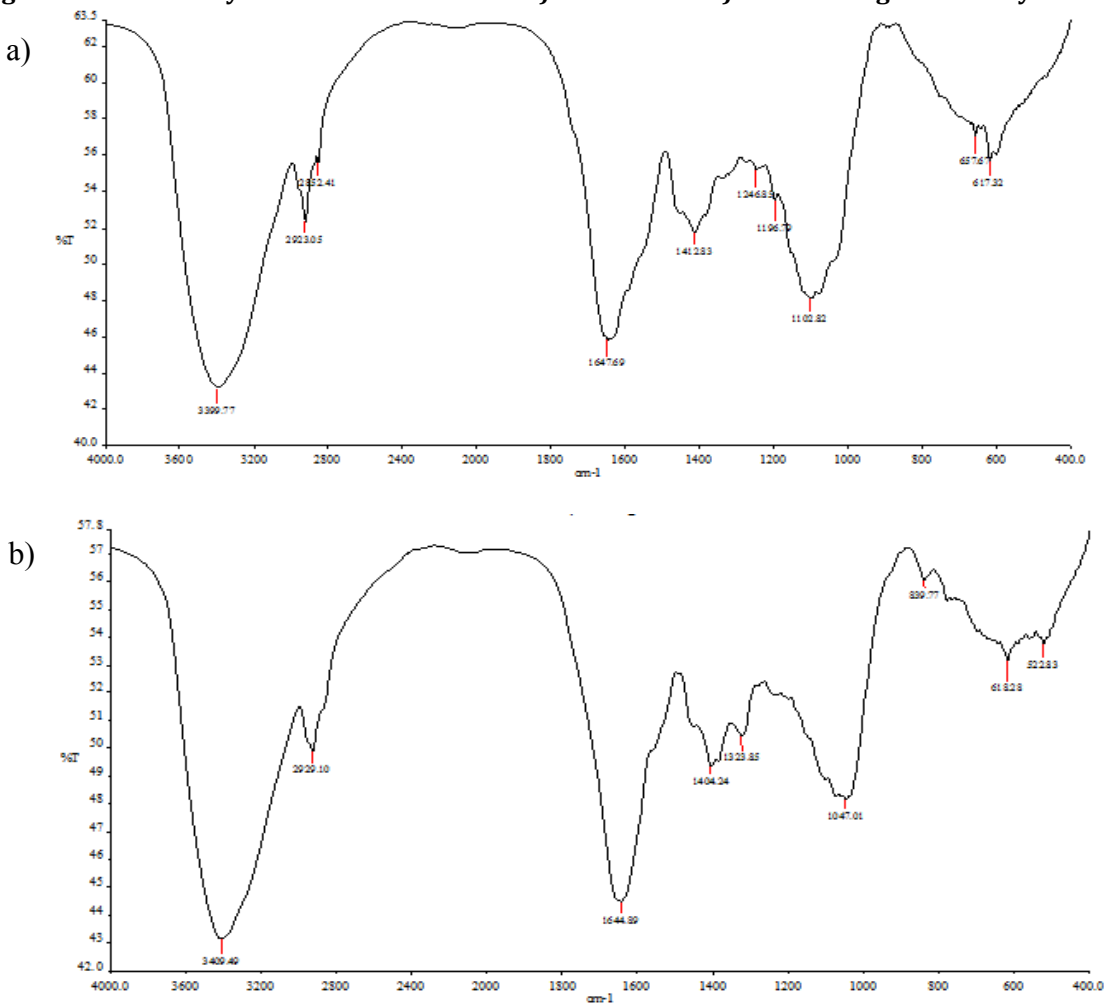


Figure 12: FTIR analysis of the Methyl violet 2B a) Control and b) After biodegradation by *S. commune*

CONCLUSIONS

In present study the ability of *S. commune* and *Pezizomycotina* sp. to decolourize Rhodamine B and Methyl violet 2B was recorded and studied for efficient decolourization. Both the fungal strains was found to degrade dye across a wide pH, temperatures, biomass production and ligninolytic enzymes, which played an important role for degradation of dyes. Among both fungi, *S. commune* was found to be more significant than *Pezizomycotina* sp. for decolourizing Rhodamine B and Methyl violet 2B. Best of our knowledge, *Pezizomycotina* sp. was first time recorded for effective decolourization of synthetic azo dye.

Furthermore both strains produced ligninolytic enzymes and higher biomass production during decolouriztion indicates their better suitability even in higher concentrations of azo dyes and wide temperature and pH. Both the dyes tested were efficiently decolourize with some differences in decolourization times depending on the dye structure and type of fungal strains. We conclude that higher decolourization efficiency was attained in the presence of only that treatments which produce higher mycelia biomass and also indicates their tolerance to higher concentration of synthetic dyes. This strategy using, a single strain was shown to be very effective in azo dye decolourization and biological system may be applied for treatment of synthetic azo dyes and their effluents in order to avoid environmental pollution.

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