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Ligninolytic Enzyme-Driven Molecular Degradation of Synthetic Dyes by Wild Mushrooms: An FTIR-Based Investigation

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Abstract
The extensive use of synthetic dyes in industries such as textiles, paper, leather, cosmetics, and pharmaceuticals has resulted in the discharge of large volumes of dye-laden effluents into the environment, posing serious ecological and health concerns. These dyes are chemically stable, highly visible even at low concentrations, and resistant to conventional physical and chemical treatment methods. In recent years, biological approaches, particularly fungal bioremediation, have gained attention as sustainable alternatives for dye degradation. Wild mushrooms, especially ligninolytic fungi, produce extracellular oxidative enzymes such as laccase, manganese peroxidase, and lignin peroxidase, which are capable of degrading complex aromatic dye structures. The present study investigates the ligninolytic enzyme-driven molecular degradation of selected synthetic dyes by wild mushroom species, with a focus on structural transformation analysis using Fourier Transform Infrared (FTIR) spectroscopy. FTIR analysis enables the identification of functional group modifications, providing insights into the breakdown of dye chromophores and confirming true biodegradation rather than mere decolorization. This research highlights the potential of indigenous wild mushrooms as eco-friendly agents for dye bioremediation and contributes to the development of sustainable wastewater treatment strategies.

Keywords: Synthetic dyes, Wild mushrooms, Ligninolytic enzymes, Mycoremediation, Dye biodegradation, FTIR analysis

1. Introduction
Synthetic dyes are widely used in industries such as textiles, paper, leather, cosmetics, pharmaceuticals, and food processing due to their color stability, vibrancy, and cost-effectiveness (Hunger, 2003) [6]. The textile industry alone accounts for more than 60% of global dye consumption, generating large volumes of dye-containing wastewater as a result of inefficient dye fixation during processing (Forgacs *et al*., 2004; Robinson *et al*., 2001) [3, 12]. Even at very low concentrations, synthetic dyes impart intense coloration to water bodies, reducing light penetration and disrupting photosynthetic activity, thereby affecting aquatic ecosystems (Yagub *et al*., 2014) [14].

Many synthetic dyes and their degradation products are toxic, mutagenic, or carcinogenic, with azo dyes capable of releasing hazardous aromatic amines under anaerobic conditions (Chung, 2001; Hessel *et al*., 2007) [2, 5]. Conventional treatment methods such as adsorption, coagulation-flocculation, membrane filtration, and chemical oxidation are often limited by high operational costs, incomplete mineralization, sludge generation, and secondary pollution (Gupta & Suhas, 2009; Zollinger, 2003) [4, 15]. These limitations necessitate the development of sustainable and environmentally benign alternatives.

Fungal bioremediation has emerged as a promising approach for the degradation of synthetic dyes. White-rot fungi and wild mushrooms are particularly effective due to their ability to produce extracellular ligninolytic enzymes, including laccase, manganese peroxidase, and lignin peroxidase, which can oxidatively cleave complex aromatic dye structures (Pointing, 2001; Wesenberg *et al*., 2003) [10, 13]. However, most studies focus on a limited number of fungal species, while the biodegradation potential of indigenous wild mushrooms remains underexplored.

Moreover, dye decolorization alone does not confirm complete degradation or detoxification. Fourier Transform Infrared (FTIR) spectroscopy is a valuable tool for identifying functional group modifications and elucidating molecular degradation pathways. Therefore, the present

study investigates ligninolytic enzyme-driven molecular degradation of synthetic dyes by wild mushrooms using FTIR analysis, aiming to contribute to sustainable wastewater treatment strategies.

2. Materials & Methods

2.1 Selection, Isolation, and Identification of Mushroom Species

Four wild mushroom species—*Pleurotus ostreatus*, *Lentinus squarrosulus*, *Schizophyllum commune*, and *Ganoderma lucidum*—were collected from forest regions of Bastar, Chhattisgarh during peak fruiting seasons. Fruiting bodies were surface sterilized, tissue-cultured on Potato Dextrose Agar, and incubated at 28±2 °C. Pure cultures were maintained through sub-culturing and preserved under refrigerated and cryogenic conditions. Morphological identification was complemented by molecular characterization using ITS-rDNA sequencing for species confirmation.

2.2 Preparation of Synthetic Dyes and Culture Media

Four synthetic dyes—Methyl Orange, Congo Red, Crystal Violet, and Malachite Green—were prepared as sterile stock solutions (1000 mg/L) and diluted to working concentrations. Various media including Mineral Salt Medium, Modified Czapek-Dox medium, Malt Extract Broth, and PDA were prepared to support fungal growth and ligninolytic enzyme production. Media pH and temperature were adjusted according to experimental requirements.

2.3 Biodegradation Experimental Setup

Batch biodegradation studies were performed in liquid culture using dye-supplemented media inoculated with actively growing fungal mycelial plugs. Experiments were conducted under static conditions at optimized pH and temperature for up to 14 days. Control sets without fungal inoculation were maintained to account for abiotic dye loss. Decolourization efficiency was quantified spectrophotometrically at dye-specific absorption maxima.

2.4 Enzyme Activity Assays

Extracellular ligninolytic enzymes—laccase, manganese peroxidase, and lignin peroxidase—were assayed from culture supernatants at regular intervals to correlate enzyme production with dye degradation efficiency.

2.5 Optimization of Degradation Parameters

Key parameters including pH, temperature, dye concentration, carbon and nitrogen sources, C:N ratio, and incubation time were optimized systematically to enhance dye degradation. All experiments were performed in triplicate, and statistical analyses were applied to determine significance.

2.6 FTIR Analysis

After biodegradation, treated dye solutions were filtered, centrifuged, and lyophilized. FTIR spectra of untreated and treated dyes were recorded in the range of 4000-400 cm^{-1} using KBr pellet technique. Changes in characteristic functional groups were analyzed to confirm molecular degradation and breakdown of chromophoric structures.

2.7 Statistical Analysis

Experimental data were analyzed using ANOVA, correlation analysis, and multivariate statistical tools to validate reproducibility and establish relationships between enzyme activity and dye degradation.

a) *Pleurotus ostreatus*

b) *Lentinus squarrosulus*

c) *Schizophyllum commune* d) *Ganoderma lucidum*

Fig 1: Representing wild ligninolytic mushroom species a) *Pleurotus ostreatus*, b) *Lentinus squarrosulus*, c) *Schizophyllum commune* and d) *Ganoderma lucidum*

3. Results and Discussion

The present study evaluated the growth behaviour, dye decolourization efficiency, enzymatic activity, and molecular degradation potential of four wild mushroom species—*Pleurotus ostreatus*, *Lentinus squarrosulus*, *Schizophyllum commune*, and *Ganoderma lucidum*—against representative synthetic dyes commonly found in industrial effluents.

3.1 Growth Tolerance of Wild Mushrooms in Dye-Containing Media

All four wild mushroom species—*Pleurotus ostreatus*, *Lentinus squarrosulus*, *Schizophyllum commune*, and *Ganoderma lucidum*—demonstrated the ability to grow in the presence of synthetic dyes, indicating inherent tolerance to dye-induced toxicity. Among them, *Pleurotus ostreatus* exhibited the highest radial growth across all dyes, particularly on Congo Red-amended media (5.0±0.2 cm), followed by Methyl Orange and Malachite Green. This superior growth suggests strong physiological adaptability and effective detoxification mechanisms, likely supported by a robust ligninolytic enzyme system.

Crystal Violet exerted the greatest inhibitory effect on all species, consistent with its known antimicrobial and fungistatic properties. Conversely, Congo Red supported comparatively higher growth, indicating partial compatibility with fungal metabolism. These findings corroborate earlier reports highlighting the resilience of white-rot fungi under xenobiotic stress (Jonathan & Fasidi, 2001; Palmieri *et al*., 2005) [8, 9].

Table 1: Radial Growth (cm) of Mushrooms on Dye-Containing Media After 7 Days Incubation

Mushroom Species	Methyl Orange	Crystal Violet	Congo Red	Malachite Green
Pleurotus ostreatus	4.8±0.2	4.2±0.3	5.0±0.2	4.6±0.3
Lentinus squarrosulus	4.4±0.1	4.1±0.2	4.7±0.2	4.3±0.3
Schizophyllum commune	4.0±0.2	3.8±0.2	4.2±0.1	4.0±0.2
Ganoderma lucidum	4.2±0.3	3.9±0.2	4.5±0.3	4.1±0.3

Data represent mean±standard deviation of three replicates.

3.2 Dye Decolourization Efficiency

Quantitative spectrophotometric analysis revealed significant decolourization of all tested dyes by the wild mushroom species after 14 days of incubation. *Pleurotus ostreatus* achieved the highest decolourization efficiencies, with maximum removal observed for Congo Red (88.7±2.3%), followed by Methyl Orange (85.3±2.1%) and Malachite Green (83.2±1.8%). *Lentinus squarrosulus* also showed high removal efficiencies, particularly for azo dyes, confirming its potential in mycoremediation applications.

Crystal Violet showed comparatively lower decolourization across all species (68.2-78.5%), reflecting its structural complexity and resistance to enzymatic attack. Nevertheless, substantial colour removal indicates that even recalcitrant triphenylmethane dyes can be degraded by ligninolytic fungi, albeit at reduced efficiency. These results are consistent with previous studies emphasizing the broad dye-degrading capacity of white-rot fungi (Forgacs *et al*., 2004; Baldrian, 2006) [3, 1].

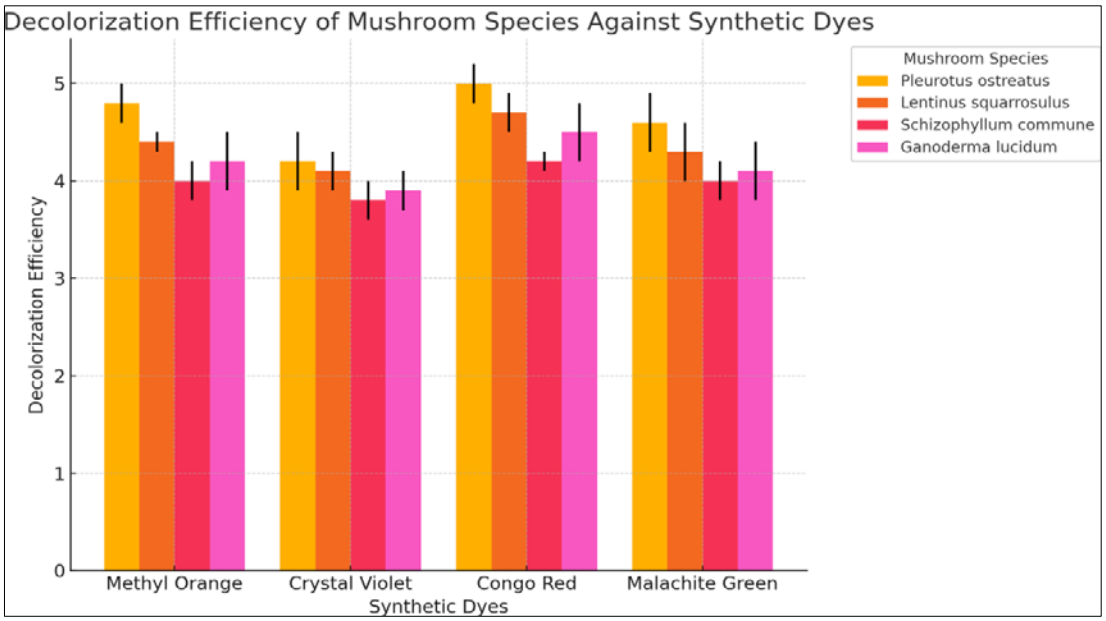

Fig 2: Representing decolorization efficiency of mushroom species against synthetic dye

3.3 Ligninolytic Enzyme Activity During Dye Degradation

The activities of key ligninolytic enzymes—laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP)—increased significantly from Day 7 to Day 14 for all mushroom species. *Pleurotus ostreatus* recorded the highest enzyme activities, with laccase reaching 285.7±6.1 U/mL on Day 14, followed by MnP (130.2±4.3 U/mL) and LiP (87.9±3.1 U/mL). This strong enzymatic response closely correlated with its superior dye decolourization performance. Laccase activity consistently exceeded that of MnP and LiP across species, underscoring its primary role in oxidative dye degradation. The induction of these enzymes in the presence of dyes suggests that synthetic dyes act as enzyme inducers, triggering oxidative stress responses that facilitate molecular breakdown of chromophoric structures (Wesenberg *et al*., 2003; Janusz *et al*., 2017) [13, 7].

Table 2: Enzyme Activity (U/mL) of Wild Mushrooms During Dye Degradation

Mushroom Species	Enzyme	Day 7 Activity	Day 14 Activity
Pleurotus ostreatus	Laccase	210.5±5.2	285.7±6.1
	MnP	98.4±3.7	130.2±4.3
	LiP	65.7±2.8	87.9±3.1
Lentinus squarrosulus	Laccase	195.3±4.8	260.4±5.8
	MnP	91.0±3.5	125.0±3.9
	LiP	60.2±2.5	80.5±2.7
Schizophyllum commune	Laccase	165.7±4.0	215.9±5.1
	MnP	85.3±3.1	110.7±3.4
	LiP	54.1±2.0	67.3±2.3
Ganoderma lucidum	Laccase	180.4±4.5	240.6±5.4
	MnP	88.6±3.3	118.4±3.7
	LiP	58.9±2.4	75.8±2.6

3.4 FTIR-Based Molecular Evidence of Dye Degradation

FTIR spectral analysis provided direct evidence of chemical transformation of dye molecules following fungal treatment. Comparative spectra (4000-400 cm^{-1}) of untreated and treated dyes revealed significant attenuation, shifts, or disappearance of characteristic functional group peaks. Notably, reductions in the O-H/N-H stretching region (~3400 cm^{-1}) and aromatic C=C or azo bond regions (~1600 cm^{-1}) indicate enzymatic cleavage of chromophoric groups. The disappearance or weakening of peaks corresponding to sulfonate (S=O) and C-N stretching further confirms degradation of complex dye substituents. These spectral changes demonstrate that dye removal was not limited to surface adsorption but involved true biochemical transformation mediated by ligninolytic enzymes. Similar FTIR-based confirmations of azo dye degradation by fungi have been reported by Wesenberg *et al*. (2003) and Revankar & Lele (2006) [13, 11].

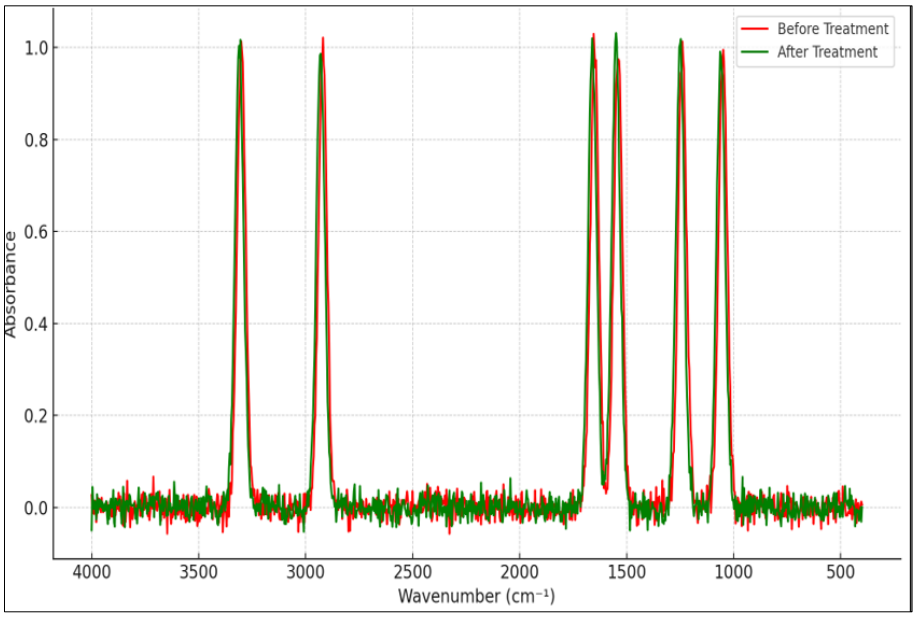

Figure 3: FTIR spectra overlay showing disappearance or shifts in major peaks post-treatment.

Table 3: Key FTIR Peak Changes Before and After Treatment with *Pleurotus ostreatus* (for Congo Red)

Wavenumber (cm^{-1})	Functional Group (Before Treatment)	Intensity Change (After Treatment)	Interpretation
3420	O-H and N-H stretching	Decreased	Breakdown of amine/hydroxyl groups
1600	Aromatic C=C stretching	Reduced	Aromatic ring cleavage
1340	S=O stretching (sulfonate group)	Reduced	Sulfonate group degradation
1110	C-N stretching	Disappeared	Degradation of azo bond

3.5 Correlation Between Enzyme Activity and Dye Degradation

Statistical analysis revealed a strong positive correlation ($r = 0.89$, $p < 0.01$) between laccase activity and dye decolourization percentage, highlighting the enzyme's central role in oxidative degradation pathways. One-way ANOVA confirmed that differences in decolourization efficiencies among mushroom species were statistically significant for all dyes ($p < 0.05$), validating inter-species variability in biodegradation performance

4. Conclusion

Wild mushroom species demonstrated effective ligninolytic enzyme-driven degradation of synthetic dyes. All fungi tolerated dye stress, with *Pleurotus ostreatus* showing the highest growth, enzyme activity, and decolourization efficiency, followed by *Lentinus squarrosulus*. Azo dyes were degraded more efficiently than Crystal Violet. Elevated laccase, manganese peroxidase, and lignin peroxidase activities correlated strongly with dye removal. FTIR analysis confirmed molecular breakdown of chromophoric functional groups, indicating true chemical degradation rather than adsorption. These findings highlight the potential of wild mushrooms, particularly *P. ostreatus*, as sustainable agents for eco-friendly treatment of dye-contaminated wastewater.

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