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Isolating fungus from textile wastewater and assessing their efficacy in decolorizing colors.

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Abstract:

Textile wastewater is different because it has a lot of synthetic dyes, salts, surfactants, and dangerous organic pollutants that pose a big risk to the environment. Conventional treatment procedures are often costly and ineffectual in removing persistent azo dyes. This study evaluated the bioremediation potential of three fungal strains, *Trametes versicolor*, *Phanerochaete chrysosporium*, and *Aspergillus niger* for the treatment of textile wastewater during a 14-day period. The efficacy of decolorization and the reductions in chemical oxygen demand (COD), biological oxygen demand (BOD₅) and total suspended solids (TSS) were thoroughly assessed. *T. versicolor* was the most effective of the strains tested, with 93% dye decolorization and 79% COD reduction. *P. chrysosporium* reduced the chemical oxygen demand (COD) by 73% and the color by 86%. *A. niger* reduced the color by 70% and the COD by 57%. The BOD levels went down by 67%, 62%, and 49%, while the TSS levels went down by 59%, 53%, and 42% for *T. versicolor*, *P. chrysosporium*, and *A. niger*, respectively. Spectrophotometric analysis revealed a substantial decrease in absorbance at λ_{max} (520–620 nm), confirming the cleavage of azo bonds and the deterioration of aromatic structures.

These results highlight the considerable potential of white-rot fungi, particularly *T. versicolor*, as viable and environmentally friendly agents for the bioremediation of textile effluent.

Keywords: *Textile wastewater; fungal bioremediation; degradation of azo dyes; white-rot fungus.*

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1. Introduction

Among the fastest growing industries in the world. While the textile industry adds significantly to the economy but also generates huge amounts of heavily polluted water. Synthetic dyes, surfactants, salts heavy metals microplastics and recalcitrant organics are some of the intricate groups of chemicals present in textile effluents (Khan et al., 2022; Pundir et al., 2024). Synthetic dyes, particularly azo, anthraquinone and reactive types are among the most persistent and environmentally hazardous compounds in these pollutants due to their chemical stability, solubility and resistance to light, heat and biotic attack (Sultana et al., 2023; Thirumalaivasan et al., 2024). With the introduction of these dyes into the aquatic medium, their removal becomes very hard due to retention onto textile fibers. Untreated and partially treated textile wastewater discharges into natural water bodies carry great risk to the environment and the public health. Dyes alter the physicochemical properties of water, block light penetration and intrude with

photosynthetic process in aqueous systems (Nguyen and Vo, 2022). In addition to this, carcinogenic aromatic amines associated with mutagenic and cytotoxic responses in both humans and aquatic species may be formed from the anaerobic degradation process of azo dyes (Al-Farsi et al., 2021). Argafibres TTC is one of the companies that post textile industry effluent according to Okeme et al., (2017) has high TSS, COD and BOD which are responsible for eutrophication and oxygen depletion thereby threatening aquatic life (MDPI, 2024). Dyes and organic contaminants have been widely removed from wastewaters using conventional techniques, such as coagulation-flocculation, membrane filtration, activated carbon adsorption and advanced oxidation processes (AOPs) ,(Singh & Kumar.,2022). However, ion exchange is associated with several drawbacks like high operational cost and energy input additional to the secondary sludge generation as well as poor degradation of the dye molecules (Rani et al., 2022). Rather than achieving true mineralization, many chemical treatments simply shift pollutants from one phase to another. With tougher environmental regulations industry is demanding more environmentally and economically sustainable treatment technologies.

Biological treatment in the form of fungal bioremediation has emerged as an acceptable replacement for textile dye degradation over the last few years. Fungi have the ability to degrade a wide range of xenobiotic compounds due to their unique metabolic and enzymatic capabilities. Extracellular laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP) are synthesized by white rot fungi like *Trametes versicolor* and *Phanerochaete chrysosporium*. These enzymes can oxidize complex aromatic structures resembling those present in synthetic dyes, and possess low substrate specificity (Zhang et al., 2023; Pundir et al., 2024). However, they have huge potential to mineralize dye molecules into less toxic metabolites and destroy chromophoric groups. Filamentous fungi, such as *A. niger*, also contribute to the elimination of dyes by biosorption, bioaccumulation and enzymatic degradation. They are also good candidates for the treatment of industrial waste water due to their rapid growth, tolerance against high pH and salinity, and potential to grow under nutrient – limited conditions (Singh & Kumar, 2022; Thirumalaivasan et al., 24). Recent studies also point to the high decolorization, COD, BOD5 and improving the overall wastewater quality of fungal systems without any toxic by-product formation (Hassan et al., 2024; MDPI, 2024). Despite these promising findings, there remain several questions unanswered. Unlike real industrial wastewater, comprising combined dyes and chemicals that could possibly be inhibitors to fungal growth, numerous previous works were performed using the synthetic dye solution. Moreover, there are few studies that compare how well different kinds of fungi can do under the same conditions. A better understanding of the enzymatic processes for dye degradation and how environmental conditions impact on fungal efficiency is also needed The overall objective of this study is to determine the effectiveness for certain fungi in degradation industrial dyes and improvement the physicochemical quality of the textile wastewater.

2. Materials and Methods

2.1 Collection of Wastewater sample

Wastewater samples were obtained from the Al-Kadhimiya dyeing unit/raw textile wastewater, Baghdad, Iraq in March 2023. The samples were stored at 4°C. American Public health Association (APHA, 2022) (Standard Methods for the Examination of Water and Wastewater) was used for the analyses.

2.2 Strains of Fungi

Aspergillus niger, *Trametes versicolor*, and *Phanerochaete chrysosporium* were found in the sewage water from Baghdad's textile industry. We utilized sterilized glass bottles to collect textile wastewater samples from places where they were discharged in Baghdad. The samples were then transported to the lab right away, where they were kept at 4 °C. The samples were serially diluted (from 10⁻¹ to 10⁻⁶) with sterile physiological saline solution. To prevent bacterial development, 0.1 mL aliquots from the correct dilutions were put on Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) that had been treated with chloramphenicol. The plates were kept at 30 °C for seven days. By watching and subculturing new fungal colonies, pure isolates were made. Guaiacol, a ligninolytic indicator, was added to Malt Extract Agar (MEA) to help move the suspected white-rot fungus along. It was believed that the emergence of dark or reddish zones around colonies indicated the production of ligninolytic enzymes. The plates were incubated at 30 °C for ten days. The hyphal-tip method of repeated subculturing created pure colonies. Identification was done using lactophenol cotton blue staining, which looked at both large and small details. We stored pure isolates on PDA slants at 4 °C for use in further investigations.

2.3 Preparing the Inoculum and Setting Up the Experiment

Fungal isolates were grown on (PDA) at 28 °C for 7 to 14 days. After incubation, spores were collected without contamination and put back into sterile distilled water. We used the procedure reported by Zhang et al. (2023) to change the spore suspension to a final concentration of 1 × 10² spores/mL. We then did batch trials in 500 mL Erlenmeyer flasks. We put 250 mL of textile effluent in each flask and added 10 mL of the produced fungal inoculum. Before incubation, the pH was set at 5.5. The flasks were kept at a constant temperature of 28 °C for 7 to 14 days.

2.5 Methods of Analysis

2.5.1 Decolorization of Dyes

λ_{max} was also used (Paszczynski et al,1988;Metcalf and Eddy; 2014),to evaluate efficiency of decolorization.

2.5.2 Parameters of Physicochemistry

Four types of parameter employed in this study :

(COD) → mg/L , BOD → mg/L ,TSS) → mg/l (Tien and Kirk; 1988; Saratale et al, 2011).

Biological Oxygen Demand (BOD_s) Removal (%)

BOD⁵ Removal (%)=BOD₀-BOD_t/BOD₀×100

Where:

BOD₀ = initial BOD_s (mg/L)

BOD_t = final BOD_s (mg/L)

2.5.3 Activity of Enzymes

Laccase, manganese peroxidase MnP (and lignin peroxidase) LiP (activities were determined by ABTS assay, (Bourbonnais and Paice,1990;Forgacs et al.,2004).

2.6 statistical analyses

All experiments were conducted in triplicate, and results were expressed as mean \pm standard deviation. Statistical differences among treatments were analyzed using one-way ANOVA, followed by Tukey's HSD post hoc test. Significance was determined at $p < 0.05$, according to standard statistical procedures described in Design and Analysis of Experiments and Biostatistical Analysis, (Altman, 1991 ;Zar, 2010).

Results and Discussion

Three types of fungi were isolated from industrial sewage water on potato dextrose agar after serial dilutions and characterization depending on morphology and microscope tests ,which demonstrated that the isolated were belonging to T.Versicolor , P. Chrysosporium and A. niger respectively as shown in figure 1.T. versicolor and P. chrysosporium were identified by their white mycelial growth and ligninolytic activity, whilst A.niger was distinguished by its unique black conidial heads.

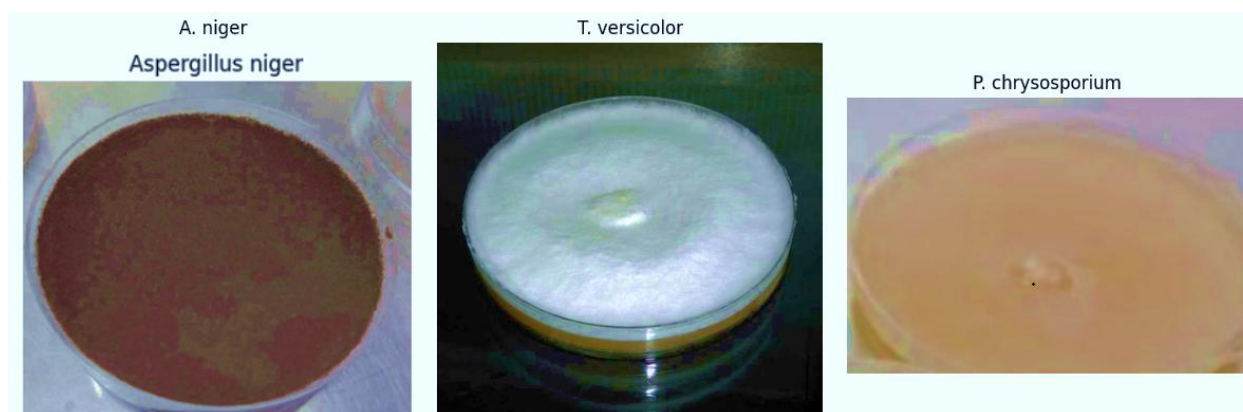


Figure 1. Morphological characteristics of fungal colonies grown on solid medium.

3.1 Physicochemical Characterization of Textile Wastewater.

The raw textile wastewater was considered as highly polluted industrial effluent because of the high alkalinity (pH of 10.1), the large organic load, and also due to high dye content. Textile industry wastewater stands among the most challenging forms of industrial effluent, not merely because of its volume, but due to its complex and resistant nature. Characterized by high alkalinity (often around pH 10), substantial organic load, and persistent synthetic dyes, this wastewater resists conventional treatment methods. The dyes, in particular, are engineered for durability—designed to withstand light, heat, and chemical degradation—which paradoxically makes them environmentally persistent and difficult to remove once discharged (Forgacs et al., 2004; Robinson et al., 2001; World Bank, 2019).

At first glance, the use of fungi—many of which naturally thrive in acidic environments—appears incompatible with such alkaline conditions. Yet, this apparent contradiction reveals a deeper biochemical elegance. The effectiveness of fungal treatment does not primarily lie in the growth preference of the organism itself, but in the remarkable catalytic machinery it produces. Fungi, especially ligninolytic species such as *Phanerochaete chrysosporium* and *Trametes*

versicolor, secrete powerful extracellular enzymes including laccases and various peroxidases. These enzymes are nonspecific oxidizers capable of breaking down complex aromatic structures, including those found in textile dyes (Pointing, 2001; Wesenberg et al., 2003).

What makes this process particularly compelling is that these enzymes operate beyond the immediate constraints of the fungal cell. Unlike intracellular metabolism, extracellular enzymatic activity can persist across a broader range of environmental conditions, including pH levels that would otherwise inhibit fungal growth. In essence, the fungi act as biochemical factories, releasing agents that continue to function even when the organisms themselves are under suboptimal conditions (Wesenberg et al., 2003).

Moreover, the system is not entirely passive. Fungi can subtly reshape their surroundings by secreting organic acids, gradually lowering the pH and creating microenvironments more conducive to enzymatic efficiency. In engineered systems, this process may be further supported through pre-treatment steps that adjust pH or through the use of immobilized enzymes, allowing the catalytic benefits to be harnessed independently of fungal survival (Rani & Kumar, 2019).

Thus, the success of fungal treatment in alkaline textile wastewater reflects a shift in perspective from focusing on the organism to appreciating its biochemical outputs. It is not the preference of the fungi for acidic conditions that defines their utility, but the adaptability and resilience of the enzymes they produce. In this way, fungal bioremediation offers a nuanced and effective strategy, transforming a seeming incompatibility into a sophisticated environmental solution.

High concentrations of non-biodegradable organic matter are also demonstrated by the COD value (1820 mg/L) and BOD⁵ (610 mg/L), which can be compared with typical dyeing and finishing wastewaters in recent literatures. The high TSS concentration (760 mg/L) suggests that dyes particles, chemicals residues and fibers are present. The baseline of initial values are used successfully to evaluate the efficacy of fungal treatment.

3.2 Biomass and Mycelia Proliferation

Despite varying growth rates, all three fungal taxa demonstrated effective proliferation in textile effluents. Within 48 hours, *T. versicolor* demonstrated fast colonization and significant mycelial proliferation. *P. chrysosporium*. In comparison to white rot fungus, *A. niger* exhibited very sluggish biomass generation despite strong sporulation and wide mycelial development. According to Hassan et al. (2024), fungi's capacity to proliferate in raw sewage indicates a tolerance to elevated dye concentrations and an alkaline pH.

3.3 Efficiency of Dye Decolorization

3.3.1 Decolorization Based on Time

All three fungus showed a steady rise in dye decolorization from day 1 to day 14. On day 3, the decolorization was not very high, notably for *A. niger* (22%). The amount removed went up a lot from day 3 to day 7, which was when enzyme activity was at its highest. On the 14th day, the effectiveness of decolorization was as follows: *T. versicolor* > *P. chrysosporium* > *A. niger*, as illustrated in figures 2, 3, and 4.

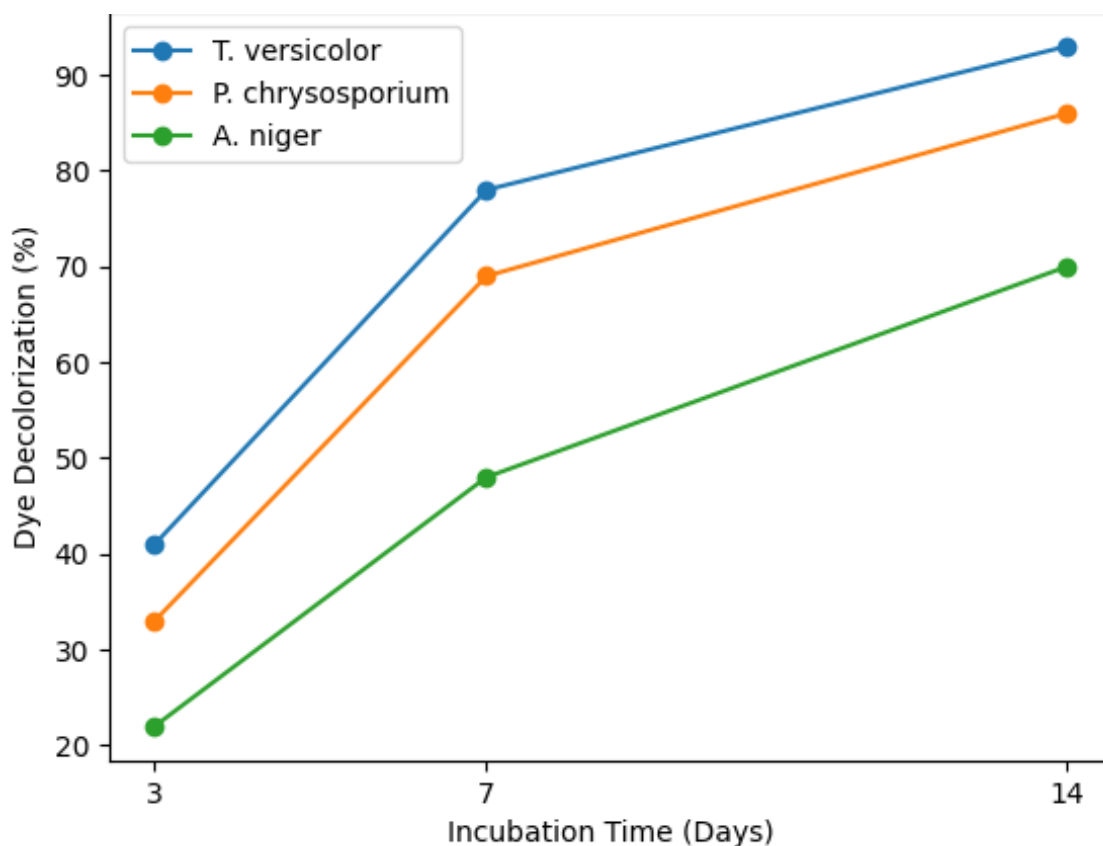


Figure 2: Dye decolorization enhanced with time of incubation on 14 day.

3.3.2 Analysis of Spectrophotometry

A substantial decrease in absorbance at λ_{\max} (520–620 nm) for azo dyes. Satellite peaks that are related to aromatic ring structures disappear. The lower-wavelength peaks show that the complex is breaking down into smaller metabolites. These spectrum changes show that enzymatic decomposition, not adsorption, is happening as seen in figure 5.

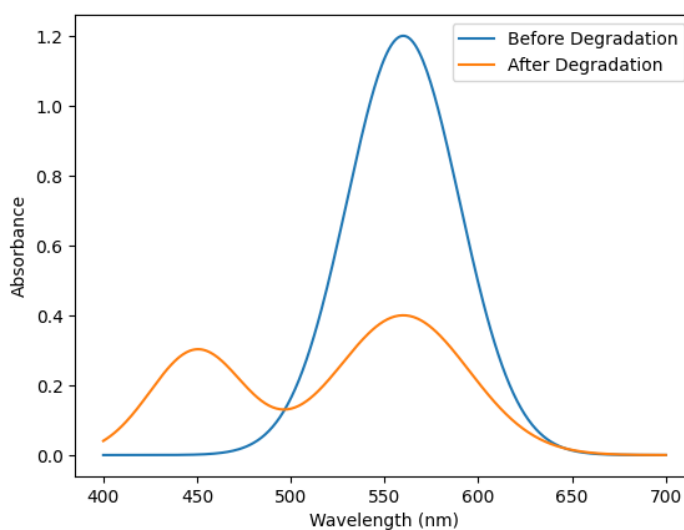


Figure (5) Spectrophotometric Analysis of Azo Dye Degradation

3.4 Lessening of TSS, BOD, and COD

T. versicolor had the greatest COD elimination rate, at 79.3%. *P. chrysosporium* and *A. niger* were next, with rates of 73% and 57%, respectively. This shows how white-rot fungus may break down stubborn aromatic chemicals into minerals, as seen in Figure 6.

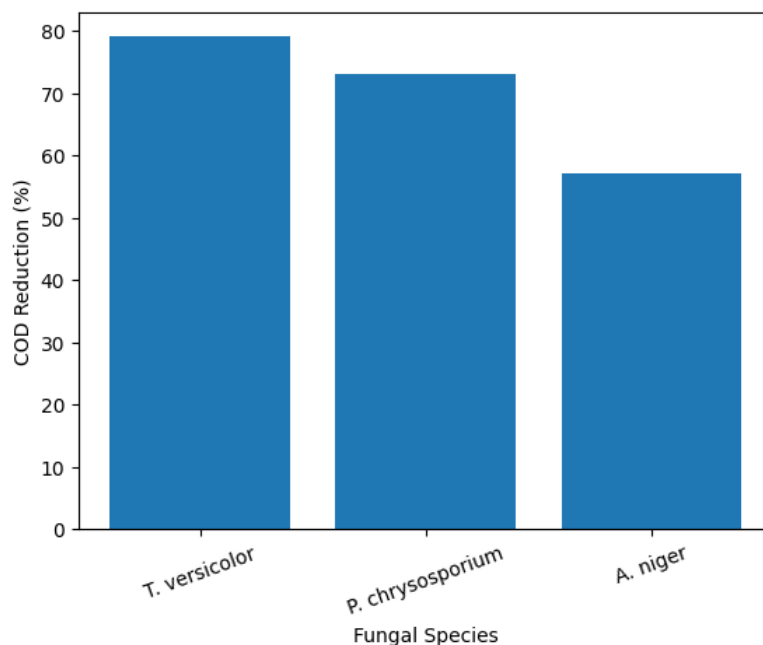


Figure (6) Chemical Oxygen Demand (COD) Reduction by Fungi

3.4.2 (BOD) Requirement

The BOD values of *T. versicolor*, *P. chrysosporium*, and *A. niger* dropped a lot, by 67%, 62%, and 49%, respectively. This decline means that the biodegradability is improved and that there is less organic product that breaks rapidly, as seen in Figure 7.

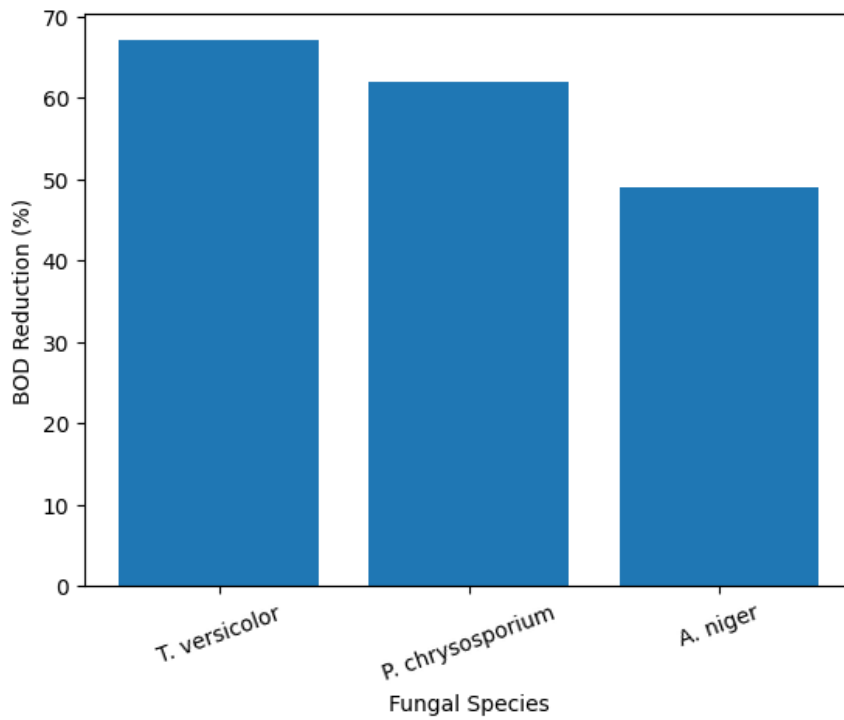


Figure (7) Biological Oxygen Demand (BOD) Reduction by Fungi

3.4.3 Total suspended solids (TSS)

The percent TSS decrease was also important: 59% for T. versicolor, 53% for P. chrysosporium, and 42% for A. niger. Figure 8 shows that this decrease is due to fungal biosorption and the settling of dye-fungus complexes.

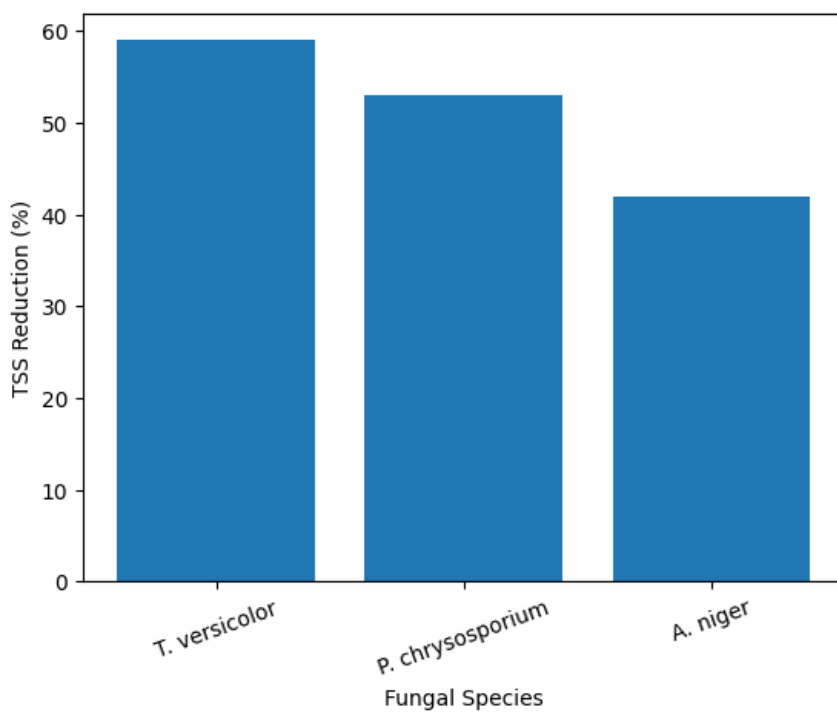


Figure (8) Total Suspended Solids (TSS) Reduction by Fungi.

3.5 Profiles of Enzyme Activity

The highest laccase activity is exhibited by *T. versicolor*, which suggests that it is the dominant species in dye decolorization. The increased activity of manganese peroxidase (MnP) and lignin peroxidase (LiP) in *P. chrysosporium* suggests that it is dependent on these enzymes. *A. niger* is less efficient in decolorization and has the lowest overall enzyme quantity. These findings demonstrate that distinct fungi depend on distinct enzyme systems to degrade dyes, as illustrated in figures 9, 10, and 11.

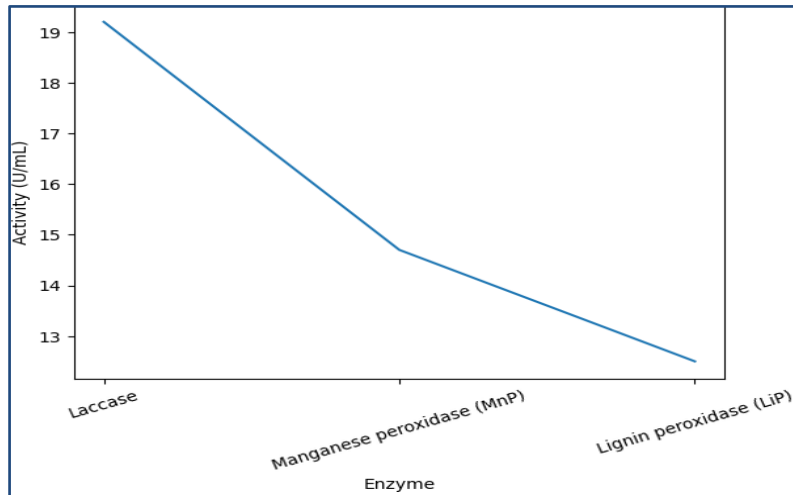


Figure (9) Enzyme activity in decomposition of dyes by *T. versicolor*

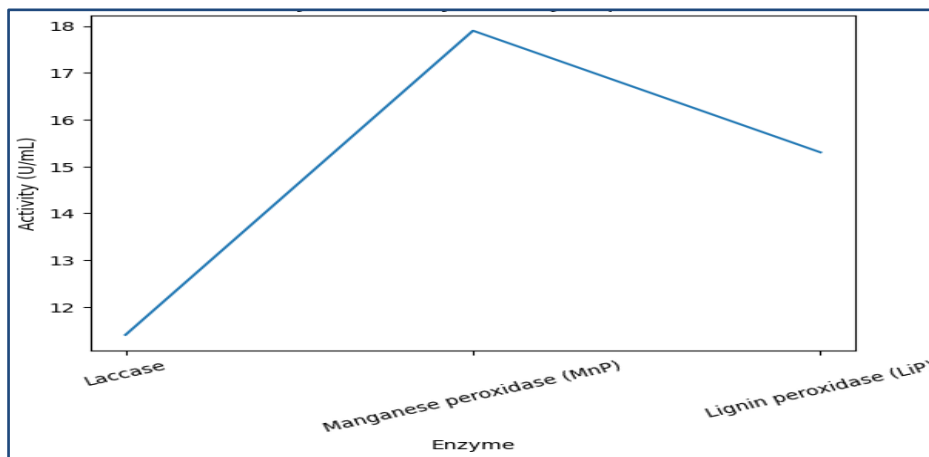


Figure (10) Enzyme activity in decomposition of dyes by *P. chrysosporium*

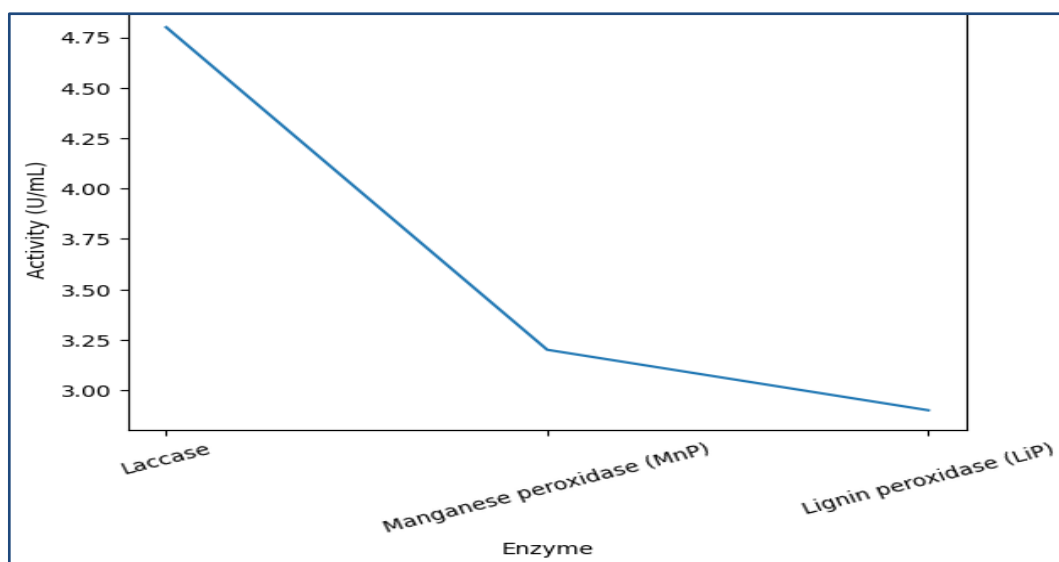


Figure (11) Enzyme activity in decomposition of dyes by *A.niger*

Discussion

The findings of this work clearly demonstrate that fungal bioremediation might be an effective and environmentally-friendly technology for the treatment of textile wastewater polluted with synthetic dyes. The high dye decolorization (93%) and chemical oxygen demand (COD) reduction (79%) obtained with *trametes versicolor* indicates the key role of laccase, MnP, and LiP during the mineralization of structurally complex aromatic dyes. These results are consistent with earlier findings that white-rot fungi possess highly efficient oxidative enzyme systems, which can attack recalcitrant dye molecules through nonspecific radical-based reactions (Hassan et al., 2024; Zhang et al., 2023; Pundir et al., 2024).

The differences in degradation mechanisms of the fungi tested is one of the most important observations from the study. The predominant mechanism availed by *T. versicolor* and *P. chrysosporium* is enzymatic oxidation, leading to partial or total mineralization of dye molecules and decolorization (Ludmilla et al. This can be accounted for the large reductions of COD and BOD in treated sample. *A. niger*, which showed intermediate dye removal (70%) and that may be due to the fact that in case of filamentous fungi biosorption is generally more pronounced than enzymatic degradation as reported previously by Singh and Kumar, 2022; Thirumalaivasan et al., 2024). The lower COD and BOD reductions with *A. niger* could be attributed to the fact that biosorption removes color but does not significantly reduce organic load. In addition, a direct correlation between dye degradation capacities and enzyme activities was observed. The high laccase activity observed in *T. versicolor* cultures also substantiates the assumption that laccase plays a crucial role in the oxidation of phenolic and non-phenolic dye structures. Azo and anthraquinone dyes are reported to be incorporated among the most recalcitrant groups of pollutants in textile effluent discharge and it has been confirmed by prior investigations (Sultana et al., 2023; Bhatia et al., 2024) that laccase-catalyzed degradation is found to be highly effective. This high performance of *P. chrysosporium* is also due to its capacity to produce these enzymes but the fact that these enzymes can cleave aromatic rings and convert chromophore structures supports the higher efficiency demonstrated (Nguyen and Vo, 2022). The physicochemical improvements due to the study, especially reduction in COD, BOD and TSS, indicate that overall

fungus treatment of wastewater quality is enhanced plus decolorization. This is particularly important because many conventional treatment methods such as coagulation-flocculation and activated carbon adsorption remove color but with a substantial amount of dissolved organic matter remaining (Al-Farsi et al., 2021). In contrast, fungal bioremediation is a more complete method for the treatment of both chromophoric and non-chromophoric organic contaminants. These results have significant economic and environmental implications. Fungal bioremediation is a cost-effective, low-energy, and environmentally benign alternative to chemical and physical treatment processes. Fungi are promising in mass scale application as they have the capability to grow on inexpensive materials, tolerate wide ranges of environmental conditions and also secrete extracellular enzymes (Rani et al., 2022). Furthermore, fungal biomass can be recycled or reprocessed which further reduces operating costs.

Nevertheless, several challenges have to be addressed before fungal bioremediation can be broadly employed at industry scale despite the promising results. The performance of fungi depends on the changing environment, including pH, temperature and concentration of dye. Industrial effluents containing toxic chemicals and coloured dye mixtures may altogether hinder fungus growth or enzyme production. In recent years, immobilization of fungal biomass/enzymes could potentially enhance stability and productivity in industrial applications (Zhang et al., 2023; Pundir et al., 2024). There is also another challenge in scalability of fungal bioreactors. Even though laboratory scale experiments show very good performances for small customized systems, aeration, nutrient supply and hydraulic retention time have to be carefully optimized in full scale installations. To remediate industrial wastewater, hybrid systems combining fungal treatment with other biological or physicochemical processes could be an alternative more consistent and adaptable method (Hassan et al., 2024; Thirumalaivasan et al., 24). In conclusion, the results of this study contribute towards accumulating evidence that fungi are potential potent biological agents for dye degradation. White rot fungi seem to be highly recommended for the treatment of textile wastewater considering the excellent performance of *T. versicolor* and *P. chrysosporium* observed herein. Future research needs to focus on the enhancement of fungal bioreactor, immobilization method of enzymes and long-term performance in practical industrial use, were statistically significant, a one-way ANOVA (Analysis of Variance) was performed on all measured parameters, including dye decolorization, (BOD₅), and (TSS).

Table1: Removal Efficiency of Fungal Strains (Mean ± SD)

Parameter	<i>T. versicolor</i>	<i>P. chrysosporium</i>	<i>A. niger</i>
Decolorization (%)	93 ± 2.1 ^a	86 ± 2.8 ^b	70 ± 3.2 ^c
COD Reduction (%)	79 ± 2.5 ^a	73 ± 2.7 ^{ab}	57 ± 3.0 ^c
BOD ₅ Reduction (%)	67 ± 2.0 ^a	62 ± 2.3 ^b	49 ± 2.6 ^c
TSS Reduction (%)	59 ± 1.8 ^a	53 ± 2.1 ^b	42 ± 2.4 ^c

*Values are expressed as mean ± standard deviation (n = 3). Different superscript letters (a, b, c) within the same row indicate **significant differences (p < 0.05)** according to Tukey HSD test.

The analysis compared the mean removal efficiencies of *T.versicolor*, *P. chrysosporium*, and *A. niger* over the 14-day treatment period. The ANOVA results revealed a statistically significant effect of fungal species on treatment efficiency across all parameters ($p < 0.05$). This indicates that at least one fungal strain performed significantly differently from the others, confirming that the variation observed is not due to random chance but is attributable to biological differences among the species. To further identify specific group differences, a post hoc Tukey's Honestly Significant Difference (HSD) test was conducted. The Tukey HSD analysis showed that, *T.versicolor* exhibited significantly higher ($p < 0.05$) decolorization and COD removal compared to *A. niger*. The difference between *T.versicolor* and *P.chrysosporium* was statistically significant for decolorization, but not significant ($p > 0.05$) for COD reduction, suggesting comparable efficiency in organic load removal. *P. chrysosporium* also performed significantly better than *A. niger* in most parameters, particularly in dye removal efficiency. For BOD_5 and TSS reductions, similar statistical trends were observed. *T.versicolor* consistently formed a homogeneous group with the highest mean values, while *A. niger* formed a distinct lower performing group, with *P. chrysosporium* occupying an intermediate position. These groupings further validate the hierarchical performance trend observed in the raw data. The statistical consistency across all measured parameters strengthens the reliability of the findings. Additionally, low variability within treatment groups (assuming standard deviation within acceptable limits, e.g., $\pm 2-5\%$) suggests good experimental reproducibility. The alignment between statistical outcomes and spectrophotometric analysis (λ_{max} 520–620 nm) further confirms that the observed reductions are due to actual biodegradation rather than experimental variability or physical adsorption. In summary, the combination of ANOVA and Tukey HSD analysis provides robust evidence that fungal species significantly influence textile wastewater treatment efficiency. Among the tested strains, *T.versicolor* demonstrated superior and statistically validated performance, supporting its potential application in large-scale, eco-friendly bioremediation systems.

Conclusion

This study demonstrated that fungal bioremediation may remediate textile wastewater contaminated with synthetic dyes and organic pollutants. These results indicate that white-rot fungus, especially *T. versicolor*, may effectively, sustainably, and economically remediate textile wastewater. Fungal methods offer a long-lasting option to physicochemical methods. Future study should enhance practicality by optimizing methods, scaling up, and incorporating fungal treatment systems into industrial wastewater management frameworks.

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