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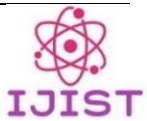

Bioremediation of Textile Disperse Dyes using White-Rot Fungi *Trametes versicolor*

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Disperse dyes, frequently used in textile dyeing processes, present a particular challenge because of their recalcitrant nature. With an emphasis on wastewater effluent treatment, white-rot fungi *Trametes versicolor* were used. The fungus was cultured on different media and optimized various biochemical parameters (temperature, pH, inoculum size, dye concentration, and culturing time). After their biomass, disperse Red-I (DR1) and disperse Blue-I (DB1), and textile wastewater were biodegraded with the fungi *T. versicolor*. The growth of *T. versicolor* is time taking but maximum degradation by *T. versicolor* (0.02 to -0.11 during 3 days) is observed. In DB1 solutions and wastewater, absorbance values started at different points. However, the efficiency of fungi was found to be more than 80%. The potential of degradation of fungi in wastewater treatment can be further maximized to reduce environmental impact.

Keywords: Disperse dyes, *Trametes versicolor*, Bioremediation, Textile, Waste water

Introduction:

Fungi possess a remarkable ability to biodegrade pollutants, thereby playing a vital role in sustaining environmental health. Among these, White-Rot Fungi (WRF), a subgroup of basidiomycetes, are especially notable for their exceptional capability to decompose non-bioavailable substances such as lignin, cellulose, and hemicellulose. These fungi are essential in natural decomposition processes and have significant potential for bioremediation applications [1]. These secrete one or more extracellular (sometimes intracellular) enzymes that directly degrade complex structures by converting them into simpler, more bioavailable forms [2]. According toauthors, nine species fall under this genus, including *T. gibbosa*, *T. junipericola*, *T. cervina*, *T. ljubarskyi*, *T. ochracea*, *T. pubescens*, *T. hirsuta*, *T. suaveolens*, and *T. versicolor*. A group known as the Coriolus group, including the widespread species *T. ochracea*, *T. hirsuta*, *T. pubescens*, and *T. versicolor*, is marked by its thin basidiocarps and a slender line [3].

T. versicolor (commonly known as *Coriolus versicolor* or Turkey Tail) is a saprophytic mushroom that stands out as one of the most extensively researched and well-characterized fungi [4]. This fungus is particularly abundant in temperate and subtropical regions and is often observed growing on decaying hardwood logs, stumps, and branches [4]. The fruiting bodies are comprised of fan-shaped structures with distinct zones of different colors ranging from brown, tan, grey, green, blue, purple, and even black [4]. The ligninolytic enzyme system in turkey tail is non-substrate specific, enabling it to degrade a huge variety of pollutants, including synthetic dyes, pesticides, and other recalcitrant compounds, making it essential for environmental cleanup [2].

Studies have demonstrated that laccase derived from *T. versicolor* can remove chlorine atoms from various compounds [5], further enhancing its potential for detoxifying chlorinated pollutants and supporting environmental remediation [6][7]. Various types of plastics, including PVC, PVA, are degraded by WRF. Among these, *T. versicolor* demonstrates exceptional efficiency in degrading Nylon-6-6, highlighting its potential for addressing plastic waste challenges [8]. *T. versicolor* is renowned for its well-characterized enzyme system, primarily dominated by laccases. Although other enzymes, such as versatile peroxidases, may also be present, they are generally produced in significantly smaller quantities. Among these enzymes, laccases produced by WRF are particularly recognized for their high efficiency [9] and oxidation of various organic compounds such as polyphenols, monophenols, aromatic amines, and non-phenolic substances by utilizing molecular oxygen [10]. During dyeing processes, a considerable amount of dye may not fix onto the fibers and remain in the wastewater. Due to their low water solubility, these dyes are resistant to natural degradation and can persist in aquatic environments, posing potential risks to aquatic organisms and disrupting ecosystems [11][12]. The chemical structures of disperse dyes have benzene rings in them, which makes them prone to degradation by WRF laccases and lignin peroxidases.

These rings serve as an attacking site for fungal enzymes. Although not limited to benzene rings, various types of carbon ring structures can potentially serve as substrates for lignocellulolytic enzymes [13]. These dyes resist biodegradation, making them harmful to aquatic flora and fauna by obstructing photosynthesis and disrupting the ecosystem. Moreover, their release into water bodies contributes to environmental pollution, affecting both aquatic life and human health [12]. Proper waste management and eco-friendly alternatives are essential to mitigate these dangers and safeguard the environment and public well-being [12][14]. In the current study, two Disperse dyes have been used, including Disperse Red-1 and Disperse Blue-1, to compare the degradation ability of fungi. Due to the similarity in structures of lignin and disperse dyes (i.e., the benzene ring), WRF enzymes can destroy their structure to make it more reactive and bioavailable [15]. By harnessing the unique enzymatic activities of this fungus, we can potentially mitigate the impacts of pollution and work toward creating a cleaner, healthier environment.

Objectives:

The objective of this study is to identify microorganisms capable of biodegrading textile disperse dyes and to develop an eco-friendly wastewater treatment process. It focused on examining the oxidative enzymes involved in the degradation. There is a growing need to explore dye-degrading microorganisms and establish sustainable bioprocesses for treating industrial wastewater. This approach can help minimize environmental pollution and lower disease risks in areas near industrial zones.

Materials and Methods:

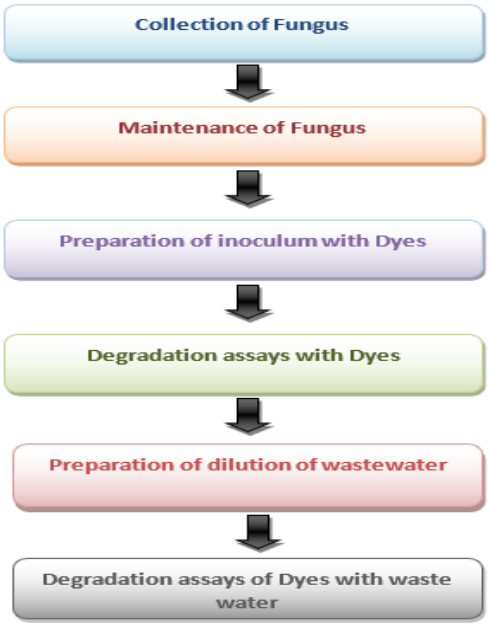

Figure 1. Methodology flow diagram

Collection:

This study explored the degradation of Disperse Red-1 and Disperse Blue-1 dyes using the fungus *T. versicolor*. The *T. versicolor* strain employed in the research was obtained from Punjab University, Lahore. This fungus grows without a stalk on the hardwood portions of tree trunks or the bark. Disperse Red-1 (DR1) and Disperse Blue-1 (DB1) dyes, used as standards in this study, were obtained in powdered form from Comfort Fabrics, Lahore, Pakistan. Wastewater from the textile industry was collected from Comfort Fabrics in Lahore, Pakistan, to be used as a growth medium for the fungi. Pure dye samples were utilized as references to determine and compare the concentration of dyes present in the wastewater.

Identification and maintenance of fungi:

T. versicolor was identified by its small, thin, leathery, and almost rounded or semicircular mushrooms, characterized by multicolored zones with alternating hairy and smooth regions. The cultures of *T. versicolor* were purified and grown on nutrient agar. Cultivation was carried out under mesophilic conditions, with a temperature range of 27–30 °C and a pH of 5–6, with a maximum incubation period of 3 days. To eliminate contamination, sub-culturing was performed multiple times. The purified culture was then transferred to a 50 mL broth medium and incubated under shaking conditions for 3 days to promote the secretion of extracellular enzymes into the medium.

Preparation of inoculum with dyes:

A 20% stock solution of the dyes was prepared in Dimethyl Sulfoxide (DMSO). Each flask contained 50 mL of broth for the experiments. In total, 12 flasks were prepared for the experiment. One flask was used as a blank, containing only the broth medium without any dye or fungal culture, while another flask served as the control, containing broth with the fungal

culture but without the addition of dye. A total of 10 flasks were specified by concentrations of the dyes, i.e., 0.01%, 0.02%, 0.03%, 0.04% and 0.05%. DR1 was added in 5 flasks, while DB1 was added in the other 5 flasks. The dilution formula was applied to determine the volume of the 20% stock solution needed to achieve the desired concentration. The formula used was:

$$C_{(stock)} \times V_{(stock)} = C_{(dilution)} \times V_{(dilution)}$$

In this formula, $C_{(stock)}$ is 20%, $C_{(dilution)}$ varies from 0.01 to 0.05%, $V_{(stock)}$ is to be found out, and $V_{(dilution)}$ is 75 mL. Considering these parameters, the volumes were calculated as shown below in Table 1.

Table 1. Volume of dyes used to prepare different dilutions

Dilution of Textile dye	Volume of Stock used
0.01%	0.0375 mL
0.02%	0.075 mL
0.03%	0.1125 mL
0.04%	0.150 mL
0.05%	0.1875 mL

These calculated volumes of dyes were added using a micropipette in the medium after 48 to 50 hours or on the 3rd day of incubation, based on the previously determined values for V (stock).

Biodegradation assays with dyes.:

A 3 mL sample was collected from Punjab University, Lahore, every 24 hours over 3 days to monitor the activity of *T. versicolor*.

The intensity of colors was qualitatively analyzed for 3 days utilizing UV-vis spectrophotometry with absorbing wavelengths of DR1 479 nm and DB1 of 615 nm. The fungal broth culture without any dye was used as the blank (auto-zero) reference for spectrophotometer readings. Positive control was also made by adding broth plus inoculum into the media. This was done to corroborate the visual observations of dye degradation and to compare them with the corresponding spectrophotometric measurements. Negative control contained broth plus dyes (highest concentration being used in the experiments, i.e., 0.05%.

Preparation of dilutions of wastewater:

Wastewater was diluted to degrade fungus effectively and remove toxicity for fungi, as wastewater also contains other chemicals like salts and surfactants (to make the disperse dyes soluble in water). Although the exact composition was unknown, it was confirmed that the sample contained only orange, red, and black disperse dyes; however, the specific types of dyes were not identified. Nearly 2% and 4% dilutions were used for fungal cultures, calculated by:

Here, $C_{(stock)}$ is taken 100% because the exact concentrations of dyes in the wastewater were not known; an approximate value was assumed for calculation purposes. Based on this approximation, the volume of wastewater used was determined and is presented in **Table 2**.

Table 2. Volumes of Wastewater used in each dilution

Dilution of wastewater	Volume (mL)	Volume (µL)
2%	1.5	1500
4%	3.0	3000

These concentrations were added after growing the fungal cultures in the broth for 72 hours, and afterwards, the wastewater was added. It was monitored for 3 days for *T. versicolor* both visually and by spectrophotometer assays. Positive and negative controls were also prepared.

Biodegradation assays of dyes in wastewater:

Wastewater collected from the industry contained Disperse Red, Orange, and Black dye of unknown concentration and unknown kinds. The maximum absorbance values of Disperse Red and Orange dyes fall within the range of 470-500nm (the maximum absorbance value of Disperse Red 1 is 479 nm; thus, it was used as a standard to measure the degradation level of Disperse Red and Orange dyes). The absorbance values of black dye were observed to fall within the range of 600–620 nm. Since Disperse Blue-1 exhibits a maximum absorbance at 615 nm, this wavelength was used as a standard reference to evaluate the degradation level of the black dye. As the specific type of disperse dye was unknown, average absorbance values for all likely dye types were calculated and used to estimate the spectrophotometric readings. The absorbance value for Disperse Red and Disperse Orange dyes was set to 480 nm for calculations, while 600 nm was used for Disperse Black dye. The broth used for growing the fungal cultures was utilized as a blank in the spectrophotometer to nullify background absorbance.

Data Automation:

To ensure accuracy and efficiency in data preparation and analysis, Python was employed for automating key aspects of the experimental workflow. This included automating dilution calculations, organizing time-series absorbance data, and generating formatted tables for spectrophotometric readings. By using Python libraries such as NumPy and Pandas, all stock-to-working solution volumes were calculated consistently using the standard dilution formula ($C_1V_1 = C_2V_2$). Experimental observations like absorbance readings over time were automatically structured into clean dataframes and exported for plotting and analysis. This automation minimized manual errors, standardized data formatting, and improved reproducibility across trials.

Results:

Growth optimization of fungi:

Fungal growth was optimized by cultivating it in various growth media, including YBD, PDA, nutrient broth, and nutrient agar. Biomass development was observed in all containers, with temperature and pH conditions maintained constant throughout the experiment.

Carbon and Nitrogen Source:

T. versicolor was first grown on three types of agar media (PDA, YBD, and Nutrient agar). The biomass after 3 days of incubation is shown in Figure 2. PDA medium contains potato extract as its primary nutrient source. YBD medium includes both yeast extract and beef extract, providing a rich supply of vitamins and proteins. The nutrient medium primarily utilizes beef extract as a nitrogen source. When a 0.1cm long media piece was cultured onto the agar plates, it was incubated for 3 days. The broth in which the fungal cultures were grown was used as a blank in the spectrophotometer to eliminate background absorbance. This approach was employed to store and maintain the fungal cultures on solid media plates before subculturing them into the broth media for further experimentation.

In PDA agar, the growth of both spores and mycelia was remarkably rapid, covering the entire plate within a 72-hour span. The issue with this rapid growth was that, during sub-culturing, it became difficult to control the size of the inoculum, as even a small area contained a dense concentration of spores and mycelia. In YBD agar, despite the presence of two nitrogen sources, fungal growth was concentrated in a single spot on the plate, producing a high number of spores while the mycelial biomass remained sparse. This also posed a challenge during subculturing, as the inoculum taken from the agar plates contained a high concentration of spores, resulting in rapid and exponential growth in the broth medium. In nutrient agar, the mycelial and spore biomass was not dense; instead, it covered the plate. This was considered the most optimum nitrogen source for *T. versicolor* as it allowed controlling the size of the inoculum. The growth has been shown in Figure 2.

(a) (b) (c)

Figure 2. Growth of *T. versicolor* on (a) PDA Agar, (b) YBD Agar, (c) Nutrient Agar

After selection of nutrient agar, the inoculum was added to 3 kinds of broth media, i.e., PDA broth, YBD broth, and Nutrient broth. Due to the higher nutrient availability in the broth medium compared to the agar medium, the fungal growth exhibited a distinct pattern, with more vigorous and accelerated development occurring in the liquid culture. In the YBD broth, since there were two types of nitrogen sources and spores were created when nutrients had been depleted, the mycelial network was remarkable. Instead of producing a large number of spores, it primarily focused on mycelial growth. In PDA broth, mycelial growth was sparse, and spore production was minimal, making it an unsuitable medium for effectively cultivating fungal biomass, as shown in Figure 3. In nutrient media, the mycelial network was again very sparse, but the spores were being created in huge amounts. The ideal nitrogen source for growth on aqueous media was considered to be a mixture of beef and yeast extract, as in YBD broth. A denser mycelial network allowed for greater dye uptake by the fungi. However, excessively rapid growth was problematic, as exponential fungal growth also led to the production of toxins in the medium, which could interfere with the binding of the dye to enzymes.

(a) (b) (c)

Figure 3. Growth of *T. versicolor* on (a) YBD Broth, (b) PDA Broth, (c) Nutrient Broth

However, a similar issue as observed with *T. versicolor* arose in the Nutrient Broth. The fungi produced more spores than mycelia, which was not ideal for biodegradation experiments, as the primary mechanism of dye degradation relies on the binding of fungal biomass, particularly mycelia with the dye molecules. PDA broth was considered the most optimal growth medium because the fungal biomass growth was just enough to be able to be incubated for about 8 days. Figure 4 is made to show the increase in biomass upon administration of all nitrogen sources, keeping the carbohydrate source constant, i.e., glucose. The temperature and pH were maintained constant throughout the experiment. It was evident

that YBD media supported the maximum fungal growth compared to the other media, as shown in Figure 4.

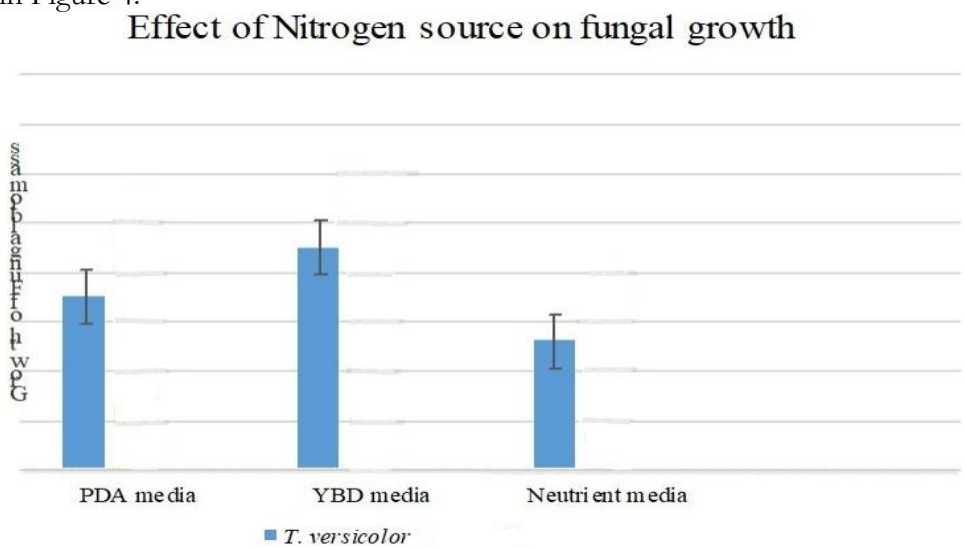

Figure 4. Effect of Nitrogen Source on Fungal Growth

The carbon sources for *T. versicolor* were not optimized due to the unavailability of the necessary chemicals in the laboratory. However, glucose was used as the carbon source in all experiments and showed acceptable performance across all media; nevertheless, the results remain inconclusive.

Temperature and pH:

However, glucose was used as the carbon source in all experiments and showed acceptable performance across all media; nevertheless, the results remain inconclusive. A total of 3 samples were incubated at 25 °C, 28 °C, 30 °C, and 37 °C while keeping the pH constant, that is, at pH 6. The growth duration was monitored to determine how quickly the fungal biomass increased and initiated spore formation, as the appearance of spores indicates that the available nutrients in the medium have been consumed and that the fungus has reached its maximum growth phase. Nutrient consumption varied with temperature, as at 25 °C, the fungi were able to colonize the entire vessel within approximately 72 to 96 hours. At 28 °C, the fungi were able to fully colonize the vessel in less than 72 hours, as shown in Figure 5. However, at 30 °C, fungal growth was significantly slower, taking up to 5 days to achieve a noticeable increase in biomass. At 37 °C, no fungal growth was observed, indicating that both mycelia and spores were unable to proliferate under normal bodily conditions (Figure 5). This observation supports the GRAS (Generally Recognized as Safe) status of *T. versicolor*. The optimal pH for *T. versicolor* is between 5.5 and 6.0, as shown in Figure 6. The experiments were carried out to check the growth at different pH values. A total of two batches of *T. versicolor* were cultivated, one at pH 5.5 and the other at pH 6.

Growth Curve of Fungi:

The resulting curve (Figure 7) illustrates the rate of biomass growth over time, with the number of days represented on the x-axis. This visual representation highlights the progression of fungal development across the observed period. Starting from day 5, a decline in mycelial biomass was observed as spore formation began, which explains the drop in the growth rate between 96 and 108 hours shown in Figure 7.

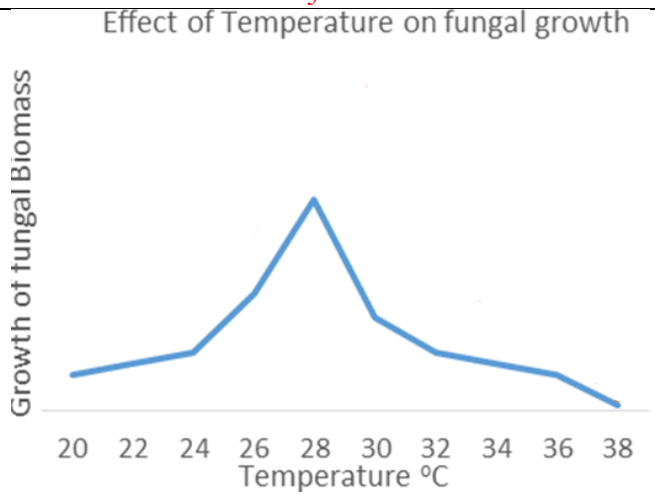

Figure 5. Effect of Temperature on Fungal Growth

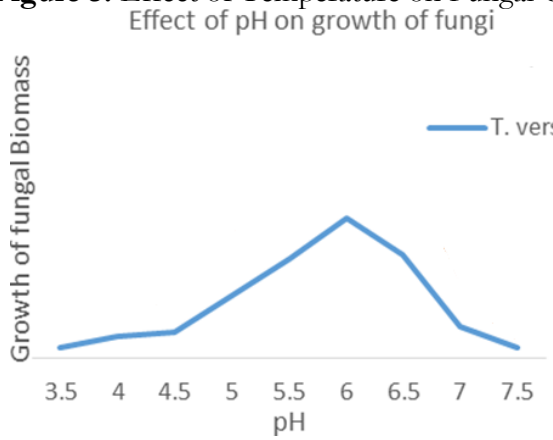

Figure 6. Effect of pH on fungal growth

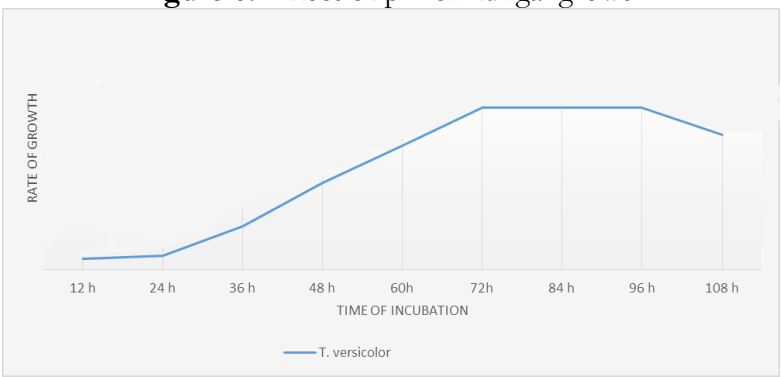

Figure 7. Growth Curve of *T. versicolor*

Biodegradation of Textile Dyes:

After successful optimization of growth conditions, the biodegradation was carried out. The degradation rate was measured by UV-vis spectroscopy after every 24 hours. To carry out this experiment, first, the fungal culture was kept in shaking incubation for 3 days at optimal conditions until the biomass had been produced. This step was carried out to enable the fungi to secrete extracellular ligninolytic enzymes into the medium, which would then be utilized for the degradation of the dyes. After 3 days of incubation, the dyes were added into the containers at varying concentrations from their 20% stock solution. Five different dye concentrations were prepared, each with increasing levels of Disperse Red-1 and Disperse Blue-1. Spectrophotometric readings were recorded for both dyes at each concentration to assess their absorbance profiles. For red dye, maximum absorbance was kept at 479 nm, while

for blue dye, the value was 615 nm. These dyes were treated as standards in the experiments with wastewater.

Biodegradation by *T. versicolor*:

When the dyes were added to the media, the spectrophotometric values were observed for 6 days after every 24 hours. The results revealed that on day between 24 to 48 hours, the values decreased, but they started to increase. Although the color of the dyes was being decolorized, the increase in absorbance values suggested that degradation was occurring. This implied the production of other compounds, primarily intermediates of the degraded dye molecules. PDA broth used as a medium was taken for auto-zero in the spectrophotometer at 479nm. The value obtained was 0.30. This setup was also treated as a positive control, as the ideal outcome for dye degradation would result in complete breakdown of the dye, leaving the solution entirely clear. The value for negative control was calculated as 1.63 (it had dye equal to the concentration of 0.05% solution; therefore, it was similar to the value of C5 for day 1. Any values between positive and negative controls were considered to be accurate between days 1 to 3. After that point, the absorbance values began to increase, making it difficult to compare the results with those of the positive and negative controls. Figure 8 clearly shows that the degradation rate was extremely fast. *T. versicolor* degraded dye in 3 days of incubation. *T. versicolor* successfully adsorbed all the dye molecules onto its mycelia within 12 hours of incubation, as shown in Figure 8. Following adsorption, the degradation of the dye molecules progressed gradually over the subsequent days. Additionally, *T. versicolor* was able to bind the intermediate compounds formed during the dye breakdown process. However, Figure 8 indicates that there was some degree of 'leakage' of dye or its intermediates back into the medium, suggesting that not all compounds remained bound to the fungal biomass throughout the degradation process.

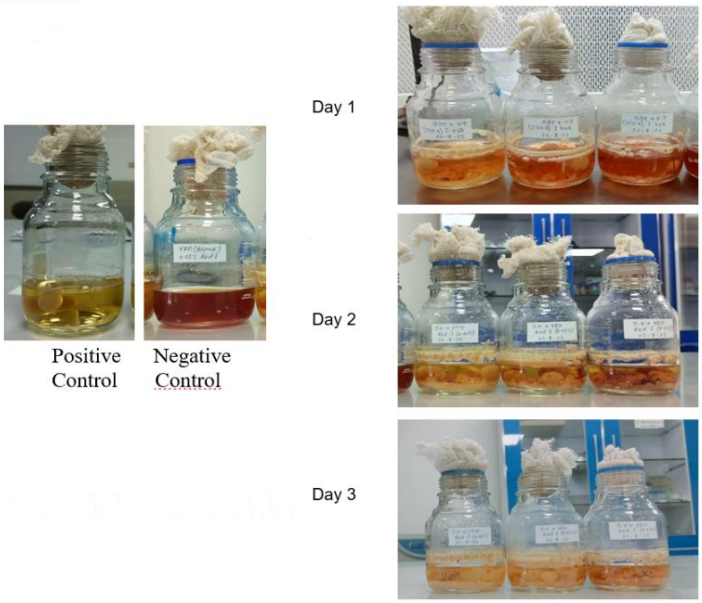

Figure 8. Visual Results of Degradation of Red-I by *T. versicolor* along with positive and negative controls

The degradation rate was monitored by calculating the absorbance values, and surprisingly, the absorbance of the broth became negative. The absorbance of Broth (Blank) =0.20, Negative control=1.38, Positive control=-0.04 as shown in Figure 9. Blank was used for auto-zeroing of the spectrophotometer. The occurrence of negative values can be attributed to the limited solubility of the dye molecules in the broth medium, which prevented a measurable increase in absorbance. Additionally, as the degradation process progressed over time, the values continued to decrease, becoming increasingly negative, likely due to further

breakdown or removal of dye molecules from the solution. This was because the nutrients kept depleting from the growth media. Table 3 shows the degradation of DR1 by *T. versicolor* below.

Table 3. Degradation of Disperse Red-I by *T. versicolor*

Days of observation	C1	C2	C3	C4	C5
	0.01%	0.02%	0.03%	0.04%	0.05%
	Absorbance at 479nm				
D1	-0.13	-0.155	-0.18	-0.08	0.02
D2	-0.15	-0.14	-0.13	-0.13	-0.13
D3	-0.09	-0.105	-0.12	-0.115	-0.11

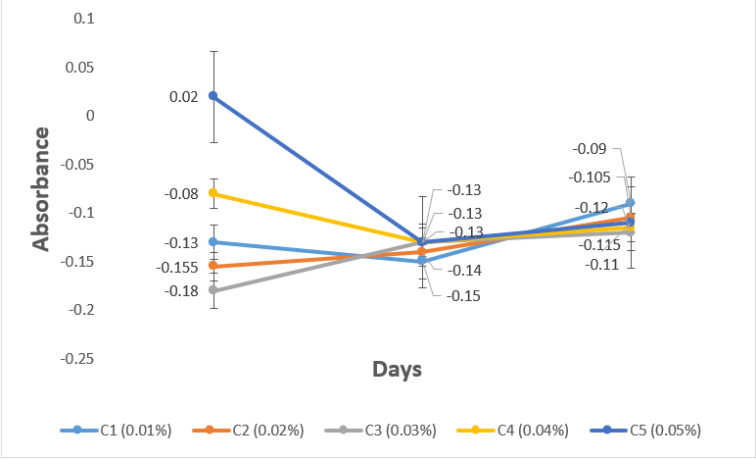

Figure 9. Degradation of Red-I by *T. versicolor*

Figure 9 demonstrates that the absorbance values continued to decrease, becoming increasingly negative and remaining relatively constant until day 3. As a result, further monitoring was deemed unnecessary. In Figure 10 below, the degradation results of DB1 are shown. Similar to DR1, within 12 hours of incubation, the dye all adsorbed onto the fungal biomass, and upon reaching day 3 of incubation, the color stayed the same. However, it became a bit more yellow, showing the accumulation of substances by the fungi.

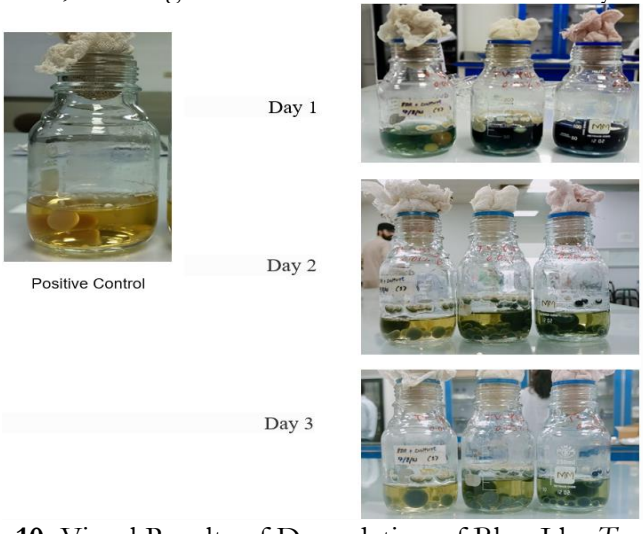

Figure 10. Visual Results of Degradation of Blue-I by *T. versicolor*

Degradation of DB1 had been more efficient than DR1, as shown in Figure 10. This is because the oxygen atoms in the side chains of disperse dyes make them more or less negative. And since laccases are acidic and protonated in their active form, the more basic the dye will be easier its degradation will be. Table 4 states the values of different concentrations

of disperse dyes over the days. The degradation was so efficient that within 24 hours, all the dye molecules cleared from the solution, and the absorbance values stayed the same. The values of Blank, negative, and positive control were almost similar to the disperse red-1 degradation because i.e., Blank = 0.20, Negative control = 1.30, Positive control =-0.04, as shown in Figure 11. Values between the negative and positive controls were considered accurate.

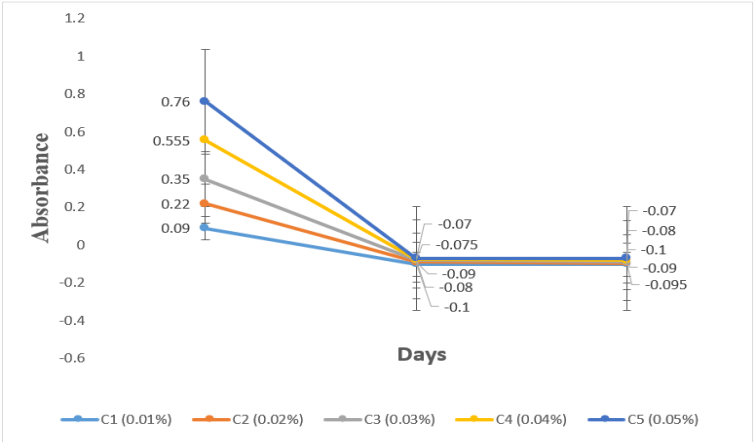

Figure 11. Degradation of Blue-I by *T. versicolor*

Figure 11 had been plotted using the values mentioned in Table 4 below. Table 4 gives us the absorbance of different concentrations of dyes on different days of observation.

Table 4. Degradation of Disperse Blue-I by *T. versicolor*

Days of observation	C1	C2	C3	C4	C5
	0.01%	0.02%	0.03%	0.04%	0.05%
	Absorbance at 479nm				
D1	0.09	0.22	0.35	0.555	0.76
D2	-0.1	-0.09	-0.08	-0.075	-0.07
D3	-0.1	-0.095	-0.09	-0.08	-0.07

Experiments with wastewater demonstrated that *T. versicolor* was effective in degrading the wastewater during the Day 3 incubation, as shown in the positive control in Figure 12 below.

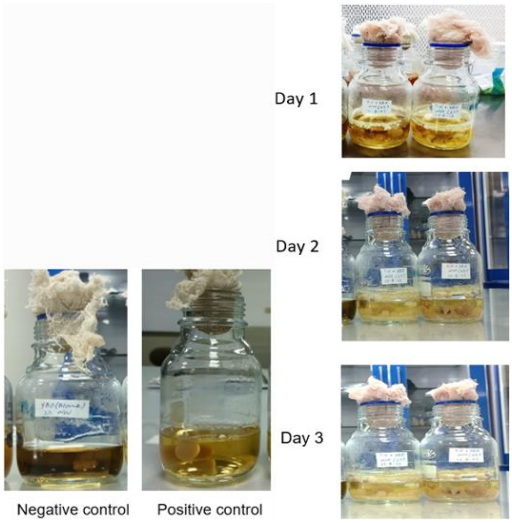

Figure 12. Biodegradation of Textile Wastewater by *T. versicolor* over 3 days

The following table (Table 5) was recorded by taking the absorbance values of the broth at 480 and 600 nm. The table was constructed for two values of absorbance for different types of dyes.

Table 5. Absorbance of Textile wastewater by *T. versicolor* over 3 days

Days of Observation	Concentration C1		Concentration C2	
	2%		4%	
	480nm	600nm	480nm	600nm
D1	-0.14	-0.04	-0.05	-0.01
D2	-0.27	-0.11	-0.14	-0.12
D3	-0.17	-0.11	-0.18	-0.12

Figure 13 shows the degradation rate of Red and orange disperse dyes, and Figure 14 shows the degradation rate of disperse Black in the wastewater.

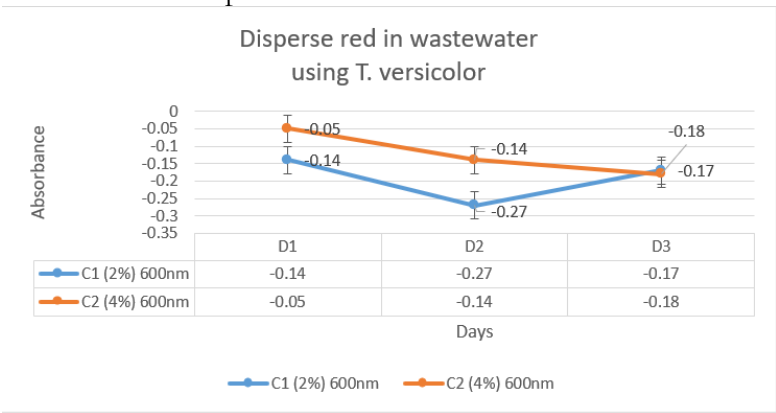

Figure 13. Degradation of Disperse Red and Orange Dyes in Wastewater

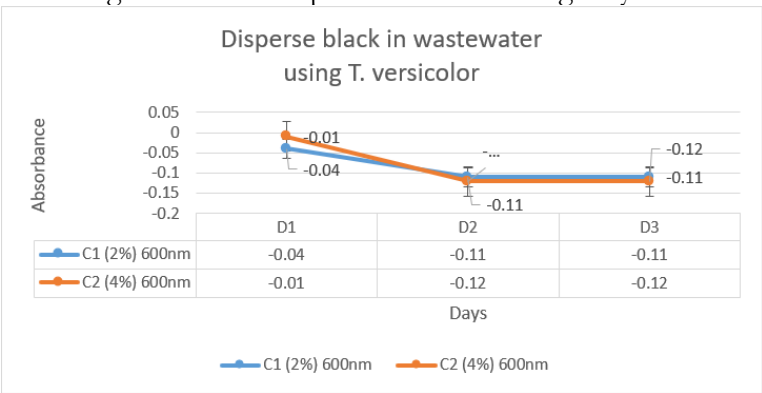

Figure 14. Degradation of Disperse Black in Wastewater

The use of Python for data automation significantly enhanced the accuracy and efficiency of the experimental process. Dilution volumes for all five concentrations of Disperse Red-I and Disperse Blue-I were automatically calculated using a Python script based on the $C_1V_1 = C_2V_2$ formula, ensuring precise and repeatable preparation of working solutions. This reduced the likelihood of human error in pipetting and standardization. Additionally, absorbance values recorded at multiple time points (0, 24, 48, and 72 hours) were logged, structured, and exported using Python's Pandas library, enabling organized tracking of degradation trends across all concentrations. The resulting data tables, automatically generated and exported in Excel format, facilitated direct analysis and visualization without the need for manual data transcription. This automation helped maintain data integrity and allowed seamless integration of results into figures and statistical summaries.

Discussion:

Author investigated the lignocellulolytic enzyme system produced by white-rot fungi, which included enzymes such as glutathione S-transferase (GST), manganese peroxidase, laccase, and cytochrome P450 (CYP450). When textile dyes are released into the water bodies, they can precipitate and can be a potential threat to wildlife, as textile wastewater has a ton of

ingredients mixed within. Authors summarized in a review about the characteristics of textile industry wastewater effluent and how it is impacting microbial and aquatic life [16]. WRF enzymes are able to degrade textile disperse dyes. The focus of this research was to first optimize the growth conditions of *T. versicolor* and then assess its ability to degrade dispersed dye-containing wastewater. There is plenty of research on *T. versicolor*, and many have suggested optimal conditions for growth, which were in accordance with our findings. The findings by author suggested that the most effective growth of *T. versicolor* is at 28 °C temperature, which is by our findings. The most significant system of enzymes in T. *versicolor* is the laccase system. Our findings suggest that at 25-28 °C the activity of fungal laccase was so high that it was able to take up whole dye and from the growth media in less than 24 hours [17].

The optimal pH of *T. versicolor* was kept at 6; however, in various other research, the optimal pH was found to be 4.5 to 5.0 [18]. Another study found that the optimal pH for *T. versicolor* was 5.51. This was observed to optimize the removal of Cu (II) from aqueous media [19]. Authors found out that the optimal pH of *T. versicolor* laccase activity was 6, which is following our findings. It implied that the different conditions of pH had different effects on the growth as well as the activity of enzymes. The difference in the optimal pH and temperature between our findings and those reported in the cited research suggests that the growth conditions of the fungus significantly influence the activity of its enzyme systems. As, authors discovered that dye adsorption in a liquid medium is influenced by the presence of charged molecules on the cell walls of the mycelia, which play a key role in the binding interactions between the fungal biomass and dye molecules.

In our experiments, the pH was maintained at 6 for fungi, which has been proven to be the most optimal in literature [20][21]. *T. versicolor* is very well-researched in terms of bioremediation and using its enzymes to biodegrade pollutants. The laccase system of *T. versicolor* is extremely efficient, and many researchers have been working to use it for the biodegradation of various effluents. author demonstrated that by using a crude extract of *T. versicolor* laccase, the degradation of benzo[a]pyrene increased by 42.21% by keeping it under optimal conditions of 28°C, pH 6, and presence of enzyme mediator Cu2+ [17]. Another study done by author in 2017 studied the efficiency of laccase in degrading various Reactive dyes. The study estimated that decolorization ranged from 51% to 80% in 9 days. However, compared to our results, *T. versicolor* was able to adsorb and bind all the dye molecules onto its mycelia from the medium, rendering the solution completely clear within 24 hours. The highest concentration of disperse dyes used in our experiment was 0.05% i.e., 0.1875 mL/75 mL (2500 mg/L), while in another author study, the concentration was 500-700 mg/L. Our research results justify that *T. versicolor* is extremely efficient in degrading the effluents in textile wastewater. Research in which laccase was extracted and used for bioremediation proved to be less efficient [17][22][23] than the research that used whole cell cultures [24]. Therefore, the growth optimization step has been the biggest factor in enhancing the efficiency of effluent degradability. As the results show, wastewater from the medium was completely cleared out when treated with *T. versicolor*. Due to the binding ability of *T. versicolor*, it bound all the dyes as well as the intermediates with its mycelia. The slightly acidic proteins also exist on the *T. versicolor* cell wall; therefore, it can adsorb the dye. Also, protonation of the laccase active site creates a cationic charge on it. This cationic-anionic interaction facilitates the formation of the Enzyme-Substrate complex [20][21]. However, it was found out by author that the fungal growth and enzyme activation are basically better at slightly lower pH, i.e., 6. To maintain this pH, we used HCl, while author used acetic acid, as it is also a weak acid. Due to this reason, *T. versicolor* culture was so efficient at the degradation of the dyes. The nature and chemical formulas of the intermediates could not be examined. [25][26] It should have been examined by GC-MS or FTIR, so that we could have known what kinds of bonds were attached by the enzymes of fungi.

Conclusion:

The research project focused on the ability of WRF, particularly *T. versicolor*, for bioremediation of wastewater by degrading dispersed textile dyes. *T. versicolor* was cultured on different agar and broth media. The incubation period and a number of growth parameters, including pH, temperature, carbon and nitrogen supplies, were optimized to enhance the production of fungal biomass and enzymes. The *T. versicolor* could degrade dispersed Red-I and Blue-I dyes as well as dyes frequently present in industrial effluent. In particular, *T. versicolor* was discovered to have a high dye degradation efficiency, which was demonstrated by the spectrophotometric analyses. The results show that wastewater contaminated with textile dyes can be treated effectively and sustainably by employing white-rot fungi. The study concludes that *T. versicolor* has strong potential to degrade dispersed dyes by over 80%, making it a promising candidate for treating industrial effluent containing these dyes. Further studies could also investigate its effectiveness in degrading other types of toxic dyes and industrial wastewater.

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