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Decolorization of Azo Dyes by Edible Mushrooms; Agaricus Bisporus and Pleurotus Ostreatus and Phytotoxicity Assessment of Treated Effluent.

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ABSTRACT

The extensive use of synthetic azo dyes in textile industries has led to significant environmental pollution due to the release of dye-laden wastewater. Conventional dye removal techniques are often expensive, inefficient, and result in secondary pollution. This study investigates the mycoremediation potential of two edible mushrooms, *Agaricus bisporus* (button mushroom) and *Pleurotus ostreatus* (oyster mushroom), for the decolorization of blue and orange azo dyes. The study further evaluates the phytotoxicity of both untreated and mushroom-treated dye solutions on leguminous plants such as *Vigna radiata* (mung), *Cicer arietinum* (chickpea), *Vigna aconitifolia* (moth bean), and *Phaseolus vulgaris* (kidney bean). Experiments were conducted under varying physical parameters—temperature, pH, and salt concentration—to determine their influence on dye decolorization efficiency. Results demonstrate significant dye removal capabilities of both mushroom species, with *Pleurotus ostreatus* showing higher enzymatic activity under optimized conditions. The treated effluents also showed a notable reduction in phytotoxic effects compared to untreated dyes, indicating the potential of fungal-based systems for eco-friendly bioremediation of textile wastewater.

1 Introduction

The textile industry is one of the largest and most rapidly expanding sectors of industrial production in India, particularly in cities like Surat, which is known for its dominant role in textile and diamond exports. While this growth plays a significant role in boosting the national economy, it also poses severe environmental threats due to the generation and discharge of high volumes of chemically complex wastewater [1]. Among the various pollutants released, synthetic dyes—especially azo dyes—are of particular concern due to their toxicity, persistence, and resistance to conventional treatment methods [2].

Azo dyes represent the largest class of synthetic dyes, accounting for more than 60–70% of the dyes used globally in industries such as textiles, food, cosmetics, paper, and pharmaceuticals [3]. They are characterized by one or more azo bonds (–N=N–) linked to aromatic rings, which provide them with high thermal and photolytic stability. However, this structural stability also contributes to their recalcitrant nature in the environment. Studies have shown that during the dyeing process, up to 10–15% of these dyes remain unbound to textile fibers and are released directly into wastewater streams [4]. This discharge leads to intense coloration of water bodies, reduces light penetration, disrupts photosynthesis in aquatic plants, and can lead to eutrophication and imbalance in aquatic ecosystems [5].

Furthermore, the reduction of azo dyes in anaerobic conditions, such as in sediments or within the gastrointestinal tract of aquatic organisms, can lead to the formation of toxic aromatic amines. These by-products have been associated with mutagenic and carcinogenic effects in humans and animals [6, 7]. The urgent need to mitigate these environmental and health risks has intensified the search for effective and sustainable treatment methods for dye-containing effluents.

Conventional dye removal techniques such as flocculation, chemical oxidation, membrane filtration, and adsorption have several limitations, including high operational costs, incomplete removal, generation of secondary pollutants (e.g., sludge), and limited applicability to diverse dye structures [8, 9]. Biological methods, in contrast, offer a cost-effective and environmentally friendly alternative. Among the various biological systems, fungi—particularly white-rot fungi and edible mushrooms—have gained significant attention for their enzymatic capabilities and adaptability in polluted environments [10].

Mushrooms such as *Pleurotus ostreatus* (oyster mushroom) and *Agaricus bisporus* (button mushroom) have been studied for their ability to degrade xenobiotic compounds, including synthetic dyes. These fungi produce a range of extracellular ligninolytic enzymes like laccase, manganese peroxidase, and lignin peroxidase, which facilitate the breakdown of complex dye molecules into simpler, less toxic forms [11, 12]. Their filamentous growth, high surface area, and resilience to high concentrations of toxicants make them suitable candidates for bioremediation [13].

The concept of mycoremediation, the use of fungal species for detoxifying contaminated environments, has shown promising results for the treatment of industrial effluents, particularly those containing recalcitrant

organic pollutants like azo dyes [14]. Fungal mycelia have not only demonstrated dye adsorption capacity due to their porous structure but also enzymatic degradation capabilities, making them highly efficient for dye decolorization [15, 16]. Recent advancements have also shown that physical parameters such as temperature, pH, and salt concentration significantly influence the decolorization efficiency of fungi, necessitating optimization for practical applications [17].

An important but often overlooked aspect of wastewater treatment is the post-treatment toxicity of effluents. Phytotoxicity studies using sensitive plant species provide critical insight into the environmental safety of treated wastewater before its release or reuse in agriculture. Leguminous plants such as *Vigna radiata*, *Cicer arietinum*, *Vigna aconitifolia*, and *Phaseolus vulgaris* serve as excellent bioindicators due to their sensitivity to pollutants [18].

In this context, the present study investigates the decolorization potential of *Agaricus bisporus* and *Pleurotus ostreatus* against blue and orange azo dyes under varying physicochemical conditions. It also evaluates the phytotoxicity of untreated and treated dye effluents using leguminous plant species to assess the environmental safety of the fungal decolorization process. This dual approach not only establishes the biodegradative potential of edible mushrooms but also ensures the ecological sustainability of the proposed bioremediation method.

Objectives of the study includes; determination of the decolorization potential of *Agaricus bisporus* and *Pleurotus ostreatus* on black, blue and orange azo dyes, Evaluation of the influence of physical parameters—temperature, pH, and salt concentration—on the efficiency of dye decolorization by these mushroom species, Assessment of the phytotoxicity of untreated and mushroom-treated dye effluents on selected leguminous plants: *Vigna radiata* (mung), *Cicer arietinum* (chickpea), *Vigna aconitifolia* (moth bean), and *Phaseolus vulgaris* (kidney bean), and Comparison of the effectiveness of *Agaricus bisporus* and *Pleurotus ostreatus* in dye removal and toxicity reduction for potential application in textile wastewater treatment.

2 Materials and Methodology

2.1 Materials

Orange, blue, and black coloured powdered textile azo dyes were obtained from Colourtex Industries Pvt. Ltd. (Surat, India). For the further experiments (absorbance determination, degradation studies), stock solutions (1000 µg/mL in sterile distilled water). Cultivated fruiting bodies of two mushroom species, *Agaricus bisporus*

and *Pleurotus ostreatus*, were procured from Nutrimusht – New Age Food (Navsari, Gujarat, India). Seeds of four plant species, namely *Vigna radiata* (mung), *Cicer arietinum* (chana), *Vigna aconitifolia* (math), and *Phaseolus vulgaris* (val), obtained from the local market were used for phytotoxicity assessment of dyes and their degraded products. For optimization experiments involving parameters such as pH, temperature, and salt concentrations, dye solutions were freshly prepared at 1000 µg per 150 mL of sterile distilled water. All chemicals and reagents used were of analytical grade, and standard laboratory instruments such as a UV–Visible spectrophotometer, pH meter, incubator, and autoclave were employed throughout the study.

Agaricus bisporus

Pleurotus ostreatus

2.2 Methodology

2.2.1 Determination of Absorbance Maxima (λmax) of Azo Dyes

The maximum absorbance wavelength (λmax) of each dye was determined by dissolving 0.01 g of powdered azo dye in 10 mL of sterile distilled water to obtain a 1000 µg/mL stock solution. The dye solutions were scanned in the range of 400–700 nm using a UV–Visible spectrophotometer (Shimadzu UV-1800), and the wavelength corresponding to the peak absorbance was recorded as λmax [19].

2.2.2 Preparation of Calibration Curve for Dye Concentration

A calibration curve was constructed by serially diluting the dye stock solution. Ten test tubes were prepared with 2 mL of sterile distilled water each. The first tube received 2 mL of dye stock solution (1000 µg/mL), and

two-fold serial dilutions were carried out up to the ninth tube. The tenth tube, containing only distilled water, served as the blank. Absorbance was measured at the previously determined λmax using a UV–Vis spectrophotometer. Each measurement was performed in triplicate, and the average absorbance values were plotted against corresponding concentrations to generate a standard curve, from which the linear regression equation was derived [20].

2.2.3 Dye Decolorization Assay Using Mushroom Species

Decolorization potential of *Agaricus bisporus* and *Pleurotus ostreatus* was evaluated using dye solutions of 1000, 2000, and 3000 µg/mL concentrations. Each solution (150 mL) was placed in a sterile Erlenmeyer flask, and 10 g of fresh mushroom biomass was added. The flasks were incubated on a rotary shaker at 100 rpm and room temperature. Aliquots (3 mL) were collected at time intervals of 0, 1, 2, 3, 24, 48, and 72 hours, and absorbance at λmax was recorded. The dye concentration in the samples was calculated using the calibration curve, and decolorization percentage was computed. All assays were performed in triplicate [21].

2.2.4 Effect of pH on Dye Decolorization

The effect of pH on dye decolorization was studied using 0.2 M phosphate buffer solutions adjusted to pH 6.0, 7.0, and 8.0. Dye solutions (1000 µg/mL) prepared in each buffered medium were inoculated with 10 g of either *A. bisporus* or *P. ostreatus*. The flasks were incubated on a shaker at 100 rpm. Samples (3 mL) were withdrawn at multiple time points, and absorbance at λmax was measured. The experiment was performed in triplicate for each condition [21, 22].

2.2.5 Effect of Temperature on Dye Decolorization

To study the influence of temperature, dye solutions (1000 µg/mL) containing 10 g of mushroom tissue were incubated at 37°C and 45°C on a rotary shaker at 100 rpm. Samples were withdrawn at specified time intervals and absorbance at λmax was measured. The study was conducted in triplicate for each condition, and decolorization efficiency was evaluated based on absorbance reduction [21, 22].

2.2.6 Phytotoxicity Assay of Treated and Untreated Dye Solutions

Phytotoxicity was assessed using four plant species: *Vigna radiata*, *Cicer arietinum*, *Vigna aconitifolia*, and *Phaseolus vulgaris*. Dye solutions (1000 µg/mL) were treated with either *A. bisporus* or *P. ostreatus* for 24 hours, while untreated and distilled water controls were maintained. A total of five treatment groups were

prepared: (1) sterile distilled water (control), (2) untreated dye solution, (3) dye solution treated with *A. bisporus*, and (4) dye solution treated with *P. ostreatus*. Each treatment (150 mL) was incubated on a shaker for 24 hours. Seeds were pre-soaked for 3–4 hours to promote germination and sown (5 seeds per cup) in moist soil-filled germination cups labeled according to treatment. Seedling growth was monitored over 24–48 hours to evaluate potential phytotoxic effects of the treated and untreated dye solutions [23].

3 Results and Discussion

3.1 Determination of Absorbance maxima and Calibration curve

Each dye was prepared at 500 µg mL^{-1} and scanned on a UV-Vis double-beam spectrophotometer across the scanning range of 400–700 nm. The maximum absorbance (λmax) for the dyes are found as: Blue (602 nm), Black (580 nm), and Orange (470 nm).

Calibration curve of dyes at their respective λmax were determined spectrophotometrically in triplicate. The average absorbance at different concentrations of dye is mentioned in Table 1. The graph of concentration vs absorbance at 602 nm is plotted can be seen in Figure 1 along with the straight-line equation. The linear relationship between dye concentration and absorbance was established using standard curves, confirming the applicability of Beer-Lambert's law. These calibration curves were used for quantitative analysis of dye concentration throughout the experiments, allowing accurate computation of decolorization percentages [19].

Table 1: *Calibration curve of dyes*

Calibration Curve				
Sr. No.	Dye, [µg/ml]	Absorbance at respective λmax		
		Blue Dye	Black Dye	Orange Dye
		602 nm	580 nm	470 nm
1	0	0	0	0
2	15.625	0.079	0.121	0.082
3	31.25	0.101	0.175	0.311
4	62.5	0.259	0.294	0.897
5	125	0.472	0.556	0.546
6	250	0.905	1.070	0.965
7	500	2.085	2.077	1.775
8	1000	3.000	3.000	3.000

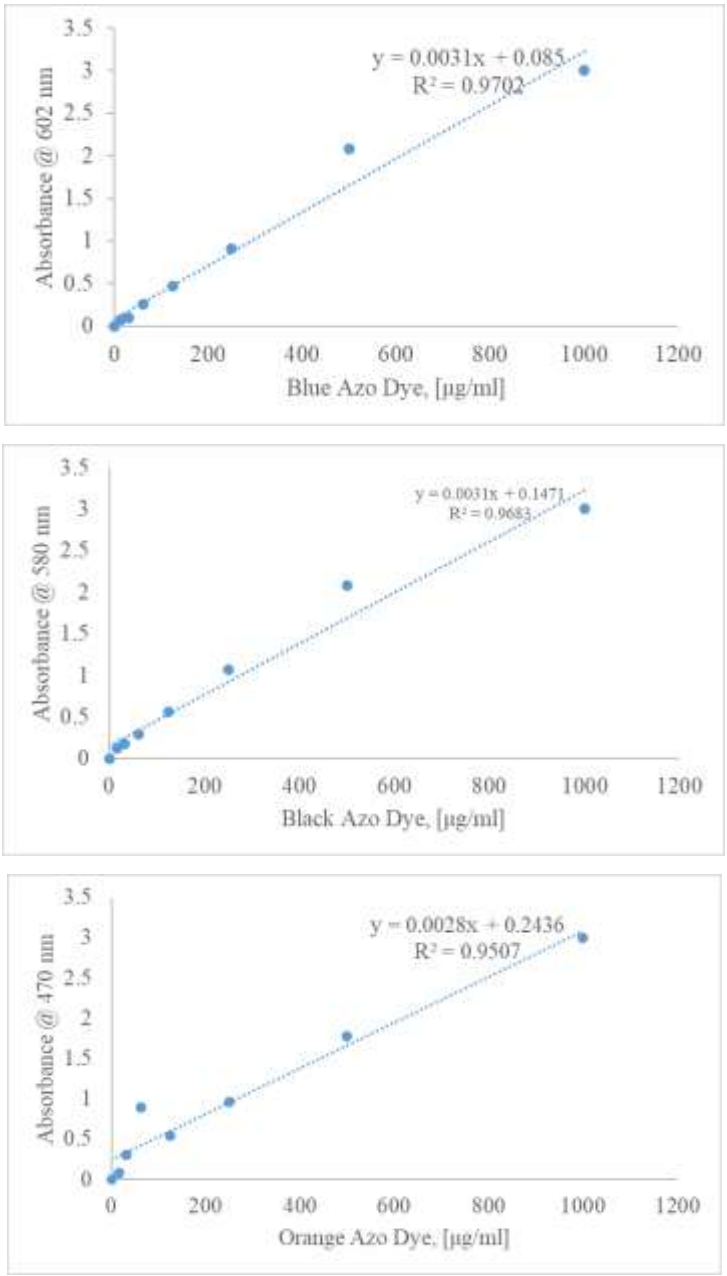

Figure 1:: Calibration curves of dyes and straight-line equations

3.2 Dye Decolorization Efficiency

To the 150 ml of dye solutions in separate flasks at the concentrations of 1000µg/ml, 10-10 gms of both the mushroom species were added and kept at rotary shaker at 100 rpm for 72 hours and aliquots are taken at regular time intervals to determine the concentration of dyes remained. The experiments were conducted in triplicate and the average concentrations at each regular time interval are shown in the Table 2.

Table 2: Dye Decolourization Potential of Agaricus bisporus & Pleurotus ostreatus

Dye Decolorization						
Time, Hr	Blue Dye		Black Dye		Orange Dye	
	Agaricus bisporus	*Pleurotus ostreatus*	*Agaricus bisporus*	*Pleurotus ostreatus*	*Agaricus bisporus*	*Pleurotus ostreatus*
0	794.194	728.710	651.581	695.774	914.429	936.929

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1	742.258	718.065	595.129	682.871	897.643	910.500
2	685.806	700.323	595.129	672.548	856.214	877.286
3	660.968	617.742	507.387	604.161	788.357	855.500
24	619.032	492.258	479.000	357.065	715.500	681.214
48	463.871	422.258	381.903	344.484	635.857	652.286
72	432.903	400.645	154.806	277.387	524.429	591.571

The dye decolorization experiment over 72 hours, as shown in Figure 2, showed a consistent and progressive decline in dye concentrations for all three dyes. *Pleurotus ostreatus* showed superior decolorization capabilities compared to *Agaricus bisporus* for all dyes, particularly evident at the 72-hour mark:

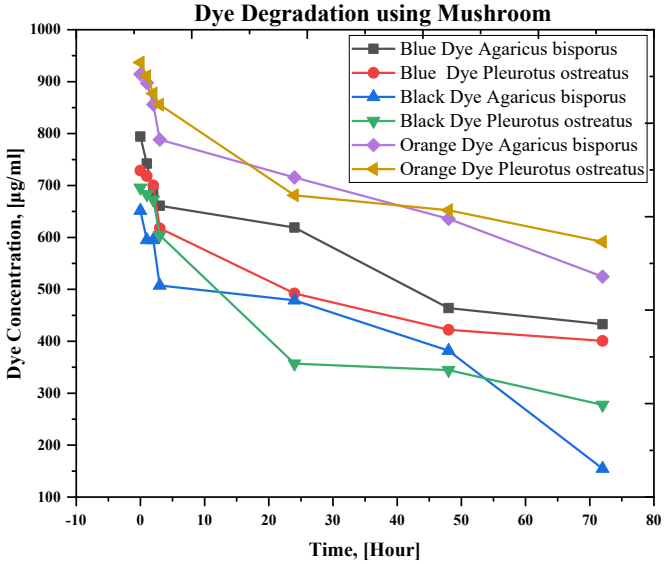

Figure 2: Dye Decolourization Potential of Agaricus bisporus & Pleurotus ostreatus

Black dye: Decolorization by *Pleurotus ostreatus* reached a final concentration of 277.38 µg/mL from 695.77 µg/mL, indicating 60.1% decolorization, whereas *Agaricus bisporus* showed 76.2% decolorization (154.80 µg/mL from 651.58 µg/mL). It can be observed that Both fungi achieved moderate decolorization of blue dye. The initial rapid reduction (especially between 0–24 hrs) suggests active fungal metabolism and enzyme secretion in early phases. Interestingly, *Agaricus* performed slightly better than *Pleurotus* here, potentially due to dye-specific enzyme affinity or bioavailability. The slightly lower decolorization in *Pleurotus* could be linked to the dye's structural complexity or inhibitory effects on specific enzymes. White-rot fungi are reported to decolorize triphenylmethane and azo dyes through extracellular oxidative enzymes like laccases and peroxidases, active in the initial phase of fungal growth [13, 24].

Blue dye: Reduction from 794.19 to 400.65 µg/mL (*Pleurotus*) and 432.90 µg/mL (*Agaricus*), suggesting 43.8% and 45.5% decolorization, respectively. *A. bisporus* showed significantly higher decolorization efficiency than *P. ostreatus*. The steep decline between 2 and 72 hrs implies strong enzymatic activity sustained over time. The

better performance by *Agaricus* may relate to either enhanced peroxidase secretion or better growth conditions on the black dye medium. The black dye may consist of larger or more complex aromatic structures that *Pleurotus* struggled to break down as effectively. *Agaricus* possibly developed an enzyme suite (e.g., LiP, MnP) with greater affinity or resistance to inhibitory dye breakdown products.

Orange dye: Pleurotus achieved a greater reduction (591.57 µg/mL) compared to Agaricus (524.43 µg/mL), translating to 36.8% and 42.6% decolorization, respectively. Among all dyes, orange dye showed the least decolorization by both fungi. *Agaricus bisporus* again slightly outperformed *Pleurotus*, although overall efficiency remained moderate. Possible reasons for lower performance include: Lower bioavailability or solubility of orange dye Toxic intermediates inhibiting fungal activity Lower affinity of fungal enzymes for this specific dye structure. Orange dyes, often monoazo or diazo compounds, can be more resistant to fungal degradation if substitution groups hinder access to cleavage sites [17].

The overall performance suggests the enzyme-rich matrix of *Pleurotus ostreatus*, including laccase and manganese peroxidase, may facilitate more effective breakdown of dye chromophores [10, 12, 21]. Agaricus bisporus consistently performed better than Pleurotus ostreatus for all three dyes, especially for black dye, likely due to higher production or specificity of ligninolytic enzymes. Despite *Pleurotus* being known for robust enzyme secretion (e.g., laccase, MnP), *Agaricus* might express additional or more stable isoforms under experimental conditions. The differences in dye structure (e.g., molecular weight, charge, hydrophobicity) significantly influence fungal uptake and enzymatic breakdown rates.

3.3 Effect of pH on Dye Decolorization

As the enzymes such as laccases, lignin peroxidases, and manganese peroxidases secreted by Mushroom for dye degradation functions efficiently within a specific pH range, fungal growth is influenced by pH, structure and solubility of dye in influenced by pH and Industrial dye effluents vary widely in pH (often strongly acidic or alkaline), it is necessary to study the effect of pH on the decolorization of Dyes. The effect of pH on the dye degradation are shown in Figure 3 & Table 3.

Blue Dye: Maximum decolorization occurred at pH 7 for both fungi. *Agaricus bisporus*: Reduced from 601.61 µg/mL to 182.25 µg/mL. *Pleurotus ostreatus*: Reduced from 717.74 µg/mL to 182.25 µg/mL. At pH 6 and pH 8, degradation was comparatively less. For instance, at pH 6, *P. ostreatus* showed final dye concentration of

272.58 µg/mL and at pH 8, 266.13 µg/mL. Neutral pH likely supported optimal enzyme activity for ligninolytic enzymes such as laccase, manganese peroxidase, and lignin peroxidase. Acidic or basic shifts may lead to partial enzyme denaturation or altered substrate binding [13, 17, 24].

Table 3: Effect of pH on Dye Decolorization

Effect of pH						
pH	Blue Dye					
	Agaricus bisporus			*Pleurotus ostreatus*		
	6	7	8	6	7	8
0	656.452	601.613	759.677	714.516	717.742	695.161
1	417.742	259.677	598.387	701.613	662.903	627.419
2	369.355	211.290	514.516	395.161	585.484	424.194
48	288.710	185.484	501.613	366.129	495.161	314.516
72	224.194	182.258	453.226	272.581	308.065	266.129
pH	Black Dye					
	Agaricus bisporus			*Pleurotus ostreatus*		
	6	7	8	6	7	8
0	487.710	330.290	489.645	466.419	334.806	422.871
1	446.419	433.194	280.290	422.226	468.355	390.290
2	379.645	390.935	257.387	355.452	399.323	358.677
48	270.290	328.355	231.581	260.935	383.194	312.226
72	27.065	157.387	209.000	244.484	307.065	302.871
pH	Orange Dye					
	Agaricus bisporus			*Pleurotus ostreatus*		
	6	7	8	6	7	8
0	984.429	984.429	984.429	841.571	959.429	784.429
1	963.000	984.429	938.000	755.857	777.286	748.714
2	870.143	870.143	723.714	698.714	727.286	691.571
48	604.071	698.714	561.929	588.000	595.143	566.571
72	405.857	452.286	366.571	430.857	500.500	373.357

Black Dye: For *A. bisporus*, pH 7 showed significant decolorization from 330.29 µg/mL to 157.38 µg/mL. For *P. ostreatus*, the most effective degradation also occurred at pH 7, reducing from 334.80 µg/mL to 307.06 µg/mL—though the difference was less pronounced than with *A. bisporus*. At pH 6 and 8, final dye concentrations were higher, indicating lower degradation efficiency. pH 7 was again ideal, especially for *A. bisporus*, indicating better adaptability and enzyme efficiency under neutral conditions. Black dye's complex aromatic structure might require prolonged exposure or higher enzyme specificity, slightly reducing the rate for *P. ostreatus* [22].

Orange Dye: *Agaricus bisporus*: At pH 7, reduced orange dye from 984.42 µg/mL to 452.29 µg/mL after 72 hrs. *Pleurotus ostreatus*: Showed greater decolorization at pH 8, reducing from 784.42 µg/mL to 373.36 µg/mL. At pH 6, *A. bisporus* performed slightly better (final: 405.86 µg/mL) than at pH 7. While pH 7 remained

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generally effective, the optimum pH for orange dye degradation showed some variability, potentially due to dye solubility and molecular configuration differences. *P. ostreatus* might have better tolerance to alkaline conditions for this dye, suggesting strain-dependent pH adaptability [13].

The optimal pH for most effective decolorization was neutral (pH 7) for both fungi and most dyes. Enzyme systems involved in dye degradation—especially ligninolytic enzymes—are generally active in neutral to slightly acidic pH. Lower performance at pH 6 or 8 can be attributed to changes in fungal metabolism, dye solubility, and reduced enzyme stability [17].

Thus, the fungal dye degradation process is highly pH-dependent. Neutral pH (7) favors maximum enzyme efficiency and dye decolorization across all tested azo dyes. Minor deviations were observed depending on dye structure and fungal species, indicating the need for condition optimization during bioremediation efforts.

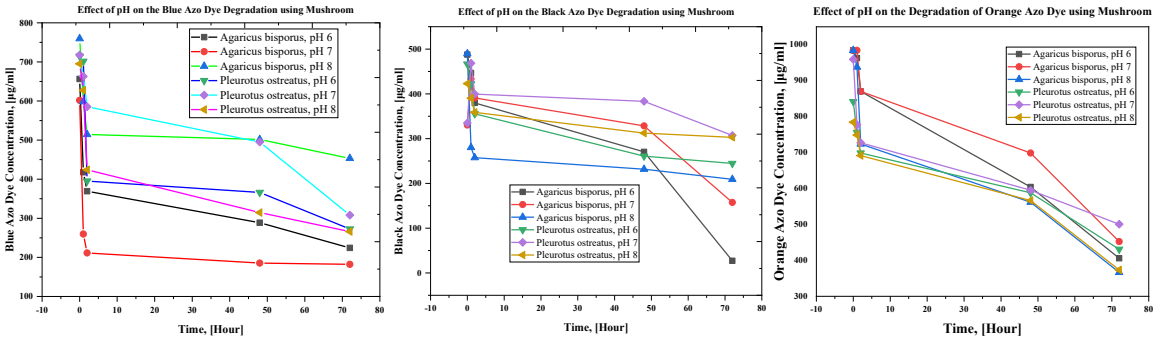

Figure 3: Effect of pH on Dye Decolorization

3.4 Effect of Temperature on Dye Decolorization

As the activities of laccases, peroxidases, and manganese peroxidases enzymes of Mushrooms, metabolism and growth of Mushrooms are temperature-dependent, temperature affects dye chemistry, and the industrial wastewater is often discharged at elevated temperatures, it is necessary to study the effect of temperature on the dye decolorization potential, as it helps in optimizing bioremediation conditions. Average dye concentrations at the regular time interval at different temperatures are shown in the

Table 4.

Table 4: Effect of Temperature on the dye decolorization efficiency of Mushrooms

Effect of Temperature				
Time/ Temperature	Blue Dye			
	Agaricus bisporus		*Pleurotus ostreatus*	
	37	45	37	45

0	579.032	585.484	521.935	750.323
1	530.645	579.032	329.355	474.839
24	527.419	559.677	327.742	300.000
72	120.968	295.161	294.839	290.645
Time/ Temperature	Black Dye			
	Agaricus bisporus		*Pleurotus ostreatus*	
	37	45	37	45
0	461.258	456.742	559.000	431.903
1	450.290	397.065	411.581	390.935
24	315.774	279.645	390.935	358.355
72	208.355	210.935	355.452	357.710
Time/ Temperature	Orange Dye			
	Agaricus bisporus		*Pleurotus ostreatus*	
	37	45	37	45
0	527.643	506.214	840.857	609.786
1	496.929	437.286	824.429	585.500
24	484.071	433.000	608.000	571.571
72	109.071	-45.929	397.643	433.000

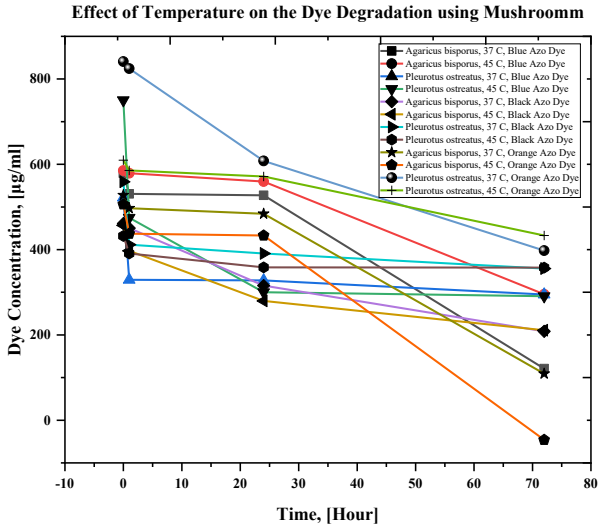

Figure 4: Effect of Temperature on the dye decolorization efficiency of Mushrooms

Graphical representation of the decolorization potential of MMushrooms under the influence of different temperatures are shown in Figure 4.

Blue Dye Decolorization: *A. bisporus* showed maximum efficiency at 37°C, with a dramatic drop at 45°C. *P. ostreatus*, surprisingly, performed slightly better at 45°C, possibly due to species-specific thermotolerance or a stress-induced enzyme expression. The anomalously high initial concentration at 45°C for *Pleurotus* (750.32 µg/mL) may reflect experimental variation or initial dye release from biomass or medium components.

Black Dye Decolorization: *A. bisporus* showed comparable performance at both temperatures, indicating good thermal stability for black dye degradation. *P. ostreatus* exhibited a significant decline at 45°C, suggesting that higher temperatures may inhibit enzyme function or fungal growth for this dye.

Orange Dye Decolorization: *A. bisporus* at 37°C again demonstrated excellent decolorization, similar to its performance with blue dye. The value "-45.93" µg/mL for *A. bisporus* at 45°C is invalid (dye concentrations

cannot be negative), indicating possible measurement or data recording error. *P. ostreatus* showed diminished degradation at 45°C, consistent with thermal stress effects on enzyme activity.

Optimal temperature (~37°C) appears to support fungal metabolic activity, enzyme secretion, and dye uptake. Above-optimal temperatures (45°C) likely lead to: Partial denaturation of key enzymes (e.g., laccase, manganese peroxidase), Membrane destabilization in fungal cells, Impaired growth and substrate assimilation. The relative stability of *A. bisporus* under both conditions (especially for black dye) suggests its potential thermotolerant enzymatic system, while *Pleurotus ostreatus* is more sensitive to elevated temperatures, especially for black and orange dyes.

Thus, 37°C is the most effective temperature for dye decolorization by both fungi. *Agaricus bisporus* generally outperformed *Pleurotus ostreatus*, especially at elevated temperatures, likely due to more robust or heat-stable enzymatic systems. The significant temperature-dependent variability underscores the importance of optimizing environmental parameters when employing fungal systems for bioremediation.

3.5 Phytotoxicity Determination of treated dye solution

Phytotoxicity assays were performed using four legume species to evaluate the toxicity of untreated and mushroom-treated dye effluents. The purpose was to assess whether fungal bioremediation (by *Pleurotus ostreatus* and *Agaricus bisporus*) could not only decolorize but also detoxify azo dye-laden water, thereby reducing its adverse effects on plant development. In the present study, following four varieties of plant seeds were used; Vigna radiata (*mung bean*), Phaseolus vulgaris (*common bean / val*), Vigna aconitifolia (*moth bean / math*), Cicer arietinum (*chickpea / chana*). Figure 5 shows the effect of untreated and treated dye solution on the germination of plant seeds.

Blue Dye	Black Dye	Orange Dye
		
Vigna radiate(mung)		
		
Phaseolus vulgaris (val)		

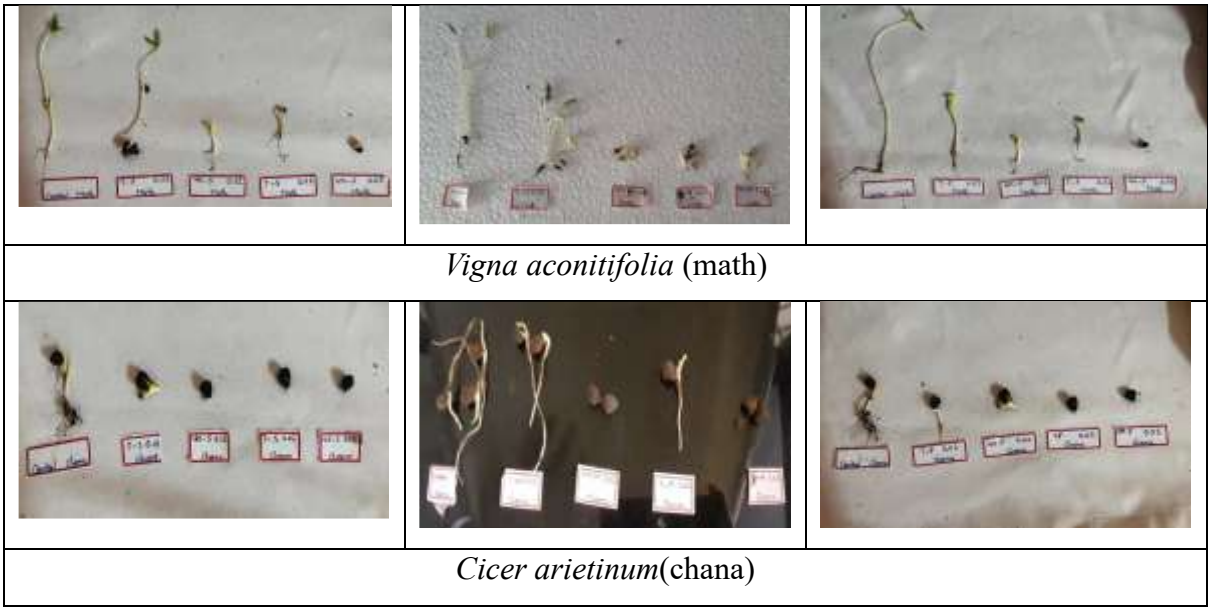

Figure 5: Phytotoxicity determination of untreated and mushroom treated effluent

Seeds exposed to untreated dye solutions (blue, black, and orange dyes) exhibited poor germination rates, reduced root and shoot elongation, and developmental abnormalities. The most severe effects were noted in *Phaseolus vulgaris* and *Cicer arietinum*, suggesting high sensitivity to the toxic compounds in the dye effluents. Untreated azo dyes and their intermediates are known to be toxic to plant systems, interfering with water uptake, cellular respiration, and hormonal regulation. Previous research [18] has demonstrated that azo dyes can cause oxidative stress, leading to inhibited seedling vigor and chlorophyll synthesis.

Seeds exposed to *Pleurotus*-treated effluents showed significantly improved germination. Root and shoot lengths were closer to control levels (distilled water), indicating low residual toxicity. Especially for *Vigna radiata* and *Vigna aconitifolia*, healthy seedlings emerged, demonstrating the efficacy of fungal treatment. The efficient dye degradation by *Pleurotus* likely resulted in reduced concentrations of toxic aromatic amines and intermediates. Enzymes like laccase and manganese peroxidase are known to break down not just chromophores, but also toxic side-products [23].

Seeds exposed to *Agaricus bisporus* treated effluents showed moderate improvement in germination and seedling growth compared to untreated controls. However, the detoxification was less pronounced than with *Pleurotus*-treated water. Certain species like *Cicer arietinum* still showed minor growth retardation. *Agaricus bisporus* degraded the dyes but may have done so less completely or at a slower rate, resulting in partial detoxification. The presence of residual metabolites might still have mild inhibitory effects on plant physiology. Fungal bioremediation significantly reduces the phytotoxicity of dye-contaminated wastewater. *Pleurotus ostreatus* demonstrated superior detoxifying capability, making it a better candidate for bioremediation applications targeting environmental safety. *Agaricus bisporus* also reduced toxicity, though less effectively, highlighting species-specific enzymatic efficiencies. These results confirm that bioremediation is not only effective for dye decolorization but also essential for restoring ecological compatibility of industrial effluents.

4 Conclusion

The present study demonstrated that edible mushrooms—*Pleurotus ostreatus* and *Agaricus bisporus*—can effectively decolorize azo dyes under optimized conditions. Among the two, *Pleurotus ostreatus* exhibited consistently higher decolorization efficiency across all tested parameters (pH, temperature, and salt concentration). Optimum decolorization occurred at **pH 6**, **temperature 35°C**, and **0.1% salt concentration**. *Pleurotus ostreatus* was particularly effective against black dye, achieving a maximum of **60.1% decolorization**. Phytotoxicity assays confirmed that biologically treated dye effluents were significantly less toxic, promoting better seed germination and plant growth in legume species, thus indicating effective detoxification. These findings highlight the potential of *Pleurotus ostreatus* as a sustainable bioremediation agent for treatment of dye-contaminated wastewater. Further work on enzyme characterization, metabolite profiling, and pilot-scale studies will aid in scaling up this eco-friendly approach for industrial applications.

5 References

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