

Production of laccase by *Pleurotus ostreatus* through submerged fermentation and its decolourisation potential

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Abstract

Laccases are multicopper oxidase enzymes with significant industrial and environmental applications, including bioremediation and the degradation of textile dyes. This study investigated laccase production by *Pleurotus ostreatus* using submerged fermentation and assessed its decolourisation potential. Lignocellulosic wastes, including sugarcane bagasse, rice straw, maize cob, conifer litters, and maize straw, were evaluated as substrates for laccase production. Parameters such as carbon and nitrogen sources, pH, inoculum size, and incubation period were optimised. Enzyme activity was quantified using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a substrate, characterised with respect to metal ions, pH, temperature, and time, and decolourisation ability was determined using various dyes. The highest laccase yield (435.04 μ U/L) was recorded in a medium containing 1% of maize straw, supplemented with 1% glucose as the carbon source and 0.1% peptone as the nitrogen source, at an initial pH of 7.0, inoculated with four circular 7 mm *Pleurotus ostreatus* plugs, and incubated for 6 days. Metal ions affected laccase activity. Activity was stable across a wide pH range (3.6–9.5), with the highest activity recorded at pH 8.0. Laccase activity was also stable across temperatures (25–60°C) and time periods (10–60 minutes). *Pleurotus ostreatus* laccase decolourised malachite green and Leishman blue by 21.8% and 21.4%, respectively. Laccase production by *Pleurotus ostreatus* was optimised through submerged fermentation, promoting the utilisation of agro-wastes and offering a scalable method for industrial enzyme production. Dyes were decolourised by laccase, demonstrating the enzyme's potential and its application in the textile industry.

Keywords: Dye decolourisation, Fungi, Lignin-degrading enzyme, Lignocellulosic substrates, Optimisation

1.0 Introduction

Enzymes are biological catalysts which increase the rate of reaction. Laccase is a multicopper oxidase enzyme that is widely recognised for its broad substrate specificity and environmentally friendly catalytic mechanism. It catalyses the oxidation of a wide range of phenolic and non-phenolic substrates with molecular oxygen, producing water as the only by-product, making it an ideal candidate for green chemistry. Laccases are important in industrial biotechnology for their ability to decolourise textile dyes, delignify pulp, detoxify phenolic compounds, and synthesise pharmaceutical precursors [1,2,3]. Laccases are used as alternatives to chemical catalysts for

oxidative coupling and cleavage reactions because they often function under mild conditions. Laccases are utilised in environmental remediation for the degradation of toxic pollutants such as polycyclic aromatic hydrocarbons, endocrine-disrupting compounds and industrial dyes [3,4,5,6,7]. A major advantage over conventional treatment systems is the ability to convert complex xenobiotics into less toxic derivatives without secondary pollution.

Advances in immobilisation techniques and laccase-mediator systems have expanded laccase application in continuous treatment processes and bioreactors [8]. Additionally, laccase has the potential to degrade microplastics and

pharmaceutical pollutants in water bodies [9]. The enzyme can be employed as a major tool in sustainable biotechnological applications due to its economically viable, environmentally friendly and biodegradative efficiencies. Incorporating it into industrial pipelines aligns with global goals for sustainable development and green technology. As the global biocatalyst demand increases, laccase production needs to be optimised using low-cost fungal sources and agro-industry waste substrates. Fungi, particularly white-rot basidiomycetes, produce laccase abundantly, with *Pleurotus ostreatus* being a strong candidate because it produces laccase in high quantities, is non-pathogenic, and can grow on a broad range of lignocellulosic substrates [10,11,12].

Pleurotus ostreatus (oyster mushroom) is a saprotrophic fungus, well adapted to decomposing lignin-rich biomass, and naturally produces laccase as part of its ligninolytic enzyme complex [13,14,15]. Its laccase production is significantly higher when induced by lignocellulosic or supplemented with copper, making it biotechnologically appealing for large-scale enzyme production. *Pleurotus ostreatus* is widely explored for the fermentation of agrowaste such as sawdust, sugarcane bagasse, wheat bran, and corn cobs, which lowers production costs while promoting waste valorisation [14,15,16]. Moreover, it offers the flexibility of operating in either solid-state or submerged fermentation systems due to its genetic stability.

The increasing demand for laccase, bio-sourced from fungi, especially white-rot fungi, has numerous applications in diverse industrial and environmental sectors. However, high production cost, low enzyme stability, and the use of expensive substrates, among other factors, impede large-scale commercialisation. Many fungal strains produce laccase at low levels, and synthetic production methods are often unsustainable. *Pleurotus ostreatus* is a promising alternative because it yields high laccase quantities on agrowaste. Optimal production conditions, substrate specificity, and process parameters remain inadequate. Addressing these gaps is essential for developing cost-effective, sustainable laccase production systems. This work aimed to optimise the production of laccase using *Pleurotus ostreatus*, characterise the laccase and utilise it in the decolourisation of dyes.

2.0 Materials and Methods

2.1 Collection and Screening of *Pleurotus ostreatus*

Morphologically identified *Pleurotus ostreatus* was collected from the Department of Microbiology and Biotechnology, Ajayi Crowther University, Oyo-Town. The fungus was screened for its ability to produce laccase on potato dextrose medium supplemented with 0.1% of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [13]. The appearance of green or purple colour on plates indicates the laccase-producing potential of the organism [13].

2.2 Selection of Lignocellulosic Substrates

Five lignocellulosic substrates (rice straw, sugarcane bagasse, maize straw, corn cob and conifer litters) used in this work were collected locally and were dried and milled. The nutrient basal medium was prepared by weighing the following components in g/L: glucose (10), (NH₄)₂SO₄ (1), MgSO₄·7H₂O (0.5), KCl (0.5), FeSO₄ (0.01), and MnSO₄ (0.01). Based on preliminary studies, 0.15g of each lignocellulosic substrate was added to 15 mL basal medium, sterilised and allowed to cool. Four circular fungal plugs (7 mm each) were inoculated into each of the media and incubated at room temperature (28±2°C) for 10 days. After fermentation, 40mL of sterile distilled water was added to each fermented medium, mixed, and then filtered using Whatman No. 1 filter paper. The supernatant was used as a crude enzyme in further tests.

2.3 Laccase Assay

Laccase activity was assayed using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a substrate. The reaction mixture consisted of 1.5 mL of 0.1 M sodium acetate buffer at pH 5.0, 60 µL of 1 mM ABTS, and 0.5mL of the crude enzyme. The reaction mixture was incubated at room temperature (28±2°C) for 10 minutes, then 20µL of Trichloroacetic acid (TCA) was added to stop the reaction [17]. A blank was also prepared containing 0.5 mL of distilled water instead of the crude enzyme. The enzyme activity was measured using a spectrophotometer at a 420 nm wavelength, expressed in U/mL. One unit of enzyme activity is defined as the amount of enzyme required to oxidise one micromole of ABTS [17].

2.4 Optimisation of Laccase Production Using One-Factor-at-a-Time

Various carbon sources (mannitol, maltose, sucrose, glucose, and fructose) were added to the basal medium at a concentration of 10 g/L. The best lignocellulosic substrate (0.15 g) was added to 15 mL of basal medium, sterilised, allowed to cool, inoculated with 4 circular fungal plugs (7 mm each) of *Pleurotus ostreatus*, and incubated for 10 days. After fermentation, 40 mL of sterile distilled water was added. The mixture was thoroughly mixed and filtered through Whatman No. 1 filter paper. The supernatant was used as the crude enzyme. Laccase yield was determined from crude enzyme activity. To determine the effect of nitrogen source on laccase yield, the best lignocellulosic substrate was used with basal medium containing the best carbon source (10 g/L) and different nitrogen sources ((NH₄)₂SO₄, yeast, NH₄Cl, malt extract, peptone, and NH₄NO₃ – 1 g/L) for laccase production. To determine the effect of initial pH, the lignocellulosic substrate with optimal production was added to the basal medium containing the optimal carbon (10 g/L) and nitrogen sources (1 g/L), with different 0.1 M buffers (sodium citrate dihydrate – 3.0, 4.0, 5.0, and 6.0; potassium phosphate – 7.0 and 8.0; and carbonate-bicarbonate – 9.0). The media were used to produce laccase, and the best medium was inoculated with different numbers of 7 mm fungal plugs (1, 2, 3, 4, 5, and 6) to determine the effect of inoculum size. The effect of different incubation periods (2, 4, 6, 8, and 10 days) was determined using the best inoculum size on the best medium.

2.5 Characterisation of *Pleurotus ostreatus* Laccase

2.5.1 Effect of pH on Laccase Activity

The effect of pH on the activity of *P. ostreatus* laccase was performed by using 1.5 mL of various pH (0.1 M acetate buffer: 3.6, 5.0; 0.1 M phosphate buffer: 6.5, 8.0; 0.1 M carbonate-bicarbonate buffer: 9.5) in the reaction mixture, and laccase activity was determined as explained earlier for the laccase assay.

2.5.2 Effect of Metal Ions on Laccase Activity

The effect of metal ions was determined by adding 0.25 mL of different metal ions (K⁺, Mg²⁺, Fe²⁺, Ca²⁺, Na⁺ and Mn²⁺) to 0.25 mL of crude enzyme. One and a half millilitres (1.5 mL) of

sodium acetate buffer (0.1 M; pH 5) was added to the mixture, followed by the addition of 1 mM ABTS (60 μL) and incubated for 10 minutes. The reaction was terminated by the addition of TCA (20 μL). Enzyme activity was determined as described earlier for the laccase assay.

2.5.3 Effect of Temperature on Laccase Activity

Crude laccase (0.5 mL) was added to 1.5 mL of sodium acetate buffer (0.1 M; pH 5.0), followed by the addition of 60 μL of ABTS (1 mM). The reaction was incubated at different temperatures (25, 37, 50, and 60 °C) for 1 hour at 10-minute intervals. The reaction was terminated after incubating for each specific time by adding 20 μL of TCA. Laccase activity was determined as previously explained for the laccase assay.

2.6 Decolourisation Experiment

To study the decolourisation ability of the laccase produced by *P. ostreatus*, 0.5 mL of crude laccase was added to 2.0 mL of various dyes (Congo red, Leishman blue and malachite green) in 100 mg/L solutions and incubated for 2 hours. Absorbance was taken before and after decolourisation at the following wavelengths: 520 nm (Congo red), 595 nm (Leishman blue) and 619 nm (malachite green). The percentage of dye decolourisation was calculated according to Forootanfar *et al.* [1].

$$\text{Decolourisation (\%)} =$$

$$\frac{\text{Initial absorb. of the dye} - \text{Final absorb. of the dye}}{\text{Initial absorb. of the dye}} \times 100$$

2.7 Statistical Analysis

The data obtained are mean values of two replicates. The experimental data were analysed using one-way Analysis of Variance (ANOVA), and the level of significance was set at $p < 0.05$.

3.0 Results

Figure 1 shows the laccase-producing ability of *P. ostreatus*. The oxidised zone changed from light yellow to purple, while the unoxidised region maintained its initial colour. The appearance of purple colour around the organism indicated its ability to produce laccase.

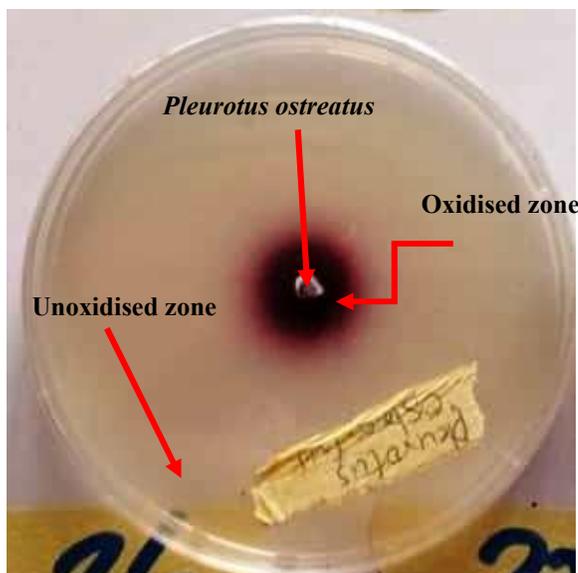


Figure 1: The Oxidation of potato dextrose agar supplemented with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) by laccase-producing *Pleurotus ostreatus*

Agrowastes affected laccase production by *Pleurotus ostreatus*. *Pleurotus ostreatus* laccase yield ranged from 208.87 $\mu\text{U}/\text{mL}$ in medium with corn cob to 257.42 $\mu\text{U}/\text{mL}$ in medium with maize straw. The laccase yields from different media used were not significantly different from each other at $p > 0.05$. Therefore, maize straw with the highest yield was selected for further study.

Table 1: Effect of agrowastes on laccase production by *Pleurotus ostreatus*

Agrowastes	Laccase yield ($\mu\text{U}/\text{mL}$)
Corn cob	208.87 ^a
Colifer litters	229.54 ^a
Rice straw	244.92 ^a
Sugarcane bagasse	247.81 ^a
Maize straw	257.42 ^a

Mean values with the same alphabetical superscripts along the column are not significantly different at $p > 0.05$

When the effect of carbon sources was examined, it was observed that the highest laccase yield (262.47 $\mu\text{U}/\text{mL}$) was recorded with glucose as the source of carbon, and this was closely followed by mannitol (255.98 $\mu\text{U}/\text{mL}$), as shown in Figure 2. The least yield (138.93 $\mu\text{U}/\text{mL}$) was recorded with fructose as the carbon source. However, different yields recorded by various carbon sources were not significantly different from each

other ($p > 0.05$). Glucose with the highest yield was selected for further work.

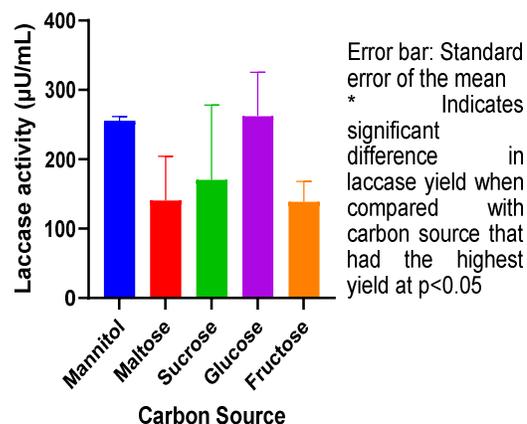


Figure 2: Effect of Carbon Sources on Laccase Yield

It was observed that when different nitrogen sources were used in the fermentation medium, the laccase yield of *Pleurotus ostreatus* was affected, as shown in Figure 3. The highest yield of laccase (425.67 $\mu\text{U}/\text{mL}$) was recorded with peptone as the nitrogen source, while the lowest yield (250.45 $\mu\text{U}/\text{mL}$) was recorded with ammonium sulphate ($(\text{NH}_4)_2\text{SO}_4$) as the nitrogen source. The highest laccase yield of *Pleurotus ostreatus* with peptone (250.45 $\mu\text{U}/\text{mL}$) was not significantly different from the yield (406.44 $\mu\text{U}/\text{mL}$) from malt extract but was significantly different from the yields recorded by other sources of nitrogen ($p < 0.05$). Peptone was selected for further work.

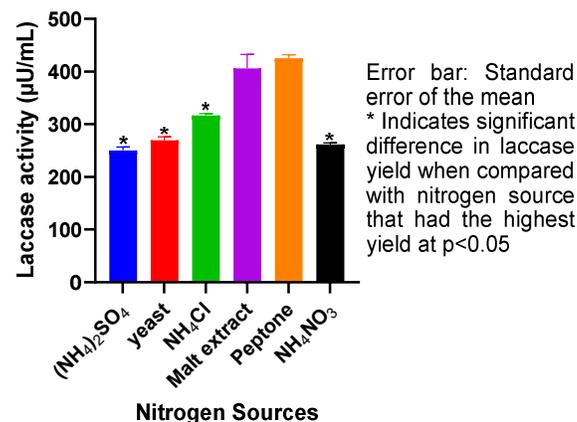


Figure 3: Effect of Nitrogen Sources on Laccase Yield

The effect of the initial pH of the fermentation medium on laccase yield of *Pleurotus ostreatus* is shown in Figure 4. There was an increase in laccase yield with an increase in pH from 298.52 $\mu\text{U/mL}$ at pH 3.0 to 429.28 $\mu\text{U/mL}$ at pH 7.0. Thereafter, a decline in laccase yield was observed with an increase in pH at pH 8.0. The highest yield (429.28 $\mu\text{U/mL}$) recorded at pH 7.0 was not significantly different from the laccase yield observed at pH 6.0, 8.0 and 9.0 ($p>0.05$). The pH of 7.0 was selected for further work.

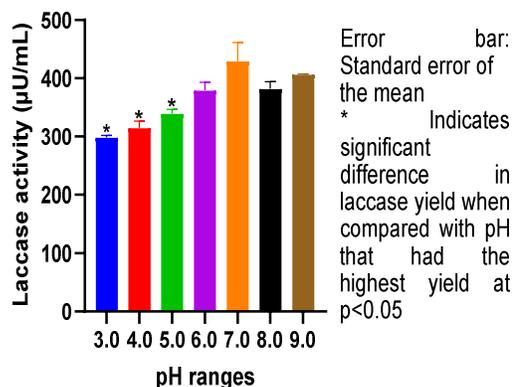


Figure 4: Effect of Initial pH on Laccase Yield

Inoculum load of *Pleurotus ostreatus* affected laccase yield as shown in Figure 5. The highest yield (423.99 $\mu\text{U/mL}$) was recorded with four fungal plugs, and the lowest (223.77 $\mu\text{U/mL}$) was observed when one fungal plug was used. The yield observed with four fungal plugs was significantly different from the yields obtained from one (223.77 $\mu\text{U/mL}$) and three (303.33 $\mu\text{U/mL}$) fungal plugs. An inoculum size of four fungal plugs was selected for further work.

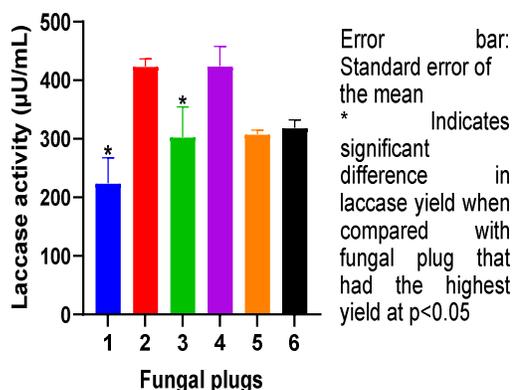


Figure 5: Effect of Inoculum Load on Laccase Yield

Figure 6 shows the effect of the incubation period on laccase yield. Laccase yield increased during the first six days of fermentation, and as the incubation period extended, a decline in yield was observed on the 8th day. The yield ranged from 369.19 $\mu\text{U/mL}$ (day 2) to 435.04 $\mu\text{U/mL}$ (day 6). There was no significant difference in yield across different fermentation periods ($p>0.05$). Day 6, with the highest laccase activity, was selected for further work.

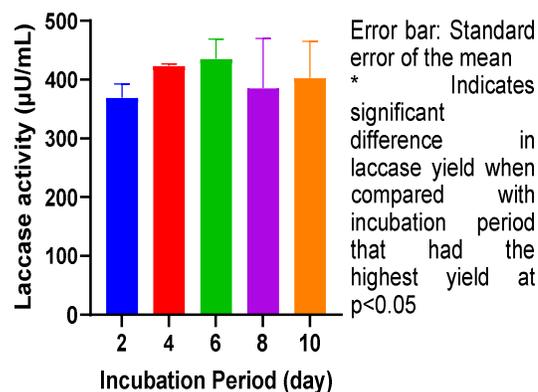


Figure 6: Effect of Incubation Period on Laccase Yield

The effect of metal ions on the activities of laccase is shown in Figure 7a. The activities of laccase produced by *Pleurotus ostreatus* were higher with Fe^{2+} (628.29 $\mu\text{U/mL}$), Na^+ (487.44 $\mu\text{U/mL}$), K^+ (482.15 $\mu\text{U/mL}$), and Mn^{2+} (436.01 $\mu\text{U/mL}$) than the control (431.20 $\mu\text{U/mL}$). However, activities recorded with Mg^{2+} (412.45 $\mu\text{U/mL}$) and Ca^{2+} (420.62 $\mu\text{U/mL}$) were lower than the value obtained in the control. There was, however, no significant difference in the activities obtained with various metal ions and control at $p>0.05$. The effect of pH on laccase activity is shown in Figure 7b. There was an initial increase in laccase activity with an increase in pH from 370.63 $\mu\text{U/mL}$ (pH 3.6) to 853.98 $\mu\text{U/mL}$ (pH 8.0) before a decline to 763.37 $\mu\text{U/mL}$ was observed at pH 9.5. The laccase activity observed at pH 8.0 was not significantly different from the activity recorded at 9.5.

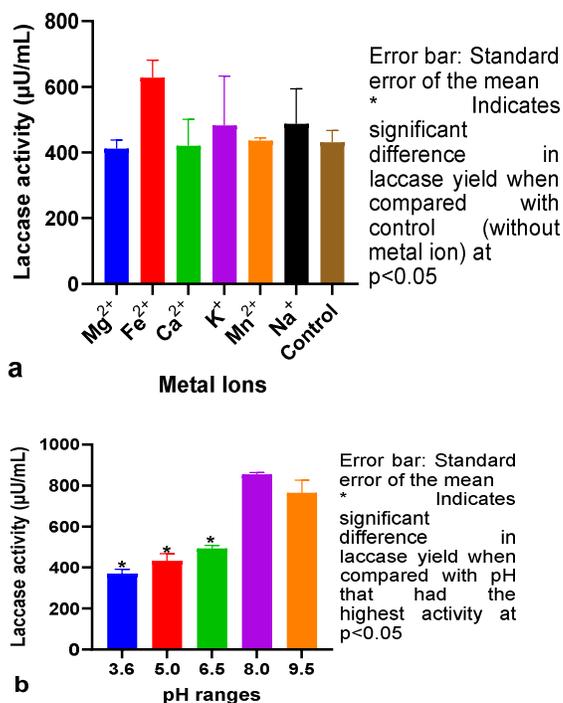


Figure 7: Effect of (a) Metal Ions and (b) pH on Laccase Activity

Laccase activity at 25 °C ranged from 439.13 µU/mL (10 minutes) to 495.85 µU/mL (20 minutes). There was an increase in laccase activity within the first 30 minutes at 37°C from 381.68 µU/mL to 465.33 µU/mL. A further increase in the incubation period resulted in a decline in activity from 465.33 µU/mL at 30 minutes to 446.82 µU/mL at 40 minutes. Incubation time did not have a significant effect on laccase activity at 37 °C ($p > 0.05$). At 50 °C, laccase activity ranged from 430.72 µU/mL (20 minutes) to 476.38 µU/mL (60 minutes). There was no significant difference in the laccase activity recorded across various incubation periods at 50 °C ($p > 0.05$). At 60 °C, there was an increase in laccase activity with an increase in incubation period, from 451.87 µU/mL at 10 minutes to 512.20 µU/mL at 60 minutes. However, the activities recorded at different incubation periods at 60 °C were not significantly different from each other ($p > 0.05$).

The decolourising potential of *Pleurotus ostreatus* laccase is shown in Figure 8. Congo red, Leishman blue, and malachite green were decolourised by 9.40%, 21.38%, and 21.76%, respectively.

Table 1: Effect of Temperature and Time on Activity of *Pleurotus ostreatus* Laccase

Temperature (°C)	Time (minutes)	Laccase activity (µU/mL)
25	10	439.13 ^{abc}
	20	495.85 ^{bc}
	30	449.94 ^{abc}
	40	448.98 ^{abc}
	50	442.98 ^{abc}
	60	448.02 ^{abc}
37	10	381.68 ^a
	20	408.36 ^{ab}
	30	465.33 ^{abc}
	40	446.82 ^{abc}
	50	404.52 ^{ab}
	60	452.35 ^{abc}
50	10	438.17 ^{abc}
	20	430.72 ^{abc}
	30	467.25 ^{abc}
	40	449.22 ^{abc}
	50	470.86 ^{abc}
	60	476.38 ^{abc}
60	10	451.87 ^{abc}
	20	456.92 ^{abc}
	30	493.21 ^{bc}
	40	499.70 ^{bc}
	50	497.54 ^{bc}
	60	512.20 ^c

Mean values with the same alphabetical superscripts along the column are not significantly different at $p > 0.05$

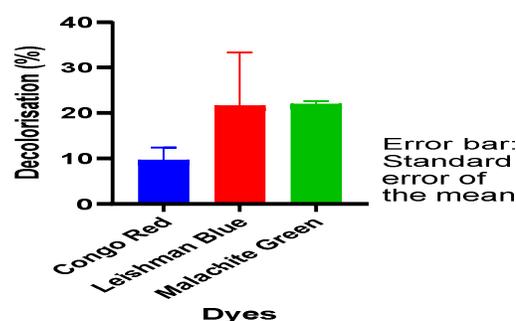


Figure 8: Decolourisation Potential of *Pleurotus ostreatus*' Laccase

4.0 Discussion

The appearance of purple colour on potato dextrose agar supplemented with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) confirmed the laccase-producing ability of

Pleurotus ostreatus. The purple colouration results from the oxidation of colourless ABTS to purple ABTS²⁺ [13]. Many studies have confirmed that *Pleurotus ostreatus* produces laccase [11,12,13].

Lignocellulosic substrates contain lignin, which can act as an inducer for laccase production. Differences in laccase yields among various substrates may reflect variations in lignin content. *Pleurotus ostreatus* has been reported to produce lignocellulose-degrading enzymes involved in the breakdown of agricultural wastes [13,14]. Agrowastes were used to produce laccase in this study, and their utilisation for laccase production is a means of turning waste into wealth. Many researchers have converted waste into value-added products, such as ethanol [18,19,20] and xylanase [21]. Hasan *et al.* [22] used different agrowastes, including conifer litters, sugarcane bagasse, rice straw, corn cob, and maize straw, for the production of laccase. Fasiku *et al.* [21] utilised sawdust, sugarcane bagasse, maize straw, conifer litters, corn cobs, and rice straw for xylanase production. Utilising waste in the environment for the production of value-added products will create a safe environment and improve the economy. Among the lignocellulosic substrates used in this work, maize straw supported the highest enzyme yield. This is in line with the work of Fasiku *et al.* [19] and Fasiku *et al.* [21] where maize straw was the best among different agrowastes screened for the production of ethanol and xylanase, respectively.

Laccase production is affected by cultural and nutritional parameters [12,23]. The carbon source affected laccase production in this work. This is in line with the studies reporting that the carbon source utilised by some *Pleurotus* species affected laccase production [23,24]. The nitrogen source is important in enzyme production and affected laccase yield in this work. A similar observation was recorded by Stajic *et al.* [24], who reported that nitrogen sources affected laccase yields of some *Pleurotus* species. The highest laccase yield in this work was observed with peptone as the nitrogen source, consistent with the report of Mikiashvili *et al.* [25], who reported that two strains of *Pleurotus ostreatus* had their highest laccase production when peptone was used as the nitrogen source.

The initial pH of the fermentation medium affects the production of metabolites [20,26]. The highest laccase yield was recorded with an initial pH of

7.0 in this work, while Bakratsas *et al.* [26] reported their highest laccase production by *Pleurotus ostreatus* at an initial pH of 6.0. However, laccase yields of *Pleurotus ostreatus* in this work at both pH 6.0 and 7.0 were not significantly different ($p > 0.05$) from one another. Inoculum size is another factor that affects metabolite yields [20,23], and this affected laccase yield in this work. Laccase yield was affected by the incubation period, and similar reports on how the incubation period affected *Pleurotus ostreatus* laccase yield have been documented [12,24,27].

In this study, the highest laccase activity of *Pleurotus ostreatus* was recorded at 60°C after 60 min of incubation, which is consistent with the work of Othman and Angelina [3], who reported that the optimal laccase activity of the *Agaricus bisporus* CU13 strain was observed at 60 °C. This result demonstrates the thermotolerant potential of *Pleurotus ostreatus* and highlights its suitability for diverse biotechnological applications operating under moderate to high temperatures. The enzyme's stability at elevated temperatures suggests that its tertiary and quaternary protein structures are resistant to denaturation, a desirable trait for industrial biocatalysts. Enzymes that retain high activity at elevated temperatures are particularly useful in processes such as biobleaching, bioethanol production, and dye decolourisation, where reaction rates and substrate solubility improve with heat [28,29,30].

The optimum pH range observed in this study further supports the enzyme's adaptability. The laccase retained substantial activity from pH 3.5 to 9.0, with a maximum at pH 8.0, which falls within the alkaline range. Bakratsas *et al.* [26] also reported the highest laccase activity at pH 8.0, which decreased with decreasing pH as observed in this work. This wide operational range is significant because it demonstrates that the enzyme can function in both mildly acidic and basic environments. This flexibility expands its usability in various industries. The enzyme's resilience across broad pH levels may be attributed to the stabilising influence of surface-exposed acidic and basic residues that preserve its electrostatic balance and conformational integrity. Similar findings were reported by Patel *et al.* [31] that *P. ostreatus* laccase displayed exceptional stability across a wide pH range.

The influence of metal ions in this study also provided key insights into the catalytic

mechanism of the enzyme. The addition of Fe^{2+} and Na^+ enhanced enzyme activity. The improvement in enzyme performance in the presence of metal ions can be attributed to their ability to stimulate and stabilise the active site [21]. These findings correspond with those of Czyrko *et al.* [32], who demonstrated that trace metals not only act as cofactors but also induce conformational changes that enhance enzyme-substrate affinity. This stimulatory role of cations is crucial when designing industrial-scale laccase processes since wastewater and bioreactor systems often contain metal residues that can influence enzyme efficiency.

The decolourisation experiment conducted in this study underscores the enzyme's practical potential in environmental bioremediation. The laccase effectively degraded Leishman blue, malachite green, and Congo red, with the highest decolourisation observed in malachite green. This result aligns with the reports of Zhuo *et al.* [2], who found that laccase from *P. ostreatus* was capable of achieving more than 90% dye removal within hours of incubation. The observed efficiency can be linked to the enzyme's non-specific oxidative mechanism, which generates free radicals that attack aromatic rings, leading to cleavage of chromophoric groups and discoloration. The ability to degrade dyes also indicates that *P. ostreatus* laccase could serve as an eco-friendly alternative to chemical oxidants in treating dye-laden industrial effluents. Additionally, the use of agro-waste substrates such as maize straw and sugarcane bagasse in laccase production makes the overall process more sustainable and cost-effective, further aligning with green chemistry principles.

From an environmental perspective, utilising agricultural residues as fermentation substrates provides dual benefits: waste valorisation and pollution mitigation. Agricultural waste disposal is a significant environmental challenge, especially in developing countries. Converting such residues into value-added products like enzymes not only reduces environmental pollution but also provides economic opportunities for rural communities [19,20]. The successful use of maize straw and sugarcane bagasse in this study confirms that *P. ostreatus* can effectively convert lignocellulosic materials into functional biocatalysts. These findings corroborate those of Hasan *et al.* [22], who emphasised that fungal conversion of lignin-rich biomass enhances

resource recovery and promotes circular bioeconomy models.

5.0 Conclusion

This study establishes *Pleurotus ostreatus* as a potent and reliable source of thermostable, pH-tolerant, and metal-activated laccase enzyme. Its efficiency in degrading multiple dyes and its ability to thrive on agro-waste substrates make it a promising candidate for biotechnological innovations in wastewater treatment, paper processing, and sustainable bio-manufacturing. The findings not only validate the enzyme's industrial significance but also contribute to ongoing global efforts toward cleaner production technologies and circular bioeconomy development. Future research should explore enzyme immobilisation and purification techniques to enhance reusability, along with molecular characterisation to identify the active isoforms responsible for such high catalytic performance.

6.0 Acknowledgment

We acknowledge Mr. M. A. Bello for his technical assistance during this research.

7.0 Conflict of interest

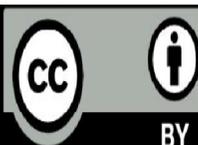
The authors declare that there is no conflict of interest.

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