

Discovery

To Cite:

Fasiku SA, Oladunni AA, Fasiku TB, Ernest GU, Adeogun OJ, Afolabi FJ. Optimisation of laccase production by *Curvularia verruculosa* UDY through solid-state fermentation using response surface methodology. *Discovery* 2026; 62: e7d3235
doi:

Author Affiliation:

¹Department of Microbiology and Biotechnology, Ajayi Crowther University, Oyo-Town, Nigeria

²Department of Chemical and Petroleum Engineering, Abiola Ajimobi Technical University, Ibadan, Nigeria

³Department of Physics, Ajayi Crowther University, Oyo-Town, Nigeria

*Corresponding author:

Samuel Adedayo Fasiku,
Department of Microbiology and Biotechnology, Ajayi Crowther University, Oyo-Town, Nigeria,
Email: samfash4@yahoo.com

Peer-Review History

Received: 07 April 2025

Reviewed & Revised: 18/May/2025 to 25/February/2026

Accepted: 03 March 2026

Published: 16 March 2026

Peer-Review Model

External peer-review was done through double-blind method.

Discovery

pISSN 2278–5469; eISSN 2278–5450



© The Author(s) 2026. Open Access. This article is licensed under a Creative Commons Attribution License 4.0 (CC BY 4.0), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

Optimisation of laccase production by *Curvularia verruculosa* UDY through solid-state fermentation using response surface methodology

Samuel Adedayo Fasiku^{1*}, Atilade Amos Oladunni², Taiwo Bukola Fasiku³, Godswill Uduak Ernest¹, Opeyemi Janet Adeogun¹, Femi Johnson Afolabi¹

ABSTRACT

Laccases are multicopper oxidase enzymes with significant industrial and environmental applications, including bioremediation, textile dye degradation, and biosensor development. This study aimed to optimise laccase production under solid-state fermentation using Response Surface Methodology (RSM) and to utilise the produced laccase for dye decolourisation. The laccase-producing fungus was molecularly identified. Parameters, including incubation period, pH, substrate concentration, inoculum size, and carbon sources, were optimised using RSM. The model equation was generated using Design Expert, and the model's statistical significance was evaluated using analysis of variance (ANOVA). Laccase was quantified using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) as a substrate. The effect of environmental conditions on laccase activity was determined, and the enzyme was used to decolourise dyes. The laccase-producing fungus was identified as *Curvularia verruculosa* UDY (accession number PV669996). The optimum laccase yield (735 µU/L) was observed with sugarcane bagasse as the substrate on a basal medium containing a glucose carbon source, at an initial pH of 3, with an inoculum size of 2 fungal plugs, over a fermentation period of 10.5 days. All cations tested enhanced laccase activity, with Mn²⁺ producing the greatest stimulation (1056 µU/mL). At various pH levels, the highest laccase activity (898 µU/mL) of *C. verruculosa* UDY was observed at pH 9.5, while activity at different temperatures peaked (943 µU/mL) at 60°C after 40 min. Laccase decolourised Congo red by 22% within 2 hours. The production of laccase by *C. verruculosa* UDY through solid-state fermentation was optimised. Cations, pH, temperature, and time affected laccase activity, and the produced laccase decolourised dyes, which could promote the utilisation of agrowastes for industrial enzyme production as well as laccase decolourisation application in the textile industry.

Keywords: Dye decolourisation, Fungi, Lignin-degrading enzyme, Microbial fermentation, Sugarcane bagasse

1. INTRODUCTION

Laccases belong to a group of multicopper polyphenol oxidases, a broad class of

enzymes that oxidise primarily phenolic compounds via one-electron transfer (Fasiku et al., 2023a). They contain copper atoms in their catalytic centre and simultaneously reduce oxygen to water. Laccases oxidise aromatic and aliphatic amines as well as phenols, provided these substrates are small enough to fit into their active centre and have a lower redox potential than the enzyme itself (Rodriguez-Couto, 2018). Laccases are categorised into two groups based on their redox potential: low- and high-potential enzymes. Enzymes with low redox potential are found in bacteria, plants, and insects, whereas fungi contain laccases with high redox potential, which are responsible for the breakdown of lignin and humus in laccase-catalysed catabolic reactions. Without the need for extra chemicals, the redox potential of plant, bacterial, and insect laccases enables thermodynamic radical coupling reactions in anabolic processes (Janusz et al., 2020).

Laccases are produced by several fungi belonging to Deuteromycetes, Ascomycetes, and Basidiomycetes. Some of the white-rot fungi are extensively explored and are involved in lignin metabolism, and they are particularly rich in laccase, that is, they exhibit the highest laccase activity (Ansari et al., 2021). It has also been shown that laccase activity is present in prokaryotes, including the following species: *Bacillus subtilis*, *B. pumilus*, *Haloferax volcanii*, *B. licheniformis*, *S. lavendulae*, *S. griseus*, *Oscillatoria boryana*, *Escherichia coli*, *P. syringae*, *Thermus thermophilus*, and *Marinomonas mediterranea* (Janusz et al., 2020). Laccase production has been identified in some insects; different genera of insects that produce laccase include *Bombyx*, *Drosophila*, *Lucilia*, *Diptoptera*, *Oryctes*, *Papilio*, *Manduca*, *Musca*, *Phormia*, *Rhodnius*, *Sarcophaga*, *Calliphora*, and *Schistocerca* (Singh and Gupta, 2020).

Laccases can use molecular oxygen for the oxidation of a broad range of organic compounds, such as lignin, aromatic amines, phenols, and polyphenols. They are also applicable in sectors like bioremediation, pulp and paper, textiles, food, and pharmaceuticals (Wadhwa et al., 2023). They are useful in lignin modification, food industries, textile treatment, paper and pulp wastewater, and petrochemical effluents and can also be used as a bioremediation agent for pesticides and herbicides. They are useful in removing toxic compounds from both aquatic and terrestrial systems. They are used in beverage production, biotransformations of specific regions, aerobic oxidation of benzyl alcohols, biosensors and analytical tools. Laccases are involved in the industrial polymerisation of lignosulfates for additional uses as dispersants, surfactants, and plasticisers within the cement and concrete industry. It is also a promising enzyme for decontaminating and biotechnological applications in phenol-polluted systems (Kyomuhimbo and Brink, 2023; Fasiku et al., 2026). This work aimed to optimise laccase production through response surface methodology and utilised the produced laccase for the decolourisation of dyes.

2. MATERIALS AND METHODS

Collection and maintenance of the microbe

The laccase-producing fungus used for this research was obtained from the Department of Microbiology and Biotechnology at Ajayi Crowther University, Oyo Town, Nigeria, and grown on potato dextrose agar.

Molecular identification of the fungus

After DNA extraction, the PCR cocktail mix includes 2.5 μ L of 10x PCR buffer, 1 μ L of 25 mM MgCl₂, 1 μ L of forward and reverse primers, 1 μ L of DMSO, 2 μ L of 2.5 mM dNTPs, 0.1 μ L of 5 u/ μ L Taq DNA polymerase, and 3 μ L of 10 ng/ μ L DNA. The total reaction volume was increased to 25 μ L by adding 13.4 μ L of nuclease-free water. The primers used are ITS 1: TCCGTAGGTGAACCTGCGG and ITS 4: TCCTCCGCTTATTGATATGS. Initial denaturation at 94°C for 5 minutes, then 36 cycles of denaturation at 94°C for 30 secs, annealing at 54°C for 30 secs, and elongation at 72°C for 45 secs. The final elongation phase is at 72°C for 7 minutes, with a holding temperature of 10°C. The amplified fragments were visualised on 1.5% agarose electrophoresis gels stained with SafeView. The ABI 3500 equipment was used for the sequencing process. The cycle sequencing phase was performed using 25 ng of the PCR result. The phylogenetic tree was created with the Mega 11 software (Kumar et al., 2018).

Experimental Design

Design Expert® 11, file version 11.1.2.0, was employed in the design of the experiment. The design type was D-Optimal with the point exchange algorithm, the model was quadratic, the study type was response surface, and the sub-type was split plot. Five factors: substrate concentration, pH, inoculum size, incubation period, and carbon source moisture content were investigated for their effects on laccase production yield. While other factors were numeric, the carbon sources used were specified as a categorical variable, as shown in Table 1, and the total experimental runs generated was 40, as shown in Table 2.

Statistical analysis and optimisation

The experimental data generated were statistically analysed in the Design Expert software using REML (Restricted Maximum Likelihood) to determine the optimal fermentation conditions for maximising laccase production yield. As shown in Table 1, the effect of five factors was investigated. These factors are substrate concentration, pH, inoculum size, incubation period, and carbon source moisture content. Table 2 shows the experimental runs for the optimisation of laccase production using Design Expert with the independent variables in Table 2.

The basal medium was prepared by weighing the following: 10 g of carbon source (glucose, sucrose, fructose, galactose, maltose or mannitol), 1 g of $(\text{NH}_4)_2\text{SO}_4$, 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g of KCl, 0.01 g of FeSO_4 and 0.01 g of MnSO_4 per litre. Four to six grams (4-6 g) of sugarcane bagasse was weighed into polythene bags, mixed with 15 mL of the basal medium, sterilised and allowed to cool. Two to ten (2-10) circular plugs (7 mm) of the laccase-producing fungus were inoculated into each bag and incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 7 to 14 days. Different buffers of pH 3 to 10 were used to prepare the basal medium. The variation in carbon source, sugarcane bagasse concentration, initial pH, inoculum size and incubation period depends on the experimental runs as shown in Table 2. After the incubation period, 40 mL of sterile distilled water was added to each bag, mixed and filtered using No. 1 Whatman filter paper. The filtrates were used as crude enzymes.

Determination of enzyme activity

Laccase activity was determined using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) [ABTS] as a substrate. The reaction mixture contained 0.5 mL of the crude enzyme, 1.5 mL sodium acetate buffer (0.1 M, pH 5.0) and 60 μL of 1 mM ABTS. The reaction mixture was incubated for 10 minutes. The reaction was terminated by adding 20 μL of trichloroacetic acid. A blank was prepared with 0.5 mL of sterile distilled water instead of the crude enzyme. Optical density was measured at 420 nm using a spectrophotometer and expressed in U/mL. One unit of enzyme activity is the amount of enzyme required for oxidising one micromole of ABTS (Mongkoltharuk et al., 2012).

Table 1: Independent factors and coding

Factor	Name	Units	Change	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Substrate concentration	g/L	Easy	Numeric	4.00	6.00	-1 ↔ 4.00	+1 ↔ 6.00	5.05	0.9594
B	pH		Hard	Numeric	3.00	10.00	-1 ↔ 3.00	+1 ↔ 10.00	6.59	2.91
C	Inoculum size	plugs	Easy	Numeric	2.00	10.00	-1 ↔ 2.00	+1 ↔ 10.00	6.25	3.85
D	Incubation Period	days	Easy	Numeric	7.00	14.00	-1 ↔ 7.00	+1 ↔ 14.00	10.50	3.36
E	Carbon Source	g/L	Easy	Categoric	Glucose	Galactose			Levels:	6

Table 2: Experimental design of independent variables for optimization

		Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Group	Run	A: Substrate concentration (g/L)	b: pH	C: Inoculum size (plugs)	D: Incubation Period (Days)	E: Carbon Source (g/L)
1	1	6	6.5	2	14	Mannitol
1	2	5	6.5	8	10.5	Sucrose
1	3	4	6.5	10	14	Galactose
1	4	4	6.5	2	14	Sucrose
1	5	4	6.5	2	7	Fructose
1	6	6	6.5	2	14	Galactose

1	7	4	6.5	2	7	Glucose
1	8	6	6.5	2	14	Glucose
1	9	6	6.5	2	14	Maltose
1	10	6	6.5	10	7	Glucose
1	11	4	6.5	10	14	Glucose
1	12	4	6.5	6	10.5	Mannitol
1	13	4	6.5	2	7	Maltose
2	14	4	3	10	14	Maltose
2	15	4	3	10	7	Galactose
2	16	6	3	6	7	Maltose
2	17	6	3	2	14	Fructose
2	18	4	3	10	7	Sucrose
2	19	5	3	10	7	Mannitol
2	20	4	3	10	14	Fructose
2	21	6	3	10	14	Sucrose
2	22	4	3	2	14	Galactose
2	23	6	3	10	7	Fructose
2	24	6	3	2	7	Galactose
2	25	6	3	2	10.5	Glucose
2	26	6	3	10	14	Galactose
3	27	6	10	2	7	Fructose
3	28	6	10	10	10.5	Glucose
3	29	5	10	2	7	Mannitol
3	30	4	10	10	14	Sucrose
3	31	6	10	2	7	Sucrose
3	32	6	10	10	7	Galactose
3	33	6	10	10	7	Maltose
3	34	6	10	10	14	Fructose
3	35	4	10	2	14	Fructose
3	36	4	10	2	7	Galactose
3	37	4	10	6	14	Maltose
3	38	4	10	10	7	Fructose
3	39	6	10	10	7	Mannitol
3	40	5	10	10	14	Mannitol

Characterisation of laccase

Laccase was produced using optimised conditions, and the effects of pH, metal ions, temperature and time on the enzyme were determined.

Effect of pH

The effect of pH on the activity of laccase was examined using 1.5 mL of different buffers with varying 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 for the laccase assay. Laccase activity was determined as earlier explained in the laccase assay.

Effect of temperature

To examine the effect of temperature and time on laccase activity, the reaction mixture in the laccase assay was incubated at different temperatures (25, 37, 50 and 60°C) for 50 minutes at 10-minute intervals. Each reaction was terminated after the specified time, and laccase activity was determined as earlier explained in the laccase assay.

Effect of metal ions

Metal ions (K^+ , Mg^{2+} , Fe^{2+} , Ca^{2+} , Na^+ and Mn^{2+}) in 5 mM were incubated with crude laccase to determine the effects of cations on laccase activity. Laccase activity was determined as earlier explained in the laccase assay.

Decolourisation experiment

A decolourisation experiment was carried out by adding 0.5 mL of crude laccase to 2 mL of dyes (crystal violet and Congo red, 100 mg/L). They were incubated at 28 ± 2 °C for 3 hours. Absorbance was read at 590 nm for crystal violet and 520 nm for Congo red. The percentage of decolourisation was calculated according to Forootanfar et al., (2012).

$$\text{Decolourisation (\%)} = \frac{\text{Initial absorbance of the dye before incubation} - \text{Final absorbance of the dye after incubation}}{\text{Initial absorbance of the dye before incubation}} \times 100$$

3. RESULTS

Molecular identification of *C. verruculosa* UDY

Laccase-producing fungus was identified as *Curvularia verruculosa* UDY and accession No. PV669996 was assigned to it at the National Center for Biotechnology Information (NCBI) with a link <https://www.ncbi.nlm.nih.gov/nucleotide/PV669996>. The phylogenetic tree of *Curvularia verruculosa* UDY is shown in Figure 1. *Curvularia verruculosa* UDY is more closely related to *Curvularia* CVP2 than to *Curvularia lunata* AAL1. It is, however, distantly associated with *Pleurotus ostreatus* PGGP2599.

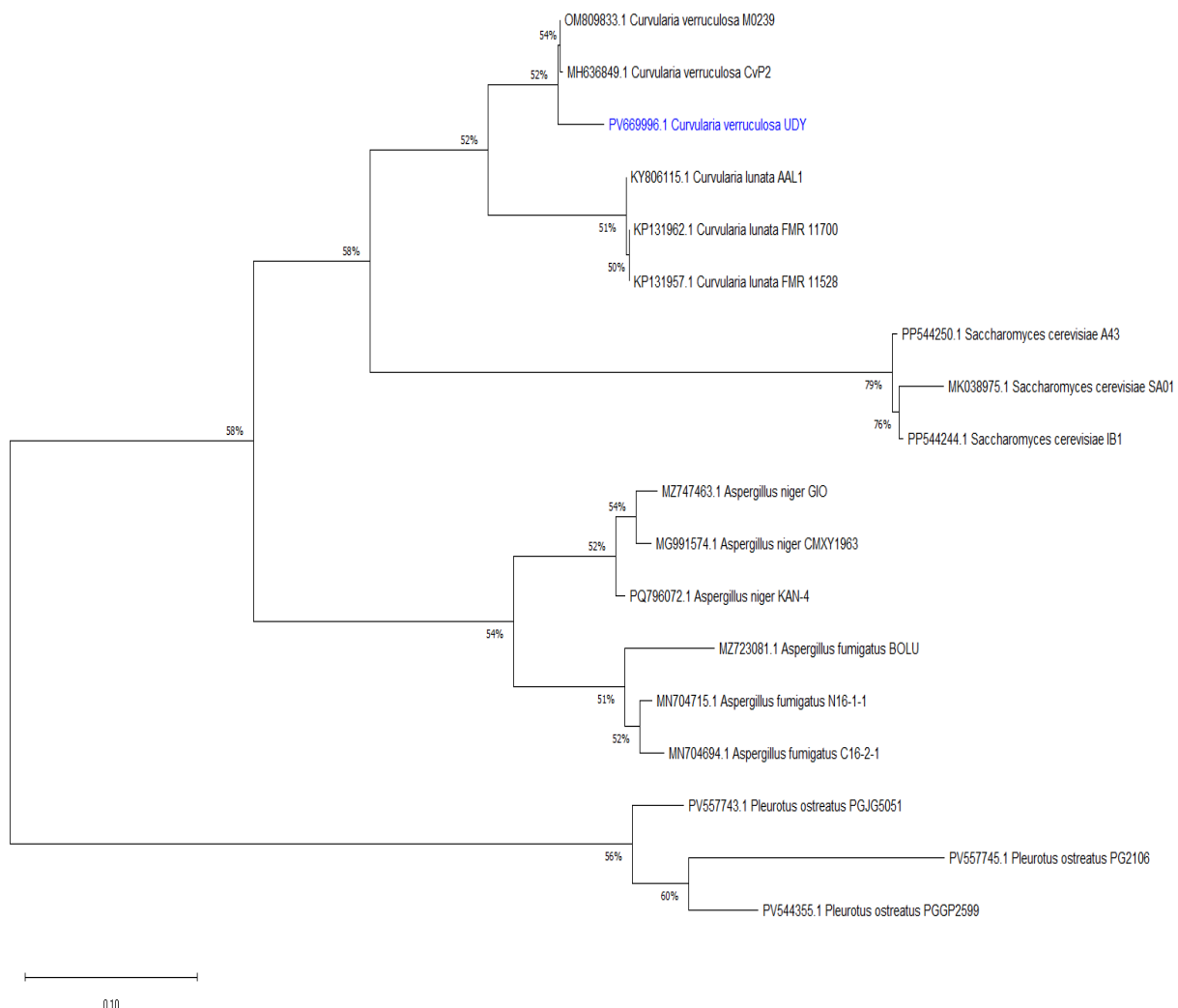


Figure 1: Phylogenetic analysis of *Curvularia verruculosa* UDY

Laccase Production

Figure 2 shows the laccase yield for different experimental runs. Experimental run 40 had the highest laccase yield (1597.78 μ U/mL) while the lowest yield (331.11 μ U/mL) was recorded at experimental run 22. The highest yield was achieved with mannitol as the carbon source, 5 g of sugarcane bagasse, an initial pH of 10, an inoculum size of 10 plugs (7 mm), and an incubation period of 14 days, as shown in the experimental design (Table 2).

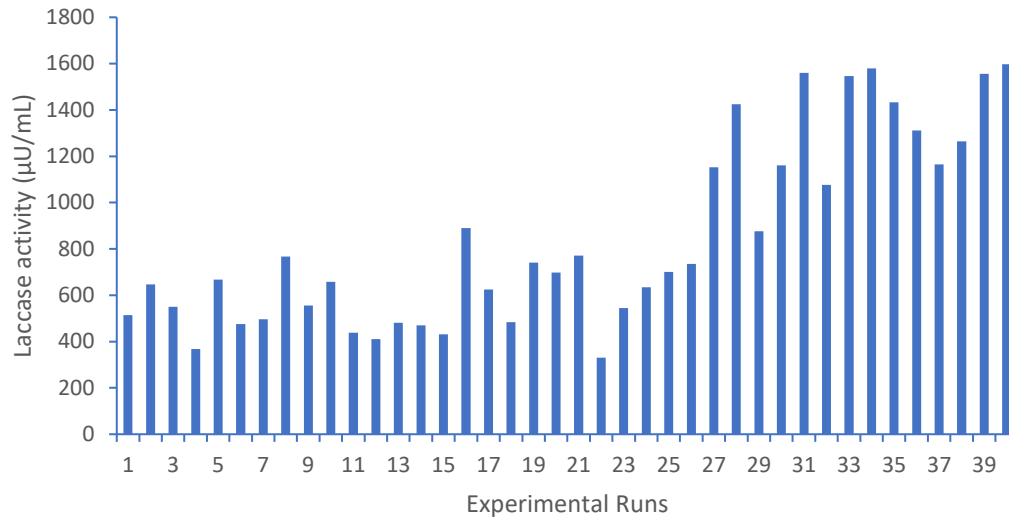


Figure 2. Laccase yield at different experimental runs

Design-Expert® Software

Factor Coding: Actual

R1

○ Design points below predicted value

0.000331111 0.00159778

X1 = A: Substrate concentration
X2 = b: pH

Actual Factors

C: Inoculum size = 2
D: Incubation Period = 10.5
E: Carbon Source = Glucose

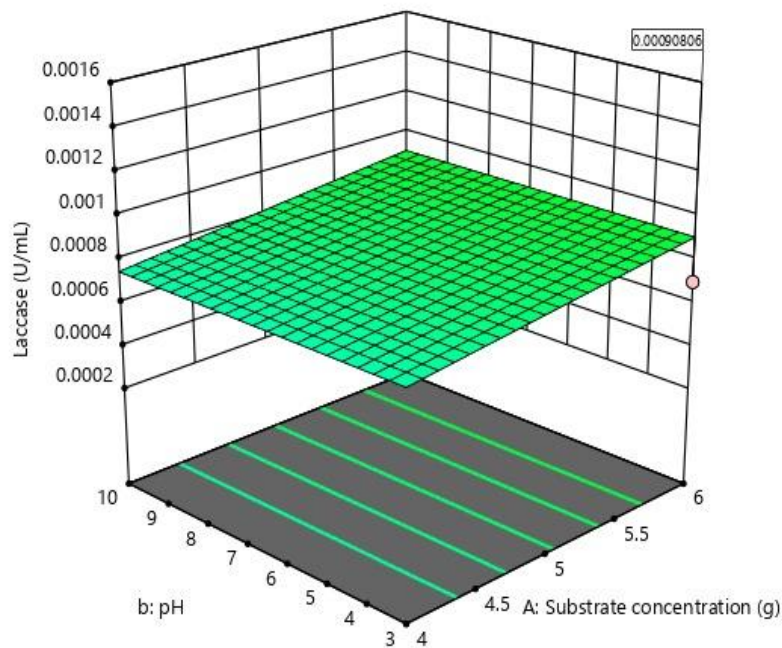


Figure 3. Optimum factors for the production of laccase.

Figure 3 shows the optimal conditions to maximise the yield of laccase. Design Expert predicted a sugarcane bagasse concentration of 6 g in 15 mL of basal medium with glucose as a carbon source at an initial pH of 3 and inoculum size of 2 plugs, with an incubation period of 10.5 days. The predicted maximal yield was 908.06 $\mu\text{U/mL}$, while the experimental highest yield was 735 $\mu\text{U/mL}$. The prediction was validated using 6 g of sugarcane bagasse in 15 mL of basal medium containing glucose as the carbon source, with an initial pH of 6.5, an inoculum size of 6 plugs of *Curvularia verruculosa*, and an incubation period of 10.5 days as suggested by response surface methodology.

As shown in Table 3, the REML analysis based on D-optimal design and Kenward-Roger p-values identified the subplot as significant and substrate concentration as the only statistically significant term in the model equation within a 95% confidence level. This is because REML focuses on calculating the precise contribution of the most relevant factors. The other variables have been eliminated because they have p-values too high (higher than 0.05) to be considered statistically significant or not properly testable with the current power of the experiment. The coded model equation is as displayed in Equation 1. The fit statistics showed that the coefficient of estimate R^2 is 0.841, and it implies the model can explain 84.1% of the data. The adjusted R^2 value of 0.8282 is also sufficiently high to validate the high significance of the model.

$$R1 = 0.0008 + 0.0001A \quad (1)$$

Where R1 is the response and A is the substrate concentration

Table 3. REML (Restricted Maximum Likelihood) analysis and Kenward-Roger p-values

Source	Term df	Error df	F-value	p-value	
Subplot	1	36.02	8.78	0.0054	Significant
A-Substrate concentration	1	36.02	8.78	0.0054	

Characterisation of the produced laccase

Effect of temperature and incubation time

The effects of temperature and time on laccase activity are shown in Table 4. At 25 °C, there was an increase in laccase activity in the first 30 minutes from 735 $\mu\text{U/mL}$ to 833 $\mu\text{U/mL}$, and thereafter a decrease in activity was observed with an increase in time. At 37 °C, laccase activities ranged from 595 $\mu\text{U/mL}$ (20 minutes) to 800 $\mu\text{U/mL}$ (10 minutes). At 50 °C, there was an increase in laccase activity from 10 minutes (613 $\mu\text{U/mL}$) to 30 minutes (672 $\mu\text{U/mL}$) before a decline in laccase activity with an increase in time was observed at 40 minutes. At 60 °C, there was an increase in laccase activity with time, from 526 $\mu\text{U/mL}$ to 943 $\mu\text{U/mL}$ within the first 40 minutes.

Effect of pH

Figure 4 shows the effect of pH on the laccase activity of *C. verruculosa* UDY. Laccase activity of *C. verruculosa* UDY showed an increase in its activity from pH 3.6 (595.5 $\mu\text{U/mL}$) to pH 6.5 (688 $\mu\text{U/mL}$), then a slight decrease to 683.5 $\mu\text{U/mL}$ was recorded at pH 8.0 before reaching its peak (897.5 $\mu\text{U/mL}$) at pH 9.5. This shows that an increase in pH is directly proportional to an increase in laccase activity.

Effect of metal ions

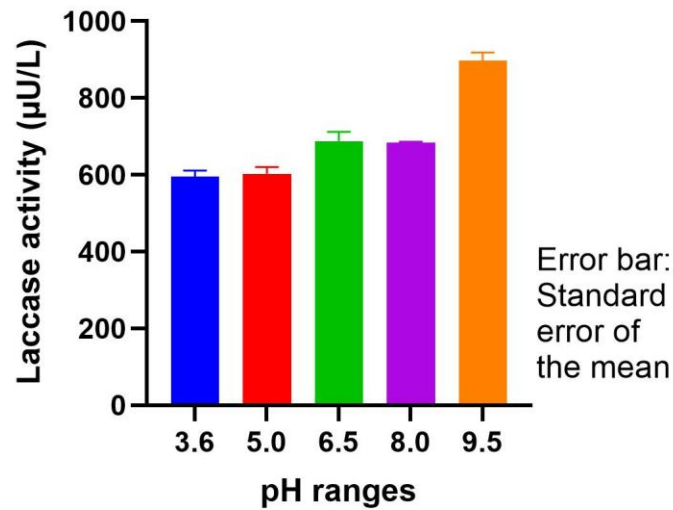
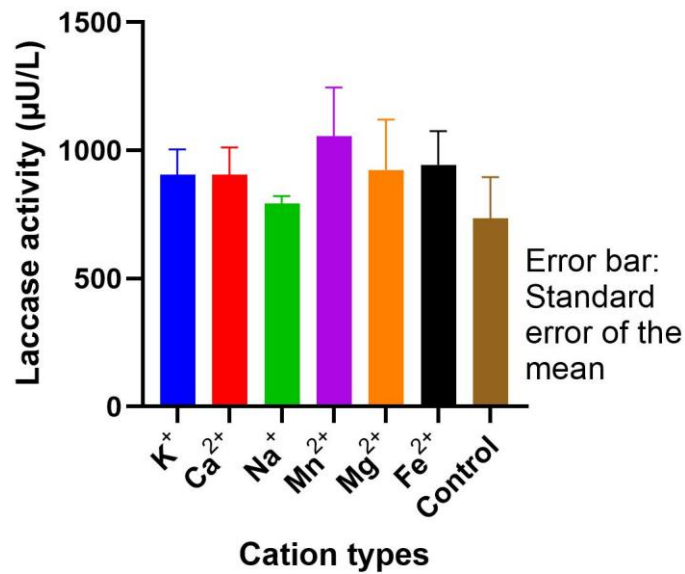
The addition of metal ions enhanced the enzymatic activity of laccase produced by *C. verruculosa* UDY, as shown in Figure 5. The highest enzyme activity (1056 $\mu\text{U/mL}$) of *C. verruculosa* UDY laccase was recorded with the addition of Mn^{2+} . The metal ion with the lowest enhancement was Na^+ , with an activity of 791.5 $\mu\text{U/mL}$, which was higher than the activity (735 $\mu\text{U/mL}$) recorded without any metal ion (control).

Dye Decolourisation

As shown in Figure 6, the laccase of *C. verruculosa* UDY decolourised Congo red by 22% and crystal violet by 5% after 2 hours of treatment.

Table 4. Effect of temperature and time on laccase activity ($\mu\text{U/ml}$) of *Curvularia verruculosa* UDY

Temperature ($^{\circ}\text{C}$)	Time (minutes)				
	10	20	30	40	50
25	735	787	833	724	569
37	800	595	737	732	649
50	613	649	672	611	620
60	526	602	676	943	808

**Figure 4.** Effect of pH on laccase activity of *Curvularia verruculosa* UDY.**Figure 5.** Effect of metal ions on laccase activity of *Curvularia verruculosa* UDY

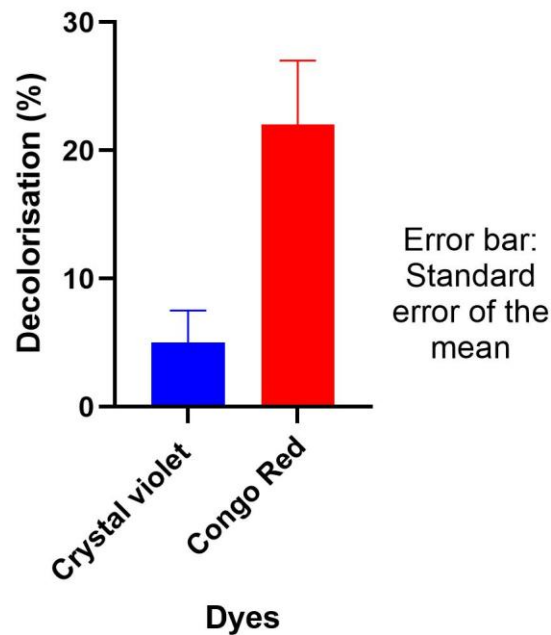


Figure 6. Decolourisation of dyes by laccase of *Curvularia verruculosa* UDY

4. DISCUSSION

Curvularia species have been known to produce lignocellulose-degrading enzymes that help break down agricultural wastes (Bello and Hussaini 2022; Mehta et al., 2022). Enzyme production using sugarcane bagasse, a waste, in this study is a means of generating wealth from waste. Numerous researchers have produced value-added products from the conversion of wastes to enzymes (Fasiku et al., 2023b; Odjogi et al., 2026), ethanol (Mohammad et al., 2021; Prasertsan et al., 2021; Fasiku and Wakil, 2021; 2022), and others. Different agrowastes (sugarcane bagasse, rice bran, reed grass, wheat straw, wheat bran, corn cobs, guava leaves and peanut husk) were used by Hasan et al. (2023) for laccase production. Waste utilisation in the environment to produce value-added products will create a better and safer environment and improve the state of the economy. Previous studies showed that the *Curvularia* species can generate laccase from sugarcane bagasse (Vazquez et al., 2024) during solid-state fermentation.

As substrates can affect laccase yield, substrate concentration is another factor that can have a significant impact on laccase yield. Substrate concentration has a significant effect on laccase production in this work. The source of carbon is another factor that can influence laccase production. There is a required carbon-to-nitrogen ratio that supports the production of metabolites, which can be influenced by the genetic makeup of the organisms used, the metabolite of interest, and other environmental factors (Fasiku and Wakil, 2022).

Acidity/alkalinity (pH) affects laccase production (Oloye et al., 2025). The optimum pH for laccase production in this work was 3. Hamed et al. (2024) and Bello et al. (2020) confirmed that pH affected the laccase yield of a *Curvularia* spp., with their optimal production at pH 5. The difference in optimum pH recorded in this work from theirs could be as a result of different substrates and different species of *Curvularia*. Hamed et al. (2024) and Bello et al. (2020) made use of *Curvularia lunata*, whereas *Curvularia verruculosa* was used in this work.

Inoculum sizes affected laccase yield in this work. Oloye et al. (2025) reported that inoculum size had a significant effect on laccase production. Bello et al. (2020) recorded that inoculum size affected the laccase yield of *Curvularia lunata*. Fasiku and Wakil (2022) reported that inoculum sizes affect metabolite yield. The incubation period is another factor that has an effect on laccase production (Oloye et al., 2025). The optimum incubation period in this work was 10.5 days. Hamed et al. (2024) recorded optimal production of laccase by a *Curvularia* sp. on the fifth day of incubation. Bello et al. (2020) reported 6 days as the optimum period for laccase production by a *Curvularia* sp. The optimal laccase production on both sugarcane bagasse and wheat bran was recorded after 168 hours of fermentation (Vazquez et al., 2024). Differences in substrates used and organisms for laccase production could be responsible for the different optimal production periods recorded by different researchers.

The highest laccase activity of *C. verruculosa* UDY recorded at 60°C in this study is similar to the work of Othman and Flaifil (2025), who reported the optimal laccase activity being produced by *Agaricus bisporus* CU13 fungal strain at 60 °C. Nadaroglu and Tasgin (2013) and Tišma et al. (2020) recorded the highest laccase activity for their microorganisms at 50 °C and 55 °C, respectively. Alshammary et al. (2025) recorded the highest laccase activity at 50 °C, while Bello and Hussaini (2022) recorded the highest laccase activity at 35 °C. This served as an indication of the thermostable properties of the laccase produced. Generally, the optimal temperature for laccase activity depends on the organism producing it, and laccase activity is sensitive to temperature.

Laccase activity is significantly influenced by pH due to the enzyme's structure and stability, substrate ionisation and redox behaviour (Oloye et al., 2025; Othman and Flaifil, 2025). It was reported that acidic pH (3.0–5.5) favoured the activities of laccase-producing fungi (Yin et al., 2019). Umar and Ahmed (2022) reported an optimal pH level for laccase activity to be at pH 3.0, Nadaroglu and Tasgin (2013) reported theirs to be at an optimum pH level of pH 4.0. Although recently laccases have been discovered to be active at higher, alkaline pH values, this happens due to direct evolution of the enzyme, as reported by Yin et al. (2019). The optimal pH for the laccase activity of *C. verruculosa* UDY was recorded at pH 9.5. The results of this study indicate that an increase in pH levels is directly proportional to an increase in enzymatic activity. Alshammary et al. (2025) recorded their highest laccase activity at pH 6.

All metal ions used in this work had positive influence on laccase activity, with Mn²⁺ having the most impact. Hamed et al. (2024) also reported Mn²⁺ as the most effective in stimulating activity of laccase produced by *Curvularia lunata* MY3. Nadaroglu and Tasgin (2013) reported a high inhibitory effect of Mn²⁺. The disparity between this study and theirs could be due to factors such as the fungal species, substrate used and Mn²⁺ concentrations. However, some metal ions such as Na⁺, Ca²⁺ and K⁺ that stimulated activity of laccase of *Curvularia verruculosa* UDY in this work retarded activity of *Curvularia lunata* MY3 laccase in the work of Hamed et al. (2024). This might be as a result of the genetic makeup of these organisms; they are of different species, or the concentration of metal ions used. Concentration affects the stimulatory effect of metal ions on enzymes (Alshammary et al., 2025).

Laccase has shown potential for decolourising and degrading crystal violet and other synthetic dyes (Zhang et al., 2020). In this study, the laccase produced by *C. verruculosa* UDY was able to decolourise Congo red, which is in accordance with what Forootanfar et al. (2012) reported. *Curvularia clavata* has been utilised in the decolourisation of palm oil mill effluent (Neoh et al., 2014) and recalcitrant dye (Neoh et al., 2015; Bello and Hussaini, 2022).

5. CONCLUSION

Optimum production of laccase (735 µU/mL) by *Curvularia verruculosa* UDY was achieved with sugarcane bagasse as an agro-waste substrate at a concentration of 6 g per 15 mL of basal medium, which contained a source of carbon (glucose), at an initial pH of 3 with an inoculum size of 2 plugs of 7 mm during 10.5 days of fermentation period. Environmental factors such as pH, temperature, and metal ions affected the activities of laccase produced by *Curvularia verruculosa* UDY. The laccase produced exhibited dye decolourisation capabilities, indicating its applicability in the treatment of dye-laden industrial effluents, and future studies should evaluate the dye decolourisation efficiency of the laccase enzyme in real textile and industrial effluents to assess its practical applicability under realistic conditions. Industrial-scale studies should be undertaken to assess the feasibility of large-scale laccase production and its economic viability for industrial applications.

Acknowledgement

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

Author Contributions

Samuel Adedayo Fasiku: Conceptualisation, design, investigation, data acquisition, data analysis and interpretation, writing original draft, review and editing/critical revision.

Atilade Amos Oladunni: design, investigation, data acquisition, data analysis and interpretation, writing original draft, review and editing/critical revision.

Taiwo Bukola Fasiku: design, investigation, data acquisition, data analysis and interpretation, writing original draft, review and editing/critical revision.

Godswill Uduak Ernest: investigation, data acquisition, writing original draft.

Opeyemi Janet Adeogun: writing original draft.

Femi Johnson Afolabi: writing original draft, review and editing/critical revision.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that they have no conflicts of interest, competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethical approval & declaration

Not applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

This research did not receive any external funding like specific grant from funding agencies in the public, commercial, or nonprofit sectors.

Data and materials availability

Data that support the findings of this study are embedded within the manuscript.

REFERENCES

- Alshammary M, Kotb E, Ababutain IM, Alabdall AH, Aldakeel SA, Alsanie SI, Alhamad S, Alshwyeh H, Ahmed M. Albarrag. Production, Biochemical Characterization, and Application of Laccase from Halophilic *Curvularia lunata* MLK46 Recovered from Mangrove Rhizosphere. *Biology*, 2025; 14(4): 402. doi: 10.3390/biology14040402
- Ansari MKA, Lastochkina O, Iqbal M, Ansari AA, Fatma T, Rodriguez-Couto S, Owens G. Laccase - The Wonder Enzyme for a Variety of Industries, *Acta Scientific Microbiology*, 2021; 4(12): 52-66.
- Bello A, Hussaini IM. Evaluation of Dye Decolourization ability of Laccase Produced *Curvularia lunata* SS17. *UMYU Journal of Microbiology Research*, 2022; 7(2): 1-9. doi: 10.47430/ujmr.2272.001
- Bello A, Machido DA, Mohammed-Dabo AI, Ado SA. Optimisation of laccase production by *Curvularia lunata* using maize cob as substrate. *FUDMA Journal of Science*, 2020; 4(4):460-468. doi: 10.33003/fjs-2020-0404-503
- Fasiku SA, Afolabi FJ, Egbeleke TA, Fashogbon RO. Applications of Microbial Enzymes in Industries. *Journal of Multidisciplinary Science: MIKAILALSYS*, 2026; 4(1): 26-40. doi: 10.58578/mikailalsys.v4i1.8137
- Fasiku SA, Bello MA, Odeniyi OA. Production of xylanase by *Aspergillus niger* GIO and *Bacillus megaterium* through solid-state fermentation. *Access Microbiology*, 2023b; 5, 000506.v5. doi: 10.1099/acmi.0.000506.v5
- Fasiku SA, Wakil SM, Alao OK. Screening for lignocellulolytic enzymes-producing white rot fungi. *Asian Journal of Research in Botany*, 2023a; 9(2): 1-7.
- Fasiku SA, Wakil SM. Pretreatment of maize straw with *Pleurotus ostreatus* and *Lentinus squarrosulus* for bioethanol production using *Saccharomyces cerevisiae*. *Novel Research in Microbiology Journal*, 2021; 5(6), 1480–1493. doi: 10.21608/nrmj.2021.209731
- Fasiku SA, Wakil SM. Screening of factors responsible for conversion of maize straw into bioethanol. *Journal of Microbiology, Biotechnology and Food Sciences*, 2022; 12(2), e5901. doi: 10.55251/jmbfs.5901
- Forootanfar H, Atefeh M, Marzieh A, Yasaman M, Alieh A, Farhad N, Mohammad AF. Synthetic dye decolorization by three sources of fungal laccase, *Journal of Environmental Health Sciences & Engineering* 2012; 9:27. doi: 10.1186/1735-2746-9-27
- Hamed AA, Abd-Elaziz AM, Ghanem MME, ElAwady ME, Abdel-Aziz MS. Production of laccase enzyme from *Curvularia lunata* MY3: purification and characterization, *Folia Microbiologica*, 2024; 69:221–234. doi: 10.1007/s12223-023-01088-2
- Hasan S, Anwar Z, Khalid W, Afzal F, Zafar M, Ali U, Refai MY, Afifi M, Al-Farga, A, Aljobair MO. Laccase Production from Local Biomass Using Solid State Fermentation. *Fermentation*, 2023;9(2), 179. doi: 10.3390/fermentation9020179
- Janusz G, Pawlik A, Swiderska-Burek U, Polak J, Sulej J, Jarosz-Wilkolazka A, Paszczyński A. Laccase Properties, Physiological Functions, and Evolution, *international journal of molecular science*, 2020; 21: 966. doi: 10.3390/ijms21030966
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing

- platforms. *Molecular Biology and Evolution*, 2018; 35: 1547-1549. doi: 10.1093/molbev/msy096
15. Kyomuhimbo HD, Brink HG. Applications and immobilization strategies of the copper-centred laccase enzyme; a review, *Heliyon*, 2023; 9: e13156. doi: 10.1016/j.heliyon.2023.e13156
16. Mehta T, Meena M, Nagda A. Bioactive compounds of *Curvularia* species as a source of various biological activities and biotechnological applications. *Frontiers in Microbiology*, 2022; 13: 1069095. doi: 10.3389/fmicb.2022.1069095
17. Mohammad S, Baidurah S, Kamimura N, Matsuda S, Bakar NASA, Muhamad NNI, Ahmad AH, Dominic D, Kobayashi, T. Fermentation of palm oil mill effluent in the presence of *Lysinibacillus* sp. LC 556247 to produce alternative biomass fuel. *Sustainability*, 2021; 13:11915. doi: 10.3390/su132111915
18. Mongkolthanaruk W, Tongbopi S, Bhoonobtong A. Independent behaviour of bacterial laccases to induce and metal ions during production and activity. *African Journal of Biotechnology*. 2012; 11(39): 9391-9398. doi: 10.5897/AJB11.3042
19. Nadaroglu H, Tasgin E. Purification and characterisation of laccase from *Lactarius volemus* and its application in removal of phenolic compounds from fruit juice, *Journal of Food, Agriculture & Environment*, 2013; 11 (3&4):109-114.3. doi: 10.12691/jfmr-2-12-13
20. Neoh CH, Lam CY, Lim CK, Yahya A, Bay HH, Ibrahim Z, Noor ZZ. Biodecolorization of recalcitrant dye as the sole source of nutrition using *Curvularia clavata* NZ2 and decolorization ability of its crude enzymes. *Environmental Science and Pollution Research*, 2015; 22(15): 11669-11678. doi: 10.1007/s11356-015-4436-4
21. Neoh CH, Lam CY, Lim CK, Yahya A, Ibrahim Z. Decolorization of palm oil mill effluent using growing cultures of *Curvularia clavata*. *Environmental Science and Pollution Research*, 2014; 21(6): 4397-4408. doi: 10.1007/s11356-013-2350-1
22. Odjogi EA, Fasiku SA, Alao OK, Salawu KO, Dada MT, Odeniyi OA, Wakil SM. Amylase production by *Streptomyces* species and its application in orange juice clarification. *Trakya University of Journal of Natural Sciences* 2026; 27(1). doi: 10.23902/trkjnat.202562
23. Oloye NR, Oyedeji BA, Taiwo MO, Adebajo SO, Akintokun AK, Akamo JA, Folarin BT. Multifaceted screening and optimization of laccase-producing rhizospheric yeast for enhanced biosynthesis of laccase enzyme. *The Microbe*, 2025; 100419. doi: 10.1016/j.microb.2025.100419
24. Othman AM, Flaifil AG. Characterization and evaluation of the immobilized laccase enzyme potential in dye degradation via one factor and response surface methodology approaches. *Scientific Reports*, 2025; 15(1): 735. doi: 10.1038/s41598-024-82310-0
25. Prasertsan P, Leamdum C, Chantong S, Mamimin C, Kongjan P, O-Thong S. Enhanced biogas production by co-digestion of crude glycerol and ethanol with palm oil mill effluent and microbial community analysis. *Biomass and Bioenergy* 2021; 148:106037.
26. Rodriguez-Couto S. Solid-State Fermentation for Laccases Production and Their Applications, *Current Developments in Biotechnology and Bioengineering*, 2018; 211-234. doi: 10.1016/B978-0-444-63990-5.00011-6
27. Singh D, Gupta N. Microbial Laccase: a robust enzyme and its industrial applications, *Biologia* 2020; 75: 1183-1193. doi: 10.2478/s11756-019-00414-9
28. Tišma M, Šalić A, Planinić M, Zelić B, Potočnik M, Šelo G, Bucić-Kojić A. Production, characterisation and immobilization of laccase for an efficient aniline-based dye decolourization. *Journal of water process engineering*, 2020; 36: 101327. doi: 10.1016/j.jwpe.2020.101327
29. Umar A, Ahmed S. Optimization, purification and characterization of laccase from *Ganoderma leucocontextum* along with its phylogenetic relationship. *Sci Rep* 2022; 12: 2416. doi: 10.1038/s41598-022-06111-z
30. Vazquez MA, Saa LR, Valiño E, Torta L, Laudicina VA. Microbiological Aspects and Enzymatic Characterization of *Curvularia kusanoi* L7: Ascomycete with Great Biomass Degradation Potentialities. *Journal of Fungi*, 2024; 10(12): 807. doi: 10.3390/jof10120807
31. Wadhwa H, Singh R, Chopra C. Occurrence and Applications of Fungal Laccases: A Comprehensive Biotechnological Review. *Biological Forum – An International Journal*, 2023; 15(4): 463-469.
32. Yin Q, Zhou G, Peng C, Zhang Y, Kües U, Liu J, Xiao Y, Fang Z. The first fungal laccase with an alkaline pH optimum obtained by directed evolution and its application in indigo dye decolorization. *AMB express*, 2019; 9:151 doi: 10.1186/s13568-019-0878-2
33. Zhang W, Yang Q, Luo Q, Shi L, Meng S. Laccase-Carbon nanotube nanocomposites for enhancing dyes removal. *J. Clean. Product*. 2020; 242: 118425. doi: 10.1016/j.jclepro.2019.118425