Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-024-06263-0

ORIGINAL ARTICLE

Optimization of laccase production by *Pleurotus pulmonarius* through solid substrate fermentation of tender coconut fiber: enhanced laccase production and biomass delignification

Geethu Suresh[1] · **Ragunathan R**[1] · **Jesteena Johney**[1]

Received: 13 June 2024 / Revised: 1 October 2024 / Accepted: 15 October 2024

Abstract
Tender coconut fiber, an abundant agro-waste, requires chemical or biological pretreatment to enhance its potential for value-added applications due to its complex lignocellulosic composition. Pretreatment of tender coconut fiber with laccase-producing white rot fungi presents a sustainable strategy for effective delignification and waste management. This study investigated the biological pretreatment of tender coconut fiber using the white rot fungus, *Pleurotus pulmonarius*, for delignification through enhanced laccase production via solid-state fermentation. This research involved exploring the influence of different factors on laccase production and understanding the relationships between these factors through response surface methodology (RSM) to obtain the maximum laccase production. A central composite design was employed to optimize the process parameters, including pH, temperature, incubation time, and concentration of corn steep liquor (CSL). The optimized process parameters were a pH of 5.6, a temperature of 27 °C, an incubation time of 21 days, and a CSL concentration of 3%. This optimization resulted in a remarkable increase in enzyme activity, reaching 663.76 ± 10.67 IU/ml, indicating a substantial 13.16-fold increase after the optimization process. Scanning electron microscopy (SEM) and FTIR analysis were performed to observe the changes in the surface structures of the raw and delignified fiber. The enhanced laccase activity suggests the potential for tender coconut fiber to be used to produce laccase enzymes, which has not been explored before. This approach offers a solution for managing tender coconut fiber and unlocks the potential for valorization due to the enhanced properties of the modified fiber.

Keywords Laccase · Tender coconut fiber · Corn steep liquor · *Pleurotus pulmonarius* · Response surface methodology · Central composite design (CCD) · Lignocellulose

1 Introduction

Laccases (benzenediol: oxygen reductases; EC 1.10.3.2) are versatile enzymes that have garnered significant attention in various fields of biotechnology with their remarkable ability to catalyze the oxidation of a wide range of organic compounds. Laccases are multi-copper oxidases classified based on their copper centers: Type 1 (T1) is responsible for the enzyme's blue color and accepts electrons, Type 2 (T2) is involved in electron transfer but lacks color, and Type 3 (T3) consists of two copper atoms that reduce oxygen to water. Together, these copper centers allow laccases to oxidize a wide range of substrates, making them potential biocatalysts in various biotechnological applications [1]. Laccases have found applications in diverse areas including biopulping, pulp bleaching [2–4], wastewater treatment [5], fuel cells [6], and bioremediation [1] due to their eco-friendly nature and ability to work under mild conditions.

Laccase enzymes can be synthesized by a diverse array of microorganisms, including both bacteria and fungi. However, white rot fungi are particularly emphasized because of their remarkable capacity to generate laccases in substantial quantities, rendering them highly promising for scaling up the production process [7]. *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotus* mushrooms are some of the well-studied white rot fungi for their lignin-degrading ability [8]. Recently, there has been mounting interest in

✉ Geethu Suresh
ammuge@gmail.com

[1] Department of Biotechnology, Centre for Bioscience and Nanoscience Research, Tamil Nadu, Coimbatore 641021, India

Published online: 23 October 2024

researching lignin-modifying enzymes with a wider range of WRF, driven by scientific and practical applications. The genus Pleuorotus, commonly known as oyster mushrooms, comprises a group of white rot fungi with excellent biotechnological applications. Their remarkable adaptability makes them suitable for cultivation on a wide range of substrates. Tender coconut fiber is a widely available lignocellulosic waste material that is a major contributor to municipal solid waste in India. Coconuts are cultivated in more than 90 tropical Asian countries. The records of the Coconut Development Board of India state that India has 2178.74 ha of coconut cultivation that produces 21,384.33 million tons of nuts with a productivity of 9815 nuts per hectare [9]. The underutilization of this natural fiber can be attributed to several factors, including its low cellulose content and high lignin content. Notably, while coconut fibers possess physical and chemical properties with significant potential for various applications, they are presently discarded in landfills. In response to societal and environmental concerns, there is ongoing research on potential ways to utilize this waste more effectively [10].

Coir fibers have experienced a surge in diverse applications such as hydroponics, container gardening, and biocomposite materials. This natural material primarily consists of lignin, which is a complex and inflexible polymer. When coir fibers are subjected to treatment with enzymes that modify lignin, such as laccase, they become more flexible and exhibit enhanced properties which makes coir fiber a suitable choice for a wide range of applications. Converting waste materials into valuable resources and reducing the environmental impact can play a pivotal role in the circular economy [11]. The use of tender coconut fiber as a substrate for laccase enzyme production, addressing the challenge of solid waste disposal, aligns with the principles of sustainable development and is highly commendable.

Solid substrate fermentation (SSF) processes have been reported as suitable methods for the production of lignin-modifying enzymes, particularly by filamentous fungi because they replicate the natural environments in which these fungi thrive [12]. Laccase production primarily occurs through fermentation. The effectiveness of this process is influenced by several factors that affect the growth and development of the fungus responsible for laccase production. Therefore, it is imperative to optimize laccase production to expand its potential applications in biotechnology. Many studies have addressed the optimization of process parameters for laccase production using white rot fungi. However, when dealing with a multitude of variables, the conventional one-factor-at-a-time approach can be both labor-intensive and time-consuming. The application of a statistical approach proves valuable for elucidating the impact and interactions of various factors. This not only enhances product yield but also minimizes process variability and shortens development time, all of which hold significant importance in laccase production [13].

Response surface methodology (RSM) is a robust and sophisticated collection of mathematical and statistical techniques tailored for the development, improvement, and optimization of production processes. RSM is invaluable for enhancing the production of microbial enzymes by analyzing the multifactorial influences on enzyme yield. By meticulously designing experiments that systematically vary input variables such as pH, temperature, incubation time, and substrate concentration, RSM enables a comprehensive understanding of their interactions and effects on enzyme production [14]. The methodology involves fitting polynomial models to the experimental data, which aids in identifying the optimal conditions for maximizing enzyme yield, thereby determining the precise combination of variables that result in the highest production levels.

Enhanced laccase production using RSM optimization has been achieved with various organisms and a variety of agro-wastes. For instance, *Pleurotus floridanus* has been used with microalgal biomass [15], *Hexagonia hirta* with coir pith waste [16], *Trametes versicolor* with pineapple waste [17], *Trametes hirsuta* with tea residues [13], and *Pleurotus sajor-caju* with wheat straw [18]. However, optimization studies for laccase production using *P. pulmonarius* utilizing coir fiber as a substrate are scarce. It is necessary to explore this underutilized agro-waste and design strategies for enhanced laccase production to achieve increased delignification.

To the best of our knowledge, this is the first attempt to optimize the process parameters for laccase production using solid-state fermentation (SSF) on tender coconut fibers. The objectives of this research were to statistically optimize the laccase production process for enhanced production and the delignification of tender coconut fiber. This study was designed to identify the optimal conditions for the fermentation process by employing central composite design (CCD), to analyze the interactions between various process parameters using response surface methodology (RSM), and to achieve effective delignification of the biomass. The effect of enhanced laccase production on the delignification process was studied by analyzing the structural changes that occur in the fibers during delignification using scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy.

2 Materials and methods

2.1 Sample collection and preparation

The tender coconut husks were obtained from a local vendor. These husks were manually defibered and carefully washed. The fibers were soaked in water for 2 h and then repeatedly rinsed until the rinse water was clear, ensuring the removal of dirt and debris. Subsequently, the fibers were dried in an oven at 60 °C

until a consistent weight was achieved. Once dried, the fibers were cut into 1–2 cm pieces using a standard laboratory cutter.

2.2 Fungal strain and culture conditions

The fungus *Pleurotus pulmonarius* was obtained from the Directorate of Mushroom Research, ICAR, and maintained by frequent subculture on malt extract medium.

The fungal pretreatment of the lignocellulosic substrate was performed using 100 ml flasks. Two grams of tender coconut fiber (dry basis) cut to a length of 1–2 cm was used as a solid substrate for the cultures. Minimal salt media comprising (w/v) 0.1% KH_2PO_4, 0.1% $MgSO_4.7H_2O$, and 0.1% peptone was used as fermentation media [19]. The medium containing the substrate was autoclaved at 121 °C for 20 min. Following sterilization, the medium was allowed to cool and inoculated with 2 ml of mycelial suspension in an aseptic manner.

Corn steep liquor (CSL) was demonstrated to be an efficient laccase inducer [20], and its effect was analyzed during preliminary studies (one factor at a time) to select factors and levels. Corn steep liquor was introduced into the medium at varying levels based on the design of experiments (DoE) provided by Stat-Ease software, and the trials were conducted accordingly.

2.3 Laccase enzyme assay

After the incubation period, the enzyme was extracted by adding 30 ml of 0.1 M sodium citrate buffer (pH 5) to the culture. The crude enzyme solution was then filtered using Whatman No. 1 filter paper, and the culture supernatant was obtained by centrifugation at 8000 rpm for 10 min.

Laccase activity was assessed spectrophotometrically by employing ABTS (2,2-azino-bis- 3-ethylbenzothiazoline-6-sulfonic acid) as the substrate [21]. The oxidation of ABTS was measured at a wavelength of 420 nm, (ε420 of ABTS 36,000 $M-1\ cm-1$) using a UV–visible spectrophotometer (Labtronics, India). One unit of laccase activity (IU/ml) was defined as the quantity of laccase needed to oxidize 1 µmol of substrate per minute. Three parallel replicate setups were conducted in the study, and the average of the results was calculated.

2.4 Optimization of culture conditions for laccase production

2.4.1 Experimental design and statistical analysis

The most influential physiological factors affecting laccase production were identified through preliminary studies conducted using the one-factor-at-a-time (OFAT) approach. These factors, including incubation temperature (20–40 °C), pH (4–7), incubation time (7–21 days), and the influence of corn steep liquor (CSL) at concentrations ranging from 1 to 3% per dry weight of the substrate, were selected for further optimization using response surface methodology (RSM) (Table 1). This optimization process was carried out by applying a central composite design (CCD) within the design of the experiment framework. A quadratic model was employed for the design, and 30 experimental runs were conducted (Table 2).

Table 1 Experimental range and levels of the independent factors studied

Factor	Name	Low		High	
		Coded	Uncoded	Coded	Uncoded
A	pH	−1	4.00	+1	7.00
B	Temperature (°C)	−1	20 °C	+1	40 °C
C	Incubation time (days)	−1	7 days	+1	21 days
D	CSL (%)	−1	1%	+1	3%

The laccase activity (IU/ml), denoted as the measured response Y, was modeled using a second-order polynomial equation:

$$y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_5 AB + \beta_6 AC + \beta_7 AD + \beta_8 BC + \beta_9 BD + \beta_{10} CD + \beta_{11} A2 + \beta_{12} B2 + \beta_{13} C2 + \beta_{14} D2$$

A, B, C, and D represent the coded values for pH, temperature, incubation time, and corn steep liquor concentration, respectively. β_0 is the intercept; β_1, β_2, β_3, and β_4 are the linear coefficients; β_5, β_6, β_7, β_8, β_9, and β_{10} are the interaction coefficients; and β_{11}, β_{12}, β_{13}, and β_{14} are the quadratic coefficients.

The design of the experiment was created and analyzed using Design-Expert 13.0 software (Stat-Ease, Minneapolis, USA). The trials, as per the design of experiments, were conducted, and the response (laccase activity) was recorded for analysis. Statistical analysis of the data and the creation of response surface plots were both carried out using the same software. The significance of the model terms was examined statistically using analysis of variance. To assess the significance of the model, Fisher's F test was employed, along with its corresponding probability p-value. The model's accuracy was statistically predicted using the coefficients of determination, R^2 and adjusted R^2. The response data were used to derive the optimal conditions for laccase production and to investigate the interactions among the individual factors.

Table 2 Design of experiment using CCD, indicating four independent variables for laccase production by *Pleurotus pulmonarius* under SSF

Runs	A: pH	B: temperature (°C)	C: incubation period (days)	D: CSL (%)	Laccase activity (IU/ml)	
					Observed	Predicted
1	4	40	7	3	138.46	91.38
2	5.5	30	14	2	550.48	561.68
3	5.5	50	14	2	49.47	41.82
4	5.4	30	14	2	585.14	561.68
5	7	40	21	3	375.14	359.27
6	5.5	30	14	2	578.21	561.68
7	7	40	21	1	247.49	274.08
8	4	20	7	3	194.37	192.46
9	4	40	7	1	146.14	160.07
10	7	20	21	1	298.47	318.85
11	5.5	30	14	2	580.00	561.68
12	5.5	30	7	2	324.79	380.19
13	7	20	21	3	495	500.87
14	5.5	30	28	2	598	594.13
15	4	20	21	3	458.45	454.6
16	5.5	10	14	2	178	187.67
17	5.5	30	14	2	539.1	561.68
18	5.5	30	14	2	587	561.68
19	7	20	0	3	1.78	−8.83
20	4	40	21	3	315.37	337.86
21	7	40	7	1	53.48	30.63
22	8.5	30	14	2	52	24.92
23	7	20	7	1	57.53	59.73
24	5.5	30	14	0	483.17	483.93
25	2.5	30	14	2	79	108.1
26	4	40	21	1	446.83	436.05
27	5.5	30	14	4	596	597.26
28	4	20	7	1	175.14	164.31
29	4	20	21	1	478.14	455.96
30	7	40	7	3	98.46	145.32

2.4.2 Validation of the optimum

The adequacy of the model was experimentally verified by conducting a fermentation process under the optimized factor levels in triplicate. Laccase activity was assessed using the method described in Sect. 2.3

2.5 Biomass delignification and analysis

The solid substrate fermentation of tender coconut fiber was carried out under the optimized conditions obtained via statistical optimization. Uninoculated medium served as a control. The lignin loss was calculated as a percentage of delignification. The functional group variations and morphological changes of the delignified and control fiber samples were then examined by FTIR analysis and SEM analysis, respectively.

2.5.1 Quantification of delignification

After the delignification process, the solid residue was washed and dried to estimate the percentage of delignification [22]. Klason lignin was quantified by hydrolyzing the biomass with 72% sulfuric acid, followed by dilution to 3% and subsequent heating. The resulting residue was then filtered, dried, and weighed to determine the acid-insoluble lignin content. The percentage of delignification was calculated using the formula:

$$\%Delignification = \frac{\text{Initial lignin} - \text{Final lignin}}{\text{Initial lignin}} \times 100 \times \text{solid recovery fraction}$$

2.5.2 Scanning electron microscopy (SEM) analysis

The alterations in the microstructure of both untreated and laccase-producing *Pleurotus pulmonarius*-delignified coir fiber samples were assessed through scanning electron microscopy (Zeiss EVO 18; Carl Zeiss AG, Oberkochen, Germany). After being thoroughly cleaned and dried, the samples were coated with a layer of gold palladium. The images were examined at different magnifications [23].

2.5.3 FTIR analysis

FTIR PerkinElmer Spectrum IR version 10.7.2 (PerkinElmer Inc., Waltham, MA, USA) was utilized to investigate the functional group variations during the delignification process in both untreated and biologically pretreated coir fibers. Samples were prepared for the FTIR analysis using the attenuated total reflection (ATR) technique with a diamond crystal accessory. The sample spectrum was obtained within a scanning range of 450–4000 cm^{-1}.

3 Results and discussion

3.1 Design of experiments

This study aimed to optimize laccase production using *Pleurotus pulmonarius* and tender coconut fiber as the substrate. We investigated the influence of various factors on laccase production and examined the interactions between these factors. The physiological parameters that were found to be significant for laccase production were pH, temperature, incubation time, and the addition of corn steep liquor (Table 2).

The quadratic equation describing the correlation between enzyme activity and individual factors is as follows:

$$\begin{aligned}\text{Laccase (IU/ml)} = {} & -2463.61 + 553.495 \times A + 71.2987 \times B + 57.5828 \times C \\ & + -31.2977 \times D + -0.414267 \times AB + -0.77438 \times AC \\ & + 30.5633 \times AD + -0.055968 \times BC \\ & + -2.42087 \times BD + -1.05368 \times CD \\ & + -55.0195 \times A^2 + -1.11735 \times B^2 \\ & + -1.12429 \times C^2 + -5.27261 \times D^2\end{aligned}$$

where A, B, C, and D are the coded values of pH, temperature, incubation time, and concentration of CSL, respectively. The equation enables predictions about the response for given levels of each factor.

3.2 Analysis of variance (ANOVA)

The analysis of variance for the quadratic response surface model is presented in Table 3. The Model F-value of 88.70 suggests the significance of the model. The probability of obtaining an F-value of this magnitude owing to random noise was only 0.01%.

p-values below 0.0500 ($p < 0.05$) indicate that the model terms were statistically significant.

Table 3 ANOVA for the response surface quadratic model for the production of laccase

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	1.254E+06	14	89,587.45	88.70	<0.0001	Significant
A-pH	10,202.90	1	10,202.90	10.10	0.0062	
B-temperature	31,373.23	1	31,373.23	31.06	<0.0001	
C-incubation time	3.571E+05	1	3.571E+05	353.55	<0.0001	
D-CSL	18,943.14	1	18,943.14	18.75	0.0006	
AB	602.44	1	602.44	0.5965	0.4519	
AC	1128.62	1	1128.62	1.12	0.3072	
AD	32,791.12	1	32,791.12	32.47	<0.0001	
BC	262.02	1	262.02	0.2594	0.6179	
BD	9143.60	1	9143.60	9.05	0.0088	
CD	928.69	1	928.69	0.9195	0.3528	
A^2	4.280E+05	1	4.280E+05	423.75	<0.0001	
B^2	3.487E+05	1	3.487E+05	345.22	<0.0001	
C^2	67,350.30	1	67,350.30	66.68	<0.0001	
D^2	776.43	1	776.43	0.7687	0.3944	
Residual	15,150.61	15	1010.04			
Lack of fit	13,126.39	10	1312.64	3.24	0.1031	Not significant
Pure error	2024.23	5	404.85			
Cor total	1.269E+06	29				

In this model, the terms A, B, C, D, AD, BD, A^2, B^2, and C^2 were found to be significant. Conversely, values exceeding 0.1000 indicate that the model terms lack significance.

The lack of fit F-value of 3.24 indicates that the lack of fit is not statistically significant compared to the pure error. This lack of significance implies that the quadratic model employed in this study is valid.

The model demonstrated its highest significance with a correlation coefficient of $R^2 = 0.9881$. The predicted R^2, which is 0.9451, aligns reasonably well with the adjusted R^2, which is 0.9769. In other words, the difference between these values is less than 0.2. This suggested a strong correlation between the predicted and evaluated values, indicating a high level of agreement.

3.3 Response surface plots and interactions between variables

The perturbation plot illustrates how the response changes when each factor is adjusted from the selected reference point while keeping all other factors constant at their reference values. Figure 1 shows that laccase activity was most strongly influenced by pH, followed by temperature and incubation time. An increase in pH and temperature up to their optimal coded values resulted in an enhancement in laccase yield. However, further increases beyond the optimal values (pH 5.5 and temperature 30 °C) led to a decline in laccase production, indicating diminishing returns at higher levels. Corn steep liquor concentration, while having a smaller influence on laccase activity, showed a slight inhibitory effect at higher concentrations. Overall, the interaction between pH and corn steep liquor also contributed to the variability in laccase production.

Exploring the interactions among two variables provides a more profound understanding of the overall process analysis. A factor can have complex interconnections with any or all of the other factors, potentially leading to a multitude of interactions. To delve into these interactions and determine the optimal concentration of individual factors for achieving the highest laccase activity, three-dimensional response surface plots were created.

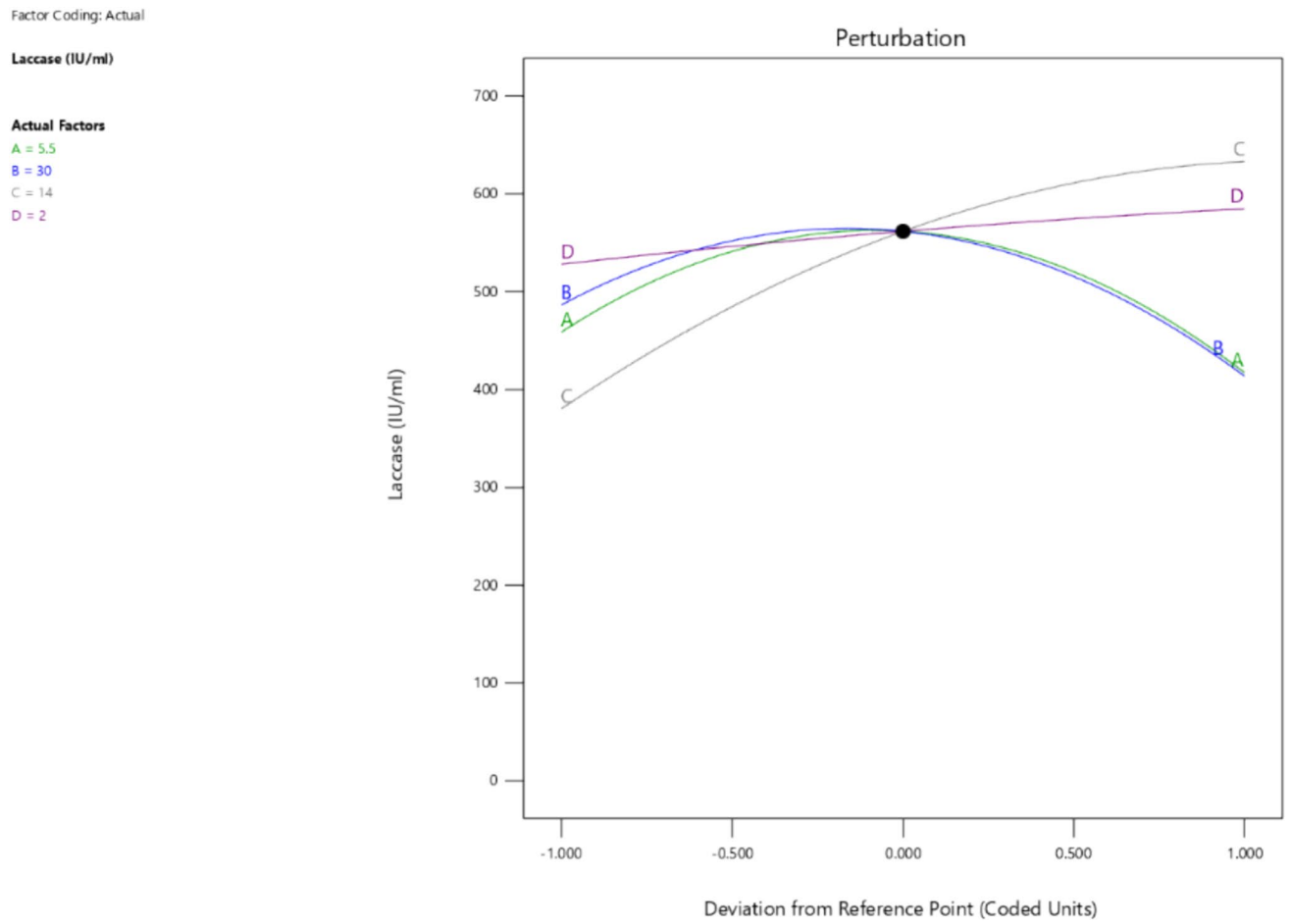

Fig. 1 Perturbation plot comparing the effect of each factor from the reference point

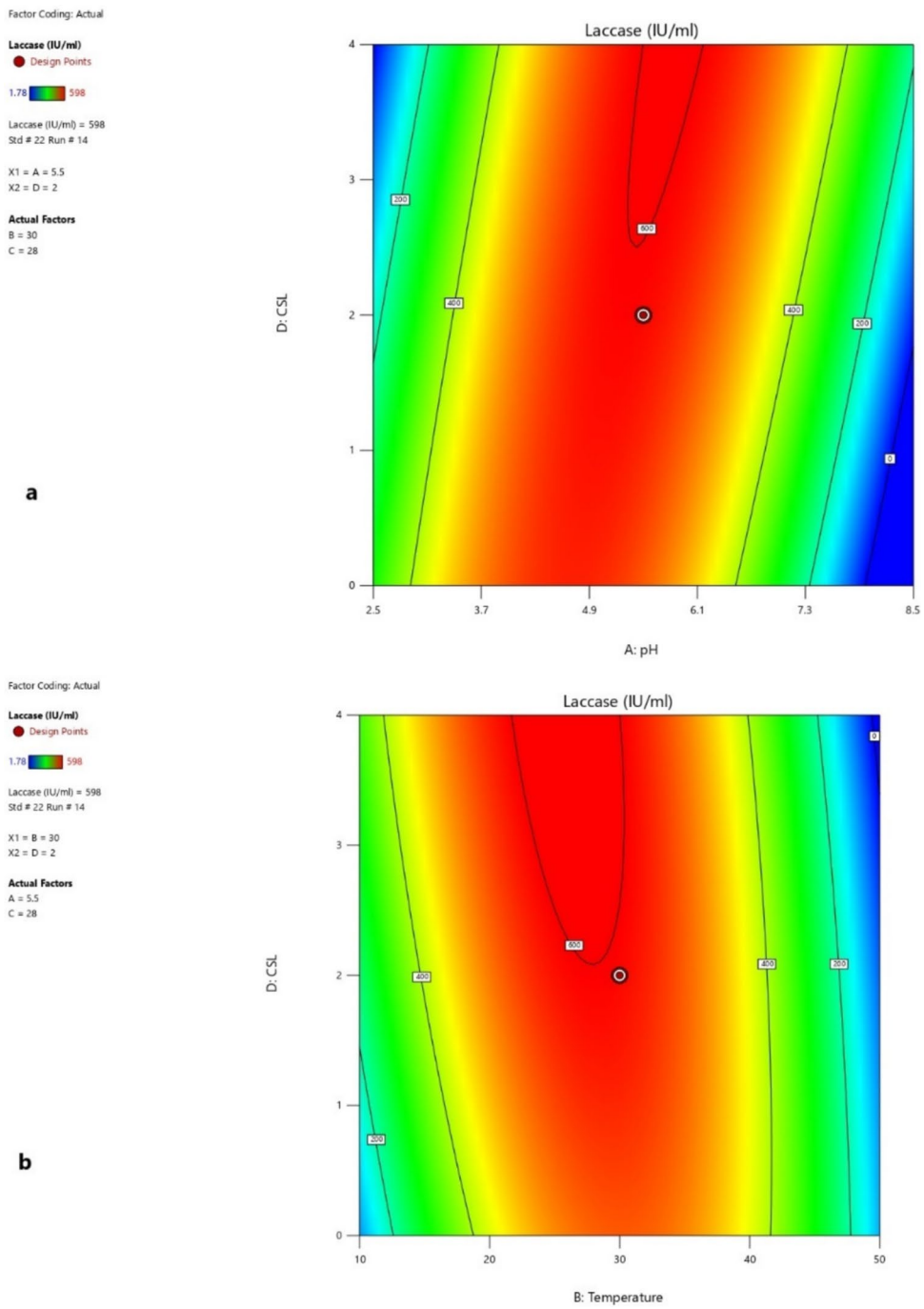

Fig. 2 Contour plots illustrating interactions among independent variables. **a** pH vs CSL and (**b**) temperature vs CSL influencing laccase production (IU/ml)

Analysis of variance revealed that the interactions between AD and BD were significant, while the interactions between other individual factors were deemed insignificant ($p > 0.05$). The peak laccase activity was observed when the pH was set at 5.5, and the corn steep liquor (CSL) concentration was 2–3%, as depicted in Fig. 2A. A temperature of 27–33 °C combined with a CSL concentration of 2–3% yielded higher laccase activity, as shown in Fig. 2B. Following the analysis of the interactions between the influencing factors at the level suggested by the software, the optimum laccase production was identified at a pH of 5.6, temperature of 27 °C, incubation time of 21 days, and corn steep liquor (CSL) concentration of 3%.

3.4 Validation of the optimum

The trials were conducted in triplicate with the optimized factors set at their recommended levels, including a pH of 5.6, a temperature of 27 °C, an incubation time of 21 days, and a corn steep liquor (CSL) concentration of 3%. The

observed laccase activity reached 663.76 ± 10.67 IU/ml, which closely aligns with the predicted value of 658.721 IU/ml. Remarkably, the enzyme activity demonstrated a substantial increase, with a remarkable 13.16-fold improvement, following statistical optimization using response surface methodology.

3.5 SEM analysis

The alterations in the structure of coir fibers following enzymatic delignification under optimal conditions determined by response surface methodology (RSM) were evaluated through SEM studies. The delignification process induced the formation of cracks and pores on the substrate, which was attributed to the removal of lignin. This was distinctly observed in the SEM images of both untreated and delignified biomass (Fig. 3).

3.6 FTIR analysis

FTIR spectra of untreated and delignified samples were examined to identify changes in functional groups and structural modifications. The peaks associated with lignin, cellulose, hemicellulose, and CH2 deformations provide crucial information for analyzing lignin degradation in biomass. Specifically, the peaks at 1249 and 1639 cm^{-1} are characteristic of lignin, while the bands between 1050 and 1150 cm^{-1} are indicative of cellulose [22] [24]. An analysis

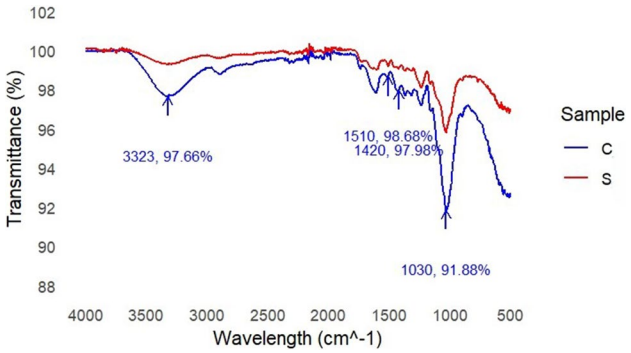

Fig. 4 FTIR spectra of untreated (**a**) and delignified (**b**) fibers

of the FTIR spectra of the untreated and delignified coconut fibers revealed significant increases in intensity at 3398 cm^{-1}, 2894 cm^{-1}, 1740 cm^{-1}, 1615 cm^{-1}, 1506 cm^{-1}, 1246 cm^{-1}, 1257 cm^{-1}, 1111 cm^{-1}, and 1030 cm^{-1} (Fig. 4). The observed increase in intensities for the treated coir, compared to the control, suggests significant degradation of the lignin component. The functional groups associated with these changes in intensity are listed in Table 4. The FTIR spectra analysis and band assignments were studied according to [22, 24–29].

The characteristic peaks of lignin in coconut fibers at 1246 and 1615 cm^{-1} correspond to aromatic C-O or C–C stretching in lignin and C = O stretching vibrations in the conjugated carbonyl of lignin, respectively [24]. The

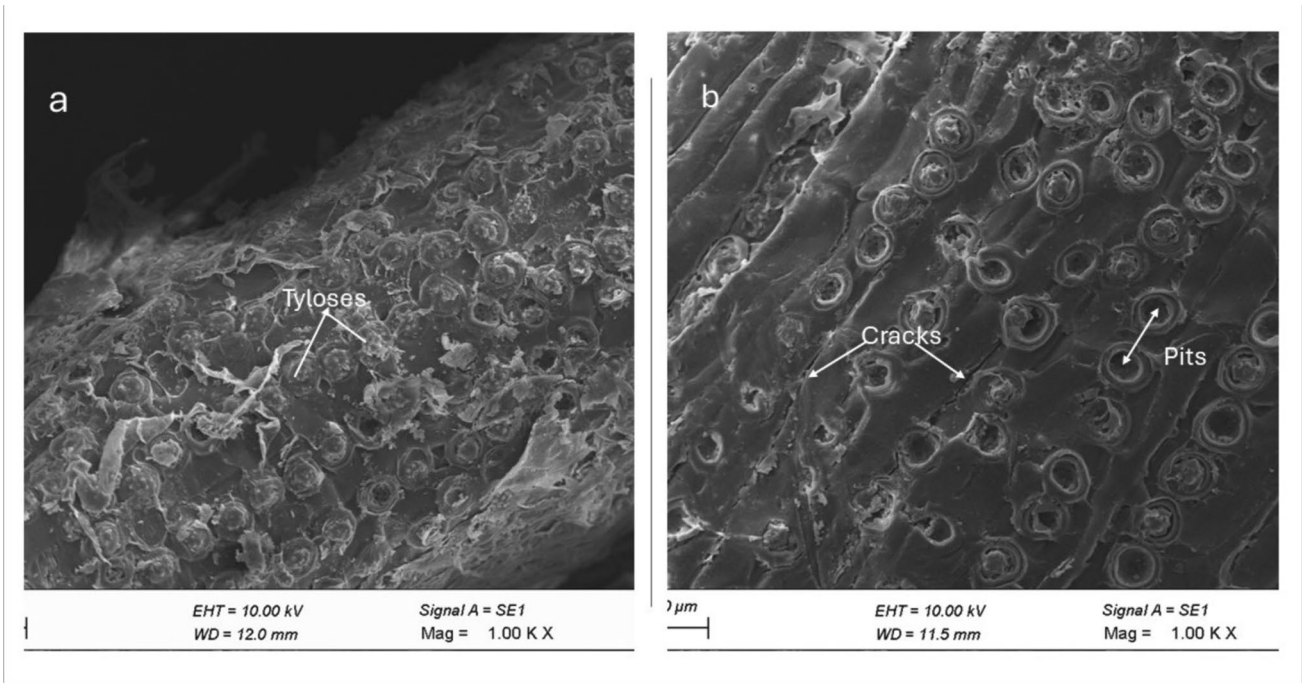

Fig. 3 SEM analysis of untreated coconut fiber (**a**) and laccase-producing *P. pulmonarius*-treated coconut fiber. The treated sample exhibits visible cracks and pores, attributed to the delignification process

Table 4 FTIR spectral analysis of lignocellulose biomass

Wavenumber (cm^{-1})	Functional group	Lignocellulose component corresponding to functional group
665	Aromatic C–H bending	Lignin
1030	C–O, C = C, and C–C–O stretching	Cellulose, hemicellulose
1111	C–C-O stretch	Cellulose, hemicellulose
1246	Syringyl ring breathing and aromatic C-O or C–C stretching in lignin	Lignin
1257	Syringyl ring breathing and aromatic C-O or C–C stretching in lignin	Lignin
1430	Aromatic skeletal vibrations combined with C-H in-plane deformation	Lignin
1506	C = C stretching vibrations of aromatic rings of lignin	Lignin
1615	C = O stretching vibration in conjugated carbonyl of lignin	Lignin
1740	C = O stretching of unconjugated ketone and carboxyl groups	Hemicellulose
2894	C-H stretch	Lignin
3398	O–H stretch	Lignin

Table 5 Lignin/carbohydrate band intensity ratio obtained from FTIR spectral analysis

Sample	I_{1510}/I_{1420}	I_{1510}/I_{1158}	I_{1510}/I_{1030}	I_{1510}/I_{895}
Control	0.651	0.423	0.156	0.387
Treated	0.406	0.294	0.161	0.220

untreated sample exhibits a strong aromatic ring structure, and the increased intensity in the treated sample indicates the effective removal of lignin. The peaks observed around 1500–1550 cm^{-1} correspond to the C = C stretching vibrations of the aromatic rings of lignin, which disappear in the spectrum of the treated sample, indicating successful delignification. Additionally, the bands in the vicinity of 1450–1500 cm^{-1} represent C-H in-plane deformations, which diminished in the treated sample.

The peak at 1740 cm^{-1} is associated with hemicellulose, and its reduction in the treated sample suggests the solubilization of hemicellulose. Furthermore, the appearance of another peak near 1740 cm^{-1} after treatment indicates the formation of functional groups such as aldehydes and ketones, likely due to the action of laccase on the aromatic structure of lignin [25]. The peak around 3300–3400 cm^{-1}, corresponding to OH bond stretching vibrations of alpha-cellulose, is lowered in the treated sample, indicating the exposure of the hydroxyl groups of cellulose. The increased intensity in this region also reflects the OH stretching of aromatic hydroxyl groups present in lignin [24].

To understand the variations in the chemical components of the fiber after delignification, relative intensity variations of key chemical compounds were analyzed (Table 5). Characteristic bands associated with cellulose and hemicellulose at 1420, 1158, 1030, and 895 cm^{-1} were observed, while the 1510 cm^{-1} band, characteristic of lignin, was selected as the reference. The ratio I1510/I1420, which indicates aromatic skeletal vibrations linked to lignin, shows a significant reduction in the treated sample (0.406) compared to the control (0.651). This pronounced decrease suggests substantial lignin degradation following treatment, serving as a critical marker of effective delignification. Additionally, the I1510/I895 ratio, indicative of changes in cellulose crystallinity, also declines from 0.387 in the control to 0.220 in the treated sample. This suggests that the treatment may have altered the cellulose structure, potentially improving its accessibility. The ratios I1510/I1158 and I1510/I1030, which reflect polysaccharide degradation, exhibit smaller variations, with the control showing values of 0.423 and 0.156, respectively, and the treated sample showing 0.294 and 0.161. These minimal changes indicate that polysaccharide integrity was largely preserved during the treatment process. The FTIR spectra clearly illustrate the delignification effect of the biological pretreatment facilitated by enhanced laccase production from *P. pulmonarius*, demonstrating its potential to effectively pretreat recalcitrant biomass.

4 Discussion

Laccases are widely recognized for their extensive industrial applications, including pulp and paper bleaching, wastewater treatment, the food industry, and the textile sector. In the food industry, laccases are utilized for processes such as brewing and color enhancement. In the pulp and paper industry, they play a crucial role in pulp bleaching and delignification. Additionally, in the textile industry, laccases have proven particularly effective in bleaching indigo-stained denim, where they have found significant application [1].

The optimization of culture conditions is an integral aspect of enhancing enzyme production. Studies utilizing

response surface methodology (RSM) and central composite design (CCD) have proven highly effective in optimizing laccase production [25]. Optimizing the physical parameters is crucial for increasing laccase production during solid substrate fermentation of agro-waste, especially when using a minimal medium composition.

Corn steep liquor has been shown to be a highly effective nutrient for laccase enzyme production, primarily because it acts as both a nitrogen source and a laccase inducer. CSL is a nutrient-rich substance, enriched with essential minerals and cofactors that support cell growth. [20] reported that *Trametes versicolor* increased laccase production when corn steep liquor (CSL) was utilized as an inducer. Our studies with *Pleurotus pulmonarius* yielded similar results. The addition of corn steep liquor (CSL) effectively boosted laccase production and contributed to reducing the time required to attain enhanced laccase production.

The optimal pH for laccase production by white rot fungi varies within the range of pH 4 to 8. For *Pleurotus sajor-caju* and *Pleurotus ostreatus*, previous studies indicated an optimal pH of 5 [30, 31] [32]. However, in the current study, the highest laccase activity was achieved at pH 5.5. The findings of this study indicated that the optimal temperature for laccase production was 27 °C. Studies involving *Pleurotus nebrodensis* and *Pleurotus pulmonarius* reported an optimum temperature of 30 °C [33] [34]. Within the temperature range of 25–30 °C, no significant change in laccase enzyme production was observed. However, beyond this temperature range, a noticeable decline in enzyme production was observed (Fig. 5). A study focusing on laccase production by *Pleurotus pulmonarius* on wheat bran yielded similar findings [34]. The insufficient fungal growth at temperatures exceeding 35 °C may have contributed to the limited laccase production.

The incubation time also plays a crucial role in laccase enzyme production, particularly when the fungus is cultivated on complex lignocellulose materials. Previous findings indicate that P. ostreatus exhibited its peak laccase production on the 25th day of cultivation, whereas other species (*P. florida*, *P. flabellatus*, *P. sajor-caju*, and *P. pulmonarius*) reached their optimal laccase activity on the 26th day [35]. Asha Singh et al. [36] reported that *Pleurotus ostreatus* cultured on paddy straw produced maximum laccase activity (45,968.9 ± 347.2 U/g) on the 28th day, while on sugarcane bagasse, it produced maximum laccase activity (10,146 ± 92.4 U/g) on the 21st day. In contrast, another study documented that *Pleurotus sajor-caju* achieved its highest laccase production (14 IU/ml) on the 8th day of cultivation when grown on a basal media supplemented with inducers [31]. Furthermore, a study on laccase production by *Phanerocheate chrysosporium*, utilizing coir waste as a substrate, reported the highest enzyme activity (5.1 IU/ml) on the 35th day [37]. It is worth noting that the choice of substrate significantly influences laccase production. Coir fiber, due to its elevated lignin content and rigid nature, may pose challenges for fungal assimilation. However, in the current study, through the optimization of parameters using RSM, it was possible to achieve the maximum laccase production on the 21st day.

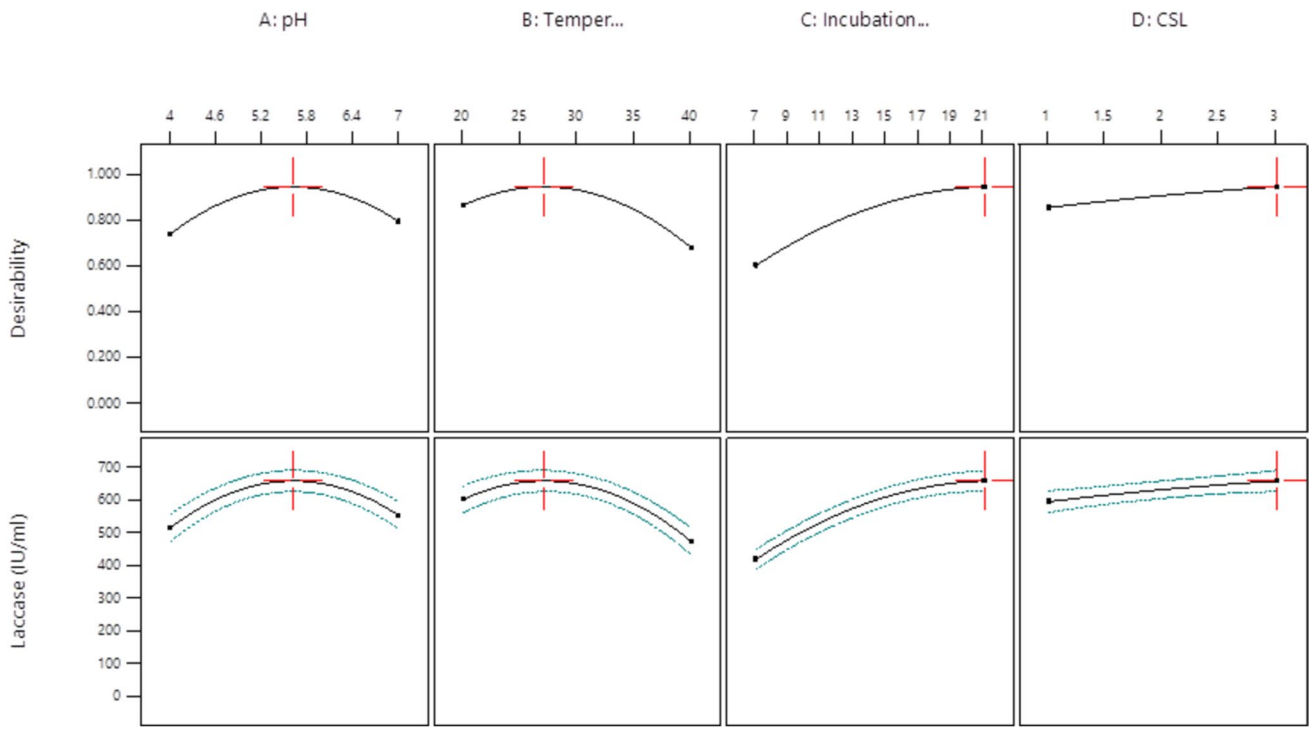

Fig. 5 Graph illustrating the desirability and behavior of laccase activity in the proximity of optimal levels of individual factors

Table 6 Comparative analysis of laccase production in white rot fungi in previous studies

Sl no	Organism	Substrate	Enzyme activity	Reference
1	*Pleurotus* eryngii	Peach waste	43,761.33 ± 3845 U/L	[39]
2	*Pleurotus sajor-caju*	Wheat straw	1450 U/g	[18]
3	*Pleurotus* florida	Paddy straw	40,989 ± 872 U/g	[36]
4	*Pleurotus pulmonarius*	Sugarcane baggase	8743.2 ± 130 U/g	[36]
5	Schizophyllum commune	Banana stalk	345 IU/ml	[40]
6	*Coriolus versicolor IBL-04*	Sugar cane baggase	675 ± 9.1 IU/ml	[41]
7	Ganoderma lucidem	Rice straw	338 IU/ml	[42]
8	*Marasmiellus palmivorus LA1*	Pineapple leaf	667.4 + 13 IU/ml	[43]
9	*Pleurotus sajor-caju*	Luffa cylindrica	80 IU/ml	[44]
10	*Pleurotus nebrodensis*	Wheat straw	171.63 IU/ml	[33]
11	*Ganoderma lucidum*	Pineapple leaves	472.3 IU/ml	[45]
12	*Trametes versicolor*	Wheat bran	1200 U/ml	[46]
13	*Trametes versicolor*	Coconut fiber	95 IU/ml	[46]
14	Co culture of *Phanerocheate chrysosporium* and Rhizopus stolonifer	Coir waste	5.1 IU/ml	[37]
15	*Pleurotus pulmonarius*	Tender coconut fiber	663.76 ± 10.67 IU/ml	Present study

The optimized process parameters were validated and applied to the delignification of tender coconut fiber. It was observed that enhanced laccase production led to increased delignification of the coir fiber, as evidenced by SEM and FTIR analyses. Similar studies, such as those by Hafid et al. [29]. and Karp et al. [38], have demonstrated that enhanced laccase production can significantly improve the delignification of OPEFB and sugarcane bagasse biomass. The process of delignification is complex, involving the coordinated action of both laccases and peroxidases. While laccases play a direct role in lignin degradation by oxidizing phenolic structures, their production levels do not always correlate directly with the rate of delignification [38]. Lignin breakdown can continue even in the later stages of fermentation, despite peak enzymatic activity occurring earlier. In the case of *Pleurotus pulmonarius*, which demonstrated high laccase production on tender coconut fiber during 21 days of solid-state fermentation, a significant delignification efficiency was observed, highlighting the fungus' potential for effective lignin degradation. The statistical optimization significantly increased laccase production (Table 6) by *P. pulmonarius* through solid-state fermentation of tender coconut fiber, facilitating its effective use in substrate delignification. Validation of these optimal process parameters offers valuable insights for large-scale bioprocess applications, potentially reducing the cost and time.

5 Conclusion

This study focused on optimizing laccase production via solid-state fermentation using *Pleurotus pulmonarius* on tender coconut fiber. This was achieved through the refinement of physical process parameters employing a central composite design. The peak laccase activity, observed at a pH of 5.5 with a 3% CSL inducer, a temperature of 27 °C, and an incubation period of 21 days, provided insights into the individual contributions of factors and their interactions. By utilizing a minimal medium and reducing additional components, this study emphasized the importance of optimizing physical parameters to enhance laccase production, thereby significantly reducing both time and costs. Enhancing laccase production can significantly improve the delignification of biomass during biological pretreatment, as demonstrated by SEM and FTIR analyses. This study, marking the initial exploration of using agro-waste tender coconut fiber as substrate, yielded a remarkable 13.16-fold increase in laccase production. Such progress holds promise for potential applications in treating the fiber, rendering it adaptable for various uses.

Acknowledgements The authors express their acknowledgments to the Centre for Bioscience and Nanoscience Research for providing all the research facilities.

Author contribution GS: investigation, methodology, data curation, visualization, formal analysis, validation, software, and writing—original draft. RR: conceptualization, supervision, methodology, validation, data curation, and writing—review and editing. JJ: writing—review and editing.

Data Availability The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Unuofin JO, Falade AO, Aladekoyi OJ (2021) Chapter 21 - Applications of microbial laccases in bioremediation of environmental pollutants: potential issues, challenges, and prospects. https://doi.org/10.1016/B978-0-12-820524-2.00021-3
2. Singh G, Ahuja N, Batish M, Capalash N, Sharma P et al (2008) Biobleaching of wheat straw-rich soda pulp with alkalophilic laccase from γ-proteobacterium JB: optimization of process parameters using response surface methodology. Biores Technol 99(16):7472–7479. https://doi.org/10.1016/j.biortech.2008.02.023
3. Sharma D, Chaudhary R, Kaur J, Arya SK et al (2020) Greener approach for pulp and paper industry by xylanase and laccase. Biocatal Agric Biotechnol 25:101604. https://doi.org/10.1016/j.bcab.2020.101604
4. Ibarra D, Romero J, Mart´ınez MJ, Mart´ınez AT, Camarero S (2006) Exploring the enzymatic parameters for optimal delignification of eucalypt pulp by laccase-mediator. Enzyme Microb Technol 39(6):1319–1327. https://doi.org/10.1016/j.enzmictec.2006.03.019
5. Sadhasivam S, Savitha S, Swaminathan K (2010) Deployment of *Trichoderma harzianum WL1* laccase in pulp bleaching and paper industry effluent treatment. J Clean Prod 18(8):799–806. https://doi.org/10.1016/j.jclepro.2009.11.014
6. Ghosh B, Saha R, Bhattacharya D, Mukhopadhyay M (2019) Laccase and its source of sustainability in an enzymatic biofuel cell. Bioresour Technol Rep 6:268–278. https://doi.org/10.1016/j.biteb.2019.03.013
7. Vela´squez-Quintero C, Merino-Restrepo A, Hormaza-Anaguano A (2022) Production, extraction, and quantification of laccase obtained from an optimized solid-state fermentation of corncob with white-rot fungi. J Clean Prod 370:133598. https://doi.org/10.1016/j.jclepro.2022.133598
8. Suryadi H, Judono JJ, Putri MR, Eclessia AD, Ulhaq JM, Agustina DN, Sumiati T (2022) Biodelignification of lignocellulose using ligninolytic enzymes from white-rot fungi. Heliyon 8(2):e08865. https://doi.org/10.1016/j.heliyon.2022
9. James A, Yadav D (2021) Valorization of coconut waste for facile treatment of contaminated water: a comprehensive review (2010–2021). Environ Technol Innov 24:102075. https://doi.org/10.1016/j.eti.2021.102075
10. Br´ıgida AIS, Calado VMA, Gonc¸alves LRB, Coelho MAZ (2010) Effect of chemical treatments on properties of green coconut fiber. Carbohydr Polym 79(4):832–838. https://doi.org/10.1016/j.carbpol.2009.10.005
11. Mahmud MA, Abir N, Anannya FR, Khan AN, Rahman AM, Jamine N (2023) Coir fiber as thermal insulator and its performance as reinforcing material in biocomposite production. Heliyon 9(5):e15597. https://doi.org/10.1016/j.heliyon.2023.e15597
12. Levin L, Herrmann C, Papinutti VL (2008) Optimization of lignocellulolytic enzyme production by the white-rot fungus *Trametes trogii* in solid-state fermentation using response surface methodology. Biochem Eng J 39(1):207–214. https://doi.org/10.1016/j.bej.2007.09.004
13. Xu L, Sun K, Wang F, Zhao L, Hu J, Ma H, Ding Z (2020) Laccase production by *Trametes versicolor* in solid-state fermentation using tea residues as substrate and its application in dye decolorization. J Environ Manag 270:110904. https://doi.org/10.1016/j.jenvman.2020.110904
14. Sondhi S, Saini K (2019) Response surface based optimization of laccase production from *Bacillus sp.* MSK-01 using fruit juice waste as an effective substrate. Heliyon 5(5):e01718. https://doi.org/10.1016/j.heliyon.2019.e01718
15. Chenthamara D, Sivaramakrishnan M, Ramakrishnan SG, Esakkimuthu S, Kothandan R, Subramaniam S (2022) Improved laccase production from *Pleurotus floridanus* using deoiled microalgal biomass: statistical and hybrid swarm-based neural networks modeling approach. 3 Biotech 12(12):346. https://doi.org/10.1007/s13205-022-03404-y
16. Arumugam DP, Uthandi S (2024) Optimization and characterization of laccase (LccH) produced by *Hexagonia hirta* MSF2 in solid-state fermentation using coir pith wastes (CPW). J Environ Manag 356:120625. https://doi.org/10.1016/j.jenvman.2024.120625
17. da Silva TBV, Uber TM, Pasquarelli DL, Bracht A, Peralta RM (2022) Production of fungal laccase on pineapple waste and application in detoxification of malachite green. J Environ Sci Health Part B 57(2):90–101. https://doi.org/10.1080/03601234.2022.2025739
18. Sharma S, Murty DS (2021) Enhancement of laccase production by optimizing the cultural conditions for *Pleurotus sajor-caju* in solid-state fermentation. J Pure Appl Microbiol 15(2). https://doi.org/10.22207/JPAM.15.2.54
19. Wang J, Li L, Hongmin Xu, Zhang Y, Liu Y, Zhang F, Shen G (2022) Construction of a fungal consortium for effective degradation of rice straw lignin and potential application in bio-pulping. Biores Technol 344:126168. https://doi.org/10.1016/j.biortech.2021.126168
20. Wang F, Hu JH, Guo C, Liu CZ (2014) Enhanced laccase production by *Trametes versicolor* using corn steep liquor as both nitrogen source and inducer. Biores Technol 166:602–605. https://doi.org/10.1016/j.biortech.2014.05.068
21. Li A, Zhu Y, Xu L, Zhu W, Tian X et al (2008) Comparative study on the determination of assay for laccase of *Trametes sp.* Afr J Biochem Res 2(8):181–183
22. Banerjee R, Chintagunta AD, Ray S (2019) Laccase mediated delignification of pineapple leaf waste: an ecofriendly sustainable attempt towards valorization. BMC Chem 13:1–11. https://doi.org/10.1186/s13065-019-0576-9
23. Ravindranath AD (2010) Bio-softening of coir yarn for ecofriendly wet processing. Cord 26(2):10–10
24. Abraham E, Deepa B, Pothen LA, Cintil J, Thomas S, John MJ, Anandjiwala R, Narine SS (2013) Environmental friendly method for the extraction of coir fibre and isolation of nanofibre. Carbohyd Polym 92(2):1477–1483. https://doi.org/10.1016/j.carbpol.2012.10.056
25. Bagewadi ZK, Mulla SI, Ninnekar HZ (2017) Optimization of laccase production and its application in delignification of biomass. Int J Recycling Org Waste Agric 6:351–365. https://doi.org/10.1007/s40093-017-0184-4
26. Gabhane J, Kumar S, Sarma AK (2020) Effect of glycerol thermal and hydrothermal pretreatments on lignin degradation and enzymatic hydrolysis in paddy straw. Renew Energy 154:1304–1313. https://doi.org/10.1016/j.renene.2020.03.035
27. Haque MA, Ashik MA, Aktar S, Akter MS, Halilu A, Haque MA, Islam MR, Abdullah-Al-Mamun M, Nahar MNEN, Das SR et al (2021) Rapid deconstruction of cotton, coir, areca, and banana fibers recalcitrant structure using a bacterial consortium with enhanced saccharification. Waste Biomass Valorization 12:4001–4018. https://doi.org/10.1007/s12649-020-01294-w
28. Singh N, Devi A, Jaryal R, Rani K (2018) An ecofriendly and efficient strategy for costeffective production of lignocellulotic enzymes. Waste Biomass Valorization 9:891–898. https://doi.org/10.1007/s12649-017-9861-9

29. Hafid HS, Baharuddin AS, Mokhtar MN, Omar FN, Mohammed MAP, Wakisaka M (2021) Enhanced laccase production for oil palm biomass delignification using biological pretreatment and its estimation at biorefinary scale. Biomass Bioenergy 144:105904. https://doi.org/10.1016/j.biombioe.2020.105904
30. Elsayed MA, Hassan MM, Elshafei AM, Haroun BM, Othman AM (2012) Optimization of cultural and nutritional parameters for the production of laccase by *Pleurotus ostreatus* ARC280. Br Biotechnol J 2(3):115
31. Murugesan K, Arulmani M, Nam IH, Kim YM, Chang YS, Kalaichelvan PT (2006) Purification and characterization of laccase produced by a white rot fungus *Pleurotus sajor-caju* under submerged culture condition and its potential in decolorization of azo dyes. Appl Microbiol Biotechnol 72:939–946. https://doi.org/10.1007/s00253-006-0403-9
32. de Souza CGM, Peralta RM (2003) Purification and characterization of the main laccase produced by the white-rot fungus *Pleurotus pulmonarius* on wheat bran solid state medium. J Basic Microbiol 43(4):278–286. https://doi.org/10.1002/jobm.200390031
33. Aslam S, Asgher M, Hussain F, Khan NA (2016) Exploration of optimum operating conditions for enhanced laccase enzyme production by *Pleurotus nebrodensis* WC 850 through response surface methodology. JAPS: J Anim Plant Sci 26(3):794–804
34. De Souza CGM, Zilly A, Peralta RM (2002) Production of laccase as the sole phenoloxidase by a Brazilian strain of *Pleurotus pulmonarius* in solid state fermentation. J Basic Microbiol 42(2):83–90. https://doi.org/10.1002/1521-4028(200205)42:2(83::AID-JOBM83)3.0.CO;2-Z
35. Das N, Naskar S, Chowdhury P, Pasman B, Adhikari D (2011) Experimental evidence for presence of a growth regulating extracellular laccase in some *Pleurotus* species. Res J Microbiol 6:496–502. https://doi.org/10.3923/jm.2011.496.502
36. Rajavat A, Shrivastava N, Choudhary P, Pandiyan K, Chakdar H, Vellaichamy M, Karthikeyan N, Agnihotri A (2023) Valorization of agro-residues for production of ligninolytic enzymes from *Pleurotus spp.* and their deployment in dye decolorisation. Biomass Conversion and Biorefinery 1–17. https://doi.org/10.1007/s13399-023–05004-z
37. Kanmani P, Karuppasamy P (2009) Studies on lignocellulose biodegradation of coir waste in solid state fermentation using *Phanerocheate chrysosporium* and Rhizopus stolonifer. Afr J Biotechnol 8(24):6880–6887
38. Karp SG, Faraco V, Amore A, Letti LAJ, Soccol VT, Soccol CR (2015) Statistical optimization of laccase production and delignification of sugarcane bagasse by *Pleurotus* ostreatus in solid-state fermentation. BioMed Res Int 2015:181204
39. Akpinar M, Urek RO (2017) Induction of fungal laccase production under solid state bioprocessing of new agroindustrial waste and its application on dye decolorization. 3 Biotech 7:1–10. https://doi.org/10.1007/s13205-017-0742-5
40. Irshad M, Asgher M (2011) Production and optimization of ligninolytic enzymes by white rot fungus *Schizophyllum commune IBL-06* in solid state medium banana stalks. Afr J Biotech 10(79):18234–18242
41. Moqueem E, Asgher M, Munir N, Tahir IM, Riaz M (2017) Development of liquid state fermentation process for the production of ligninolytic enzymes by *Coriolus versicolor ibl-04* using lignin containing substrate. Pak J Med Biol Sci 1(2)
42. Asgher M, Sharif Y, Bhatti HN (2010) Enhanced production of ligninolytic enzymes by *Ganoderma lucidum* IBL-06 using lignocellulosic agricultural wastes. Int J Chem React Eng 8(1). https://doi.org/10.2202/1542-6580.2203
43. Chenthamarakshan A, Parambayil N, Miziriya N, Soumya PS, Lakshmi MK, Ramgopal A, Dileep PN (2017) Optimization of laccase production from *Marasmiellus palmivorus LA1* by Taguchi method of design of experiments. BMC Biotechnol 17:1–10. https://doi.org/10.1186/s12896-017-0333-x
44. Patrick F, Mtui G, Mshandete AM, Kivaisi A (2011) Optimization of laccase and manganese peroxidase production in submerged culture of *Pleurotus sajor-caju*. Afr J Biotech 10(50):10166–10177
45. Muhammed M, Nambisan P (2012) Optimization of lignin peroxidase, manganese peroxidase, and lac production from *Ganoderma lucidum* under solid state fermentation of pineapple leaf. BioResources 8. https://doi.org/10.15376/biores.8.1.250-271
46. Atilano-Camino MM, Alvarez Valencia LH, Garcıa-Gonza´lez A, Garcıa-Reyes RB (2020) Improving laccase production from *Trametes versicolor* using lignocellulosic residues as cosubstrates and evaluation of enzymes for blue wastewater biodegradation. J Environ Manag 275:111231. https://doi.org/10.1016/j.jenvman.2020.111231