Journal of Experimental Biology and Agricultural Sciences, August - 2022; Volume – 10(4) page 818 – 830

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Ameliorating Direct Blue Dye Degradation Using *Trametes versicolor* Derived Laccase Enzyme Optimized through Box–Behnken Design (BBD) via Submerged Fermentation

Umamaheswari Ramasamy[1], Ramkumar Lakshmanan[1*], Mythili Ravichandran[1,3], Prabu Periasamy[2], Shanmugam Sengodan[1]

[1]Department of Microbiology, K.S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode, Namakkal, India
[2]Department of Biotechnology, Periyar University PG Extension Center, Dharmapuri, India
[3]Department of Microbiology, Vivekanandha Arts and Science College for Women, Sankari, Namakkal, India

Received – April 25, 2022; Revision – July 06, 2022; Accepted – July 13, 2022
Available Online – August 30, 2022

DOI: http://dx.doi.org/10.18006/2022.10(4).818.830

KEYWORDS

Laccase

Trametes versicolor

Fruit peels

Dye decolorization

Box-Behnken design

ABSTRACT

The major intend of this study was to elucidate the laccase production by *Trametes versicolor* under submerged fermentation using fruit waste peel as substrate. The textile dye was decolorized by the procured crude enzymatic extract using the response surface methodology. The submerged media with organic fruit peel waste extract (jackfruit, pineapple & kaffir) supplemented with gypsum, calcium carbonate, and nutrient broth were considered superior for laccase production. The produced laccase enzyme was used in dye decolorization at the optimum conditions using the Box-Behnken design. Subsequently, the experiment was designed with four variables (dye concentration, pH, temperature & time) with three factors to achieve the maximum direct blue dye decolorization. The highest laccase activity level was obtained from jackfruit peel extract with 3.86U/ml on 15th day at 25^{o}C with pH 5.0 when compared to the other two extracts. The maximum laccase activity with guaiacol was obtained at optimum pH 4 and 40^{o}C. The predicted value was experimentally validated by attaining 81.25% of dye color removal. From the result, the optimum conditions for direct blue color removal were: dye concentration 40ppm, pH 4.0, temperature 40^{o}C at 24 hours. From the results of this study, it was concluded that the jack fruit peel was a more suitable substrate for laccase production. The dye decolorization results were recommended that Box-Behnken design for parameters optimization. The *T. versicolor* laccase was more proficient for textile dye decolorization. The opportunity was created by using the laccase enzyme for the biological treatment of textile dyeing effluent before discharging into the environment.

* Corresponding author
E-mail: lakshmananram75@gmail.com (L. Ramkumar)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

A Phenomenon of economic expansion and urbanization progression led to the inundation of toxic materials discharged from the industry. Typically, the disposal of the complex pollutant deposition was hazardous to environmental health (Crawford et al. 2022) and also a challenging task (Shaban et al. 2017). Various options were available to control these pollutants. Unfortunately, the physical-chemical methods led to immense expenses towards investments such as the ozonation plant which was a batchwise setup (de Boer et al. 2022), the operation cost of running an anaerobic reactor for a long duration (Muthukumar et al. 2004), poor stability of electrodes (Bayramoglu et al. 2007). Even though these methods had a good beginning but faced unfavorable ends like deposits of sludge and toxic by-products many countries, including India (Szostek et al. 2022; Ali et al. 2013), impractical electrodes maintenance (Macedo et al. 2021), low efficiency besides all types of dyes (Jebapriya and Gnanadoss 2013), needed more improvement in efficiency by filtration process (Cescon and Jiang 2020), election and cleaning of filtration, and sometimes the effluent flow rate might affect the reactor (Muthukumar et al. 2004). Occasionally, the intricate matrix in the textile effluent hold noxious properties (Jalal et al. 2021) which would affect the microorganisms and lead to an elongated process (Husain 2010) and nevertheless if disintegrated the chemicals in the environment. But some reports confirmed that the metabolites obtained during degradation become more vicious than parent dye compounds (Manavalan et al. 2013). However, the biological option could make a viable one.

Over the past few decades, enzymatic treatment for textile dye decolorization was widely practiced. The major advantages of this method were it could react in a wide range of pH, different temperatures, substrate specificity, no sludge accumulation, and appropriate for various effluents (Sathishkumar et al. 2010; Songserm et al. 2012; Manavalan et al. 2013; Cordova-Villegas et al. 2019). Singh et al. (2022) also proved that biological treatment had a potential way than standard methods. Despite its dye degradation potential, the earlier reports described the predilection of laccase superiority over the physical-chemical methods. In addition to recycling, stability, and lifetime of laccase by immobilization (Shokri et al. 2021), no radical mechanisms are needed to cleave the bond of the dye structure (Upadhyay et al. 2016), and eradication of dye lethal properties (Shekher et al. 2011). Furthermore, the laccase could endure in both phenolic and non-phenolic substances, and due to high tolerance towards noxious waste led to simplifying the man-made dye (Madhavi and Lele 2009).

Laccase (EC 1.10.3.2: p-diphenol: dioxygenoxidoreductase), a blue multicopper protein belonged to an oxidase group and existed in four categories of living organisms like bacteria, and insects, fungi, and higher plants (Shekher et al. 2011; Agustin et al. 2021). Perhaps, due to attentiveness toward polyphenol group oxidation and substrate specificity, laccase was used in bioremediation, dye color removal, pulp and paper bleaching, and wastewater treatment (Okwara et al. 2021). Freshly, laccase had gained application in food processing as a wine stabilizer, juice stabilizer, and as biocatalyst (Backes et al. 2021). However, the enzyme played a crucial role in bioremediation as a multifarious mixture of chemical endocrine disruptors (Becker et al. 2017; Villalba-Rodriguez et al. 2022). *T. versicolor* was the most excellent laccase producer when compared with other white-rot fungi (Sun et al. 2021).

A massive enhancement of industrial activities led to the accumulation of waste in the surroundings. The accumulated organic wastage would become a major perplexity associated with environmental pollution. The agro-industrial waste mainly consisted of valuable components (Freitas et al. 2021) like carbohydrates, protein, a complex polysaccharide, and polyphenol components (Yusuf 2017). Owing to the poor waste management system, the deployment of this valuable component had converted into valued integrated products now established the world over (Levin et al. 2012). Thus the economy changed its way from the "take-make-dispose" to the "take-make-use" model (Russo et al. 2021). The fruit wastes from the pabulum industries were utilized as substrates for engendering the enzymes, which made it less investment for the industrialist (Yusuf 2017). Using *T.versicolor*, the fruit peel like jackfruit, pineapple, and kaffir was taken as substrates for laccase production by submerged fermentation. The main objective of this study is to obtain the laccase enzyme from the waste fruit peels and incorporate it into the textile dye to achieve higher potential decolorization.

2 Materials and Methods

2.1 Media formulation and submerged fermentation

T.versicolor (MTCC No: 138) was collected from Chandigarh, India, and was periodically subcultured and maintained at 4^{o}C on Potato Dextrose Agar (PDA) slant for further processing. For laccase screening, the five days old fungus was inoculated in the PDA plate with 2mM of guaiacol. These plates were incubated at 25^{o}C for 5 days with free guaiacol as control.

The lignocellulose organic substrates of fruit peels *viz.*, jackfruit, pineapple, and kaffir were soaked in 83.17 mM KOH solution for one hour and dehydrated at 60^{o}C in a tray drier (Rosales et al. 2002). The 10g of dried substrates were boiled separately in 500ml distilled water till they attain 100ml. Then, the substrates had undergone homogenization and filtration. A submerged fermentation culture was created in a 250ml Erlenmeyer flask each containing 100ml homogenized extract, 1.3g of nutrient broth, and 1g of both gypsum and calcium carbonate. The pH was adjusted to

5.0. Then, the flask was autoclaved at 121°C for 15 minutes, inoculated with five days old fungal mycelium, and incubated at 25°C for 30 days (Xin and Geng 2011).

2.2 Downstream process and enzyme assay

For this, 20 ml of sterile distilled water was added into the fermentation flask, mixed thoroughly for 20 minutes in the shaker, and filtered through a muslin cloth. The extraction was centrifuged at 5,000 rpm for half an hour to remove the slurry (Patel and Gupte 2016). The obtained supernatant was known as crude enzyme and was stored in the sterile container at -20ºC for further work. The laccase enzyme activity was determined using guaiacol as substrate under a double beam UV spectrophotometer at 450 nm (Desai et al. 2011).

2.3 Effect of Laccase activity at different pH and temperature

The effect of pH on laccase activity was analyzed using different pH ranges of 3 to 11 with sodium acetate buffer at 30°C for 15 minutes. The effect of temperature on laccase activity was measured by varying the incubation temperature between 20°C - 60°C at the optimum pH for 15 minutes and the residual activity was dignified using guaiacol as substrate. All the experiment was performed in triplicate.

2.4 Optimization of dye decolorization

In the present study, the decolorization was carried out in 10ml of the test tube with enzyme volume under static conditions. The dye decolorization effectiveness was achieved by the partially purified enzyme. The 5ml of the reaction mixture in the test tube consisted of an equal volume of dye solution and crude laccase enzyme and 3ml of 50 mM of sodium acetate buffer and incubated at 30°C. The percentage of color removal was detected under a UV spectrophotometer at 587 nm (Sathishkumar et al. 2010).

The direct blue azo dye decolorization was optimized by RSM based Box-Behnken method employed by Statease Design Expert software (version 11) to minimize the number of experiments carried out to analyze the data.. Dye concentration (30, 40, 50 ppm), pH (2, 4, 6), temperature (30°C, 40°C, 50°C), and time (12, 24, 36 hours) were expressed as X_1, X_2, X_3, X_4 respectively to evaluate the decolorization as a response. The four major independent variables could be approached by the quadratic model equation as given.

$$Y= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4$$

Where Y response, b_0 constant, X_1, X_2, X_3, and X_4 were dye concentration, pH, temperature, and time respectively, b_1, b_2, b_3, and b_4 were linear coefficients, b_{11}, b_{22}, b_{33}, and b_{44} were quadratic coefficients, b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, and b_{34} were cross-product coefficients. To barricade the unexpected variability in the visually examined replications the experimental runs were accomplished randomly.

The dye decolorization was recorded for the entire factors using Box-Behnken Method. Following that, it was statistically analyzed by ANOVA (Two-way table) to analyze the interaction between the independent variables. In addition, it was also assessed the fitness of the model based on their interactions.

3 Results

3.1 Medium optimization on laccase activity

T.versicolor was grown in the guaiacol plate for laccase screening test. The growth of the fungi had been deferral due to the effect of the guaiacolsubstrate destruction. After a week of incubation, the culture had developed brown color around the colony. It was indicated that the *T.versicolor* was a laccase producer. Furthermore, the *T.versicolor* was cultivated on three different lignocelluloses organic substrates along with nutrient broth, gypsum, and calcium carbonate to enhance its growth.

The laccase production by *T.versicolor* on lignocellulose organic substrates under submerged fermentation was observed and the result was recorded (Figure 1). The enzyme activity was quantified every alternative day from the third day of inoculum till the enzyme level truncates. The laccase enzyme activity was gradually incremented from the 5th day and progressively increased up to the 15th day and then the activity declined from the 16th to 22nd day. It was found that the laccase enzyme activity on jackfruit peels was 3.86 U/ml, which was higher than pineapple peel at 2.96 U/ml, and kaffir peel at 2.66 U/mlon 15th day. For further incubation after15th day, the level of the enzyme was diminished.

3.2 Effect of Laccase activity at different pH and temperature

The pH was an essential and significant factor, that influenced the extracellular laccase production during fermentation. The optimum pH was determined by changing the pH range from 3-11 in sodium acetate buffer solution. In this study, the laccase activity was increased at pH 4.0. By increasing in pH range above pH4.0, a sharp decline was reported in the enzyme activity (Figure 2).

Another important factor was the temperature which thermal stability was determined with different temperatures ranging from 20°C - 60°C with 10°C intervals at optimum pH. The enzyme activity versus temperature showed a great increased activity at 40°C when compared to 20°C and 30°C. After a prolonged increase in the temperature above 40°C, the laccase activity was diminished (Figure 3).

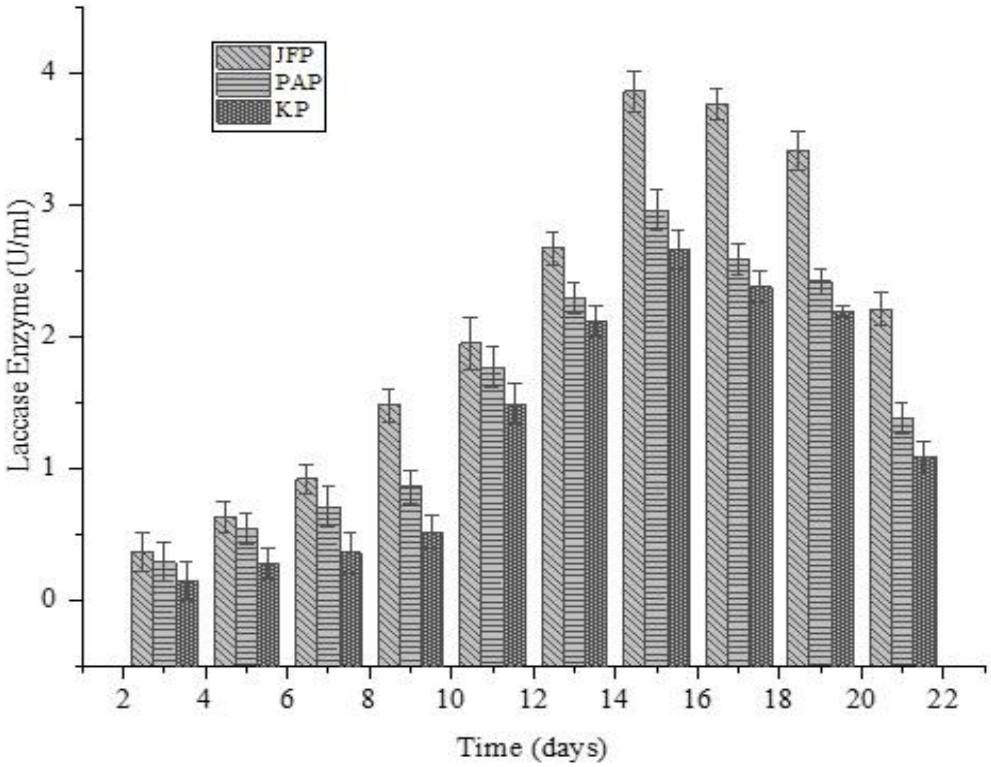


Figure 1 Production of Laccase under submerged fermentation using three different organic fruit peel wastes

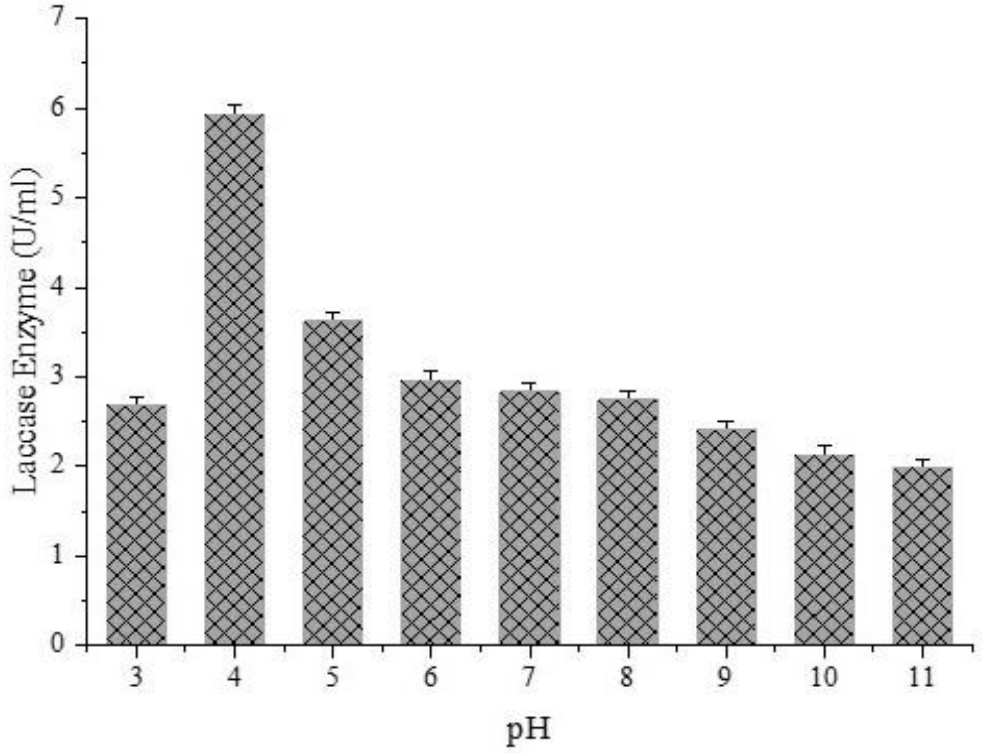


Figure 2 Effect of different pH on laccase activity

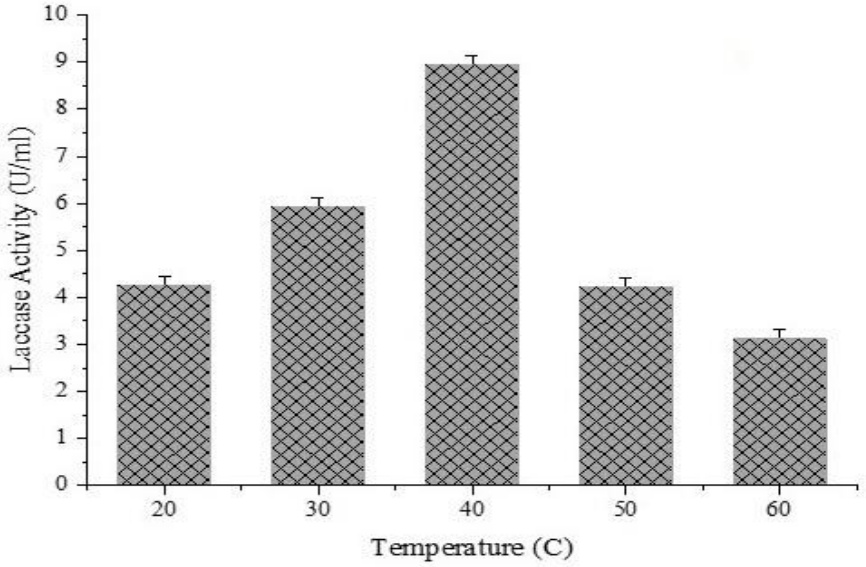


Figure 3 Effect of different temperatures on laccase activity

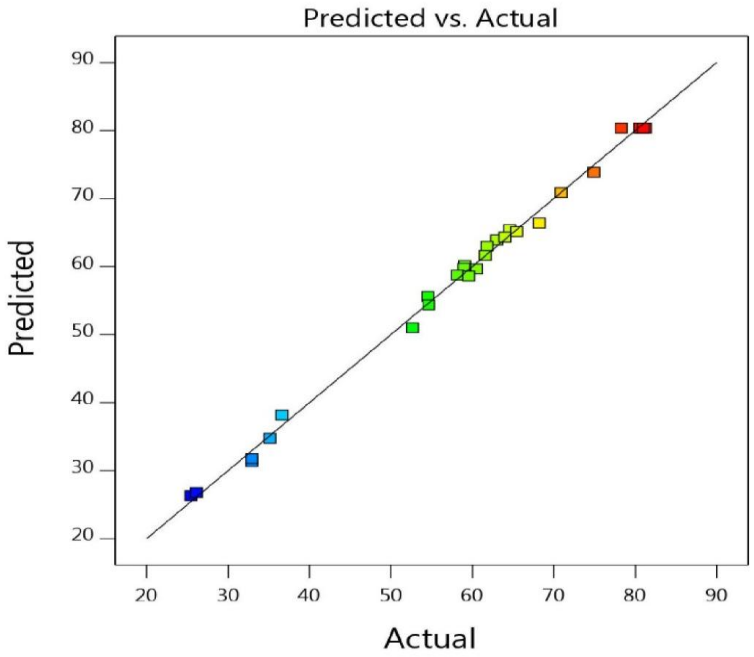


Figure 4 Box-Behnken design plot for actual versus predicted values for dye color removal

3.3 Dye decolorization Box-Behnken Method

By using the Box-Behnken design, the effect of independent variables *viz*., dye concentration (X_1), pH (X_2), temperature (X_3), and time (X_4) were systematically investigated by running 29 experiments. The level of independent variables used in the decolorizing of direct blue dye was given in table 1. In our study, the obtained result (Figure 4) was stated that, the actual and predicted response for direct blue dye decolorization. The experiment data were analyzed using ANOVA (Two-way table) to ascertain the interaction between the independent variables and the response by the quadratic model. The first and second-degree effects of all the variables were significant except for initial dye concentration, which was not omitted for the model. The second-order polynomial equation had been fitted to the data by the multiple regression procedure. This observation had been related to the response with the four factors as the below equation:

$Y(\%) = +80.36 - 0.9308X_1 + 16.13X_2 - 0.5308X_3 + 5.93X_4 - 31X_1^2 - 22.89X_2^2 - 12.28X_3^2 - 8.86X_4^2 + 0.7800X_1X_2 + 0.4275X_1X_3 + b_{14}X_1X_4 - 3.83X_2X_3 + 0.2175X_2X_4 + 1.78X_3X_4$

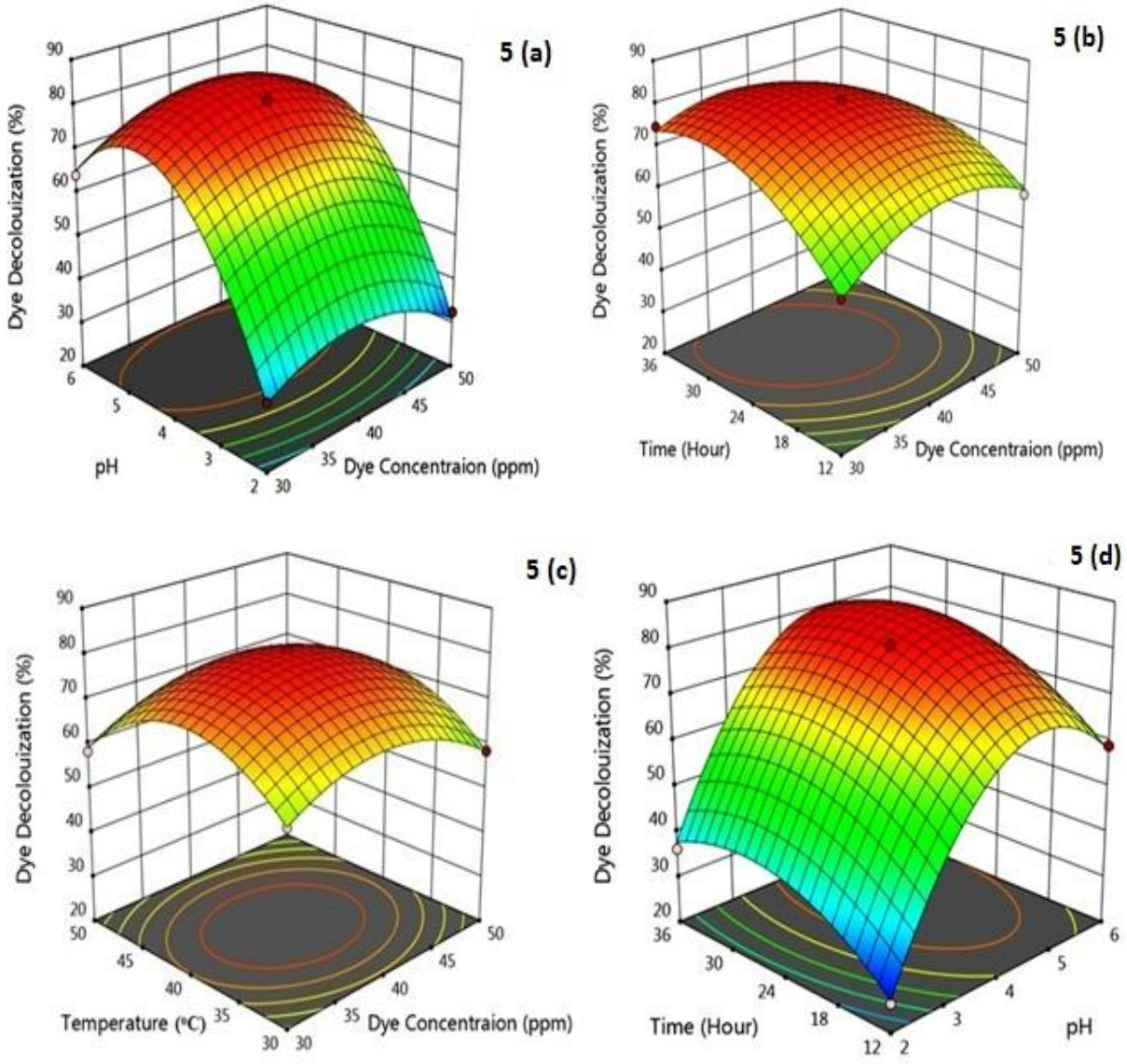
Furthermore, the ANOVA had been engaged to examine the fitness of the model. The high F-value of 264.61 and P-value < 0.0001 indicated that the model was significant which was less than 0.05 with the value of R^2= 0.9962. In addition, the experimental value of predicted R^2 = 0.9814 was coincidental with the adjusted R^2= 0.9925. The ANOVA result was satisfied with the experimental data of the quadratic model.

The ANOVA result was satisfied with the experimental data of the quadratic model for the independent variables involved in

Table 1 Box–Behnken design matrix for Direct Blue dye decolorization with a comparison of theoretical and experimental values

Run	Independent Variables				Response		
	DB Dye Concentration (ppm)	pH	Temperature (°C)	Time (Hour)	DB Dye Decolorization (%)	Predicted Value	Residue
	X_1	X_2	X_3	X_4	Y	Y^1	
1	40	6	40	36	70.89	70.89	-0.0008
2	40	2	30	24	32.91	31.79	1.1246
3	50	2	40	24	32.91	31.31	1.5963
4	40	4	40	24	81.25	80.36	0.8880
5	40	6	30	24	60.49	59.65	0.8362
6	40	6	50	24	61.79	62.99	-1.1971
7	50	4	30	24	59.06	58.94	0.1225
8	50	4	40	12	59.06	60.16	-1.1038
9	40	4	30	36	62.98	63.90	-0.9238
10	30	4	30	24	61.58	61.65	-0.0742
11	40	2	50	24	25.42	26.33	-0.9088
12	40	2	40	36	36.62	38.19	-1.5725
13	40	4	40	24	80.75	80.36	0.3880
14	40	4	40	24	78.29	80.36	-2.0720
15	40	4	50	12	52.64	50.99	1.6513
16	30	2	40	24	35.15	34.74	0.4146
17	40	4	40	24	80.54	80.36	0.1780
18	50	4	50	24	58.15	58.73	-0.5808
19	40	4	40	24	80.98	80.36	0.6180
20	40	4	50	36	68.21	66.40	1.8129
21	30	4	40	12	54.64	54.37	0.2746
22	50	6	40	24	65.45	65.14	0.3129
23	50	4	40	36	64.01	64.36	-0.3471
24	40	4	30	12	54.52	55.61	-1.0854
25	40	2	40	12	26.12	26.77	-0.6542
26	30	6	40	24	64.57	65.44	-0.8688
27	30	4	50	24	58.96	59.74	-0.7775
28	40	6	40	12	59.52	58.60	0.9175
29	30	4	40	36	74.91	73.88	1.0313

decolorization. Besides, the regression equation was characterized as three-dimensional surface plots of graphical representations. Figure 5 (a-f) represented the interaction between the two variables and the other two was constant at the central values. The central values of all the variables were dye concentration of 40 ppm, pH 4, the temperature of 40^{o}C, and the time interval of 24 Hrs. Figure 5a showed that the maximum decolorization was obtained at acidic pH whereas the percentage decreases at pH 6. The decolorization was not achieved at basic pH and also increase in dye concentration ensured a change in the color removal. The surface plot of Figure 5b was shown when the time duration increased up to 24 hours for an increase in the decolorization process due to the depletion of enzyme volume. In our study, the maximum result was obtained in 24 hours and there was no change in percentage by prolonged incubation. Figure 5c designated that the decolorization had increased when the temperature increased up to 40^{o}C. The decolorization was faintly improved at temperature versus dye concentration when compared to pH versus dye concentration. The increase in the dye concentration did not affect the decolorization as did the pH and temperature affected. Figure 5d represented the change in both reaction time and pH providing an increment in decolorization. Figure 5e illustrated that the decolorization was affected when temperature and pH increased up to a certain level. The optimum pH and temperature contributed to a better response to decolorization. Figure 5f expounded that the decolorization had increased with the time duration and it also had increased to a particular hour and then equilibrium was sorted out.



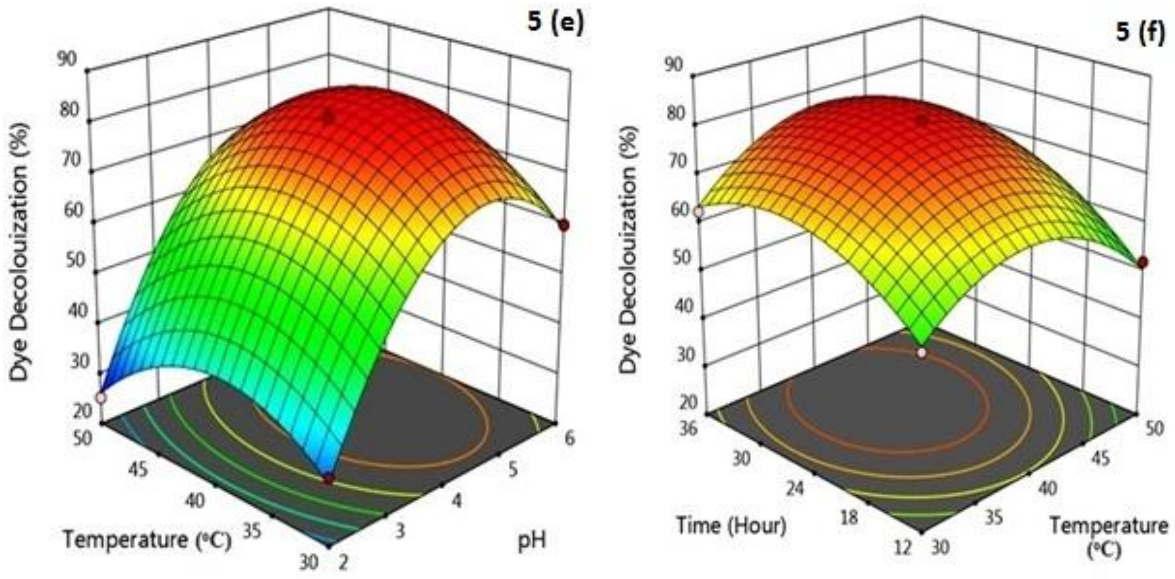


Figure 5 (a-f) Surface graph of direct blue dye decolorization displaying the interaction of the parameters like dye concentration, pH, temperature, and time interval.

The Direct Blue dye spectrum in the visible section exhibited a maximum peak at 587 nm. The UV visible scan of the treated dye showed the desertion of the band and a substantial decrease in the peak compared to the untreated band which directed the significant amendment in the dye structure due to the oxidation of guaiacol substrate as revealed in Figure 6. In this study, the optimum pH value was 4 and the detected temperature was 40°C for direct blue dye with 40 ppm dye concentration at 24 hours. The results of the study showed that the rate of decolorization of crude laccase enzyme was recorded to be 81.25% and it was obvious that there was an increased rate of decolorization than the purified one, which was recorded to be 49%.

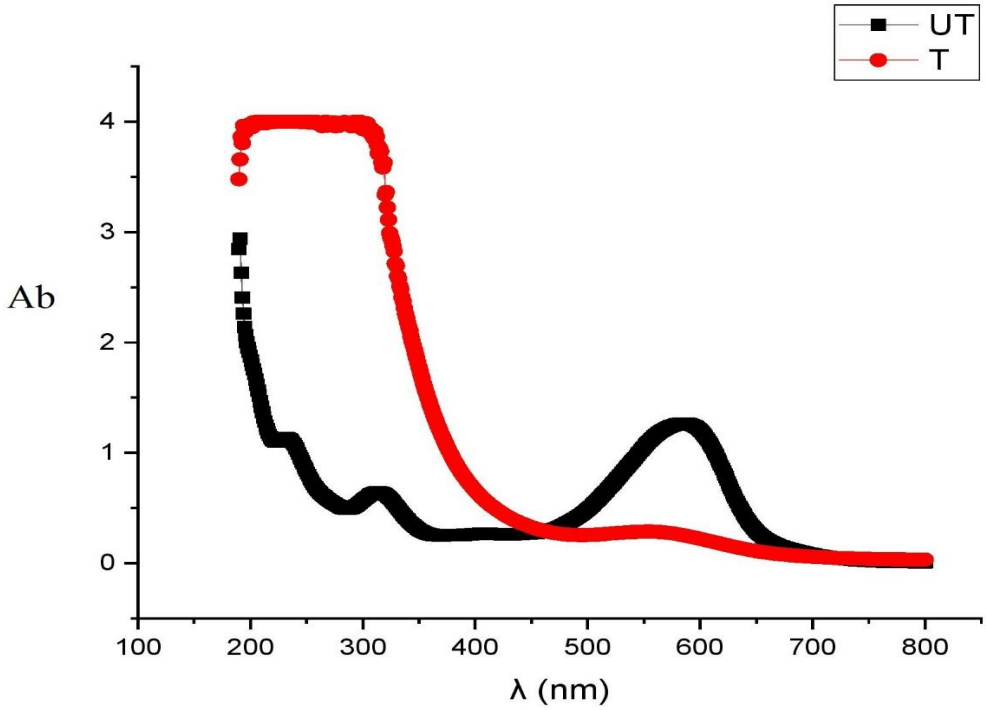


Figure 6 UV –visible absorption spectra of direct blue dye before (UT) and after (T) decolorization by crude laccase enzyme

4 Discussion

The *T.versicolor* involved in this study was proved as a laccase producer based on its efficacy. Most of the research studies proved that the *T.versicolor* was one of the potent laccase producers among the white-rot fungi (Okwara et al. 2021). Ramkumar et al. (2010, 2011) exhibited that the inorganic substrates like gypsum and calcium carbonate encouraged the high yield of enzyme when incorporated into lignocellulose organic substrate and also additionally performed as additives for the mycelium growth. The nutrient broth contained peptone, yeast extract, and beef extract which acted as nitrogen and vitamin sources that enhanced the fungal growth and thus the laccase engendered (Garzillo et al. 1992). The optimum pH range and temperature for *T.versicolor* were 4 - 6 and 25°C - 30°C respectively (Jo et al. 2010; Dos Santos Bazanella et al. 2013; Latif et al. 2022). Moreover, it was worth probing for incipient substrates like jackfruit peel, pineapple peel, and kaffir peel, especially if they were available in generously voluminous amounts, allowing the white-rot fungi to produce a maximum peak of enzymes. Romelle et al. (2016) reported that the cellulose content in jackfruit peel (27.75%) was higher than in pineapple (14.80%) and kaffir fruit peel (12.72%). Results of this study suggested that jackfruit peel could be used as a lignocellulosic organic substrate for further study. In the year 2013, Dos Santos Bazanella et al. (2013) reported that the maximum laccase activity by *Pleurotus* sp was obtained using pineapple peel when compared to wheat bran. *T.versicolor* had obtained laccase activity of 60.73 U/g using pineapple crowns as substrate (Backes et al. 2022)

The best laccase activity was determined at pH 4. It was obvious that the enzyme activity was greater under acidic conditions than in the alkaline condition. The change in the optimum pH strongly depended on the substrates involved in the fermentation media. The nutritional composition in the medium was affected due to an increase in pH which leads to low microbial growth (Braunschmid et al.2021). This was due to hydroxide anion bonding with T_2/T_3copper site subsequent inhibition in the enzyme activity which affected electron transfer during oxidation (Sousa et al. 2021). Amari et al. (2021) reported that the laccase from *T.versicolor* had an optimum pH in acidic conditions and correlated with the current result.

Like pH, the temperature was energy which played a vital role in the enzymatic reaction. The maximum laccase activity was achieved at 40°C due to an increased speed in kinetic energy and also the enzyme interaction between protein molecules and substrates to a certain temperature range (Kurniati et al. 2022). The reason for declined activity towards high temperature was due to protein denature and also loss of the three-dimensional structure. The preceding report also defined the optimum temperature for free laccase was retained at 55°C and 65°C for immobilized laccase (Amari et al. 2021). From these results, the optimum temperature obtained could be successfully used for industrial effluent treatment, which would be below 60°C (Zang et al. 2022; Ivanka et al. 2010).

Box-Behnken Method (BBM) was one of the principles of Response Surface Methodology (RSM), as a potential empirical evidence tool for decolorization. It was evaluated with mathematical and statistical methods, which related between independent variables and responses (Akar et al. 2021). The previous report of Cordova-Villegas et al. (2019) proved that the maximum removal of azo dye color was obtained at the optimum pH of 3-5. The same report was also proved by Birhanli et al. (2022) for the effective decolorization of azo dye. Generally, the fungal laccase was more active at acidic pH whereas bacterial laccase was active at basic and neutral pH (Coria-Oriundo et al. 2021) The other study reported that the maximum laccase activity was at acidic pH and low activity was observed at basic pH (Iqbal et al. 2021). However, the increase in dye concentration might affect the enzyme activity due to the toxicity of the concentration (Barathi et al. 2022; Hafshejani et al. 2014), which was directly linked to our results. The Direct blue dye was decolorized at 24 hours and no more decolorization was observed after 48 hours which was correlated with the report of Darvishi et al. (2018). The change in both reaction time and pH provides an increment in decolorization. Wikee et al. (2019) proved that the temperature for laccase stability was up to 50°C and if it increased after this the enzyme would be unstable. However, the decolorization was affected when temperature and pH were increased up to a certain level. It led to enzyme inactivation due to high temperature and the dispersion of dye molecules across the matrix due to the increase in pH (Ranimol et al. 2021). The decolorization increases with the time duration and equilibrium was sorted out and the temperatures above 40°C inhibit the enzyme activity leading to diminishing the decolorization (Iqbal et al. 2021). The antecedent reports proved that null decolorization occurs below 25°C and above 50°C (Hafshejani et al. 2014).

Based on the analysis, the pH, temperature, dye concentration, and time interval were considered as the most predominant parameters for the decolorization of the textile dye. In this study, an increase in these parameters was excepted dye concentration to brink value, led to an increase in decolorization, and proved the quadratic model. The crude laccase enzyme decolorization efficacy showed more activity than the purified one based on the typical characteristics of the laccase, which would be lacking in purified conditions (Madhavi and Lele 2009; Kandasamy et al. 2022). The results were closely related to Murugesan et al. (2007) who reported that purified laccase required mediator HBT (1-hydroxy benzotriazole) for certain dyes. Hou et al. (2004) also proved that

decolonization was increased from 66% to 90% by the addition of mediator ABTS (2,2′- Azino-di-(3-ethylbenzothialozin-6-sulfonic acid). The decolorization with purified laccase was obtained with 49%, which indicated crude laccase act as a potential candidate to degrade textile dyes in near future.

Conclusion

The obtained result of this study demonstrated the potential production of laccase enzyme through submerged fermentation by *T.versicolor* using agro-waste such as organic peels. Based on this study, the potent lignocellulose organic substrate was identified by comparing the three-waste substrate and from the results of this study, the maximum yield was obtained at 25^{o}C as 3.86U/ml by Jack fruit peel. The maximum laccase activity was recorded at pH 4 and 40^{o}C. The color removal efficiency of the Direct Blue azo dye was 81.25% at dye concentration 40 ppm, pH 4, temperature 40^{o}C at 24 hours by Box-Behnken Method. It was practically proved than a purified one. The prospects of laccase enzyme in decolorization were emphasized more than other endeavors of physical and chemical treatment. Besides the articulated needs of this laccase enzyme in bioremediation, capable of solving environmental pollutants problems like textile effluent treatment and massive application in food industries, the lucrative economic way for the enzyme production using organic waste was undoubtedly proved. The transformation of agro-industrial residues to essential substances might not only provide future aspects to researchers but it also would diminish the existing environmental hazards and enzyme production in the commercial market.

Acknowledgments

The authors wish to acknowledge the Management and Principal, K.S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode, Namakkal, Tamil Nadu, India, and the faculty of the Food Technology Department, Kongu Engineering College, Perundurai, Erode, Tamil Nadu, India for providing all the facilities during the study periods.

Conflict of Interest

The authors declare that they have no conflict of interest concerning this work.

References

Agustin, M. B., de Carvalho, D. M., Lahtinen, M. H., Hilden, K., Lundell, T., & Mikkonen, K. S. (2021). Laccase as a Tool in Building Advanced Lignin-Based Materials. *ChemSusChem*, 14(21), 4615-4635. DOI: https://doi.org/10.1002/cssc.202101169

Akar, S.T., Koc, E., Sayin, F., Kara, I., & Akar, T. (2021). Design and modeling of the decolorization characteristics of a regenerable and eco-friendly geopolymer: Batch and dynamic flow mode treatment aspects. *Journal of Environmental Management*, *298*, 113548. DOI: https://doi.org/10.1016/j.jenvman.2021.113548

Ali, L., Algaithi, R., Habib, H.M., Souka, U., Rauf, M.A.,& Ashraf, S.S. (2013). Soybean peroxidase-mediated degradation of an azo dye–a detailed mechanistic study. *BMC biochemistry*,*14*, 1-14.

Amari, A., Alzahrani, F. M., Alsaiari, N. S., Katubi, K. M., Rebah, F. B., & Tahoon, M. A. (2021). Magnetic metal organic framework immobilized laccase for wastewater decolorization. *Processes*, *9*(5), 774. DOI: https://doi.org/10.3390/pr9050774

Backes, E., Kato, C.G., da Silva, T.B., Uber, T.M., Pasquarelli, D.L., Bracht, A., & Peralta, R.M. (2022). Production of fungal laccase on pineapple waste and application in detoxification of malachite green. *Journal of Environmental Science and Health, Part B*, *57*(2), 90-101. DOI: https://doi.org/10.1080/03601234.2022.2025739

Backes, E., Kato-Schwartz, C.G., Corrêa, R.C.G., Moreira, R.d.F.P.M., et al. (2021). Laccases in food processing: Current status, bottlenecks and perspectives. *Trends in Food Science & Technology*, *115*, 445-60.DOI: https://doi.org/10.1016/j.tifs.2021.06.052

Barathi, S.,Aruljothi, K.N., Karthik, C., Padikasan, I.A., & Ashokkumar, V. (2022). Biofilm mediated decolorization and degradation of reactive red 170 dye by the bacterial consortium isolated from the dyeing industry wastewater sediments. *Chemosphere*, *286*, 131914. DOI: https://doi.org/10.1016/j.chemosphere.2021.131914

Bayramoglu, M., Eyvaz, M., & Kobya, M. (2007). Treatment of the textile wastewater by electrocoagulation: economical evaluation. *Chemical Engineering Journal*, *128*, 155-161. DOI: https://doi.org/10.1016/j.cej.2006.10.008

Becker, D., Rodriguez-Mozaz, S., Insa, S., Schoevaart, R., et al. (2017). Removal of endocrine disrupting chemicals in wastewater by enzymatic treatment with fungal laccases. *Organic Process Research & Development*, *21*, 480-491. DOI: https://doi.org/10.1021/acs.oprd.6b00361

Birhanlı, E., Noma, S.A.A., Boran, F., Ulu, A., Yeşilada, Ö., & Ateş, B. (2022). Design of laccase–metal–organic framework hybrid constructs for biocatalytic removal of textile dyes. *Chemosphere*, *292*, 133382. DOI: https://doi.org/10.1016/j.chemosphere.2021.133382

Braunschmid, V., Binder, K., Fuerst, S., Subagia, R., Danner, C., Weber, H., & Guebitz, G. M. (2021). Comparison of a fungal and a bacterial laccase for lignosulfonate polymerization. *Process*

Biochemistry, *109*, 207-213. DOI: https://doi.org/10.1016/j.procbio.2021.07.001

Cescon, A., & Jiang, J. Q. (2020). Filtration process and alternative filter media material in water treatment. *Water*, *12*(12), 3377. DOI: http://dx.doi.org/10.3390/w12123377

Cordova-Villegas, L.G., Cordova-Villegas, A.Y., Taylor, K.E., & Biswas, N. (2019). Response surface methodology for optimization of enzyme-catalyzed azo dye decolorization. *Journal of Environmental Engineering*,*145*(5), 04019013.

Coria-Oriundo, L.L., Battaglini, F., & Wirth, S.A. (2021). Efficient decolorization of recalcitrant dyes at neutral/alkaline pH by a new bacterial laccase-mediator system. *Ecotoxicology and Environmental Safety*, *217*, 112237. DOI: https://doi.org/10.1016/j.ecoenv.2021.112237

Crawford, S.E., Brinkmann, M., Ouellet, J.D., Lehmkuhl, F., Reicherter, K., Schwarzbauer, J., & Hollert, H. (2022). Remobilization of pollutants during extreme flood events poses severe risks to human and environmental health. *Journal of hazardous materials*, *421*, 126691. DOI: https://doi.org/10.1016/j.jhazmat.2021.126691

Darvishi, F., Moradi, M., Jolivalt, C., & Madzak, C. (2018). Laccase production from sucrose by recombinant Yarrowia lipolytica and its application to decolorization of environmental pollutant dyes. *Ecotoxicology and environmental safety*,*165*, 278-283. DOI: https://doi.org/10.1016/j.ecoenv.2018.09.026

de Boer, S., González-Rodríguez, J., Conde, J.J., & Moreira, M. T. (2022). Benchmarking tertiary water treatments for the removal of micropollutants and pathogens based on operational and sustainability criteria. *Journal of Water Process Engineering*, *46*, 102587. DOI:https://doi.org/10.1016/j.jwpe.2022.102587

Desai, S.S., Tennali, G.B., Channur, N., Anup, A., Deshpande, G., & Murtuza, B.A. (2011). Isolation of laccase producing fungi and partial characterization of laccase. *Biotechnol Bioinf Bioeng*, 1, 543-549.

Dos Santos Bazanella, G.C., de Souza, D.F., Castoldi, R., Oliveira, R.F., Bracht, A., & Peralta, R.M. (2013). Production of laccase and manganese peroxidase by Pleurotus pulmonarius in solid-state cultures and application in dye decolorization. *Folia microbiologica*, *58*, 641-647.

Freitas, L. C., Barbosa, J. R., da Costa, A. L. C., Bezerra, F. W. F., Pinto, R. H. H., & de Carvalho Junior, R. N. (2021). From waste to sustainable industry: How can agro-industrial wastes help in the development of new products? *Resources, Conservation and Recycling*, *169*, 105466. DOI: https://doi.org/10.1016/j.resconrec.2021.105466

Garzillo, A.M.V., Di Paolo, S., Burla, G., & Buonocore, V. (1992). Differently-induced extracellular phenol oxidases from *Pleurotus ostreatus*. *Phytochemistry*, *31*, 3685-3690. DOI: https://doi.org/10.1016/S0031-9422(00)97509-5

Hafshejani, M.K., Ogugbue, C.J., & Morad, N. (2014). Application of response surface methodology for optimization of decolorization and mineralization of triazo dye Direct Blue 71 by *Pseudomonas aeruginosa*. *3 Biotech*, 4(6), 605-619.

Hou, H., Zhou, J., Wang, J., Du, C., & Yan B. (2004). Enhancement of laccase production by *Pleurotus ostreatus* and its use for the decolorization of anthraquinone dye. *Process Biochemistry*, *39*(11), 1415-1419.DOI: https://doi.org/10.1016/S0032-9592(03)00267-X

Husain, Q. (2010). Peroxidase mediated decolorization and remediation of wastewater containing industrial dyes: a review. *Reviews in Environmental Science and Bio/Technology*,*9*, 117-140.

Iqbal, K., Nadeemm, A., & Zafar, U. (2021). Biostoning of textile effluent with laccase enzyme. *Bangladesh Journal of Scientific and Industrial Research*, *56*(2), 115-24. DOI: https://doi.org/10.3329/bjsir.v56i2.54318

Ivanka, S., Albert, K., & Veselin, S. (2010). Properties of crude laccase from *Trametes versicolor* produced by solid-substrate fermentation. *Advances in Bioscience and Biotechnology*, *1*(3), 2010. DOI: 10.4236/abb.2010.13029.

Jebapriya, G.R.,& Gnanadoss, J.J. (2013). Bioremediation of textile dye using white rot fungi: A review. *International Journal of Current Research and Review*, 5(3), 1.

Jalal, G., Abbas, N., Deeba, F., Butt, T., Jilal, S., & Sarfraz, S. (2021). Efficient removal of dyes in textile effluents using aluminum-based coagulant. Efficient Removal of Dyes in Textile Effluents Using Aluminum-Based Coagulant. *Chemistry International*, *7*(3), 197-207. DOI: https://doi.org/10.5281/zenodo.4899952

Jo, W.S., Kang, M.J., Choi, S.Y., Yoo, Y.B., Seok, S.J., & Jung, H.Y. (2010). Culture conditions for mycelial growth of *Coriolus versicolor*. *Mycobiology*,*38*(3), 195-202.DOI: https://doi.org/10.4489/MYCO.2010.38.3.195

Kandasamy, S., Ameen, F., M. Amirul, I., Sudhakar, C., & Selvankumar, T. (2022). Laccase production from *Bacillus aestuarii* KSK using Borassus flabellifer empty fruit bunch waste as substrate and assess their malachite green dye degradation.

Journal of Applied Microbiology, 1-8. DOI: https://doi.org/10.1111/jam.15670

Kurniati, A., Puspaningsih, N.N.T., Putri, K.D.A., Damayanti, M., Purwani, N.N., Rahmah, S. A., & Sanjaya, R. E. (2022). Heterologous fusion gene expression and characterization of a novel carbohydrate binding module (Cbm36) to laccase (Lcc2). *Biocatalysis and Agricultural Biotechnology*, *102377*. DOI: https://doi.org/10.1016/j.bcab.2022.102377

Latif, A., Maqbool, A., Sun, K., & Si, Y. (2022). Immobilization of *TrametesVersicolor*laccase on Cu-alginate beads for biocatalytic degradation of bisphenol A in water: Optimized immobilization, degradation and toxicity assessment. *Journal of Environmental Chemical Engineering*, *10*(1), 107089. DOI: https://doi.org/10.1016/j.jece.2021.107089

Levin, L., Diorio, L., Grassi, E., & Forchiassin, F. (2012). Grape stalks as substrate for white rot fungi, lignocellulolytic enzyme production and dye decolorization. *Revista Argentina de microbiologia*, *44*, 105-112.

Macedo, D.S., Vepsäläinen, M., Acharya, D., Wood, C.D., et al. (2021). An unusually stable solid state Ag| AgCl reference electrode for long term continuous measurements based on a crosslinked poly (vinyl acetate)/KCl composite. *Electrochimica Acta*, *368*, 137636.DOI: https://doi.org/10.1016/j.electacta.2020.137636

Madhavi, V.,& Lele, S. (2009). Laccase: properties and applications. *BioResources*, *4*, 1694-1717.

Manavalan, T., Manavalan, A., Thangavelu, K.P., & Heese, K. (2013). Characterization of optimized production, purification and application of laccase from Ganoderma lucidum. *Biochemical Engineering Journal*, *70*, 106-114.DOI: https://doi.org/10.1016/j.bej.2012.10.007

Murugesan, K., Dhamija, A., Nam, I.H., Kim, Y.M.,& Chang, Y.S. (2007). Decolourization of reactive black 5 by laccase: optimization by response surface methodology. *Dyes and Pigments*, *75*(1):176-84. DOI: https://doi.org/10.1016/j.dyepig.2006.04.020

Muthukumar, K., Sundaram, P.S., Anantharaman, N., & Basha, C.A. (2004). Treatment of textile dye wastewater by using an electrochemical bipolar disc stack reactor. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, *79*, 1135-1141. DOI: https://doi.org/10.1002/jctb.1104

Okwara, P., Afolabi, I.S., & Ahuekwe, E.F. (2021). Application of laccase in aflatoxin B1 degradation: a review. In *IOP Conference Series: Materials Science and Engineering*, *1107*(1), 012178. DOI:https://doi.org/10.1088/1757-899X/1107/1/012178

Patel, H., & Gupte, A. (2016). Optimization of different culture conditions for enhanced laccase production and its purification from Tricholoma giganteum AGHP. *Bioresources and Bioprocessing*, *3*, 1-10.

Ramkumar, L., Ramanathan, T.,& Nedumaran, T. (2011). In Vitro effect of Organic and inorganic additives from The production of radial mycelial growth and lignocellulolytic enzyme in Lentinus Edodes (berk.) SING. *Emirates Journal of Food and Agriculture*, *23*(1), 71-79.

Ramkumar, L., Thirunavukkarasu, P.,& Ramanathan, T. (2010). Development of improved technology for commercial production and preservation of shiitak mushroom (Lentinus edodes). *American-Eurasian Journal of Agricultural & Environmental Sciences*,*7*, 433-438.

Ranimol, G., Paul, C., & Sunkar, S. (2021). Optimization and efficacy studies of Laccase immobilized on Zein-Polyvinyl pyrrolidonenano fibrous membrane in decolorization of Acid Red 1. *Water Science and Technology*, *84* (10-11): 2703–2717. DOI: https://doi.org/10.2166/wst.2021.200

Romelle, F.D., Rani, A.,& Manohar, R.S. (2016). Chemical composition of some selected fruit peels. *European Journal of Food Science and Technology*,*4*, 12-21.

Rosales, E., Couto, S.R.,& Sanromán, A. (2002). New uses of food waste: application to laccase production by *Trametes hirsuta*. *Biotechnology Letters*, *24*, 701-704.

Russo, C., Maugeri, A., Lombardo, G.E., Musumeci, L., Barreca, D., Rapisarda, A., & Navarra, M. (2021). The second life of Citrus fruit waste: A valuable source of bioactive compounds. *Molecules*, *26*(19), 5991. DOI: https://doi.org/10.3390/molecules26195991

Sathishkumar, P., Murugesan, K.,& Palvannan, T. (2010). Production of laccase from *Pleurotus florida* using agro-wastes and efficient decolorization of Reactive blue 198. *Journal of basic microbiology*, *50*, 360-367.DOI: https://doi.org/10.1002/jobm.200900407

Shaban, M., Abukhadra, M.R., Ibrahim, S.S., & Shahien, M.G. (2017). Photocatalytic degradation and photo-Fenton oxidation of Congo red dye pollutants in water using natural chromite—response surface optimization. *Applied Water Science*, *7*, 4743-4756.

Shekher, R., Sehgal, S., Kamthania, M., & Kumar, A. (2011). Laccase: microbial sources, production, purification, and potential biotechnological applications. *Enzyme research*, *2011*, 217861. DOI:https://doi.org/10.4061/2011/217861

Shokri, Z., Seidi, F., Karami, S., Li, C., Saeb, M.R., & Xiao, H. (2021). Laccase immobilization onto natural polysaccharides for biosensing and biodegradation. *Carbohydrate Polymers*, *262*, 117963. DOI: https://doi.org/10.1016/j.carbpol.2021.117963

Singh, A., Pal, D.B., Mohammad, A., Alhazmi, A., Haque, S., Yoon, T., & Gupta, V.K. (2022). Biological remediation technologies for dyes and heavy metals in wastewater treatment: New insight. *Bioresource Technology*, *343*, 126154. DOI: https://doi.org/10.1016/j.biortech.2021.126154

Songserm, P., Sihanonth, P., Sangvanich, P., & Karnchanatat, A. (2012). Decolorization of textile dyes by *Polyporus seudobetulinus* and extracellular laccase. *African Journal of Microbiology Research*, *6*, 779-792. DOI: https://doi.org/10.5897/AJMR11.988

Sousa, A. C., Martins, L. O., & Robalo, M. P. (2021). Laccases: Versatile biocatalysts for the synthesis of heterocyclic cores. *Molecules*, *26*(12), 3719. DOI: https://doi.org/10.3390/molecules26123719

Sun, K., Hong, D., Liu, J., Latif, A., et al. (2021).*Trametes versicolor* laccase-assisted oxidative coupling of estrogens: Conversion kinetics, linking mechanisms, and practical applications in water purification. *Science of the Total Environment,782*, 146917. DOI: https://doi.org/10.1016/j.scitotenv.2021.146917

Szostek, M., Kosowski, P., Szpunar-Krok, E., Jańczak-Pieniążek, M., Matłok, N., Skrobacz, K., & Balawejder, M. (2022). The Usefulness of Ozone-Stabilized Municipal Sewage Sludge for Fertilization of Maize (*Zea mays* L.). *Agriculture*, *12*(3), 387. DOI:https://doi.org/10.3390/agriculture12030387

Upadhyay, P., Shrivastava, R.,& Agrawal, P.K., (2016). Bioprospecting and biotechnological applications of fungal laccase. *3 Biotech*, *6*(1), 1-12.DOI: https://doi.org/10.1007/s13205-015-0316-3

Villalba-Rodríguez, A. M., Parra-Arroyo, L., González-González, R. B., Parra-Saldívar, R., Bilal, M., & Iqbal, H. M. (2022). Laccase-assisted biosensing constructs–Robust modalities to detect and remove environmental contaminants. *Case Studies in Chemical and Environmental Engineering*, *100180*. DOI: https://doi.org/10.1016/j.cscee.2022.100180

Wikee, S., Hatton, J., Turbé-Doan, A., Mathieu, Y., et al. (2019). Characterization and dye decolorization potential of two laccases from the marine-derived fungus *Pestalotiopsis sp*. *International journal of molecular sciences*, *20*(8):1864. DOI: https://doi.org/10.3390/ijms20081864

Xin, F., & Geng, A. (2011). Utilization of horticultural waste for laccase production by *Trametes versicolor* under solid-state fermentation. *Applied biochemistry and biotechnology*,*163*, 235-246.

Yusuf, M. (2017). Agro-industrial waste materials and their recycled value-added applications. *Handbook of Ecomaterials*, 1-11. DOI: https://doi.org/10.1007/978-3-319-48281-1_48-1

Zhang, F., Lian, M.,Alhadhrami, A., Huang, M., Li, B., Mersal, G. A., & Xu, M. (2022). Laccase immobilized on functionalized cellulose nanofiber/alginate composite hydrogel for efficient bisphenolA degradation from polluted water. *Advanced Composites and Hybrid Materials*, 1-13. DOI: https://doi.org/10.1007/s42114-022-00476-5