Nigerian Journal of Technology (NIJOTECH)
Vol. 44, No. 3, September, 2025, pp.507 - 517
www.nijotech.com

Print ISSN: 0331-8443
Electronic ISSN: 2467-8821
https://doi.org/10.4314/njt.v44i3.14

EFFICIENT SYNTHETIC DYES DECOLORIZATION USING MAGNETIC NANOPARTICLE CROSS-LINKED AGGREGATE OF ASPERGILLUS FLAVUS KIGC LACCASE

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ARTICLE HISTORY:
Received: February 02, 2025.
Revised: October 02, 2025.
Accepted: October 07, 2025.
Published: October15, 2025

KEYWORDS:
synthetic dye, fermentation, laccase, nanoparticle, decolorization.

ARTICLE INCLUDES:
Peer review

DATA AVAILABILITY:
On request from author(s)
EDITORS: Chidozie Charles Nnaji

FUNDING:
None

Abstract

The study evaluated the synthesis, characterization and synthetic dye decolorization potential of iron oxide nanoparticle immobilized laccase extracted from Aspergillus sp KIGC. Laccase was extracted from fungal isolate on Day 8 of submerged fermentation process, with Bambara nut husk and soybean extract as sole carbon and nitrogen sources, respectively at pH 6.5. The enzyme was purified to 2.55 folds and 68.90% purification yield, with an SDS-PAGE gel that showed a band corresponding to 66 KDa, indicating a monomeric enzyme. The immobilization of Aspergillus sp KIGC laccase on iron oxide nanoparticle chemically modified and functionalized by amino-prolyltri-ethoxysilane/glutaraldehyde was evaluated. DLS, DSC, XRD, FTIR and SEM were the instruments used in the characterization of the synthesized and immobilized paramagnetic nanoparticles. There was effective nanoparticle synthesis and adequate laccase immobilization on the nanoparticle as evidenced by the characterization results obtained. Immobilization improved the enzyme's catalytic properties (KM (1.04 mM) and catalytic efficiency (13.308 mol/S-1) more than the free enzyme (1.971 mol/S-1), with the concomitant high dye decolorization efficiency (75% decolorization rate). The results show the production of laccases using cheap and readily available agro-residues at relatively mild submerged fermentation conditions from Aspergillus sp KIGC and the high decolorization potential of immobilized laccase on iron oxide nanoparticle, as a promising biocatalyst that could be used in treating (decolorizing) wastewater containing synthetic dyes.

1.0 INTRODUCTION

There are several sources of contaminants in the environment today due to the increase in industrialization and agricultural mechanization. Dye contamination is one of the commonly encountered in the environment due to its ease of usage and wide applicability in textile, leather industries and some personal uses. These dyes are recalcitrant in the environment due to their complex structures. There are several methods (physical and chemical) used in

HOW TO CITE:
Omeje. K. O., Ezugwu. L. C., Ossai. E. C., Eze. K. C., Ezema. B. O., Eze. S. O. O. "Evaluating Deep Learning Models For Real-Time Waste Classification In Smart Iot Environment", *Nigerian Journal of Technology*, 2025; 44(3), pp. 507 – 517; https://doi.org/10.4314/njt.v44i2.14

removing or degrading these dyes from the environment, but not without their attendant disadvantages of high costs and production of more recalcitrant compounds. Recently, Nanotechnology has become an important tool in dye decolorization by improving the process efficiency and reducing the load of effluents generated [42], making it an important green chemistry process by reducing the generation of hazardous compounds [43]. Hence, removal of these contaminants from the textile wastewater before releasing it into the ecosystem is important.

Bioremediation is adjudged a better option due to it being environmental friendliness, cost effective and high catalytic efficiency [1]. It is the use of biomaterials, including whole cells or enzymes in metabolizing or breaking down compounds into important nutrients required for their survival. Peroxidases and laccases are mostly used for this purpose due to their broad substrate specificity [2, 3]. Laccase (1.10.3.2) is an oxidase responsible for oxidating various aromatic and nonaromatic compounds using molecular oxygen [4, 5]. They also catalyze the degradation and ring cleavage of aromatic compounds using molecular oxygen [4], and are found in both microorganisms, especially fungi as well as plants [6, 7]. They have several applications in food industry [4], decolorization of fungi pigments [7], bioremediation [8, 9], decolorization of synthetic dyes [10, 11]. Laccase is one of the commonly used industrial enzymes. These common industrial applications include delignification and pulp properties improvement [49], dye removal from wastewater [50] and clarification of fruit juice and removal of phenolics from food samples [51; 52].

Laccase ability to oxidize highly recalcitrant environmental pollutants has attracted several attentions and researchers are exploiting the opportunity to solve some environmental and industrial problems [4]. The successful application of laccase in dye decolorization depends on its reusability, cost effectiveness and readily availability. One way to achieve these properties is through immobilization of laccase, which will as well improve laccase activity and stability [12]. It has been immobilized on several inert materials such as alumina [13], silica [14], zeolites [15] and Polyvinylidene fluoride membrane [16]. Some of the immobilization materials are expensive, toxic and not readily available, thus, the need to source for supporting materials that are cheap, non-toxic and readily available. Iron metallic nanoparticle is easily synthesized using Fe_2O_3 and Fe_3O_4 and functionalizes with APTES and glutarldehye [17] and does not require much expertise. Other advantages attributed to immobilizing enzymes on Iron metallic nanoparticles include large surface area, uniform morphology and ease of separation using external magnetic field.

Immobilized fungal laccases have been used in bioremediation and degradation of Bisphenol A and other recalcitrant environmental contaminants [17, 18, 19]. *Aspergillus sp. KIGC* is a new fungi strain characterized in our laboratory and deposited in the Gene data bank with GenBank Accession number MT951631. The focus of the research was to evaluate the catalytic efficiency of the immobilized enzyme with its free enzyme. Thus, iron magnetic nanoparticle immobilized fungal laccase was characterized and the decolorization of textile dyes potential was evaluated.

2.0 METHODOLOGY

2.1 Sample collection and preparations

The organism, *Aspergillus sp. KIGC* was isolated from sample of soil collected using a sterile bag from the textile laboratory dumping site, University of Nigeria and transported to the laboratory. The sample was prepared and diluted to 10^5, before spreading an aliquot on potatoes dextrose agar petri dish and placed in an incubator (37°C) and allowed to stand for 7 days. Isolated fungal strains were characterized as described by [41]. The isolates were maintained on Potato Dextrose Agar slants as stock cultures.

Four commonly available agro-waste (banana, orange peel and sugarcane bagasse) were gathered, washed and dried in the laboratory at room temperature between November and December 2024 before placing the samples in the oven for 60minutes at 45 °C on the day of milling the samples, Bambara husk (dry) inclusive.

2.2 Culture Condition and Laccase production

The enzyme production technique used was submerged fermentation in a 250 mL flask containing K_2HPO_4 (0.04%), peptone (0.3%), KH_2PO_4 (0.06%), $FeSO_4$ (0.0005%), $MnSO_4$ (0.05%) and $ZnSO_4$ (0.0001%), $MgSO_4$ (0.05%); and glucose (1%) in basal salt medium (BSM) [10]. The optimum conditions for the enzyme production were studied using sucrose, soluble starch, glucose, lactose and mannose at varying concentrations of 0.50 and 1.00 g/100 mL. The medium optimal pH at interval of 0.5 units (pH3.5-11.0) and temperature (30–45°C) were also studied. Some agro-waste (banana, orange peel, okpa chaff (Bambara husk) and sugarcane bagasse) were evaluated to serve as sole carbon source. Four commonly available agro-waste (banana, orange peel and sugarcane bagasse) were gathered, washed and

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dried in the laboratory at room temperature between November and December 2024 before placing the samples in the oven for 60minutes at 45 °C on the day of milling the samples, Bambara husk (dry) inclusive. Similarly, best nitrogen source for (peptone, soybean extract, beef extract and casein) was evaluated.
High shear homogenizer (JRJ300-SH) was used to homogenize the fermentation broth, with subsequent centrifugation at 6000 rpm for 30mins at 4 ◦C to separate the homogenate into supernatant and cell debris. The supernatant (crude laccase) was harvested, filtered and stored for future use. To ascertain the best day of laccase production by the organism, an aliquot of fermentation broth was taken every day for fifteen (15) days and assayed for laccase activity.

2.2.1 Laccase activity assay
The method of Kalra et al. [20] was adopted for the assay of laccase activity and ABTS was used as substrate. The oxidation of ABTS after incubation at 30 ◦C was studied spectroscopically (UV/Vis, Beckman/Instruments, USA). This experiment was carried out in triplicate.

2.2.2 Protein determination
The protein concentration of the crude enzyme was quantified using Bradford method [21], using ovalbumin (standard protein). This experiment was carried out in triplicate.

2.2.3 Laccase purification
Crude laccase was precipitated using ammonium sulphate, adding 400 g in 1000 mL of crude mixture, stood for 6 hr at continuous shaking, the pellet recovered after 30 mins centrifugation at 6000 rpm and saturation was subsequently brought to 60 %. The pellet was dissolved in buffer (sodium acetate; pH 6.0; 100 mM) and introduced onto DEAE-cellulose column, washed with 100 mM sodium acetate buffer (pH 6.0). The sample was eluted (NaCl gradient, 0.5 - 0.2M) and the active fractions were purified on sephadex G75 gel and fractions were collected.

2.2.4 Electrophoretic analysis
SDS-PAGE evaluation was carried out as described by Laemmli [22] on 4-12%-disc gels and stained with silver as described [23] under standard conditions.

2.3 Production of functional magnetic nanoparticles
Iron oxide magnetic nanoparticles (IOMNP) synthesis was done by the method of Ranjbakhsh et al. [24]. Briefly, $FeCl_2.4H_2O$ (1.25 g) and $FeCl_3.6H_2O$ (3.40 g) were dissolved in de-ionized (100 mL) water at 60 ºC. Subsequently, 25% $NH_3.H_2O$ (6 mL) was added and stirred for 60mins at 250 rpm. The NH_2 groups were attached to IOMNPs by incubating (300 mg, dry weight) with 2% 3-aminopropyltriethoxysilane (APTS, 30 mL) and mixed (250 rpm) at 70˚C. Glutaldehyde (2%) was added and stirred for 120mins at 350rpm [25].

2.3.1 Characterization of IOMNPs
X-ray diffraction was used for phase identification of the formulated IOMNP (XRD, Philips, The Netherlands). The size of IOMNP particle formulated was determined by dynamic light scattering (DLS, Malvern Zetasizer Nano S, Worcestershire). Fourier transforms infrared spectroscopy (FTIR, Shimadzu - Kyoto) was used to determine the functional groups therein. The thermostability of the nanoparticle was determined using differential scanning calorimetry (DSC, Shimadzu - Kyoto) at (10 ˚C from 60 - 300 °C).

2.4 Characterization of Enzyme immobilization on nanoparticles

2.4.1 Temperature and pH Characterization of free and immobilized laccase
The optimum temperature and pH of free and immobilized laccase was determined at 30 – 70 °C and pH of 3.5–11.0. The laccase residual activity was obtained using the criteria in Enzyme assay section.

2.4.2. Determination Laccase kinetics (Free and immobilized)
The rate of ABTS and O-dianisidine metabolism by laccase was carried out at 0.20 – 1.20 g/10 mL. K_M, *Vmax*, *Kcat*, Catalytic efficiency was calculated using their derivative constants.

2.4.3 Effect of organic solvents on laccase Activity
The effect of methanol, chloroform and ethanol on the laccases was studied after 60mins incubation with the organic solvents.

2.4.4 Textile dye decolorization
The potential of the free and the immobilized laccase to decolorize textile dyes was evaluated as described by Victor et al. [26]. Acid Blue 74, Azo brilliant black, Basic blue 22 and Azo Yellow 6, Vat Blue 5 dyes were used. The reaction contained 0.05 M acetate buffer (2.7 mL, pH 5.0), dye solution (0.1 mL), and the free and immobilized laccase (0.1 mL each) was incubated (37 °C and 96 hr). The control reaction contained 0.05 M acetate buffer (2.7 mL, pH 5.0), dye solution (0.1 mL), H_2O_2 (0.1 mL) was incubated (37 °C and 96 hr). At the end of the incubation, the reaction absorbance

was read at 614 nm and percentage decolorization of dye calculated as follows:

$$Change\ in\ decolorization = \frac{Ai - Af}{Ai} X\ 100 \quad (1)$$

A*i* = absorbance before incubation, A*f* = absorbance after incubation. This experiment was carried out in triplicate

3.0 RESULTS AND DISCUSSION

Carbon, nitrogen sources, temperature and pH are some essential physicochemical conditions or factors that affect laccase production from microbes. The effects of varying concentrations of mannose, glucose, lactose, sucrose and soluble starch on laccase production were evaluated as shown in Figure 1. The fermentation with mannose had the highest laccase activity at both concentrations of 0.50 and 1.00 g/100 mL (476.50 and 391.16 U/min) respectively, when compared to glucose (264.11 and 258.21 U/min) and lactose (181.87 and 217.01 U/min) at the same concentrations. The medium containing more complex carbon sources (sucrose and soluble starch) gave the least laccase activity (Figure 1a).

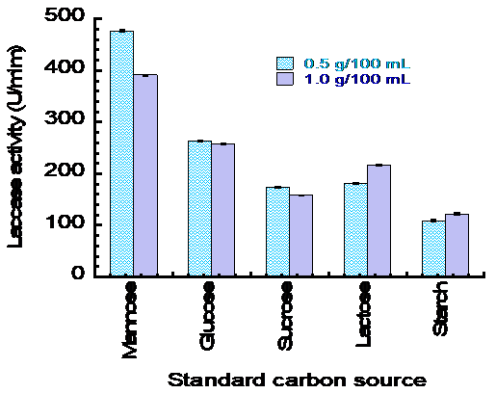

Figure 1a: Standard carbon sources on laccase production.

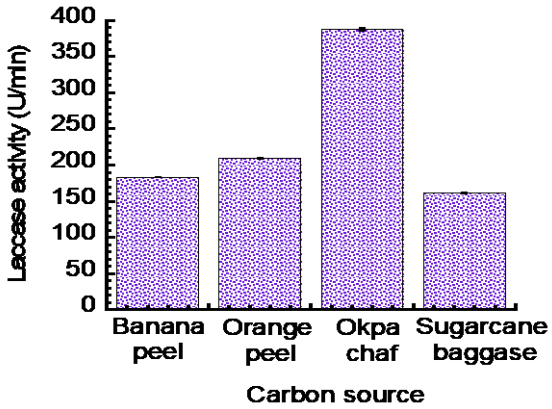

Figure 1b: Agro-waste as carbon sources in laccase production

Thus, monosaccharides supported the organism growth and concomitant laccase secretion more than disaccharides and soluble starch. The highest enzyme activities obtained using mannose as the sole carbon source in this study is supported by the report of Ire and Vinking [27], that mannose is a good inducer of enzyme in microbes. Whereas complex carbon sources (soluble starch) have been reported to reduce enzyme production in fungi [27] obtaining the highest laccase activity in medium containing lower concentrations of mannose corroborates the earlier report that excess carbon impedes the production of laccase [4]. Earlier researchers have reported glucose and fructose as carbon sources for enzyme production by fungal species [27]

In the reaction medium, the substitution of some common cellulosic agro-waste (banana and orange peels, Bambara chaff and sugarcane bagasse) as the main carbon source yielded high laccase activity (387.13 U/min) in the medium containing Bambara (okpa) chaff (Figure 1b).

Banana and orange peels containing fermentation medium, produced laccase activity of 183.41 and 209.09 U/min respectively, considerably lower than that obtained using okpa chaff, with sugarcane bagasse giving the least enzyme yield. As reported elsewhere, banana peels served as best carbon sources, with KNO_3 serving as nitrogen source for enzyme production by *Aspergillus* species [27]. Orange peel [28], mombin pulp residue [29], cassava peel [27] are other agro-residues that have been reported to enhance enzyme production by fungi. This could be due to the presence of different sugars in the residues. Also, wheat bran has been reported to improve enzyme production by *Aspergillus* species [30]. Several compounds (KNO_3 and urea) have been reported to be good sources of nitrogen for enzyme production by fungal species. This study is in agreement with the report of Kashyap et al. [31] that enzyme production is enhanced by supplementing the medium with yeast extract, peptone and ammonium sulphate as nitrogen sources, thereby supporting the findings of this report where Bambara nut chaff fermentation medium produced the highest laccase. This approach could reduce the cost of laccase production significantly [32]. Furthermore, the best pH for laccase production was evaluated between acidic and alkaline regions. At pH 6.5, highest laccase activity of 758.99 U/min was obtained (Figure 2).

There was a steady increase in laccase activity starting from the acidic region until it peaked at pH 6.5, beyond which a sharp decrease in laccase activity was

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observed in the alkaline region. Previous researchers have reported the extraction of laccases from *P. chrysosporium* [33], *P. ostreatus* [34], *T. versicolor* [35], *T. harzianum* [7, 36] and *R. vernicifera* [17] where they opined that one of the factors that enhance extracellular production of laccase was pH. The production of extracellular laccase at pH 6.5 by the organism in this study makes it a good industrial enzyme-producing candidate since this is near water pH. Previously, acidic fermentation medium (pH5.0) was optimum for enzyme production [37]. Similarly, the effects of pH on the extracellular secretion of enzymes were previously recorded by Kim et al. [38] and Nnolim et al. [39].

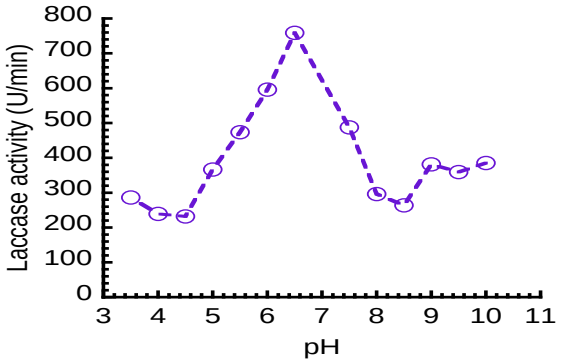

Figure 2: Effect of pH on laccase production by *Aspergillus sp KIGC*

Table 1 presents a summary of the enzyme purification processes. After three steps of laccase purification, 68.90% yield was obtained, with 2.55 purification folds and specific activity, 462.16 U/min-1. The data obtained are indications that the purification steps were adequate in purifying the enzyme.

Furthermore, graded concentrations of mannose (1.00-8.00 g/mL) were added to the enzyme production medium. The highest laccase activity (439.16 U/min) was observed and recorded at 3.00 g/100 mL. Subsequent increase in mannose concentration produced laccase activity of 209.70, 132.09 and 76.56 U/min at 5.00 - 8.00 g/100 mL respectively**.** Nitrogen is an important element for microbial growth and subsequent secretion of laccase. The sole nitrogen source was substituted in the production medium using each Soybean extract, beef extract, peptone and casein. Soybean extract was recorded as the best source of nitrogen with laccase activity of 679.72 U/min. Other nitrogen sources used in the medium induced the production of the enzyme at lower concentrations of 474.13, 344.02 and 270.80 U/min for peptone, beef extract and casein, respectively.

The molecular weight of fungal laccase as obtained from SDS-PAGE was 66 KDa (Figure 3a). Also, the single band observed was an indication that the enzyme was a monomer.

The best day for laccase production by the organism was evaluated from day 1 to day 15. On day 8, highest laccase activity of 639.18 U/min was recorded (Figure 3b), thus making Day 8 the best day for laccase production by the organism, with Bambara nut husk and soybean extract as sole carbon and nitrogen sources respectively. Further increase in the day of enzyme production led to decrease in laccase activity.

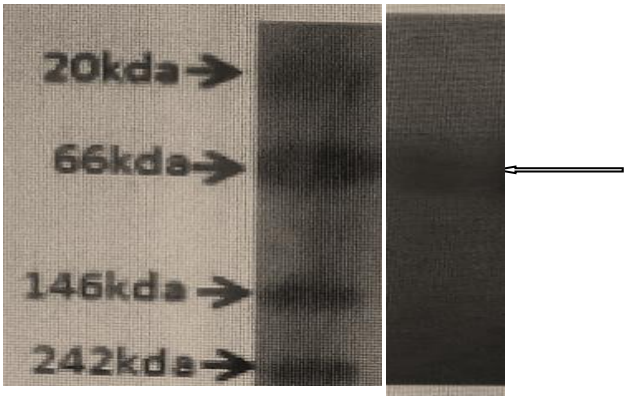

Figure 3a: SDS-PAGE of purified laccase

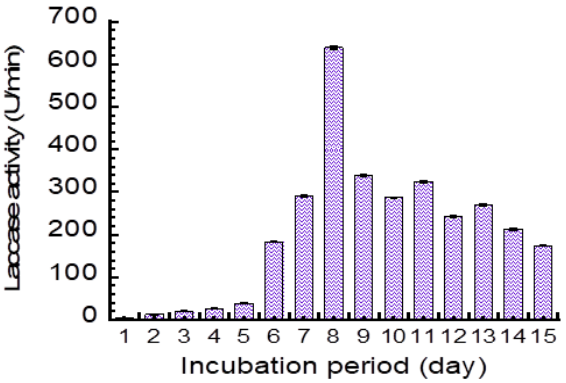

Figure 3b: Time course study of Aspergillus sp KIGC over the incubation period

Table 1: Purification profile of *KIGC* laccase

Steps	Protein (mg)	Total Activity (U/min)	Specific Activity (U/min^{-1})	Percentage Yield	Purification folds

Crude Laccase	261.06	47170.30	180.68	100	1.00
Ammonium Sulphate	156.72	40080.22	255.74	84.96	1.41
Ion exchange	99.12	36195.41	365.16	76.73	2.02
Gel filtration	70.33	32503.90	462.16	68.90	2.55

X-ray Diffraction (XRD) patterns of both IOMNP (control) and immobilized enzyme (Figure 4). There is no sharp diffraction peak in IOMNP immobilized enzyme, unlike the pure sample (the control). This suggests that the formulated nano-composition contains no crystalline phases which could be attributed to immobilization of laccase. The immobilization of the enzyme in the magnetic nanoparticle is believed to distort the lattice arrangement of the iron atoms resulting in high amorphous phases. This confirms the presence of laccases immobilized on the IONPs as was earlier reported [48].

Dynamic light scattering (DLS) was used to measure or determine the size of synthesized particles. The control nanoparticle gave peak diameter of 76.2 nm and nanoparticle immobilized laccase had three modal peaks at 49.14 nm, 304 nm and 4770 nm. The formation of a large second peak showed the presence of enzyme-nanoparticle complex aggregate. This caused reduction in the immobilized enzyme's thermostability but did not have noticeable effect on the enzyme catalytic ability. The size of the nanoparticle immobilized enzyme increased, showing that there was immobilization (Fig. 5). The use of DLS to measure and authenticate the size of nanoparticles in this study has been corroborated by the reports of several research reports on silver nanoparticles and paramagnetic nanoparticles immobilized fungal peroxidase [47; 48].

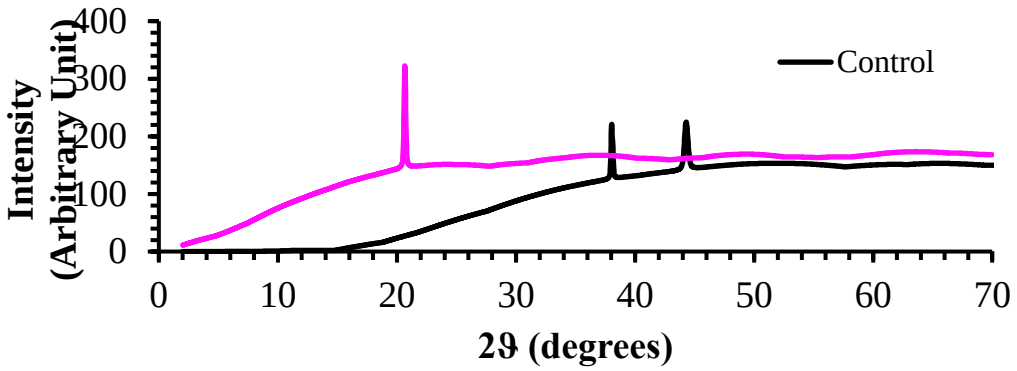

Figure 4: X-ray diffraction of magnetic nanoparticle

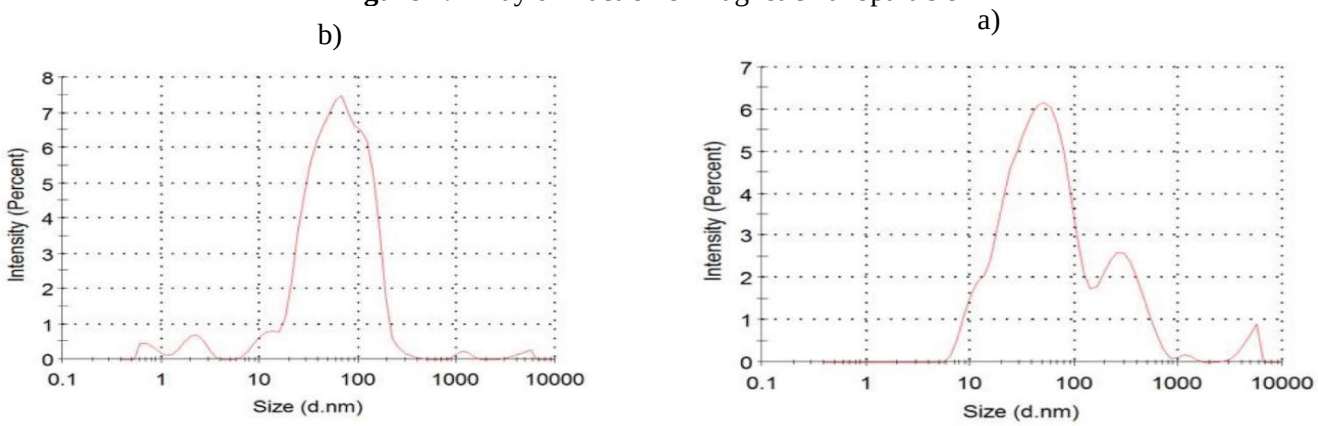

Figure 5: Dynamic light scattering data showing the intensity size distribution of (a) control (IOMNP), and (b) synthesized magnetic nanoparticle.

The thermostability of the nanoparticle of immobilized laccase was studied using DSC. The control nanoparticle had melting temperature of 193.97 °C, while the IOMNP immobilized laccase melting temperature was 110.57 °C, respectively (Fig.

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6). The immobilized enzyme's temperature reduced after the incubation to 110.57 °C. This reduction could be attributed to the presence of high concentrations of iron molecules in the reaction mixture which has led to low thermostability but, a concomitantly high magnetic property that will make it easy to separate the enzyme from their products.

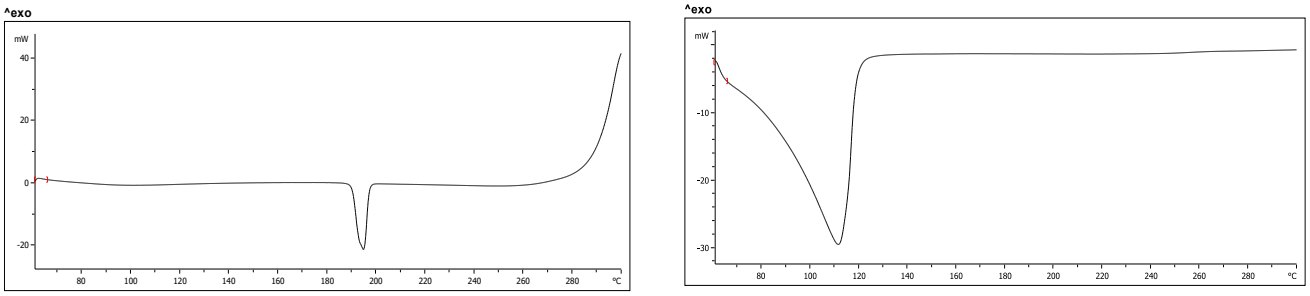

Figure 6: DSC thermogram of (a) synthesized magnetic nanoparticle (control) and (b) magnetic particle immobilizing laccase.

After immobilization, the optimum temperature obtained for both free and immobilized laccase was 55 °C, with activities of 1474.37 and 910.55 U/min respectively (Fig. 7a). Similarly, the optimum pH for immobilized laccase after immobilization on the IOMNP was 7.5 (833.10 U/min), while the free enzyme showed two peaks at pHs 5.5 and 8.0, with laccase activity of 955.81 and 894.00 U/min, respectively (Fig. 7b).

Several optimal conditions of pH and temperature have been reported for laccase depending on the source of the enzyme. Hasan [44] reported 45 °C and pH 5.5 as the optimum temperature and pH for laccase activity extracted from a strain of *A. niger*, while *T. harzianum* and *A. faecalis* laccase showed optimum activities at pH 5.5 and 30 °C and pH 8.0 and 80 °C respectively [45]. These pH values corroborate the finding of this study at both their free (pH5.5) and immobilized state (pH8.0). Laccase of *Trametes spp* origin gave its optimum activity at pH 2.7 and 60°C [46]. The vast differing optimum conditions of laccases from different sources suggest that conditions such as fungal strain and sources of fungi isolation affect their characteristics.

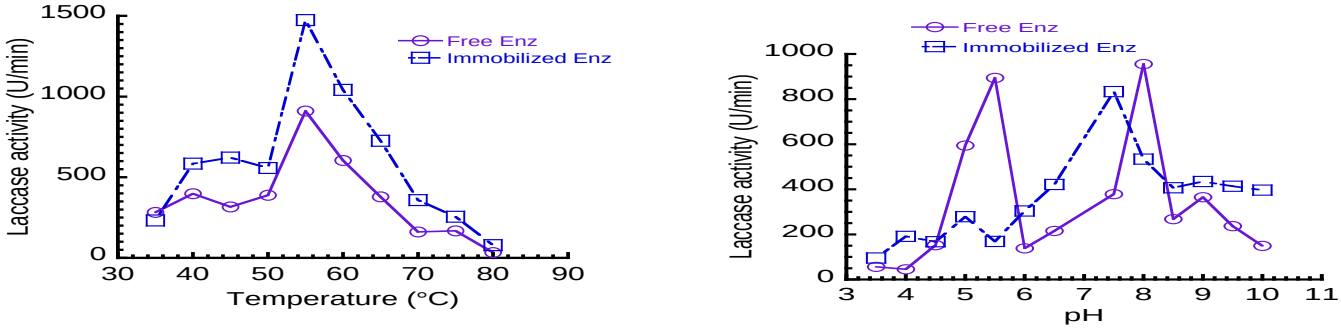

Figure 7: (a) Effect of temperature on free and immobilized laccase activity, (b) Effect of pH on free and immobilized laccase activity

The rate at which the enzymes metabolized ABTS and O-dianisidine per second was 1.971 and 13.308 mol/S^{-1}, and 110.010 and 22.817 mol/S^{-1} respectively. ABTS is the most commonly used laccase substrate due to its high sensitivity and chromatogenic potential, making it possible to read and quantify using a spectrophotometer. The Michealis constants (K_M) obtained for the free laccase when ABTS and O-dianisidine served as substrates were 1.851 and 2.941 mM and V_{max} of 133.330 and 1428.570 µmol/min. For immobilized laccase, 1.041 and 2.631 mM were the *Michealis* constants (K_M) obtained for ABTS and O-dianisidine as substrates and V_{max} of 2500 and 1310.540 µmol/min, respectively. Similarly, 114.521 and 60.033 min^{-1} were the *K*cat values obtained for immobilized laccase using ABTS and O-dianisidine as substrates, respectively.

The effect of methanol, chloroform and ethanol were evaluated at concentrations of 20, 40, 60 and 80%

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respectively on both free and immobilized laccase activity after 60 mins of incubation. At 40%, more than 70% of laccase activity was retained after 1 h incubation time (Fig. 8a). Furthermore, at 80% concentration of ethanol and methanol, the immobilized laccase retained more than 50% of the enzyme activity (Fig. 8b). Chloroform at all concentrations had more inhibitory potentials than other solvents studied, with ethanol showing more activating ability.

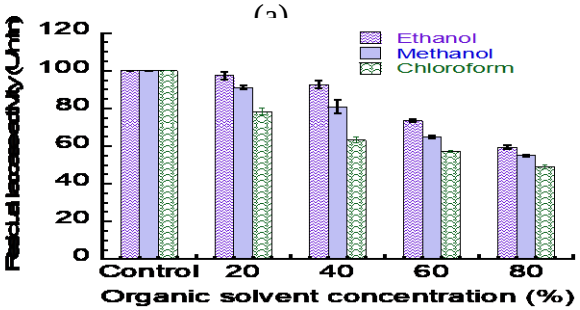

Figure 8a: Effect of organic solvents on free immobilized laccase activity

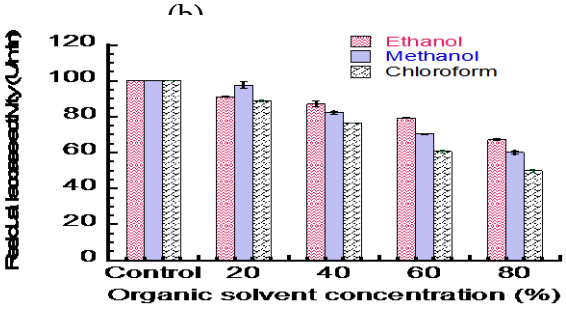

Figure 8b: Effect of organic solvents on magnetic nanoparticle immobilized laccase activity

SYNTHETIC DYE DECOLORIZATION RESULTS

The ability of laccase to decolorize some synthetic dyes was studied for a period of 96 h at varying concentration. The nanoparticle immobilized laccase showed higher percent decolorization potential among different types of dyes than the free enzyme (Figure 9). Furthermore, immobilized laccase decolorized Basic blue 22 (94 %), Azo brilliant black (90 %) and Vat Blue 5 (94 %) more than other dyes when compared to the control (H_2O_2), with more than 80% decolorization rate obtained, while the decolorization rate of other dyes (Azo yellow 6 (58 %) and Acid blue 74 (79 %) was below 80 % when compared to the control (Figure 9). There were differences at the rate at which the enzymes decolorized the synthetic dyes, especially Basic blue 22. This could be attributed to the differences in the chemical nature of the dyes. Omeje et al. [10] had reported the considerable ability of laccase in synthetic dyes decolorization, suggesting the ability of fungal KIGC laccase to be employed in the decontamination of environmental effluents especially from textile industries as these compounds are predominant [40].

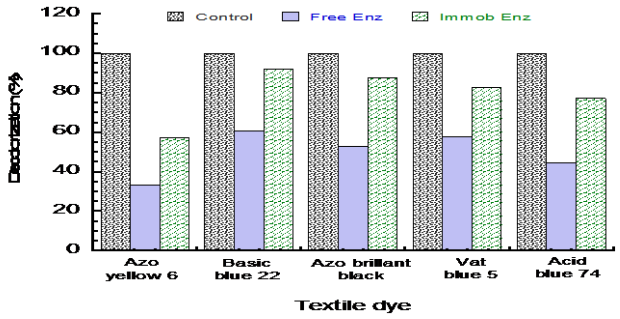

Figure 9: Decolorization of synthetic dyes by conjugated enzyme

CONCLUSION

Aspergillus sp KIGC secreted laccase of high biocatalytic activity purified to homogeneity with molecular weight 66kda when some agro waste residues served as sole carbon sources. The enzymes exhibited optimal activities at pHs 5.5/8.0 and temperature of 55.0 ◦C respectively. To maintain the recoverability and sustainability of the industrial and environmentally important enzyme, laccase was immobilized on a cheap, non-toxic and readily available iron oxide nanoparticle. There was adequate nanoparticle synthesis and laccase immobilization on the nanoparticle as evidenced by the characterization results, and the immobilization enhanced the catalytic abilities of the enzyme more than the free enzyme, with the concomitant high synthetic dye decolorization efficiency, though the thermostability of the immobilized laccase reduced. *Aspergillus sp KIGC* laccase showed considerable laccase activity (decolorization of synthetic dyes) at mild bioprocess conditions. These mild condition attributes of *Aspergillus sp KIGC* laccase suggest its potential in textile wastewater and in the process of bioremediation. Thus, future research works should study and develop protocols for the laccase gene overexpression at an industrial scale-up purposes.

REFERENCES

[1]. Gopinath, S. C. B. Anbu, P. Lakshmipriya, T. and Hilda, A "Strategies to characterize fungal lipases for applications in medicine and dairy industry", *BioMed Research International*, 154549, 2013.

[2]. Masjoudi, M. Golgoli, M. Nejad, Z. G. Sadeghzadeh, S. and Mehdi, B. S. M

https://doi.org/10.4314/njt.v44i3.14

"Pharmaceuticals removal by immobilized laccase on polyvinylidene fluoride nanocomposite with multi-walled carbon nanotubes", *Chemosphere*, 263, 128043, 2021.

[3]. Omeje, K. O. and Eze, S. O. O "Properties of partially purified peroxidase extracted from the fruit of *Solanum* spp grown in Enugu north (Variety A) and Plateau central (variety B) zones of Nigeria", *Biokemistri*, 30, p. 1–12, 2018.

[4]. Shraddha, R. S. Simran, S. Mohit, K. and Kumar, A "Laccase: microbial sources, production, purification and potential biotechnological application", *Enzyme Research*, 217861, 2011.

[5]. Reiss, R. Ihssen, J. Richter, M. Eichhorn, E. and Schilling, B "Laccase versus laccase-like multi-copper oxidase: A comparative study of similar enzymes with diverse substrate spectra" *PLoS ONE*, 8(6), e65633, 2013. journal.pone.0065633.

[6]. Kumar, P. Kamle, M. and Singh, J "Biochemical characterization of Santalum album (Chandan) leaf peroxidase" *Physiology and Molecular Biology of Plants*, 17, p. 153–159; 2011.

[7] Abd El Monssef, R.A. Hassan, E. A. and Ramadan, E. M "Production of laccase enzyme for their potential application to decolorize fungal pigments on aging paper and parchment" *Annals of Agricultural Science*, 61, p. 145–154, 2016.

[8]. Usman, M.M. Dadrasnia, A. Lim, K.T. Mahmud, A.F. and Ismail, S "Application of biosurfactants in environmental biotechnology; Remediation of oil and heavy metal" *AIMS Bioengineering*, 3, p. 289–304, 2016.

[9]. Udayasoorian, C. and Prabu, P. C. "Biodegradation of phenols by ligninolytic fungus Trametes versicolor" *Journal of Biological Sciences*, 5, p. 824-827, 2005.

[10]. Omeje, K. O. Nnolim, N. E. Ezema, B. O. Ozioko, J. N. and Eze, S. O. O "Synthetic dyes decolorization potential of agroindustrial waste-derived thermo-active laccase from Aspergillus species" *Biocatalysis and Agricultural Biotechnology*, 29, 101800, 2020.

[11]. Patel, S.K.S. Otari, S.V. Li, J. Kim, D.R. Kim, S.C. Cho, B.K. Kalia, V.C. Kang, Y.C. and Lee, J.K "Synthesis of cross-linked protein-metal hybrid nanoflowers and its application in repeated batch decolorization of synthetic dyes". *Journal of Hazardous Materials*, 347, p. 442–450, 2020.

[12]. Fernandes, A.L. Daniel, S. Ana P.M. Tavares, A. and Xavier, M. R. B "EDTA-Cu (II) chelating magnetic nanoparticles as a support for laccase immobilization Raquel A". *Chemical Engineering Science*, 158, p. 599–605, 2017.

[13]. Zille, A. Tzanov, T. Gübitz, G. M. and Cavaco-Paulo, A "Immobilized laccase for decolourization of Reactive Black 5 dyeing effluent". *Biotechnology Letters*, 25, p. 1473–1477, 2003.

[14]. Peralta-Zamora, P. Pereira, C. M. Tiburtius, E. R. L. Moraes, S. G. Rosa, M. A. Minussi, R. C. and Durán, N "Decolorization of reactive dyes by immobilized laccase," *Applied Catalysis B: Environmental*, 42, p. 131–144, 2003.

[15]. Ameri, A. Taghizadeh, T. Talebian-Kiakalaieh, A. Forootanfar, H. Mojtabavi, S. Jahandar, H. Tarighi, S. and Faramarzi, M. A "Bio-removal of phenol by the immobilized laccase on the fabricated parent and hierarchical NaY and ZSM-5 zeolites," *Journal of the Taiwan Institute of Chemical Engineers*, 120, 300312, 2021.

[16]. Masjoudi, M. Golgoli, M. Nejad, Z. G. Sadeghzadeh, S. Mehdi-Borghei, S. M "Pharmaceuticals removal by immobilized laccase on polyvinylidene fluoride nanocomposite with multi-walled carbon nanotubes," *Chemosphere*, 263, 128043, 2021.

[17]. Monier, A. A. M. Zhenbin, C. Ke, L. and Boyuan, Z "The study of Fe3 O4 @SiO2 -NH2 nano-magnetic composite modified by glutaraldehyde to immobilized penicillin G acylase," *Turkish Journal of Chemistry*, 46, p.103-115; 2022.

[18]. Patel, S. K. S. Otari, S. V. Li, J. Kim, D. R. Kim, S. C. Cho, B. K. Kalia, V. C. Kang, Y. C. and Lee, J. K "Synthesis of cross-linked protein-metal hybrid nanoflowers and its application in repeated batch decolorization of synthetic dyes," *Journal of Hazardous Materials*, 347, p. 442–450, 2018.

[19]. Qingjing, C. Xiaodan, W. Leipeng, C. Yanjuan, L. Xuan, L. Jianwen, C. Guiming, F. Yuhuan, L. and Roger, R "Green production of a yellow laccase by Coriolopsis gallica for phenolic pollutants removal," *AMB Express*, 12, p. 96, 2022.

[20]. Kalra, K. Chauhan, R. Shavez, M. and Sachdeva, S "Isolation of laccase producing Trichoderma spp. and effect of pH and temperature on its activity," *International Journal of Environmental Science and Technology*, 5, p. 2229–2235, 2013.

[21]. Bradford, M. M. "A rapid and sensitive method for the estimation of microgram quantities of

protein utilizing the principle of protein-dye binding," *Analytical Biochemistry*, 72, p. 248–254, 1976.

[22]. Laemmli, U. K "Cleavage of structural proteins during the assembly of the head of bacteriophage T4," *Nature*, 227, p. 680–685, 1970.

[23]. Switzer, R. C. Merril, C. R. and Shifrin, S. A. "Highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels," *Analytical Biochemistry*, 98, p. 231–237, 1979.

[24]. Ranjbakhsh, E. Bordbar, A.K. Abbasi, M. Khosropour, A.R. and Shams, E "Enhancement of stability and catalytic activity of immobilized lipase on silica-coated modified magnetite nanoparticles," *Chemical Engineering Journal*, 179, p. 272–276, 2012.

[25]. Cruz-Izquierdo, Á. Picó, E. A. López, C. Serra, J. L. and Llama, M. J "Magnetic cross-linked enzyme aggregates (mCLEAs) of Candida antarctica lipase: An efficient and stable biocatalyst for biodiesel synthesis," *PLoS One*, 9, p. 1–22, 2014.

[26]. Victor, C. C. Omeje, K. O. Ezugwu, A. L. Eze, S. O. O. Chilaka, F. C. Onwurah, I. N. E. and Ukoha P. O "Decolorization of synthetic dyes using partially purified peroxidase from green cabbage," *Songklanakarin Journal of Science and Technology*, 41, p. 783–787, 2019.

[27]. Ire, F. S. and Vinking, E. G "Production, purification and characterization of polygalacturonase from Aspergillus niger in solid state and submerged fermentation using banana peels," *Journal of Advances in Biology & Biotechnology*, 10(1), p. 1-15, 2016.

[28]. Maktouf, S. Neifar, M. Drira, S. J. Baklouti, S. Fendri, M. and Châabouni, S. E "Lemon juice clarification using fungal pectinolytic enzymes coupled to membrane ultrafiltration,". *Food Bioproducts Process*, 92, p. 14–19, 2014.

[29]. da Câmara Rocha, J. da Silva Araújo, J. de Paiva, W.K.V. Ribeiro, E.S.S. de Araújo Padilha, C.E. de Assis, C.F. dos Santos, E.S. de Macêdo, G.R. and de Sousa Junior, F.C "Yellow mombin pulp residue valorization for pectinases production by A. niger IOC 4003 and its application in juice clarification," *Biocatalysis and Agricultural Biotechnology*, *30*, 101876, 2020.

[30]. Dange, V. U. and Harke, S "Production and purification of Pectinase by fungal strain in solid-state fermentation using agro-industrial bioproduct," *World Journal of Microbiology and Biotechnology*, 16, p. 277–282, 2018.

[31]. Kashyap, D. R. Vohra, P. K. Chopra, S. and Tewari, R "Applications of pectinases in the commercial sector: a review". *Bioresource Technology*, 77, p. 215–227, 2001.

[32]. Puspita, K. Chiari, W. Abdulmadjid, S.N. Idroes, R. Iqhrammullah, M "Four decades of laccase research for wastewater treatment: Insights from bibliometric analysis," *International Journal of Environmental Research and Public Health*, 20, p. 308-316, 2023.

[33]. Viswanath, B. Chandra, M. S. Pallavi, H. and Reddy, B. R "Screening and assessment of laccase producing fungi isolated from different environmental samples," *African Journal of Biotechnology*, 7(8), p. 1129–1133, 2008.

[34]. Palmieri, G. Giardina, P. Bianco, C. Fontallella, B. and Sannina, G "Copper induction of laccase isoenzyme in the lignolytic fungus Pleurotus ostreatus". *Applied Microbiology and Biotechnology*, 66, p. 920–924, 2000.

[35]. Bourbonnais, R. Paice, M. G. Reid, I. D. Lanthier, P. Yaguchi, M "Lignin oxidation by laccase isozymes from *Trametes versicolor* and role of the mediator 2, 22-azinobis(3-ethylbenzthiazoline-6-sulfonate) in kraft lignin depolymerization," *Applied and Environmental Microbiology*, 61 (5), p. 1876–1880, 1995.

[36]. Holker, U. Dohse, J. and Hofer, M "Extracellular laccases ¨in ascomycetes *Trichoderma atroviride* and *Trichoderma harzianum," Folia Microbiologica*, 47, p. 423–427, 2002.

[37]. Nnolim, N. E. Ntozonke, N. Okoh, A. I. and Nwodo, U. U "Exoproduction and characterization of a detergent-stable alkaline keratinase from Arthrobacter sp. KFS-1". *Biochimie*, 177, p. 53–62, 2020.

[38]. Kim, J. M. Lim, W. J. and Suh, H. J "Feather-degrading Bacillus species from poultry waste," *Process Biochemistry*, 37, p. 287–291, 2001.

[39]. Nnolim, N. E. and Nwodo, U. U "Bacillus sp. CSK2 produced thermostable alkaline keratinase using agro-wastes: keratinolytic enzyme characterization," *BMC Biotechnology*, 20, p. 65-76, 2020.

[40]. Mandic, M. Djokic, L. Nikolaivits, E. Prodanovic, R. O'Connor, K. Jeremic, S. Topakas, E. and Nikodinovic-Runic, J "Identification and characterization of new laccase biocatalysts from Pseudomonas species suitable for degradation of synthetic textile dyes," *Catalysts*, 9, p. 629-668, 2019.

[41]. Abd El Monssef, R.A. Hassan, E.A. Ramadan, E.M "Production of laccase enzyme for their potential application to decolorize fungal

https://doi.org/10.4314/njt.v44i3.14

pigments on aging paper and parchment," *Annals of Agricultural Sciences*, 61, p. 145–154, 2016.

[42]. Anoushka, R. Anupam, C. S. and Wazed, A "Green chemistry: its opportunities and challenges in colouration and chemical finishing of textiles," *Sustainable Chemistry and Pharmacy*, 27, 100689, 2022.

[43]. Paul, A. and Eghbali, N "Green Chemistry: Principles and Practice," *Chemical Society Review*, 39, p. 301–312, 2010.

[44]. Hasan, S. Anwar, Z. Khalid, W. Afzal, F. Zafar, M. Ali, U. Refai, M.Y. Afifi, M. AL-Farga, A. Aljobair, M.O "Laccase production from local biomass using solid state fermentation". *Fermentation*, 9, p. 179-189, 2023. https://doi.org/10.3390/ fermentation9020179.

[45]. Seema, M. Sharma, S. C. and Shailendra, K. A "Isolation and characterization of an alkali and thermostable laccase from a novel *Alcaligenes faecalis* and its application in decolorization of synthetic dyes," *Biotechnology Reports*, 25, e00413, 2020.

[46]. Yang, X. Wu, Y. Zhang, Y. Yang, E. Qu, Y. Xu, H. Chen, Y. Irbis, C. and Yan, J. A "Thermo-active laccase isoenzyme from *Trametes trogii* and its potential for dye decolorization at high temperature," *Frontiers in Microbiology*, 11, p. 241, 2020. doi: 10.3389/fmicb.2020.00241.

[47]. Omeje, K.O. Nworah, F.N. Ezema, B.O. and Eze, S.O.O "Enzyme inhibition and antibiotics properties of six (6) weeks stable *C. albidum* leaf silver nanoparticles," *Engineering Proceedings*, 56, p. 196-210, 2023. https://.org/10.3390/ASEC2023-16578.

[48]. Omeje, K.O. Magbo, C. Ossai, E.C. Ozioko, J.N. Ezema, B.O. Nnolim, N.E. and Eze, S.O.O "Immobilization of fungal peroxidase on paramagnetic nanoparticles for synthetic dye decolorization". *Materials Proceedings*, 9, p. 24-32, 2022

[49]. Debnath, R. and Saha, T "An insight into the production strategies and applications of the ligninolytic enzyme laccase from bacteria and fungi," *Biocatalysis and Agricultural Biotechnology*, 26, 101645, 2020.