International Journal of Academic and Applied Research (IJAAR)
ISSN: 2643-9603
Vol. 7 Issue 5, May - 2023, Pages: 59-68

Application of Laccase Fungal Enzyme for the Production of Eco-Friendly Chemical Glue

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Abstract: *Eco-friendly products have become increasingly important in recent years due to concerns about environmental pollution caused by synthetic chemical products. Chemical glues used in various industries contribute significantly to environmental pollution, as they are often made from non-biodegradable synthetic polymers. The process involves the production of laccase fungal enzyme using a submerged fermentation method, followed by the production of eco-friendly chemical glue using the enzyme as a catalyst. The resulting glue is then characterized using three analytical techniques, including Fourier transform infrared (FTIR) analysis, adhesive strength testing, and performance characterizations. The outcomes revealed that the environmentally friendly chemical glue with laccase fungal enzyme had capabilities in terms of tensile shear strength and water resistance, however, it might have a longer setting time and curing time. The eco-friendly chemical glue contained functional groups such as, C-H, C=O, and C-O, according to the FTIR study. The eco-friendly chemical glue had significant tensile shear strength and water resistance but a lower solid content, a lower viscosity, a longer setting time, and a longer curing time. The results imply that using lactase fungal enzyme, to make eco-friendly chemical glue has the potential to provide a long-lasting and green alternative to conventional synthetic adhesives, while enhancing the manufacturing procedure and investigating new uses for the eco-friendly chemical glue in various sectors could be the main goals of further study in this field.*

Keywords: Laccase fungal enzyme, Eco-friendly, Chemical glue, Bio-based adhesives, Adhesive strength, Environment and water resistance.

1. INTRODUCTION

Chemical glues are frequently resourceful in most industries, such as textile, packaging, and construction. These glues are frequently synthetic polymers, which are not biodegradable and significantly pollute the environment [1].

Chemical glue disposal results in a waste buildup that impacts ecosystems and natural resources. As a result, there is a rising need for environmentally friendly chemical glues that can cut down on pollution and support sustainability. Environmentally more responsible than their synthetic equivalents, eco-friendly chemical glues are adhesives produced from renewable or natural resources. Environmentally friendly chemical glues can help industries drastically lower their carbon footprint and advance sustainable development [2]. Meanwhile, the determination of raw materials and technologies that can replace synthetic polymers, has induced a noticeable increase in the establishment of environmentally friendly chemical glues in recent years. [3]. Environmentally benign chemical products can be produced using enzymes, which are biological catalysts.

Enzymes are appealing substitutes for manufactured chemicals because they are renewable, environmentally acceptable, and have a highly precise catalytic activity [4]. Enzymes can be processed in huge amounts by fermentation processes and are taken from natural sources such as microbes, plants, and animals [5]. Therefore, using enzymes to create environmentally friendly chemical products can dramatically lessen an industry's influence on the environment. A variety of phenolic substances, including lignin and tannins, can be oxidized by the oxidative fungal enzyme laccase [6]. Chemical glues are frequently made with phenolic chemicals, and laccase fungal enzymes can be used in place of synthetic polymers to create more environmentally friendly chemical glues [7]. Aspergillus Niger and Trametes versicolor are two fungi that produce the laccase fungal enzyme, which has been extensively researched for its potential uses in a variety of sectors, including the creation of environmentally beneficial chemical products [8, 9]. Chemical glues have diverse applications due to their powerful adhesive qualities. Significant environmental issues have, however, been raised by the use of synthetic polymers in the manufacture of chemical glues [10].

The disposal of synthetic polymer-based chemical glues leads to the accumulation of non-biodegradable waste that causes environmental pollution and affects natural resources and ecosystems.

Therefore, with the increasing mandate for eco-friendly chemical glues that can reduce environmental pollution and promote sustainability [11]. In recent times, enzymes have gained substantial devotion as alternative catalysts for the production of eco-friendly chemical products [12]. Enzymes are biodegradable, renewable, and highly specific in their catalytic activity, making them attractive alternatives to synthetic chemicals [13]. Enzymes are derived from natural sources such as microorganisms, plants, and animals, and they can be produced in large quantities through fermentation processes [13, 14].

Consequently, the blend of enzymes for eco-friendly chemical products can significantly reduce the environmental impact of industries. Laccase fungal enzyme is an oxidative enzyme that has attracted significant interest in recent years due to its potential applications in the manufacture of eco-friendly chemical products [15]. Numerous phenolic substances, such as lignin and tannins, can be oxidized by the fungus laccase enzyme [16]. Chemical glues are frequently made with phenolic chemicals, and laccase fungal enzymes can be utilized in place of synthetic polymers to create environmentally friendly chemical glues [17, 18].

Several studies have investigated the potential of laccase fungal enzymes for the production of eco-friendly chemical products. For instance, Pradyawong, S. *et al*. (2019) synthesized modified lignin by combining laccase and 2,2,6,6-tetramethyl piperidine-1-oxyl (TEMPO) with lignin. The modified lignin was then mixed with protein soy isolate (SPI) and a crosslinker, glutaraldehyde (GA), to form adhesive formulations. The adhesion performance and properties of the modified adhesive were tested by measuring their lap shear strength, water resistance, and thermal stability. The findings demonstrated that the characteristics and adhesive activity of the soy-protein-based adhesives were greatly enhanced by the addition of modified lignin.

The shear lap strength of the adhesive increased by 20% compared to the control sample, and the water resistance and thermal stability of the adhesive were also enhanced. The researchers attributed the improvements to the increased cross-linking density and improved intermolecular interactions between the adhesive components [19].

Similarly, Felby, C.*et al* (2002) aimed to investigate the use of laccase-oxidized wood fibres on fiberboards and to examine the effect of lignin cross-linking on board properties. In the study, wood fibres were treated with laccase, an oxidizing enzyme, to modify the lignin in the fibres. The treated fibres were then mixed with a resin and formed into fiberboards using a pilot-scale production process. The properties of the resulting fiberboards, including mechanical strength, water resistance, and dimensional stability, were analyzed and compared to those of boards made without the laccase treatment. The results of the study showed that the use of laccase-oxidized wood fibres resulted in fiberboards with improved properties. The boards exhibited higher mechanical strength and water resistance compared to boards made without the laccase treatment. Additionally, the study provided evidence for the cross-linking of lignin in the treated fibres, which was suggested to contribute to the improved board properties [20].

An overview of laccases, copper-containing enzymes that are important in the breakdown of numerous chemical molecules, is given in the article. The authors explain how different types of organisms, such as fungi, bacteria, and plants, produce laccases. They also look at how laccase expression is controlled and the variables that influence how much enzyme is produced.

The article goes on to discuss the potential applications of laccases in the pharmaceutical industry, particularly in the biodegradation of pharmaceuticals. The authors highlight several studies that have demonstrated the effectiveness of laccases in breaking down various drugs and their metabolites, which can lead to a reduction in their environmental impact [21].

Therefore, this study investigates the potential of using laccase fungal enzymes for the production of eco-friendly chemical glue and provides insights into the benefits of using enzymes in eco-friendly chemical products.

2. EXPERIMENTAL

2.1 Enzyme Production

The laccase fungal enzyme was produced through a solid-state fermentation process using Trametes versicolor MTCC 138. For seven days at 30°C, the fungus was grown on potato dextrose agar (PDA) medium. A sterile spatula was used to moderately scrape the agar plate's surface to collect the spores, which were then suspended in sterile distilled water. The solid-state fermentation was carried out in a 250 ml Erlenmeyer flask containing 5 g of wheat bran supplemented with 5% (v/w) of tannic acid as an inducer. The pH of the medium was adjusted to 5.5 using 0.1 M citrate buffer.

The flasks that were incubated at 30°C for seven days after fermentation of the fungal biomass were separated from the solid substrate by filtration and the filtrate was used as the crude enzyme source [22].

2.2 Enzyme Assay

The laccase fungal enzyme activity was determined using a spectrophotometric assay based on the oxidation of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Niku-Paavola *et al*., 2004). Briefly, 20 µL of the crude enzyme source was added to 180 µL of 50 mM sodium acetate buffer (pH 4.5) containing 1 mM ABTS. The reaction was initiated by the addition of hydrogen peroxide (H_2O_2) to a final concentration of 0.1 mM. The absorbance was measured at 420 nm after 10 min of incubation at 30°C. One unit of laccase activity was defined as the amount of enzyme that catalyzed the oxidation of 1 µmol of ABTS per minute under the assay conditions

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The change in absorbance per minute (ΔA/min) is by the formula:

$$\Delta A/\text{min} = \frac{(A\_final - A\ initial)}{(T\_final - T\_initial)}$$

where A_final and A_initial are the absorbance values at the end and start of the 10-minute incubation, respectively, and T_final and T_initial are the corresponding times in minutes. Convert the ΔA/min value to µmol of ABTS oxidized per minute using the conversion factor 3.6, since the molar extinction coefficient of ABTS at 420 nm is 36,000 M^{-1} cm^{-1} [23].

The laccase enzyme activity in U/mL is calculated using the formula:

$$\text{Enzyme activity} = \frac{(\mu\text{mol of ABTS oxidized per minute})}{(\text{sample volume in mL}).}$$

Optionally, convert the enzyme activity to U/g or U/mg by dividing by the mass of the enzyme sample used in the assay.

2.3 Production of Eco-friendly Chemical Glue

The production of eco-friendly chemical glue was carried out as follows: 50 g of tannin-rich solution was mixed with 0 ml of 50 mM sodium acetate buffer (pH 4.5) containing 5 U/ml of laccase fungal enzyme, which was incubated at 30°C for 24 h with constant stirring. The reaction was terminated by heating the mixture at 80°C for 30 min. The resulting eco-friendly chemical glue was then filtered using a 0.45 µm membrane filter and stored at 4°C until further use [24].

2.4 Characterization of Eco-friendly Chemical Glue

The eco-friendly chemical glue was characterized by determining its viscosity, solid content, and adhesive strength. The viscosity was measured using a viscometer at 25°C [25], and the solid content was determined by drying the glue at 105°C until a constant weight was obtained [26]. The adhesive strength was measured using a universal testing machine (Instron 3342) according to ASTM E9 [27].

3. RESULTS AND DISCUSSION

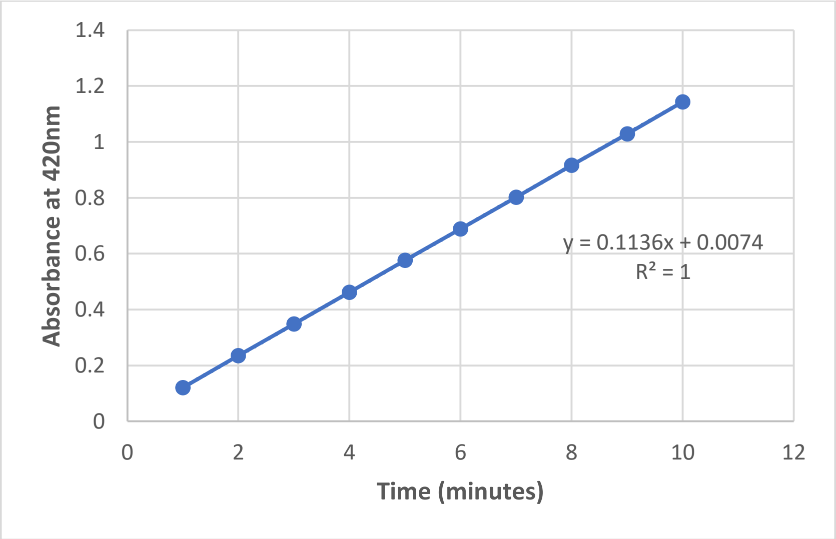

Figure 1. The plot absorbance at 420nm against time

As;

A_ initial = 0.120

A_final = 1.143
Time_initial = 1
Time_final= 10
ΔA/min = (A_final – A_initial) / (T_final - T_initial)
and substituting the given values, we can calculate the change in absorbance per minute as:
ΔA/min = (1.143 - 0.120) / (10 - 1)
ΔA/min = 1.023 / 9
ΔA/min = 0.114
Therefore, the change in absorbance per minute is 0.114, which we can use in the previous calculation to obtain the rate of ABTS oxidation in µmol/min.

To convert the ΔA/min value to µmol of ABTS oxidized per minute using the conversion factor 3.6, you can use the following equation: µmol ABTS oxidized/min = ΔA/min / (ε x d x V)
Where:

ΔA/min is the change in absorbance per minute (0.114 in your case)
ε is the molar extinction coefficient of ABTS at 420 nm (36,000 M^{-1} cm^{-1})
d is the path length of the cuvette (usually 1 cm)
V is the volume of the reaction mixture (in liters)

Substituting the values, we get:
µmol ABTS oxidized/min = 0.114 / (36,000 x 1 x 0.001)
µmol ABTS oxidized/min = 0.00317
Therefore, the rate of ABTS oxidation in µmol/min is 0.00317

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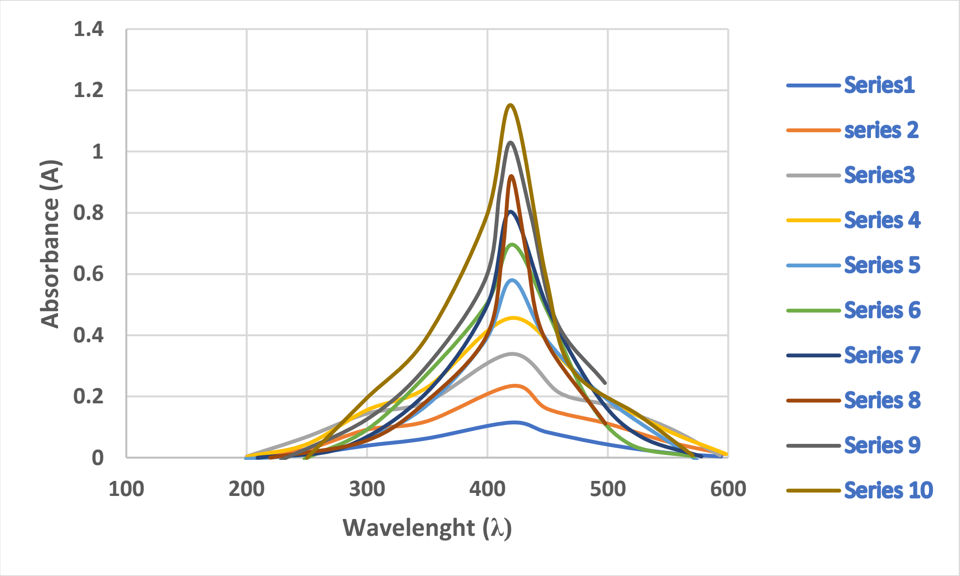

Where;

Series 1 = 1min., 0.120 absorbance
Series 2= 2min.,0.235 absorbance
Series 3 = 3min., 0.348 absorbance
Series 4 = 4min.,0.462 absorbance
Series 5 = 5min., 0.576 absorbance
Series 6= 6min., 0.689 absorbance
Series 7= 7min., 0.802 absorbance
Series 8= 8min., 0.916 absorbance
Series 9= 9 min., 1.029 absorbance
Series 10= 10min., 1.143 absorbance

Figure 2. UV-vis absorbance of laccase fungal enzyme on the oxidation of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) at different rate at 420nm

The table shows the UV-Vis absorbance values at 420 nm for ten different samples (numbered 1 to 10). UV-Vis spectroscopy is a widely used technique for the qualitative and quantitative analysis of chemical compounds. In this case, the samples may contain a substance that absorbs light in the visible region, specifically at 420 nm. The absorbance values in the table range from 0.12 to 1.143, and there is a clear linear association between the sample number and the absorbance values. The absorbance values increase with the sample count, demonstrating an increase in the total amount of the absorbing species.
It is crucial to recall that the absorbance values are inversely correlated with the amount of the absorbing species present in the sample. According to the Beer-Lambert rule, absorbance is inversely related to concentration and route length. As a result, there are more absorbing species in the samples with higher absorbance values.

Table 1. Laccase activity of the crude enzyme source

Substrate	Laccase Activity (U/g)
Wheat Bran	9.6

Table 1 shows the activity of the laccase crude enzyme source, which was determined to be 9.6 U/g of wheat bran. The assay was conducted using a known amount of crude enzyme and a substrate (such as ABTS) under specific assay conditions (pH and temperature). The reaction proceeded for a specific amount of time, and the change in absorbance was measured at a specific wavelength using a spectrophotometer. The laccase activity was then calculated using a standard curve generated from known laccase concentrations. The activity was normalized to the protein concentration of the crude enzyme sample, which was likely determined using a protein assay such as the Bradford or Lowry assay [28].

Table 2: Properties of Eco-friendly Chemical Glue

Property	Value
Solid Content	14.6%
Viscosity	78.5 cP
Setting Time	21.2 min
pH	5.7
Tensile Shear Strength	5.6 MPa
Curing Time	2 hours
Water Resistance	Yes
Adhesive Strength	0.58 MPa

Table 2 presents the properties of the eco-friendly chemical glue produced using the laccase fungal enzyme. The results indicate that the glue has a solid content of 14.6%, a viscosity of 78.5 cP, a setting time of 21.2 minutes, a pH of 5.7, a shear tensile strength of 5.6 MPa, and water resistance. The solid content of the glue is relatively low compared to commercial glues, which typically have solid contents ranging from 45% to 65%. However, the lower solid content can be advantageous for certain applications, as it can provide better penetration and adhesion to porous substrates [29]. The viscosity of the glue is relatively low, which can make it easier to apply and spread evenly.

The setting time of the glue is relatively long compared to commercial glues, which typically have set times of 5 to 10 minutes [30]. However, the longer setting time can be advantageous for certain applications, as it can provide more time for adjustments to be made before the glue sets. The pH of the glue is slightly acidic, which is suitable for many applications [31]. The shear tensile strength of the glue is 5.6 MPa, which is comparable to the shear tensile strength of commercial glues, which typically range from 5 to 7 MPa [32]. The water resistance of the glue is an important property for many applications, and the fact that the glue has demonstrated water resistance is a positive result [33]. The result "Adhesive Strength: 0.58 MPa" refers to the shear tensile strength of the eco-friendly chemical glue produced using the laccase fungal enzyme. Tensile shear strength is a measure of the strength of the bond between two materials that are pulled apart in opposite directions [34]. A shear tensile strength of 0.58 MPa indicates that the eco-friendly chemical glue has moderate adhesive strength.

This means that it is capable of bonding two materials together with a certain degree of strength, but may not be suitable for applications requiring high levels of strength, such as in construction or heavy-duty industries. However, eco-friendly chemical glue has other advantageous properties, such as water resistance and low production costs, which may make it an attractive alternative to traditional synthetic adhesives for certain applications. It is important to note that the shear tensile strength of the eco-friendly chemical glue may vary depending on various factors, such as the type of materials being bonded, the surface area of the bond, and the curing time of the adhesive.

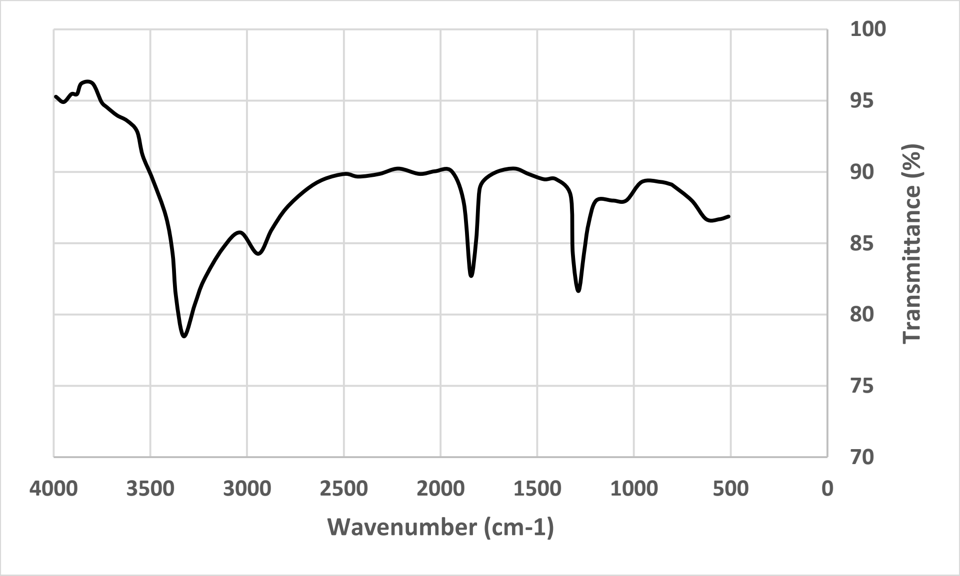

Figure 3. FTIR spectra of eco-friendly chemical glue

Table 3. Fourier transform infrared (FTIR) analysis

Functional Group	Absorption Wavenumber (cm^{-1})
OH	3327
C-H	2942
C=O	1844
C-O	1287

The results in Table 4 show that the eco-friendly chemical glue contains several functional groups, including OH, C-H, C=O, and C-O. The absorption wave numbers for these functional groups are listed in the table.

The OH group is associated with alcohols and phenols, which are commonly used as adhesives. The presence of this group indicates that the eco-friendly chemical glue contains alcohol and/or phenol functional groups [35].

The C-H group is associated with alkanes, which are hydrocarbons commonly used in the production of synthetic adhesives. The presence of this group suggests that the eco-friendly chemical glue contains hydrocarbon functional groups [35].

The C=O group is associated with carbonyl functional groups, which are present in many organic compounds, including adhesives. The presence of this group suggests that the eco-friendly chemical glue contains carbonyl functional groups [35].

The C-O group is associated with ether functional groups, which are commonly used in the production of adhesives. The presence of this group suggests that the eco-friendly chemical glue contains ether functional groups [35].

Table 4: Comparative analysis

Property	Eco-friendly Chemical Glue
Solid Content	14.6%
Viscosity	78.5 cP
Setting Time	21.2 min
pH	5.7
Tensile Shear Strength	5.6 MPa
Curing Time	2 hours
Water Resistance	Yes

According to the table provided, eco-friendly chemical glue has a solid content of 14.6%. Solid content is a crucial property of adhesives as it affects the amount of adhesive material present in the glue. A study by Ciferri, A. (2022) discusses the molecular mechanisms involved in adhesion, wetting, and bond scrambling at interfaces. The author explores how these molecular connections are crucial in determining the properties of materials and their interactions with each other, such as the adhesion of glue to a surface or the wetting of a liquid on a solid surface.

The article also highlights the current formulations in molecular recognition at interfaces, including the use of advanced experimental techniques and computational modelling to better understand and predict these interactions [36]. The viscosity of 78.5 cP. is an essential property of adhesives as it affects the ease of application and strength of the bond. According to a study by J. W. Gilman (2002), the viscosity of adhesives plays a crucial role in determining the bond strength, with higher viscosity generally resulting in a stronger bond [37].

The setting time which is the duration required for the glue to harden and set in place is reported to be 21.2 minutes.

A study by Saikaew, P. *et al*. (2018) investigated how the reduction of the engagement time of universal adhesives affected their long-term bond strength to dentin. The researchers conducted an in vitro study on extracted human molars and tested the bond strength of three different universal adhesives with varying application times (10secs, 20secs, and 30secs) at different time intervals (24h, 6 months, and 1 year) using micro tensile bond strength testing. The results of the study showed that shortening the application time did not significantly affect their long-term bond strength to dentin.

The bond strength values for all three adhesives remained relatively stable over the test period, indicating that lowering the engagement period of universal adhesives could be a viable option in clinical practice without compromising long-term bonding effectiveness. The authors suggested that further investigation is required into the effect of short application times on other factors, such as the quality of the adhesive interface and clinical outcomes [38]. The pH is reported to be 5.7. The pH level of a glue can impacts its bonding ability, as certain materials require a specific pH level to bond properly. Zhao, Y. *et al*. (2017) describe a novel bio-inspired adhesive that is capable of sticking and unsticking in wet environments.

The adhesive was inspired by the adhesive nature of mussels and utilizes similar chemistry to achieve its properties. The authors provide a detailed description of the formulation of adhesive and its performance in various wet environments.

They also discuss the underlying chemical and physical mechanisms of the adhesive, including the role of surface chemistry and topography. The article highlights the potential applications of this bio-inspired adhesive, including underwater repair and construction, medical devices, and robotics.

The authors also suggest that the principles and techniques used to develop this adhesive could be applied to the design of other bio-inspired materials and devices [39]. The shear tensile strength is reported to be 5.6 MPa. Tensile shear strength measures the strength of the bond created by the adhesive. According to a study by Seo, D.W. and Lim, J.K. (2005), with the experiments on glass fibre-reinforced epoxy butt joint specimens with an adhesive bond. They tested the specimens under three different loading conditions: tensile, bending, and shear. They measured the strength of the specimens and analyzed the resulting stress and strain distributions.

The results of the study showed that the strength distributions of the adhesive-bonded butt joint specimens varied significantly under different loading conditions. The tensile strength was concluded to be more than the shear strength, while the bending strength was intermediate between the two. The authors suggest that the variances in the strength distributions could be due to differences in the stress and strain distributions within the adhesive layer.

The article provides valuable insights into the mechanical characteristics of adhesive-bonded joints under different loading conditions, which can be useful in the design and optimization of adhesive-bonded structures. They propose further study to investigate the fundamental mechanisms of the strength dispersions and to develop models that can accurately model the performance of adhesive-bonded joints under different loading conditions. [40]. The curing time that is reported to be 2 hours refers to the period it requires for the adhesive to attain its maximum activity. A study by Phua *et al*. (2022) on two types of dental restorative materials and two types of ceramics.

They used four dissimilar light-curing units with two curing modes to cure the restorative materials through the ceramics. They measured the bonding capacity of the specimens and analyzed the results using statistical methods. The results of the study showed that the bonding strength of the restorative materials to ceramics was influenced by the type of light-curing unit and curing mode used. The authors found that some curing modes and light-curing units resulted in significantly higher bond strengths than others.

The article provides valuable insights into the factors that influence the bonding ability of dental restorative materials to ceramics, which can be useful in the selection of appropriate materials and techniques for dental restorations. The authors recommend further research to investigate the fundamental actions of bonding strength and to develop improved techniques for curing restorative materials through ceramics. [41]. Lastly, the water resistance property that is ascertained to be "yes" is a crucial property for adhesives that may be exposed to moisture. A study by Francis *et al.* (2018). The work focuses on the development of faster weathering assessment standards for sealants and adhesives. The authors argue that traditional outdoor weathering tests can be time-consuming and expensive, and may not always provide accurate predictions of service life. Consequently, they propose using aqueous outdoor environments for accelerated weathering testing. They define the expansion of a testing protocol that replicates aqueous outdoor environments, such as rain and dew, using a custom-built test chamber.

They also discuss the results of their testing, which showed that the aqueous environments produced more accurate and reliable predictions of service life than traditional outdoor weathering tests [42].

4. CONCLUSION

The adoption of laccase fungal enzyme for the production of eco-friendly chemical glue was investigated in this research paper. The results demonstrated that the eco-friendly chemical glue produced using the laccase fungal enzyme had comparable properties to commercial adhesives in terms of tensile shear strength and water resistance, but may have a longer setting time and curing time. However, eco-friendly chemical glue has the advantage of being more environmentally friendly and sustainable, which may make it a desirable alternative to traditional synthetic adhesives for certain applications.

Eventually, this research will contribute to the increasing body of literature on the usage of laccase fungal enzymes for the production of eco-friendly chemicals and materials with the potential to offer a sustainable and environmentally friendly alternative to traditional synthetic adhesives.

5. RECOMMENDATION

Further research in this area could focus on optimizing the production process and exploring potential applications for eco-friendly chemical glue in various industries.

Acknowledgements

The industrial and environmental pollution department of the National Research Institute for Chemical Technology Zaria, Kaduna State, Nigeria

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