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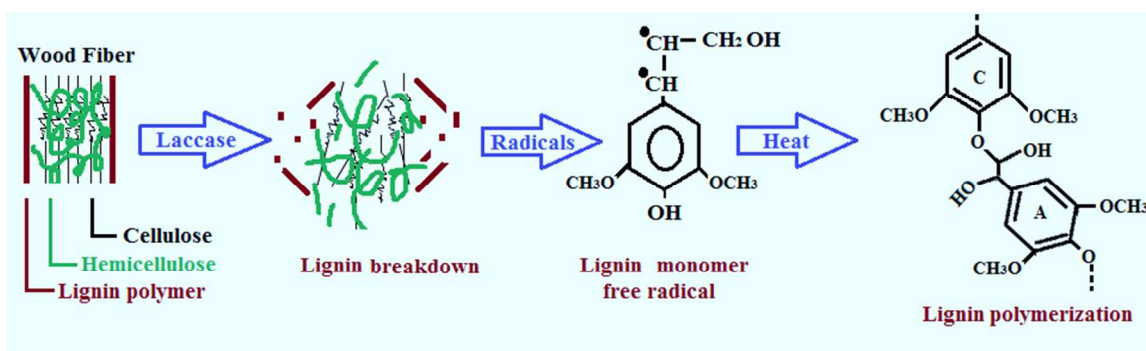
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Laccase Application in Medium Density Fibreboard to prepare a Bio-composite

Mohammed Nasir¹, Arun Gupta^{1*}, Mohammad Dalour Hossen Beg¹, Gek Kee Chua¹ and Mohd Asim²

¹Faculty of Chemical and Natural Resource Engineering, University Malaysia Pahang, Malaysia 26300;

²Department of Biocomposite Technology, INTROP, Universiti Putra Malaysia, 43400; Corresponding Email: arun@ump.edu.my, Ph: 09 549 2867, Fax: 09 549 2867



Abstract

Laccase efficacy as a biological tool for the removal of lignin in pulp industries is evident and has a scope for a wider application. In this research study, rubber wood (*Hevea brasiliensis*) fibres were treated with laccase enzyme to study its effect on the fibre surface and the enzyme hydrolysis lignin (EHL) was collected as a byproduct. Collected EHL was concentrated (con) till the solution reached a 3% solid content. Fibre surface modification was studied by FESEM, FTIR and XRD. A distinct fibre surface with an improved crystallinity index was observed. EHL and Con-EHL were analyzed on viscometer, FTIR, DSC, and TGA. Con-EHL exhibits a lower stretching energy at the benzene range as compared to EHL and a curing pattern similar to UF was reported. To evaluate the capability of modified fibre and Con-EHL, 6 mm medium density fibreboard (MDF) of 810 kg/m³ were prepared by using 10% Con-EHL solution (by weight of

fibre). The MDF boards exhibit higher mechanical strength and have passed the ASTM D1037 standard for internal bonding and modulus of rupture.

Keywords: Crystallinity index; Differential scanning calorimetry; Urea formaldehyde; Fourier transform infrared

Abbreviations

EHL: enzyme hydrolysis lignin Crl: Crystallinity index MOR: Modulus of rupture IB: Internal bonding XRD: X-ray diffraction	Con- EHL: Concentrated EHL lignin MDF: Medium density fibreboard FESEM: Field emission electron Microscope FTIR: Fourier transform infrared DSC: Differential scanning calorimetry
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1. Introduction

Wood composites are a two component system where natural wood fibre is mixed with synthetic adhesive at high temperature and pressure. The most common synthetic adhesives used are urea formaldehyde (UF) and phenol formaldehyde (PF). Since formaldehyde is supposed to be carcinogenic to humans, a continuous emission of formaldehyde from such products is a serious concern research [1]. For this reason, many developed countries have already imposed or are in process to impose regulations, limiting the emission of formaldehyde from building materials [2]. The United States Environmental Protection Agency has declared it a priority pollutant to environment [3]. The government of Korea passed a law in 2004, which stated that the use of materials with total volatile organic compound (TVOC) with emission levels above 4.0 mg/m².h (JIS A 1901, small chamber method) is prohibited [4][5].

Lignin, a natural binder in plants is considered as a potential substitute to synthetic adhesive due to its resemblance in structure with PF and huge availability as a

byproduct of pulp industries. It is a highly complex, non-uniform and second most abundant natural polymer in the world, after cellulose [6][7]. Approximately 5×10^6 million metric ton of lignin is produced as a by-product annually from different processes such as liginosulfonate and black liquor [8][9]. Most of the lignin obtained from pulp mills is either burned or utilized wastefully and only about 15-20% of the total lignin is used for various purposes [10].

In plants, lignin is a hydrophobic material and acts as a water barrier. However, when lignin undergoes hydrolysis, it breaks up into smaller structural units and becomes soluble in water. To create insoluble resins, technical lignins (liginosulfonate and black liquor) have to be additionally cross-linked [10]. Nimz (1983)[11] suggested a lignin polymerization method by radical coupling instead of condensation reactions by using hydrogen peroxide. Recently, lignin has been studied as a partial replacement of phenol for many formaldehyde based resins but a complete replacement has not been successful as yet **Error! Reference source not found.**[13].

Laccase efficacy as a biological tool for the removal of lignin in pulp industries is evident and has a scope for a wider application [14][15]. The lignin obtained by enzymatic hydrolysis contains impurities such as polyphenol and polysaccharides and the solution obtained is termed as enzyme hydrolysis lignin (EHL) [16][17]. Laccase is an oxido-reductase agent; on one hand it removes the lignin molecule from cellulosic material and on other the hand it helps in *in vivo* polymerization of lignin through free radical reactions [18][19]. Laccase catalyzes one-electron oxidation reaction, where four moles of phenoxy hydroxyl groups are oxidized in to four moles of phenoxy radical and oxygen is reduced to two molecules of water [20][21]. This hypothesis to activate lignin

of cellulosic material can be utilised to produce a lignin-based adhesives for making a bio-composite [22][23].

Laccase treatment to rubber wood fibres results in several changes in the fibre morphology. It not only removes the amorphous lignin content, but also changes the hemicellulose content and ultimately the cellulose crystallinity [24]. Fibre surface physiognomies and its interfacial contact greatly affect the physical and chemical properties of single cellulose fibres. A fibre modified with laccase/alkali treatment improved the tensile strength of individual fibre and ultimately the strength of the composites by 43 to 51% [25]. Since laccase enzymes are too large to penetrate the cell (55-80 kDa), treatment results in only surface modification of the fibres [26]. Although several researchers have studied the enzymatic hydrolysis of ligno-cellulosic materials, the changes in physical and mechanical properties of fibre are not fully understood.

In this work, laccase was used to manufacture bio-composite (medium density fibreboard). Laccase activity on rubber wood fibre was examined in two aspects: 1) fibre surface modification and 2) Preparing EHL and its modification. The MDF boards were prepared by using different ratios of modified EHL (by weight of dried fibre) and the mechanical properties of the boards were compared with ASTM D1037 and Urea formaldehyde (UF) Boards. Felby et al. 2002, 2004 used laccase treated wood fibre to prepare fibre board but no one has used con-EHL as an adhesive for MDF fabrication. Thus, this preliminary study can lead to a platform for future studies on lignin-based completely natural adhesives.

2. Experimental

2.1. Materials

Thermo-mechanical processed rubber wood fibre was provided by Robin Resources (Malaysia) Sdn. Bhd. The commercial pulp was washed with water until the pH of the filtrate became neutral. Later, the pulp was air dried before commencing all treatments and prior to lignin oxidation procedures. Klason-lignin of untreated rubberwood fibre was calculated by the method suggested by Jung et al., and found to be 30.6% and 29.8% with and without ash content respectively [27]. Laccase enzyme (53739-WA20040) was supplied by Sigma-life science, Malaysia and enzyme activity was marked with 93.6 units per mg (U/mg). UF resin was supplied by Dynea Malaysia Sdn. Bhd., with the following specifications: viscosity 140 cps, solid content 65%, pH 8.1 and density 1.2 at 30 °C.

2.2. Enzyme hydrolysis method

Two hundred grams of well-dried fibres were suspended in 3800 g of deionized water to make a solution of 5.0% consistency (mass pulp/mass suspension) in a 10 liter controlled rotary reactor. A buffer solution of sodium acetate and acetic acid was used to maintain the solution pH at 5. The suspension was stirred in a rotary reactor with free oxygen supply at a controlled temperature of 24-25 °C. 12.8 mg laccase enzyme was added (to obtain a 6 U/g enzyme activity) as per the method suggested by Felby et al. (2002)[23]. In order to allow the best enzymatic reaction, fibres were kept on uniform rotation for 120 min at 25 °C. After treatment, the pulp samples were filtered out and the solution containing mainly lignin and traces of organic materials, defined previously as enzyme hydrolysis lignin (EHL), was retained. Fibres were washed until the filtrate

became colourless and the pH was neutral and then the fibres were dried in a flash drier. **A mean value of Klason lignin content of treated fibre was calculated as 24.2% with ash content [27].**

2.3. Concentrated enzyme hydrolysis lignin (con-EHL)

After the extraction, EHL was immediately transferred to Erlenmeyer flasks and air-dried at 75-80°C, in an oven for one hour to stop further enzymatic reaction. Later, this EHL solution was kept on an electrical heater and dried for a period of 4 to 5 hours at temperature not exceeding 100 °C. The obtained EHL was contracted up to 3% solid content which was termed as con-EHL. The solid content of Con-EHL was calculated as final weight over initial weight.

Physical properties of EHL, Con-EHL and UF are summarised in table 1. The solid content of the solution was calculated using oven dry method. It's viscosity was measured by Brookfield digital Rheometer DV III at 50 rpm and 25 °C with spindle number 31.

2.4. Preparation of MDF boards

The enzyme treated fibre was well dried to a moisture content of 14-16%. Con-EHL was used as an adhesive for board making and a rotating drum type mixer was used for adhesive mixing. Con-EHL was sprayed on wood fibres using a spray gun. A loose mat of dimensions 200 mm × 200 mm was prepared and then pre-pressed to a thickness of 60 mm in cold press. The mat was then hot-pressed at 180°C for 240 seconds, between aluminum caul plates to a thickness of 6 mm at 5 MPa pressure. The target density of the board was 810 kg/m³. All the parameters of board manufacturing were kept constant for

the rest of the samples. With the objective of testing the performance of the adhesive, five replicates of each adhesive were prepared. Following pressing, the boards were cooled in an incubator and conditioned at 24 °C and 67% relative humidity.

2.5. Chemical Analysis

Fourier Transform Infrared (FTIR) measurements were performed in a Perkin-Elmer instrument by direct transmittance using a fitted universal ATR accessory. Each spectrum was recorded in the range of 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1} . Background spectra were collected before every sample.

Micro structure of the cellulosic fibre and MDF boards were analysed a field emission scanning electron microscope (FESEM) JOEL-JSM-7800F. Sample were mounted on 10mm thick, 12.5 mm diameter stub and coated with platinum.

X-ray measurements were conducted on a Rigaku MiniFlex II, a benchtop X-ray diffraction (XRD) analyzer. The fibre specimens were pressed into the shape of a tablet with rectangular dimensions of 20 × 20 mm and a thickness of 0.5 mm [28]. The X-ray diffractometer was operated at a voltage of 30 kV with a current density of 15 mA. The scanning range was from $2\theta = 10^\circ$ to 50° at a scan speed of 0.015°/s. The data was collected using “fixed time” mode with angular intervals of 0.015°. The crystallinity index (Crl) was calculated from Equation 2 as per the method suggested by Monrroy et al. [27].

$$Crl \% = \frac{(I_{002} - I_{am})}{I_{002}} \times 100 \quad \text{Equation 1}$$

Where Crl is crystallinity index fibre, I_{002} is intensity of the peak diffraction from the 002 plane around $2\theta = 22.00$ and I_{am} is the intensity of the background scatter measured around $2\theta = 18.00$ (Figure 2A).

Differential scanning calorimetry (DSC) was done on EHL, con-EHL, and UF resins. DSC was done to study the pattern of the hardening rate and its comparison with UF resin. It was carried out with a Q 1000-0567 DSC spectrometer and TA Universal Analysis 2000 software was used to analyze the data. The DSC was calibrated with a standard sample before analysis. Aluminium pans containing samples were heated from 35°C to 250°C at a heating rate of 10 °C/min to obtain the exothermic curing reaction curves.

2.6. Mechanical Tests

Modulus of rupture (MOR), Modulus of elasticity (MOE), Internal bonding (IB), and maximum load bearing capacity were the key tests done to test the performance of board. Mechanical testing of the samples was done on Shimadzu UTM AG-X plug series and results were analyzed on Trapezium X-software. Result was compared with ASTM D1037. All statistical analysis was performed by using SYSTAT 9.0 software.

Two samples for IB and two for MOR and MOE were prepared from each board. A total of 7 samples for IB and 10 samples for flexure test were done for each set of experiment. IB is tested perpendicular to the plane of the boards with a cross head speed of 1 mm/min. Flexure test is done by 3-point static bending test to determine the MOE and MOR with a cross head speed 10 mm/min. Equations 3 and 4 were used to calculate the MOR, MOE and IB, respectively.

$$\text{Modulus of Elasticity (MOE)} = \frac{\text{stress}}{\text{strain}} \quad \text{Equation 2}$$

$$\text{Modulus of Rupture (MOR)} = \frac{3 P \times L}{2 \times b \times d^2} \quad \text{Equation 3}$$

Where P is breaking load, L is the distance between knife edges on which the sample was supported, b is the average specimen breadth, and d is the average specimen depth.

$$\text{Internal Bonding (MPa)} = \frac{\text{Maximum Force Calculated at entire area (F)}}{\text{Surface Area of The Specimen(A)}} \quad \text{Equation 4}$$

3. RESULTS AND DISCUSSION

Divided into three parts i.e. fibre modification, EHL modification and mechanical analysis of MDF boards prepared from them.

3.1. Fibre Modification

3.1.1. Fourier transform infrared (FTIR) of treated Fibre

The spectrum of both treated and untreated fibres shows a typical structure of cellulosic compound as in all other wood samples. A strong, broad OH bond stretching was observed between 3300–3500 cm^{-1} , C–H bond stretching of methylene groups from 2800–3000 cm^{-1} , and narrow sharp and discrete absorptions in the region from 1000 to 1750 cm^{-1} . Figure 1, shows the changes in FTIR spectra of control fibre and laccase treated fibre for 3 h. The treated fibres show a lower intensity of guaiacyl unit (G-bands) at the range of 1247 cm^{-1} , 1191 cm^{-1} and 830 cm^{-1} , while a typical syringyl (S-bands) disappeared at 1328 cm^{-1} due to enzymatic degradation of lignin. The spectra between 1335–1316 cm^{-1} is assigned to the cellulose part of fibre, and is related to the crystallinity and amorphous content of cellulose, it shows the improvement in crystallinity [29].

Except a little modification, there are no significant differences between spectra of treated and untreated fibre. The lower intensity of the transmittance band in treated fibre may be due to esterification on the surface of fibre. Some shifts of the spectra in the

range of 2940-2900 cm^{-1} arise from CH stretching of aromatic methoxyl groups may be due to interactions between the functional groups.

3.1.2. X-ray diffraction (XRD)

Figure 2B, shows the X-ray-based measurements of crystallinity index (CrI). The results indicated that laccase treatment increases the crystallinity of the fibre. The maximum value reached after 3 h of enzyme treatment. The crystallinity index of rubber wood fibres treated for 3 h was 14% higher than that of untreated fibre. It is assumed that the improvement in crystallinity was due to removal of extracellular, amorphous lignin, which was bonded loosely to the cellulose fibre. However, when the reaction continued, its crystallinity remains constant for few hours and after that started decreasing drastically. It is expected that if the reaction was prolonged further, the intracellular lignin would begin to degrade ultimately reducing the crystallinity.

3.1.3. FESEM Analysis of Fibre

Fibre surface morphology of untreated and laccase treated fibre (for 3h) is compared in Figure 3 (A-B). Since the fibre cell wall is an aggregate of cellulosic microfibril embedded in the matrix of lignin with some patches of aliphatic and aromatic ring molecules on its surface, an untreated fibre looks rough and wrinkled (Figure 3A) [23]. When the fibre is treated with laccase enzyme, in addition to removing lignin it breaks down the aliphatic and aromatic ring molecules and makes the fibre smooth (Figure 3B)[30]. As the reaction proceeds, the dissolved lignin starts precipitating on the fibre surface again and a thin layer of lignin deposition can be seen which is also expected to help in fibre bonding [31][32]. Thus, it was concluded that an untreated fibre has a high lignin-content but with uneven distribution and rough surface. However, a laccase treated fibre gives a smooth surface with a uniform layer of lignin deposition.

3.2. EHL Modification

3.2.1. Fourier transform infrared (FTIR) of EHL and con-EHL

FTIR analysis of EHL and Con-EHL, obtained from enzyme treatment showed prominent peaks in the range of 1750-600 cm^{-1} (Figure 4). The observed peaks represent the characteristic peaks of lignin such as 1596, 1505, 1490, 1270, 1227, 1000 cm^{-1} . The observed spectra of both EHL and con-EHL were very similar but a closer inspection revealed a minor shift in transmittances frequencies of con-EHL when compared to EHL. As expected, the absorbance in the region from 1,200 to 900 cm^{-1} , which is the polysaccharide region [33][34], was strongly diminished in EHL and con EHL spectra when compared to fibre spectra (Figure 1). The carbohydrate bands at 1620 and 780 prominent in fibre spectra disappeared in the lignin spectrum of EHL and con-EHL (Figure 1).

A slight shift of lignin spectra was also observed when the wood fibre was treated with laccase enzyme. The Guaiacyl peak at 1270 cm^{-1} in the fibre spectra shifted to 1260 cm^{-1} in the lignin spectra. This shift might have been caused by inductive effects of substituent (e.g., H₃CO) in the aromatic ring system of lignin [35]. The guaiacyl peaks at 1270 cm^{-1} was found to be more prominent in con-EHL as in the EHL, whereas the syringyl ring breathing with CO stretching at 1330 cm^{-1} , was found less pronounced in con-EHL than EHL. Since higher ratio of G/S (Guaiacyl/Syringyl) is an indication of improved cross-linking, con-EHL exhibits a better chance of polymerization [36].

3.2.2. Differential scanning calorimetry (DSC)

DSC is used to analyse the change in properties of unreacted to reacted thermosets when heat is applied. In table 2, the peak temperature (T_p), onset temperature

(T_0), and rate of curing ΔT are presented which was automatically generated from TA universal analyses software. From Figure 5, it can be observed that in the UF resin, curing started and reached a peak earlier than Con-EHL and EHL. The ΔT value is 13.49 °C for the UF resin, and it decreases to 8.33 °C and 4.96 °C for Con-EHL and EHL, respectively [37]. The higher value of ΔT in the case of UF resin shows a lower rate of curing, whereas in the case of EHL and Con-EHL, curing started at a higher temperature but reached its peak earlier. Thus, it was concluded that EHL and Con-EHL had a higher rate of curing. The higher onset temperature in the case of lignin indicates greater activation energy for initiation of the curing. Once started, the curing progress proceeded very fast and finally completed in a temperature range of 4-8 °C.

Generally, UF resin exhibits an initial small exothermic peak around 84-85 °C due to poly-condensation reaction of primary amino groups of unreacted urea, followed by a large endothermic peak of water evaporation with minimum at 99 °C [38]. In contrast to UF resin, EHL and con-EHL exhibits a large endotherm peak first at 103 and 105 °C followed by small exothermic peak at 122 and 124 °C due to lignin cross-link reaction [39]. The endothermic peaks in EHL and con EHL is mainly related to water evaporation that is why it is found higher for EHL in comparison to Con-EHL [40]. It is very difficult to determine a reliable glass transition temperature (T_g) of lignin and it varies greatly according to the water content present in it [41]. T_g value has been reported between 90 °C to 180 °C for various lignin [42][43] and minimum was observed for the EHL and organosolv lignin [43]. Thus it gives strength to the hypothesis that a pure lignin adhesive would be having a lower T_g value as compared to pure PF. **Error! Reference source not found.**

3.3. Mechanical Testing

To evaluate the performance of modified fibre and Con-EHL, four types of MDF were prepared. Modified fibre (treated for 3h) was compared with untreated fibre whereas Con-EHL 10% was compared with UF 10%. Modulus of rupture (MOR) and internal bonding (IB) were the key tests done to test the performance of board.

3.3.1. Flexure Test

Figure 6(A), shows the modulus of rupture (MOR) and modulus of elasticity (MOE) prepared from treated and untreated fibre with Con-EHL and UF resin. According to ASTM D 1037, the MOR for a board with a thickness of 6 mm and density 750-800 kg/m³ should be at least 35 MPa [44]. Board prepared from untreated fibre exhibited the lowest mechanical strength in comparison to treated fibre whether it is prepared of Con-EHL or UF adhesive. MDF board fabricated from treated fibre and Con-EHL 10% presented a lower mechanical strength as compared to treated fibre with UF% but it is strong enough to pass the minimum standard for MOR. Standard deviation error bar depicted in the Figure 6(A) revealed that the difference in the mean value of treated fibre and untreated fibre is less but significant.

3.3.2. Internal bonding

Figure 6(b) shows the internal bonding test results of MDF prepared from treated and untreated fibre with Con-EHL and UF resin. Fibre modification was analyzed from MDF prepared from untreated and treated fibre with Con-EHL and UF resin. Boards prepared from treated fibre with Con-EHL and UF showed higher IB strength in comparison to the board prepared from untreated fibre. Standard deviation error bar revealed that the difference in the mean value of treated and untreated fibre was significant. According to ASTM D 1037(a), the IB standard for a board having a

thickness of 6 mm and density above 750-800 kg/m³ should be more than 0.7 MPa [44]. Treated MDF board prepared with UF 10% had the highest IB strength followed by treated fibre with Con-EHL 10%. Standard deviation error bar illustrates that the difference between Con-EHL and UF adhesive boards are significant but pass the minimum required standard.

3.3.3. FESEM Analysis OF MDF

To ascertain the interfacial bonding of adhesive, FESEM was carried out. Figure 7 (A) and (B) represent the MDF board made from 10% UF and 10% Con-EHL respectively. Adhesion between the fibres is clearly evident in both the boards. The extensive study of UF boards at various places revealed that the UF adhesive was spread more uniformly and forms a relatively thick layer around the fibre as compared to Con-EHL which is required for good adhesion. In addition to that, the interfacial voids were observed more prominent in con-EHL boards as compare to UF boards that is probably the reason for a lowering of the mechanical property of con-EHL boards [45]. This problem could be overcome by increasing the solid content of EHL that would fill up the voids and form a uniform layer around the fibre. The microscopic structure of Con-EHL board justifies the results obtained by mechanical testing.

4. CONCLUSIONS

The improved characteristics of laccase treated fibre and Con-EHL exhibited a promising possibility to prepare an innovative, eco-friendly MDF board. Board prepared from 10% Con-EHL (by weight of fibre) exhibited improved mechanical strength. In comparison to 10% UF boards, the 10% EHL boards have slightly lower mechanical strength but still strong enough to meet the ASTM D1037. From the FESEM result, it

was concluded that EHL board can be improved further, if the solid content of the EHL is increased more than 3%. MOR, MOE and IB results revealed that laccase treatment improves the crystallinity of the fibre in comparison to the untreated fibres, that further improves the mechanical strength of individual fibres and ultimately of the board.

Furthermore, a lignin-based adhesive will not only help to reduce the dependence on the petrochemical industry, but also promote the use of eco-friendly and sustainable development of the MDF production.

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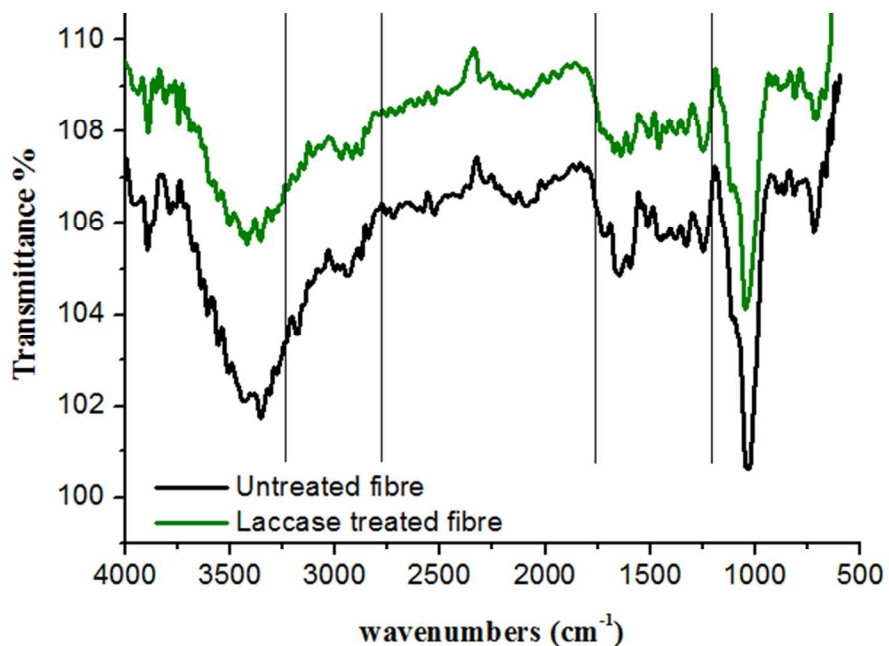


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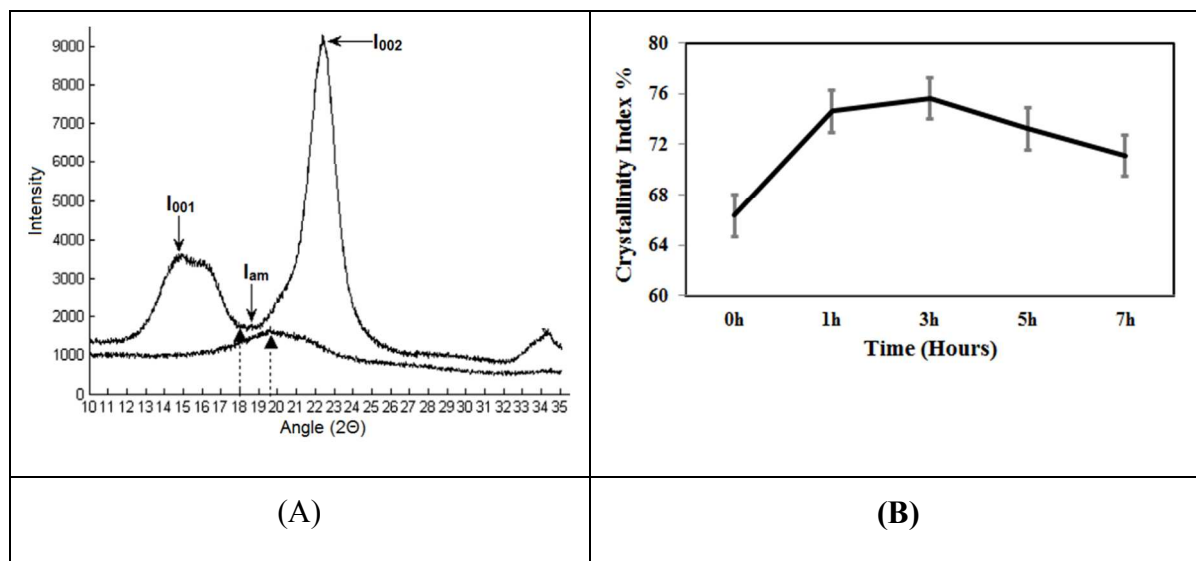


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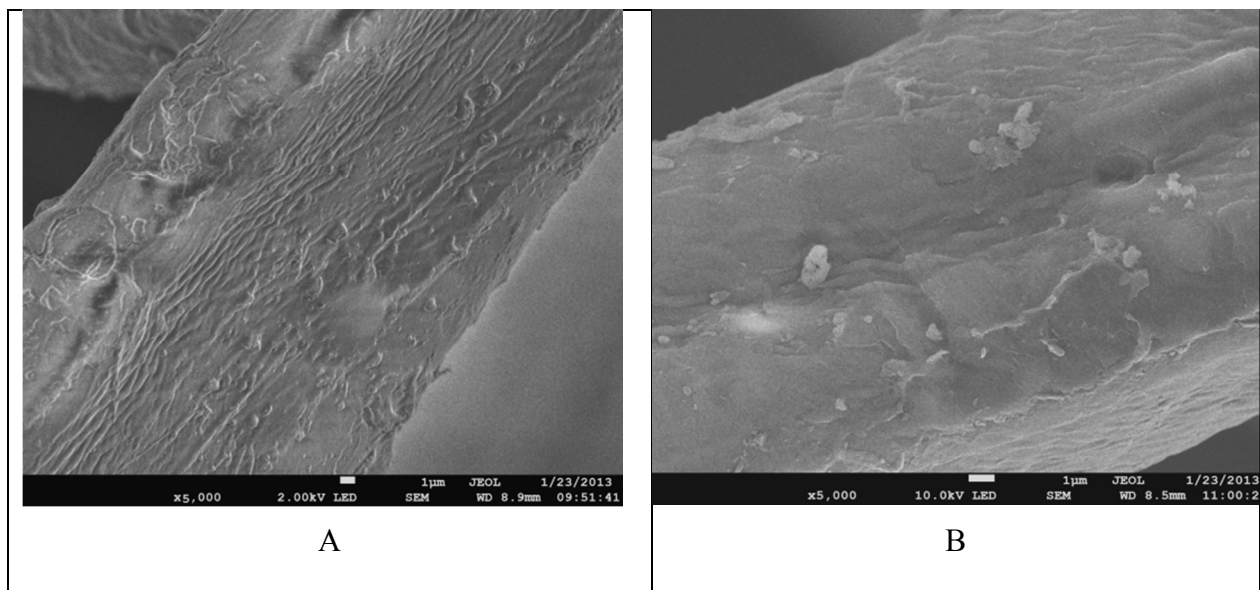


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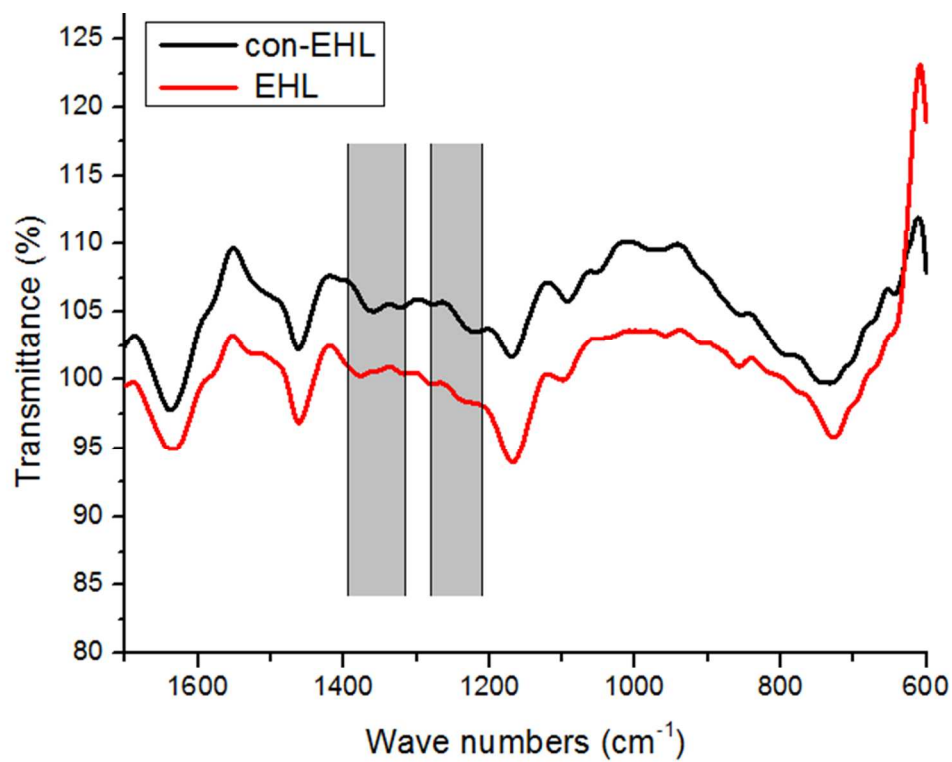


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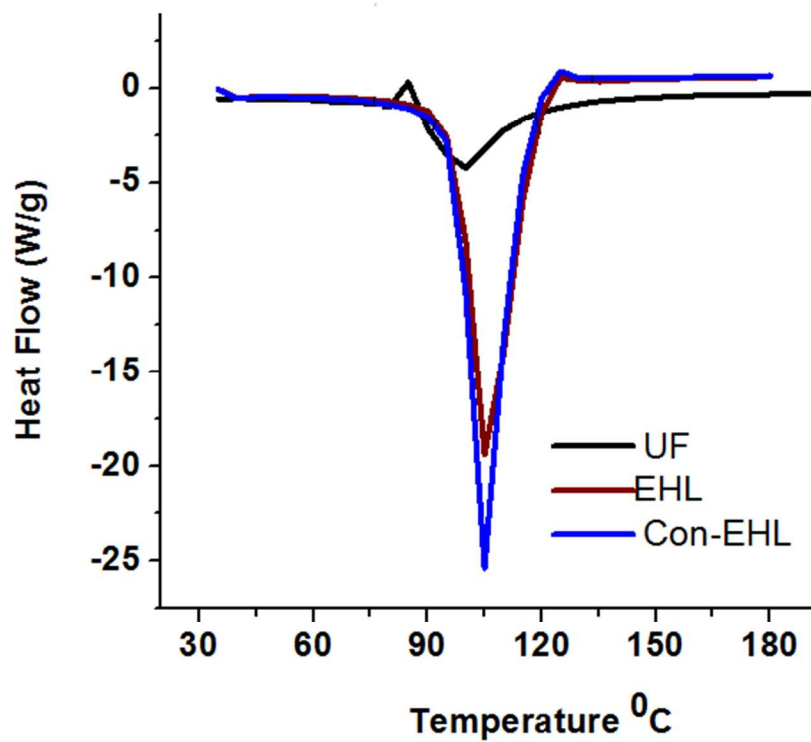
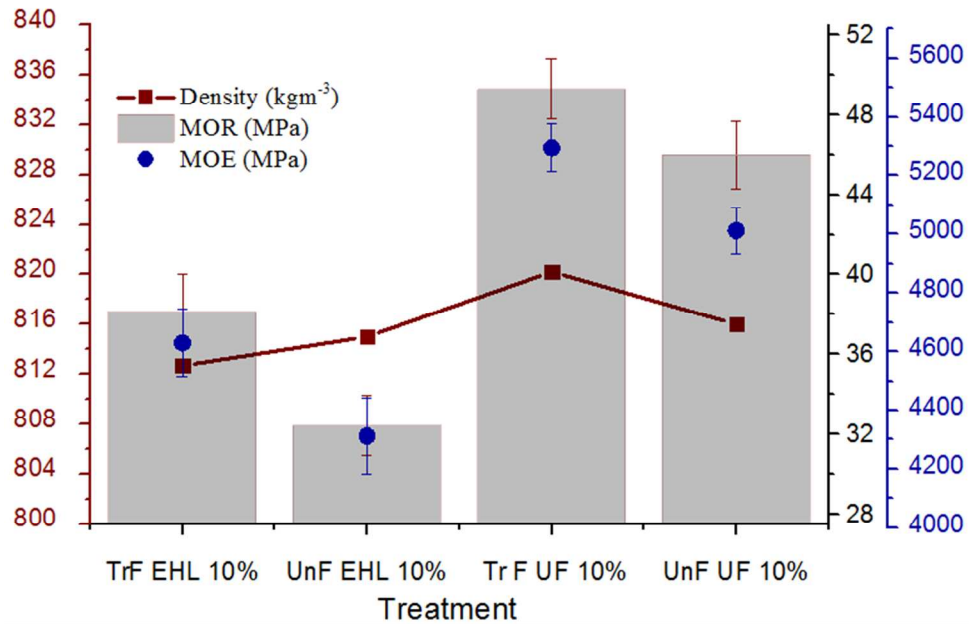
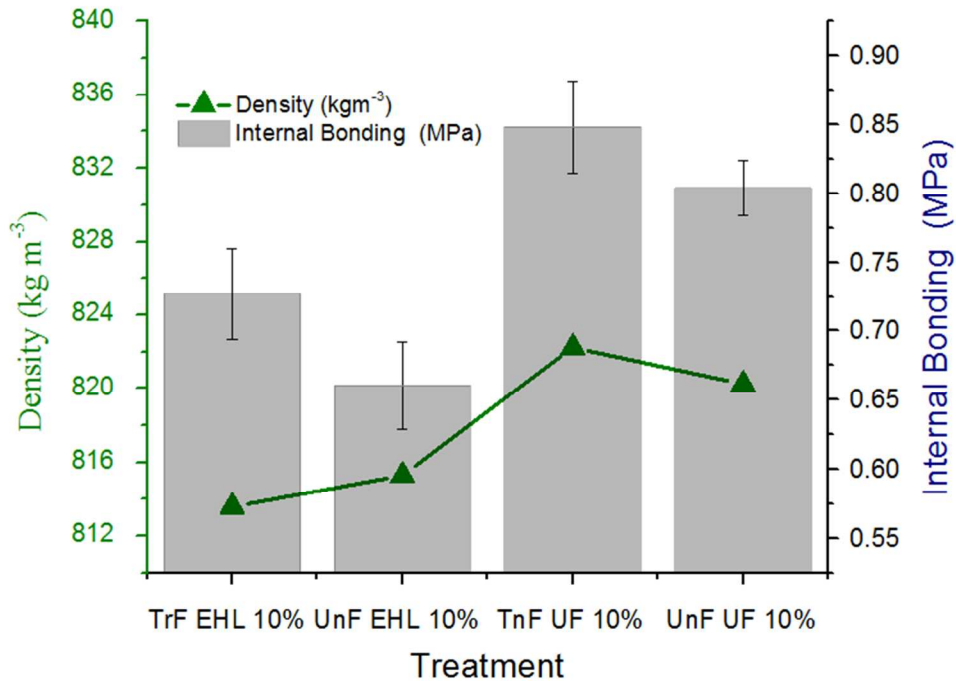


Figure 5: DSC rate of curing of EHL and con-EHL in comparison to urea formaldehyde



(A)



(B)

Figure 6: (A) Shows the MOE and MOR whereas, (B) shows the IB strength with their respective density and compared with UF resin. All values are means and error bar shows the standard deviation error. Where, TrF =Treated Fibre, UnF = Untreated Fibre.

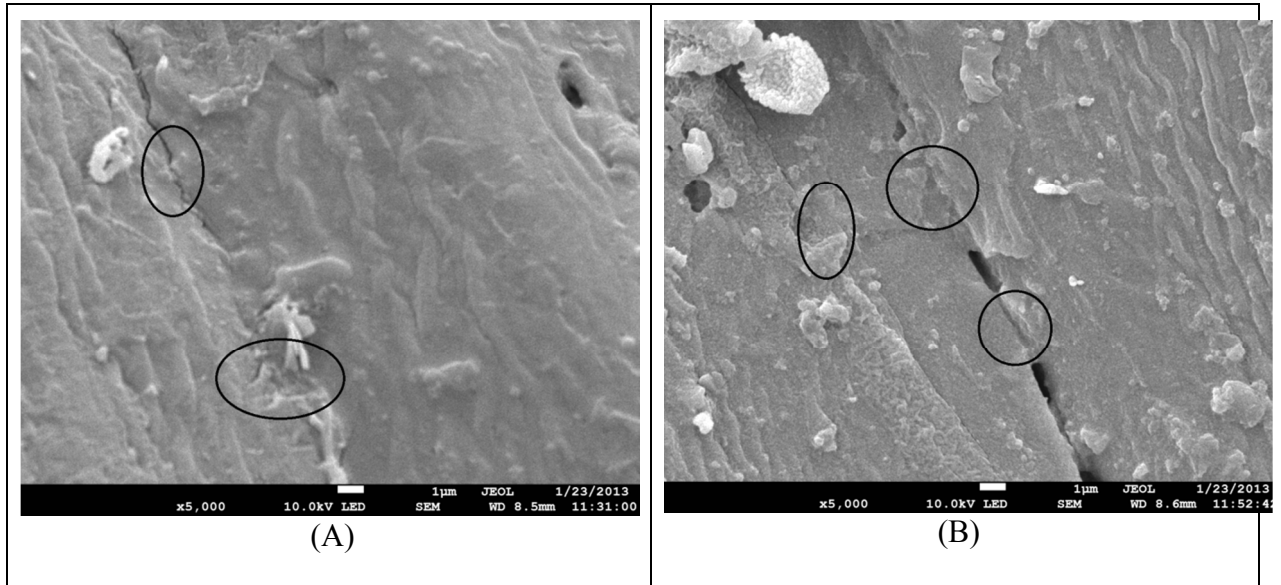


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Table 1: Physical properties of the adhesive

Adhesive	Solid Content %	Viscosity (cP)	PH	Colour
EHL	0.2-0.5%	1.8	4.5	Light brown
Con-EHL	3.0%	3.4	4.5	Dark brown
UF	65.0%	140	8.1	Colourless (White)

Table 2: Thermal properties of EHL and con-EHL in comparison with UF resin

Sample	Onset (To)	Maximum (Tp)	ΔT (Tp-To)	Stop	Area(ΔH)
	$^{\circ}\text{C}$	$^{\circ}\text{C}$	$^{\circ}\text{C}$	$^{\circ}\text{C}$	J/g
UF	85.55	99.04	13.49	196.79	472.3
EHL	98.87	103.83	4.96	124.45	1986
Con-EHL	97.46	105.79	8.33	127.96	1644

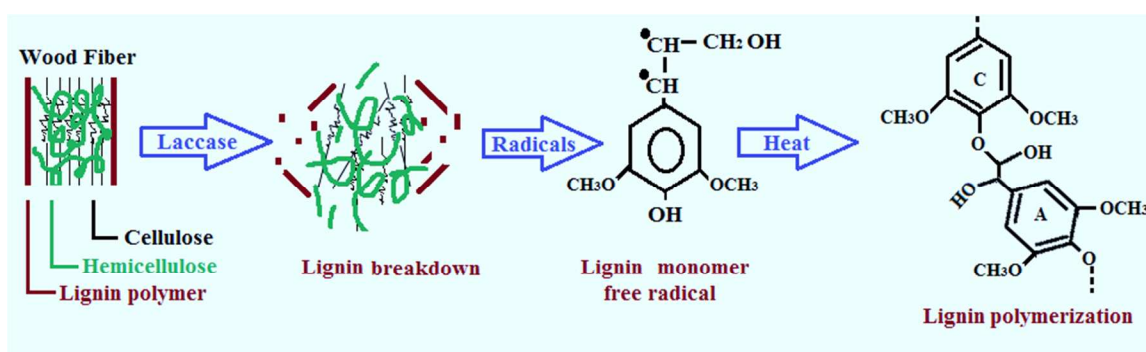
Laccase Application in Medium Density Fibreboard to prepare a Bio-composite

Mohammed Nasir¹, Arun Gupta^{1*}, Mohammad Dalour Hossen Beg¹, Gek Kee Chua¹ and Mohd Asim²

¹Faculty of Chemical and Natural Resource Engineering, University Malaysia Pahang, Malaysia 26300;

²Department of Biocomposite Technology, INTROP, Universiti Putra Malaysia, 43400;

Corresponding Email: arun@ump.edu.my, Ph: 09 549 2867, Fax: 09 549 2867



Abstract

Laccase efficacy as a biological tool for the removal of lignin in pulp industries is evident and has a scope for a wider application. In this research study, rubber wood (*Hevea brasiliensis*) fibres were treated with laccase enzyme to study its effect on the fibre surface and the enzyme hydrolysis lignin (EHL) was collected as a byproduct. Collected EHL was concentrated (con) till the solution reached a 3% solid content. Fibre surface modification was studied by FESEM, FTIR and XRD. A distinct fibre surface with an improved crystallinity index was observed. EHL and Con-EHL were analyzed on viscometer, FTIR, DSC, and TGA. Con-EHL exhibits a lower stretching energy at the benzene range as compared to EHL and a curing pattern similar to UF was reported. To evaluate the capability of modified fibre and Con-EHL, 6 mm medium density fibreboard (MDF) of 810

kg/m³ were prepared by using 10% Con-EHL solution (by weight of fibre). The MDF boards exhibit higher mechanical strength and have passed the ASTM D1037 standard for internal bonding and modulus of rupture.

Keywords: Crystallinity index; Differential scanning calorimetry; Urea formaldehyde; Fourier transform infrared