

Fabricating Eco-Friendly Binderless Fiberboard from Laccase-Treated Rubber Wood Fiber

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The emission of formaldehyde vapors from adhesives such as urea formaldehyde (UF) and phenol formaldehyde (PF) is a main concern for the wood composite industry. The ability of laccase enzymes to modify the fiber in pulp industries has given hope to the wood composite industry to prepare composites without using external adhesives. In the present work, rubber wood fiber was treated with different amounts of laccase enzymes at varying time intervals. Although laccase is known to cause changes to lignin, FTIR analysis of treated fiber revealed no significant difference in the chemical composition of cellulosic fiber as compared to the untreated fiber. SEM analysis exhibited a thin uniform layer of lignin deposition on the fiber surface that may be precipitated back when the reaction reaches equilibrium. XRD showed a 10% increase (maximum) in the crystallinity index of treated fibers as compared to untreated fiber. The treated fibers were dried and pressed at different platen temperatures for various time intervals without any adhesive. Mechanical properties such as MOE, MOR, and IB were done to analyze the performance of binderless fiberboard as per ASTM D1037. Binderless fiberboard of density 750 (± 10) MPa that was fabricated from the fiber treated at 6 U/g enzyme for 60 min and pressed at 200 °C for 6 min showed good performance.

Keywords: Modulus of rupture (MOR); Modulus of elasticity (MOE); Internal bonding (IB); Crystallinity index (CrI); X-ray diffraction (XRD); Scanning electron microscope (SEM)

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INTRODUCTION

Wood composites such as medium density fiberboard (MDF) follow one basic process: solid wood is fragmented into small strands, supplemented with binder, and pressed to form a wood-like structure again. Using this method, the anisotropy of wood is minimized and wood of small dimensions or recycled timber can be incorporated into a valuable product (Hüttermann *et al.* 2001). In this process, only one new component is added that is not already present in wood originally *i.e.* binder. At present, formaldehyde-based adhesives such as urea formaldehyde (UF) and phenol-formaldehyde (PF) are the most common binders used with some additives such as a wax in wood composites (Jin *et al.* 2010). These adhesives are associated with a dangerous gaseous emission of formaldehyde, which is carcinogenic to human health (Kim 2009). With increasing concern over formaldehyde emission, most developed countries are restricting the import of wood panels that contain high contents of formaldehyde-based adhesives (Moubarik *et al.* 2009).

In nature, wood itself is the best example of a natural composite that is mechanically strong cellulose and hemicelluloses, embedded in the matrix of lignin. To overcome the problem of synthetic adhesives, biological modification of wood fiber is being studied intensively for various applications (Garcia-Ubasart *et al.* 2012; Felby *et al.* 2004; Widsten Petri 2002; Felby *et al.* 1997). A wide range of enzymes has been reported that are able to act on cellulosic materials and change the surface structure and composition of the fiber. Enzymatic treatments are the most eco-friendly, least detrimental to fiber structure, and their conditions are often milder. Enzymatic surface modification of fibers can be achieved using glucohydrolisis or some oxidative enzymes (Witayakran and Ragauskas 2009; Kim and Holtzapple, 2006). Laccase is a multi-copper-containing oxidoreductase enzyme widely used in the pulp and paper industry for lignin removal (Moilanen *et al.* 2011). In addition to lignin, laccase can catalyze the oxidation of various phenolic substrates including phenols, di-phenols, aminophenols, polyphenols, polyamines, *etc.* and reduce oxygen to water (Moilanen *et al.* 2011).

The use of laccase enzymes to improve bonding between fibers has already been applied in the pulp and paper industry (Chandra and Ragauskas 2002; Camarero *et al.* 2007; Fillat and Roncero 2010). Laccase is a copper-containing oxidoreductase enzyme that acts on the lignin molecule of the cell wall while other components (*e.g.*, cellulose) remain almost untouched (Moniruzzaman and Ono 2012). Since it is an oxidoreductase enzyme, it causes polymerization as well as depolymerization of lignin compounds through free-radical reactions (Felby *et al.* 1997). Laccase either acts on lignin and oxidizes it to a relatively stable phenoxy radical (one-component system) or provides a surface for another component to attach to it and act as a potential cross-linking agent (two component system) (Mai *et al.* 2004). Since laccase enzymes are too large to penetrate fibers (50-70 kDa), their action results only in a surface modification (Giardina *et al.* 2010). Therefore, during fiber treatments, free phenolic groups on the fiber surface should act as potential reactive sites for laccase enzymes to create phenoxy radicals.

Based on the literature, it is apparent that laccase can be employed to generate stable lignin radicals onto the fiber surface that may undergo cross-linking reactions during hot pressing and would form a strong covalent bond. This study examines the various grafting conditions of laccase with respect to fiber surface modifications and its impact on board strength properties. Owing to recent developments in the wood-composite market, it is regarded as among the most promising developments in this field. Since laccase is commercially available and easy to produce, this technique would be economically sound and practically viable. Moreover, this technique will not only make the product eco-friendly but also reduce the cost of production, as it does not need any artificial binder, which plays a major role in cost effective production.

MATERIALS AND METHODS

Materials

Thermo-mechanically prepared fiber of rubber wood (*Havea brasiliensis*), with size ranging from 1.0 to 2.5 mm supplied by Robin Resources (Malaysia) Sdn. Bhd, was used in this study. Laccase enzyme isolated from *Trametes villosa* with enzyme activity 20 units/mg was supplied by Sigma Aldrich, Malaysia (53739 -1G-F).

Pulp Treatment

Fiber (192.0±8.0 g) was suspended at 5.0% consistency (mass fiber /mass pulp) in a controlled rotary bio-reactor and combined with sodium acetate and acetic acid as a buffering solution and maintained at pH 5. The suspension was stirred in the rotary bioreactor with free oxygen supply at room temperature (25±2°C). In order to understand the laccase activity, different concentrations of enzymes and reaction time were applied, while the pulp was continually rotated at 25 °C. After treatment, the pulp was filtered and washed until the filtrate was colorless and pH neutral. Then the fibers were dried in a flash drier to a moisture content (MC) of 14 to 18%.

Board Manufacturing

This study was based on studying one parameter at a time while keeping the rest of the parameters constant. Based on the literature, various range of parameters such as enzyme amount 3 to 12 U/g, enzyme reaction time 20 to 100 min, platen temperature 180 to 210 °C, and pressing time 4 to 10 min, were applied to fabricate a binderless board with the target density of 750 MPa.

From the dried fibers, a fluffed mat with a thickness of approximately 200 mm was produced and then pre-pressed to a thickness of 60 mm. The mat was then hot-pressed between aluminum caul plates with a steel screen to 6-mm stops. Different times and platen temperatures were used in board making. A constant pressure of 5 MPa was applied to fabricate a 6-mm S1S board with a target density of 0.75 g/cc (750 MPa). Following pressing, the boards were conditioned at 25±2 °C, and 67% relative humidity, and the boards were stored until the testing was performed. Three replicate boards were produced for each treatment. For each fiberboard, 4 samples were prepared for IB and 3 for static bending tests. Modulus of rupture (MOR) and modulus of elasticity (MOE) were determined from the 3-point static bending test; similar to interfacial bonding, they were analyzed from internal bonding (IB) results. All statistical analysis was performed using SYSTAT 9.0 software.

Analysis

FTIR measurements were performed using a Perkin-Elmer instrument by direct transmittance with a universal Attenuated Total Reflectance (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 each sample. Samples treated at different time interval and a constant enzyme amount of 6 U/g were selected for the FTIR study.

X-ray measurements were conducted on a Rigaku MiniFlex II, a benchtop X-ray diffraction (XRD) analyzer. The fiber specimens were pressed into the shape of a tablet with rectangular dimensions of 20 × 20 mm and a thickness of 0.5 mm (Kim and Holtzaple 2005). The X-ray diffractometer was operated at a voltage of 30 kV with a current density of 15 mA. The scanning range was from 2θ = 10° to 50° at a scan speed of 0.015°/s. The data was collected using the 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.* (2011),

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

where Crl is crystallinity index fiber, 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 $2\theta = 18.00$.

SEM analysis was performed on an EVO 50, Brand ZEISS, US, to investigate the morphological changes of the fibers due to hot pressing. A sample of 5 mm radius was taken from the MDF boards. The small sample was mounted on an aluminum stub using carbon tape. Silver paint was used to stub the sides to reduce charging. The sample was coated with Pt-Pd by a sputter coater. Images were taken at 3 kV of beam voltage (Koo *et al.* 2012).

Mechanical Testing

Modulus of rupture (MOR), internal bonding (IB), and maximum load bearing capacity were the key properties considered to analyze the performance of the board. Mechanical testing of the samples was done on a Shimadzu UTM AG-X plug series and results were analyzed using TRAPEZIUM X-software. All testing was performed according to the ASTM D1037 standard, and statistical analysis was performed on SYSTAT 9.0 software.

RESULT AND DISCUSSION

Fourier Transform Infrared (FTIR) of Treated Fiber

FTIR was performed to compare the changes in the chemical composition of treated fiber at different time intervals compared with untreated fiber. All the spectra, of both treated and untreated fibers, showed the typical structure of a cellulosic compound. A strong, broad OH bond stretching was observed between 3300 and 3500 cm^{-1} , C–H bond stretching of methylene groups from 2800 to 3000 cm^{-1} was observed, and narrow, sharp, and discrete absorptions in the region from 1000 to 1750 cm^{-1} were observed. Figure 1 depicts the treated fiber at various time intervals such as 20, 40, 60, and 80 min, as well as untreated fiber.

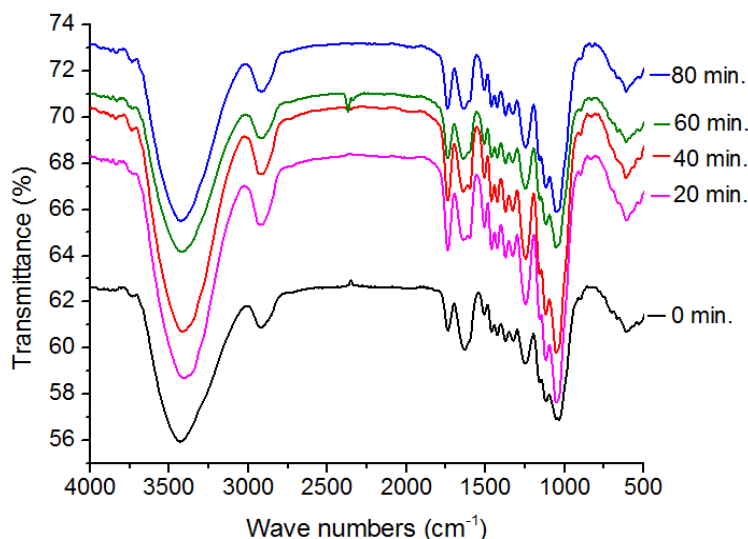


Fig. 1. Comparison of fibers treated at different time interval (in minute) and constant enzyme amount of 6 U/g with untreated fiber

The spectra showed that there was not much difference in the chemical structures of treated and untreated fiber, except for the relative proportion of different functional groups, as indicated by the intensities of the corresponding bands. The treated fibers showed a lower intensity of guaiacyl units (G-bands) in 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 and 1316 cm^{-1} is assigned to the cellulose part of the fiber and is related to the crystallinity and amorphous content of cellulose; it showed an increase in crystallinity (Wang *et al.* 2012). It was expected that the lower intensity of the transmittance band in the treated fiber might be due to esterification on the surface of the fiber. The shifts of the spectra in the range of 2940 to 2900 cm^{-1} arose from CH stretching of aromatic methoxyl groups and may be due to interactions between the functional groups.

Crystallinity Index

Figure 2 shows results of the X-ray-based measurements of crystallinity index (*Crl*) of fiber treated with different amounts of laccase for 60 min. The results reveal that a small amount of laccase treatment can cause a sharp increase in the crystallinity index of the fiber. The maximum crystallinity index was observed at 6 U/g of enzyme treatment. The crystallinity index of rubber wood fibers treated at 6 U/g was 10% higher than that of untreated fiber. It is assumed that the improvement in crystallinity was due to the removal of extracellular, amorphous lignin, which was bonded loosely to the cellulose fiber. However, when the enzyme amount is increased, the material's crystallinity started decreasing drastically. It is assumed that the increase in the enzyme amount resulted in degradation of the intracellular lignin and phenolic compounds, ultimately reducing the crystallinity of the cellulose component.

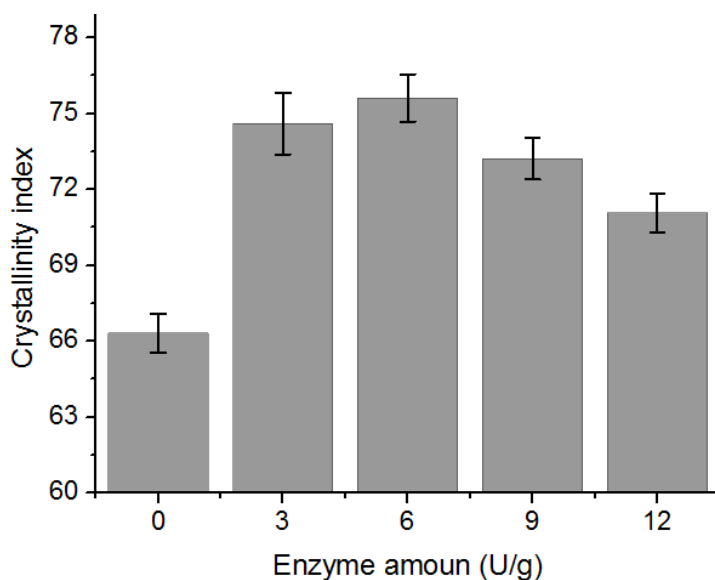


Fig. 2. Crystallinity index of cellulosic fiber treated with various amounts of laccase

SEM Analysis of Fiber Surface

Fiber surface morphology of untreated and laccase-treated fiber (6 U/g for 60 min.) is compared in Fig. 3. Since the fiber cell wall is an aggregate of cellulosic microfibrils embedded in the matrix of lignin with some patches of aliphatic and aromatic

ring molecules on its surface, the untreated fiber looked rough and wrinkled (Fig. 3A) (Peng *et al.* 2010). When the fiber is treated with the laccase enzyme, in addition to removing lignin, it breaks down the aliphatic and aromatic ring molecules and makes the fiber smooth (Fig. 3B) (Bledzki *et al.* 2010). As the reaction proceeds, the dissolved lignin precipitates back onto the fiber surface; the thin layer of lignin deposition on the fiber surface is anticipated to be helpful in fiber bonding (Maximova *et al.* 2001; Koljonen *et al.* 2004). Thus it was concluded that an untreated fiber has a high lignin-content with an uneven distribution and rough surface. However, a laccase-treated fiber gives a smooth surface with a thin and uniform layer of lignin deposition.

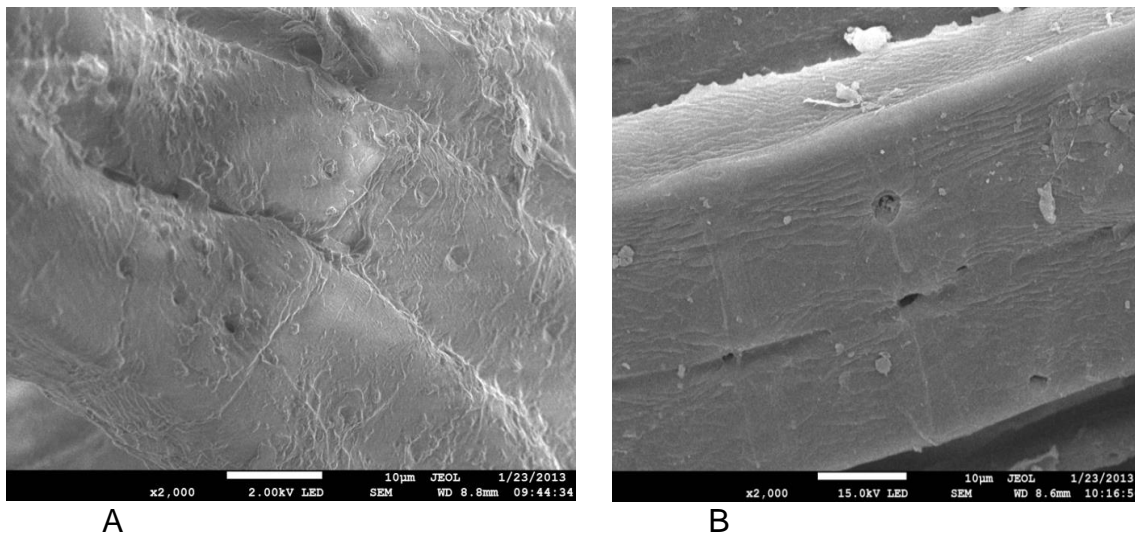


Fig. 3. SEM picture of (A) untreated and (B) treated fiber at 2000K magnifications

Mechanical Properties

Four different parameters, *i.e.* enzyme reaction time, enzyme amount, pressing temperature, and pressing time were used for this study. Based on previous work on binderless boards, four different treatments of all parameters were applied with two replicates of each. Modulus of elasticity (MOE), modulus of rupture (MOR), and internal bonding (IB) were the key properties evaluated to analyze the performance of the board for each treatment.

Four different enzyme treatments at 3, 6, 9, and 12 U/g were applied to modify the fiber surface. Figure 4(A) depicts the mechanical properties of the boards prepared from 60 min of enzyme-treated fiber, pressed at 200 °C for 4 min but different enzyme amount. It was found that a small amount of enzyme was sufficient to modify a cellulosic fiber. An improved mechanical strength (MOE, MOR, and IB) was observed when enzyme concentration reached to 6 U/g (similar to the finding of Felby *et al.* 2004) and 9 U/g. The highest value of MOE and IB was observed as 2253.80 (± 74.20) MPa and 0.72 (± 0.04) MPa, respectively, when the fiber was treated with 9 U/g of enzyme amount. On the other hand, the maximum value for MOR was observed 8.10 (± 0.21) MPa, when it was treated with 6 U/g enzyme concentration. As per the ASTM D 1037-96a Part-A, the guideline for mechanical properties of interior grade MDF, except MOR other properties can pass the standard for 130 grade of MDF (ANSI A208.2-2002). Increasing in laccase amount caused a negative effect on mechanical strength. From this result it was concluded that a higher amount of laccase may cause an excess removal of lignin from

the wood surface, which explains why internal bonding exhibited a sharp decrease in the strength.

Enzyme reaction was carried out at five different time intervals, *i.e.* 20, 40, 60, 80, and 100 min. Figure 4(B) shows the board testing result prepared from treated fiber of 6 U/g enzyme amount, pressed at 200 °C platen temperature for 4 min, but treated for different time intervals. The boards prepared from 20 min of treated fibers had the least mechanical strength, whereas it increased over time and again started decreasing if run for a longer time. The highest value of MOE and MOR was observed as 2955.73 (± 69.30) MPa and 7.95 (± 0.21) MPa, respectively when the fiber was treated with enzyme for 40 min. The maximum value for IB was observed 0.69 (± 0.03) MPa, when it was treated with enzyme for 60 min. As per the ASTM D 1037-96a Part-A, the guideline for mechanical properties of interior grade MDF, MOR is good for 120 grade MDF, but the other properties can pass the standard for 130 grade of MDF (ANSI A208.2-2002). From the graph it can be observed that laccase reaction time determined the mechanical strength by improving the crystallinity of the fiber. Since its crystallinity was found to be higher at treatment times around 40 to 80 min, its mechanical strength was also found to be higher (Fig. 2). Furthermore, with the increase in reaction time, lignin deposition also increased, forming a thin layer on the fiber surface. The deposited lignin at the surface helps in interfacial bonding of fiber when the fiber is hot pressed. Because of this reason the IB of boards remained high, whereas MOR and MOE exhibited a sharp decrease due to reduced fiber strength. Maximum strength was observed in the board prepared from fiber that was treated for 60 min.

The boards were pressed at four different pressing times of 4, 6, 8, and 10 min. Figure 4(C) presents results for the test of boards prepared from 6 U/g and 60 min enzyme-treated fiber, pressed at 200 °C, but with different pressing times employed. In Fig. 4(C) the board pressed at 4 min showed the least mechanical strength, which might be due to the incomplete bonding between wood fibers when the plasticized lignin could not be distributed uniformly. The maximum mechanical strength was obtained at 6 min of pressing time, and the mechanical strength started decreasing at higher pressing time. The highest value of MOE, MOR, and IB was observed as 3604.85 (± 161.10) MPa, 9.29 (± 0.41) MPa, and 0.67 (± 0.04) MPa, respectively when the board was pressed for 6 min. As per the ASTM D 1037-96a Part-A, the guideline for mechanical properties of interior grade MDF, except MOR, other properties can pass the standard for 150 grade of MDF (ANSI A208.2-2002). Lower strength at higher pressing time is again attributed to the effects of burning the fiber. When a board is pressed at higher temperature for longer time, it usually gives a burn mark on the surface and makes the boards brittle.

Figure 4(D) presents the results of testing the board prepared from the fiber treated at 6 U/g and 60 min enzyme treatment, pressed for 4 min but applied a different platen temperature (such as 180, 190, 200, and 210 °C). The figure depicts the board pressed at 180 °C displayed the least mechanical strength, whereas it was found to be highest at 200 °C. The highest values of MOE, MOR, and IB were observed as 2107.91 (± 81.50) MPa, 7.39 (± 0.33) MPa, and 0.67 (± 0.02) MPa, respectively, when the board was pressed at 200 °C platen temperature. As per the ASTM D 1037-96a Part-A, the guideline for mechanical properties of interior grade MDF, IB can only pass the standard for 130 grade of MDF, and the rest of the properties were sufficient only for lower grades (ANSI A208.2-2002). Lower mechanical strength at lower temperature can be explained by the plasticization behavior of lignin. Since lignin becomes plasticized at 170 °C, a higher temperature improves the bonding strength (Feldman *et al.* 2001). When such

plasticized lignin fibers are compressed under heat, various types of covalent and secondary bonds are formed by the reactive free radicals present in the molecule (Widsten *et al.* 2002). A board pressed at 210 °C again showed a lower strength, which may be due to the burning effect of fiber at higher temperature. At higher temperature the boards becomes brittle and hard; this reduces the MOE and MOR drastically whereas IB remains high.

The water absorption and thickness swelling properties of the boards were also analyzed, and boards were damaged after 3 to 4 h. Although none of the fiberboard could pass the ASTM standard for higher grade MDF, this innovative idea can lead to future work with some more improvement in technique. Moreover, this approach can be used in combination with some synthetic/natural adhesives, as it will reduce the total amount of adhesive required for board making.

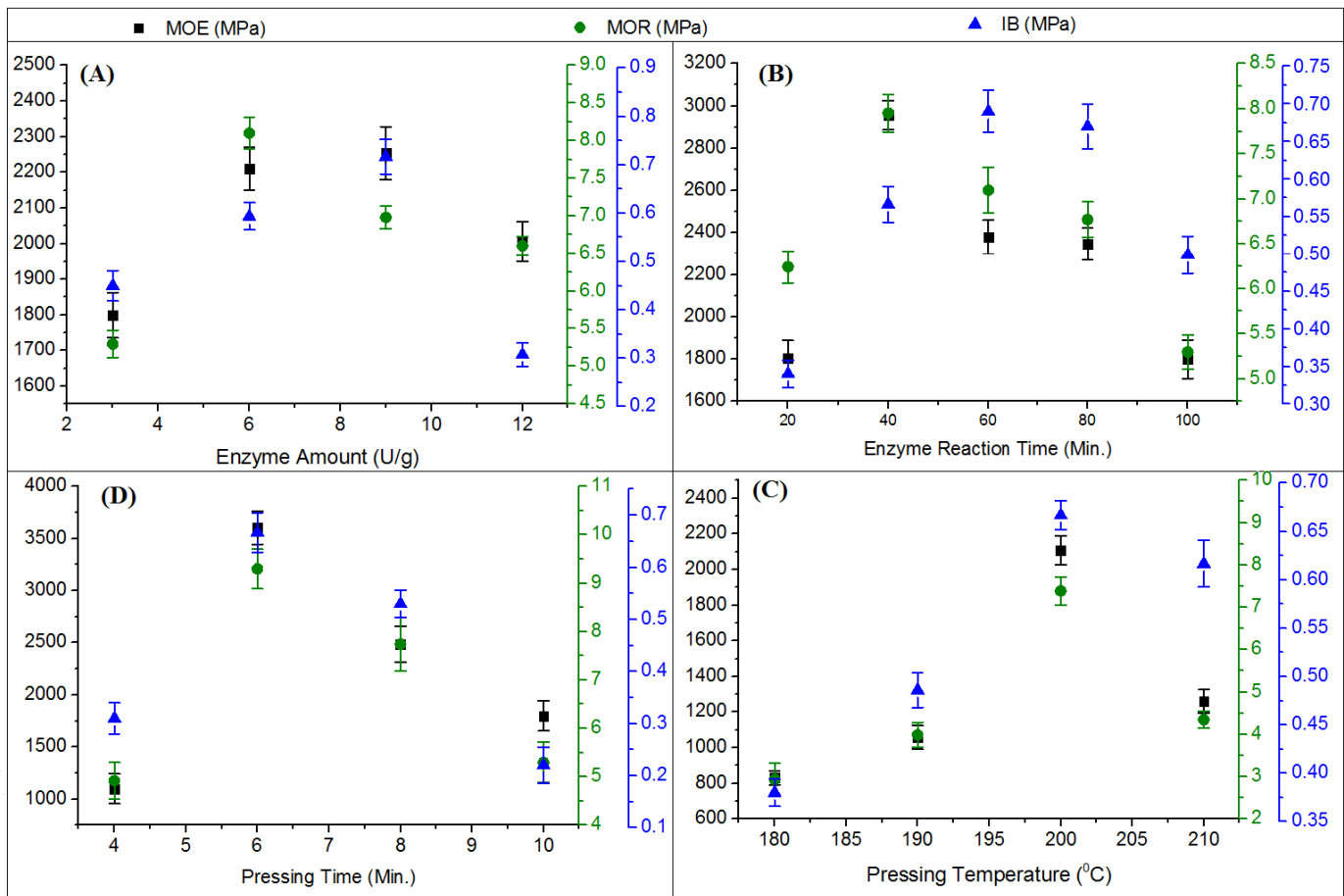


Fig. 4. Mechanical properties (MOE, MOR, and IB) of binderless boards prepared from four different treatments

CONCLUSION

A successful binderless fiberboard of density 750 (± 10) MPa was fabricated from fiber treated at 9 U/g for 60 min and pressed at 200 °C for 6 min. Laccase enzyme oxidized the lignin into a stable phenoxy radical, provided a suitable surface for another component to attach onto it, and acted as a potential cross-linking agent. It was assumed

that a thin uniform layer on wood fiber surface (as visible in SEM) was plasticized at high temperature, and free radicals of lignin formed cross-linked joints between the phenoxy molecules. Thus, laccase-treated board has potential not only to solve the formaldehyde emission problem but also to reduce the cost of production, as it does not need any artificial binder.

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