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Rubberwood Fiber Treatment by Laccase Enzyme and Its Application In Medium Density Fiberboard

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In this work physico-chemical properties of rubber wood fiber were analyzed after laccase treatment and evaluated the performance of medium density fiberboard (MDF) fabricated from the treated fiber. In a single parameter study, two different times i.e. 60 min and 120 min of laccase treatment were applied at constant temperature, enzyme concentration and pH. After the pulp treatment, fibers were sieved out and transferred in to autoclave at 121 °C for 15 min to stop further enzyme reaction. Treated fiber was dried in electric oven drier at 80-90°C for 24 hr to dry up the fiber until constant moisture content. Crystallinity index of the fiber was measured by X-ray diffraction method and it was observed maximum up to 14% higher compared to untreated fiber. The treated fiber was further analyzed for FE-SEM, TGA and compared with untreated fiber in order to evaluate its properties. Fiber treated with enzyme exhibited superiority in fiber surface structure and thermal degradation over untreated fibers. The MDF prepared from improved crystalline fiber showed improved physical and mechanical properties as compared to the MDF from untreated fiber.

Key words: Crystallinity index, FE-Scanning Electron Microscope, Thermal properties.

Medium density fiberboard (MDF) is fabricated commercially by a set process of converting wood logs into fiber and the dried fiber is blended with wood adhesive before pressing it into board at high temperature. Thus, the MDF is a two component system one is wood materials in fiber form and another is adhesive (Li *et al*., 2007). Any changes in these two component leads to change in MDF properties. The properties of individual fiber depend on species, shapes, sizes, and thickness of the cell which ultimately appear in the product (Satyanarayana *et al*., 1990). These fibers may differ, in coarseness, strength, stiffness and roughness or smoothness of the fiber surface of cell walls. Thus it is better to know the length, width type and source of the fibers which play an important role to determine the properties of MDF. Being a cellulosic material, wood fiber is a hygroscopic, dimensionally unstable and prone to biological attack. When MDF prepared from such fiber comes into contact of water, it swells up and sometime swelling is not recoverable. A thickness swelling markedly weakens panel products and reduces the appearance.

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Wood cell walls are composed of cellulose, hemicelluloses and lignin (Stefanidis *et al*., 2014). The mechanical property of wood fibers is mainly determined by cellulose and hemicelluloses, whereas lignin acts as an adhesive between the fibers (Gea *et al*., 2011; Winandy & Rowell, 2005). Cellulose is a long polymer chain of linear crystalline interrupted with regular amorphous region, primarily responsible for strength in the wood fiber (Muhammad Shahid Nazir & Mohd Azmuddin, 2013; Stefanidis *et al*., 2014). Cellulose crystallinity directly affects the physico-chemical behavior of the individual fiber such as modulus, hardness, stiffness, tensile, and swelling-shrinkage properties of individual fiber. Hemicelluloses are amorphous, highly branched polymer compared to cellulose (Zhou *et al*., 2009), acts as a linkage between cellulose and lignin. Lignin, on the other hand, is an amorphous phenolic compound that functions as a cementing material and stiffening agent for the cellulose molecules within the fiber cell wall (El Mansouri *et al*., 2007). Most of the lignin is found inside the cell wall as an intra-cellular lignin (70%), the rest are found in the middle lamella as extracellular lignin (Kunamneni *et al*., 2007; Tuor *et al*., 1995).

Laccase is a well-studied oxido-reductase enzyme, it take part in polymerization as well as depolymerization of lignin compound through free radical reaction(Nasir *et al*., 2013). Since Laccase enzymes are too large to penetrate into fibers (55-80 kDa), it modify the amorphous extracellular component only(Gochev & Krastanov, 2007). Hence, due to a size exclusion reasons, laccase treatment is only a surface modification and it alters extracellular lignin without affecting the cellulose fiber. Although several researchers have studied the enzymatic hydrolysis of lingo-cellulosic fiber but the changes in physical and mechanical properties of fiber is still not fully understood (Kharazipour *et al*., 1997; Yaropolov *et al*., 1994). Thus this study deals the pretreatment reaction of laccase at two different times, on rubber wood fiber and the result was compared with untreated fiber. Such improved fibers can be used in many industrial applications like fiber board, pulp and paper industries etc (Nasir *et al*., 2013; Virk *et al*., 2012; Wu *et al*., 2011).

MATERIALS AND METHODS

A thermo-mechanically processed pulp of rubber wood fibers was supplied by Robin Resources (Malaysia) Sdn. Bhd. The pulp was then air dried to moisture content of 15-20% before applying any treatment. Laccase enzyme (Novo WA 20040) was supplied by Novozyme, Malaysia.

Laccase Hydrolysis

About 25 g of oven dried wood fibers of was suspended in 475 g of deionized water to make a solution of 5.0% consistency (mass pulp/mass suspension) in a 2 L Erlenmeyer flasks. A buffer solution of sodium acetate and acetic acid was used to maintain the pH. Two different reaction time (60 min and 120 min) were applied at constant temperature of 25 °C, enzyme concentration of 7 U/g and 5 pH(Nasir *et al*., 2014). The two treatment were named as FT1 (Fiber treated) FT2 when treated at 60 min and 120 min respectively, whereas untreated was named as UT in later part of result and discussion. After each pulp treatment, fibers were sieved out immediately from the solution and transferred in to autoclave at 121 °C for 15 min to stop further enzyme reaction. Treated fiber was dried in electric oven drier at 80-90 °C for 24 hr to dye up the fiber until zero moisture content.

Preparation of medium density fiber boards

Air-dried treated fibre was used for preparation of MDF board. A total of 195 g fibre (185 + 5% allowance) was used for making the board with target panel density of 770 kg m^{-3}. Soy–lignin adhesive was spread over fibres using air pressure gun. A locally designed rotating drum was used to mix the fibre and urea formaldehyde adhesive. A mat of dimension 220 mm × 220 mm was prepared after adding adhesive by weight of the fibre and pre-pressed to a thickness of 60 mm in cold press. The mat was then hot-pressed to a 6 mm thickness. Total pressing time was 240 s and applied pressure was 5 MPa and both these parameters were kept constant for all samples. After hot pressing, the boards were cooled in an incubator, conditioned at 25 °C and 67% relative humidity. From each board, two samples were prepared for internal bonding, two for thickness swelling test and two for modulud of rupture (MOR) and modulud of elasticity (MOE) tests (Figure 1).

Crystallinity Index

X-Ray measurements were conducted on a Rigaku MiniFlex II, bench top X-ray diffractometer (XRD) analyzers. The fiber specimens were pressed at 10 tone hydraulic pressure into a circular shape of a tablet with 14 mm diameter and 0.5 mm of thickness. The X-ray diffractometer was operated at a voltage of 30 kV with a current density of 15 mA. The scanning range was from 2q = 10° to 50° at a scan speed of 0.015°/s. The data was collected using a fixed time mode with angular intervals of 0.015°. The method adopted was based on Kim and Holtzapple (2005). Crystallinity Index (*CrI*) was calculated from Equation 1.

$$\text{Crystallinity Index (CrI)} = [(I_{002} - I_{am})/I_{002}] \times 100 \quad ...(1)$$

Where, I_{002} is the intensity of the diffraction from the 002 plane 2è = 22.0-23.0, and I_{am} is the intensity of the background scatter measured at 2è = 16.0-18.0.

Medium density fiber boards (MDF) performance Test

Modulus of rupture, internal bonding and maximum load bearing capacity were used to determine the performance of the board. Mechanical testing of samples was done using a universal testing machine and results were analysed using TRAPEZIUM X-software. All testing was performed according to ASTM D1037-06a (ASTM 2006) and statistical analysis was performed on SYSTAT 9.0 software.

RESULTS AND DISCUSSION

Physical Properties of Treated Fiber

An initial experiment was cried out to understand the real effect of laccase treatment on rubber wood fiber. Figure 2 exhibited, the untreated fibers having the least crystallinity index of 65.5% whereas it was improved in treated fibers to 74.4% and 72.87% in FT1 and FT2, respectively. The X-ray-based measurement of crystallinity index (CrI) indicates that laccase treatment increased the

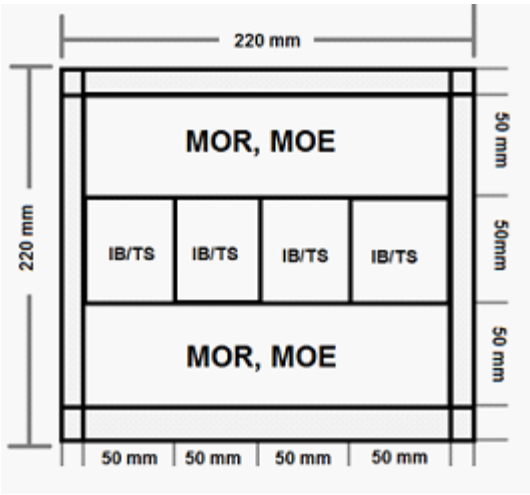

Fig. 1. A sketch showing the cutting pattern of sample prepared for performance test (the dimensions are not exact to the scale).

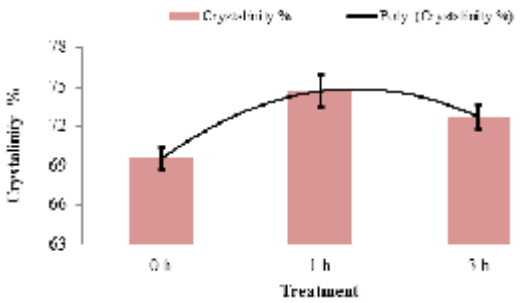

Fig. 2. Fiber crystallinity index of treated at enzyme concentration of 7 U/g and 5 pH

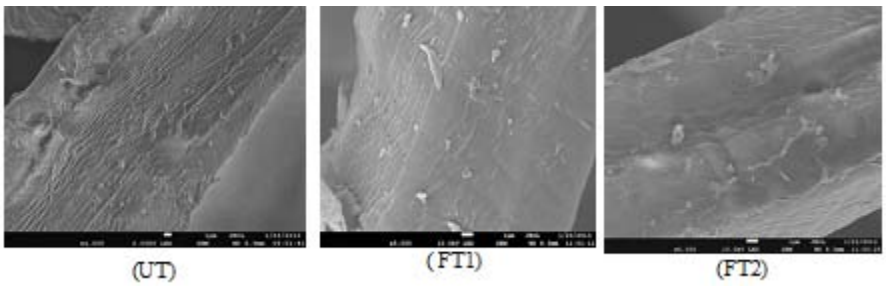

Fig. 3. FE-SEM of (UT) untreated fibers and (FT1, FT2) treated fibers at different reaction condition at 5000x magnification

crystallinity of rubber wood and maximum was observed at 1h of treatment time. However, the CrI started to reduce when the reaction was prolonged for 2h. The change in crystallinity index was due to the removal of extracellular lignin from the fiber surface that ultimately improves the crystallinity (Lionetto *et al*., 2012; Wu *et al*., 2011), But in the next two hour fiber showed a decreasing trend. It was expected that a prolong treatment, lignin started to precipitate back on the fiber surface that results in the fall of crystallinity which is supported by the FE-SEM result (Kumar *et al*., 2009).

Figure 3 depicted the FE-SEM picture of treated and untreated fiber at 5000x magnification. From the close view of micrograph it was obvious that an enzyme treatment brought a distinguish change in the fiber surface. An untreated fiber (UT) had uneven furrowed surface whereas a treated fibers have a very smooth surface. The uneven surface of wood fiber could be due to extra-cellular lignin found naturally on fiber surface which was either removed in lignification process or become even surface due to deposition. Furthermore FT2 fiber exhibits slightly thicker layer of deposition as compared to the FT1 treated fiber. Although laccase act specifically on lignin, the deposition composition should be studied thoroughly. Thus it was concluded that the smoothness of the fiber surface could be due to the removal of loosely bonded lignin and then precipitation of the hydrolyzed lignin, as a smooth layer on the fiber surface which is similar to the finding of Kumar *et al.* (2009).

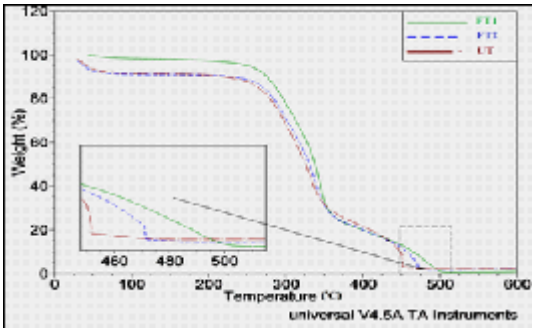

Fig. 4. Thermo-gravimetric curve, showing a two-step decomposition of fiber

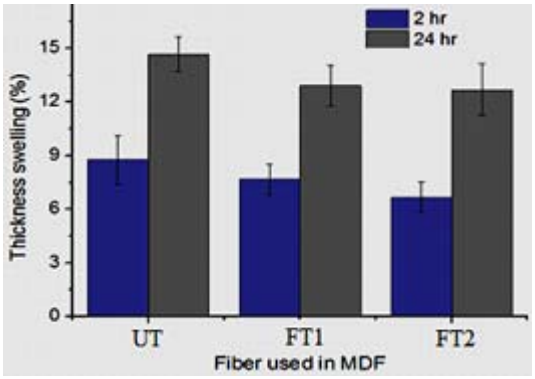

Fig. 5. Water absorption properties of MDF made from untreated fiber (UT), treated fibers (FT1 and FT2).

Thermal Properties of Treated Fiber

Figure 4 shows the Thermo-gravimetric curves were all wood fibers (either treated or untreated fibers) exhibited two exothermic peaks overlapping with regions of weight loss(Jawaid & Abdul Khalil, 2011). Thermal stability of treated and untreated fibers was analyzed on TGA. Thermal degradation of wood fibers occurs in two stages, it initiates from the amorphous cellulose at around 300°C followed by the crystalline cellulose degradation at a higher temperature around 350°C(Quintana *et al*., 2015). It was observed that initial degradation of untreated fiber (UT) was started at temperature 331°C whereas this temperature was found higher for treated fibers (FT1 and FT2). Final degradation temperature of treated fiber was also increased from 449 °C of untreated fiber to 491 °C of treated fiber. Among the treated fibers FT1 exhibits higher degradation temperature as compared to FT2 due to its higher crystallinity index (CrI). It was observed that Tg value of treated fiber was higher and increases with the increase in crystallinity of the fiber. Therefore it was concluded that a treated fiber were having a higher decomposition temperature as compared to untreated fiber(Li & Pickering, 2008).

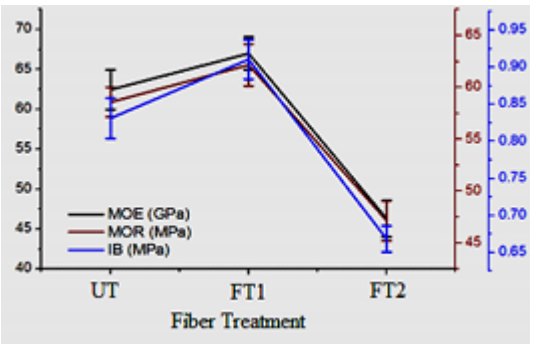

Fig. 6. Mechanical Properties of MDF made from untreated fiber (UT), treated fibers (FT1 and FT2).

Medium Density Fiber Board Performance Analysis

Thickness swelling results of the MDF observed at 2 hr and 24 hr is depicted in Figure 5. A treated fiber exhibits greater dimensional stability as compared to untreated fiber. A lower thickness swelling in treated fiber could be due to the change in surface morphology of the fiber. Since FT1 fiber had least free hydroxyl group due to interfacial bonding between fibers, it exhibits the lowest thickness swelling. Whereas in case of FT2, lignin formed a thin layer over the fiber, it reduces the accessibility of water to the fiber, which ultimately improves the dimensional stability of the fiber. However, the standard deviation error bar shows an overlapping trend that explains that the difference was least significant.

Figure 6 shows the mechanical test (MOE, MOR and IB) results of MDF prepared from different fibers. A very significant and unexpected change in properties of the board is visible when fiber is treated at different reaction condition. The figure demonstrates an increase in the tensile and bending propertied when the fiber FT1 is treated at optimum condition. The increment in bonding strength of the fiber is probably because of the exposed hydroxyl groups on the fiber surface due to removal of lignin from the fiber that helps in interfacial bonding between fibers (Li & Pickering, 2008). Moreover, the strength of MDF reduces drastically and exhibited lower than the untreated fiber when the fiber FT2 was treated for prolonged time. It was assumed that in a prolong enzymatic reaction, lignin molecules starts precipitating and form a uniform layer on wood fiber surface that mask the free hydroxyl groups on wood fiber surface (Figure 3, FT2). That ultimately hindered the interfacial bonding between the fibers. However the strength of FT2 boards below the untreated fiber (UT) boards is still need to be investigated.

CONCLUSION

In the preliminary study of laccase effect natural fibers were carried on for two different times. The crystallinity index was observed maximum up to 74.4 %, at 1 hr enzyme reaction condition then after it decreased considerably and remained constant for a wide range of around 71-72%. Fiber treated with enzyme exhibited superiority in fiber surface structure and thermal degradation over other treated and untreated fibers. A treated fiber with improved crystallinity exhibited improved individual performance and collectively improved the performance of MDF. However in this work only one parameter was considered for study but to obtain a highly crystalline and mechanically strong fiber, a thorough study of various reaction parameters such as time, concentration and temperature of the reaction is recommended. Furthermore, the fiber with improve mechanical strength and crystalline structure can be utilized for various other purposes also in order to produce high quality product.

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