Preparation of Medium Density Fiberboard from Wood Fibers Catalytically Oxidized by Laccase-Mediator System

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Laccase, an enzyme capable of degrading lignin, has become an effective agent for green processing and has great significance for the protection of the environment and the development of a low-carbon economy. In this work, wood fibers were selected as the raw material, and with activation by a laccase-mediator system, lignin was changed to a natural adhesive material, and the high temperature/high pressure method was used to prepare medium density fiberboard (MDF). The bonding mechanism was explored with measurements of Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), environmental scanning electron microscope (ESEM), and X-ray diffraction (XRD). It was found that the self-adhesive effect was realized through esterification, hydrogen bonding, polycondensation, coupling, and a Schiff base reaction, among which coupling and polycondensation were the primary reactions. The chemical bonds between the MDF interior and surface varied because of the mobility of the lignin during the reaction.

Keywords: Laccase-mediator system; Medium density fiberboard; Mechanism

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INTRODUCTION

The traditional use of formaldehyde-type adhesives for preparing MDF involves not only biohazard issues associated with free formaldehyde, but also issues involved in the use of non-renewable resources such as oil (Kurt *et al*. 2011; Zhang *et al*. 2014). Yet the biggest issue facing the petroleum-based adhesives would likely be attributable to the adhesives themselves when they are being recycled or discarded. Due to the action of laccase, great changes can take place in the bonding system, and such changes can make a significant contribution to environmental protection (Barsberg and Thygesen 1999; Felby *et al*. 2004; Duan *et al*. 2007; González-García *et al*. 2011). Meanwhile, based on the preliminary experiments, the MDF prepared from natural lignin activated through laccase-mediator system may have better prospects for carbon sequestration.

However, the growth process of lignocellulosic material shows that lignin, hemicellulose, and some carbohydrates serve as the plant's own natural crosslinking agents (Altaner *et al*. 2007). Lignin is a complex net-like polymer containing many active functional groups, such as phenolic hydroxyls, alcoholic hydroxyls, carboxyls, and esters (Nord and Schubert 1959; Kilpelainen *et al*. 2007). Under certain conditions, crosslinking reactions can proceed between lignin and cellulose or hemicellulose, where lignin plays a role similar to that of cement in reinforced concrete. In general, wood with relatively high tangential and radial direction mechanical strength has relatively high lignin content. Currently, the preparation of lignin-based adhesives, such as the well-known lignin sulfonate adhesive from modified natural lignin, has become a research focus (Simon and Pizzi 2003). The hemicellulose cross-linking effect, in which hemicellulose behaves similarly to hot gel, can be achieved by the production of aldehydes, ketones, and phenolic and other substances during the hot-pressing of lignocellulosic material (Azhar *et al*. 2015; Kacik *et al*. 2015). Therefore, increasing the active functional groups of lignin and reducing the loss of hemicellulose during the lignin modification process can be expected to effectively increase the MDF crosslink density.

As an oxidoreductase, laccase can intensify catalytic oxidation of phenols, polyamines, lignin, *etc*. (Burton 2003; Galli and Gentili 2004; Madhavi and Lele 2009; Witayakran and Ragauskas 2009). The catalytic oxidation of phenolic lignin primarily results in the formation of phenoxy radicals, which can change into quinone compounds through a disproportionation reaction under different conditions. Meanwhile, C_α oxidation, aromatic ring cleavage, alkyl-aryl group bond cleavage, and C_α-C_β bond cleavage also occur during the reaction (Baldrian 2006; Kalliola *et al*. 2014). The oxidation-reduction potential of laccase is between 450 and 790 mV, and the active site (Cu1) determines the catalytic efficiency of laccase. Considering the steric hindrance in the reaction between the active site and lignin, a natural mediator (vanillin) and artificial mediators (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), abbreviated ABTS) were used in this study. With the radical displacement reaction, these small-molecule compounds oxidize non-phenolic lignin, but do not occupy the active site of laccase (Camarero *et al*. 2005; Lahtinen *et al*. 2009; Nousiainen *et al*. 2009). Thus, the catalytic efficiency of laccase can be enhanced and the physical and chemical properties of MDF can be improved. The preparation of MDF from wood fibers activated by a laccase-mediator system was studied based on the physical and mechanical properties, cellulose crystallinity, and functional groups.

EXPERIMENTAL

Preparation of MDF

Specimen

Myceliophthora thermophila laccase solution (60 U/mL) supplied by Wuhan Yuancheng Technology Development Co., Ltd and mediator solution made of ABTS (Sigma-Aldrich Co.) and vanillin (Adamas Reagent Co.) were prepared first. Then, 50 mL of an acetic acid/sodium acetate buffer solution (0.2 M, pH=5) was added to the laccase solution. The molar concentrations of ABTS and vanillin in the mediator solution were 0.28 and 1.66 mM, respectively. The laccase solution and mediator solution were mixed, and then weighed wood fiber was also added and mixed. The fiber was a miscellaneous blend with poplar and birth, and the volume fraction of wood fiber was 4.0%. Oxygen was continuously fed to the mixture during the reaction process, and the reaction time was set at 55 min at 50 °C. The moisture content of wood fiber was set at 20% ± 3%. A total of 1.2% chitosan as waterproof agent was mixed with the wood fiber. After preloading for 60 s, the mixture was hot-pressed. The pressure, temperature, and time of the hot-press were set at 10 MPa, 160 °C, and 512 s, respectively. The experiment was repeated 20 times. The samples of approximately 200 mm × 200 mm × 3.2 mm (length × width × height) with 0.8 g/cm^3 was suitable for furniture grade under dry condition.

Control sample

Control samples were fabricated using the same process, only using deionized water as the solvent without adding laccase or mediator during the system treatment. Chitosan was not added before the hot-pressing. The same tests were repeated 20 times.

Mechanical Properties

The specimen and control samples were kept for 48 h in an environment with constant temperature and humidity. The thickness expansion rate during water absorption was measured according to the People's Republic of China National Standard GB/T 11718-2009 (2009). Then, static bending strength, elastic modulus, and internal bond strength were measured using a universal testing machine (Kunshan Creator Testing Instrument Co., Ltd, Jiangsu Province).

Fourier Transform Infrared Spectroscopy

The pressed MDF specimen and control sample were made into small samples with dimensions of 10 mm × 10 mm × 3.2 mm. FTIR spectra were obtained using a gas chromatograph - Fourier transform infrared spectrometer (GC-FTIR, MAGNA-IR560 E.S.P). The baseline correction was conducted on the obtained spectral line using OMNIC ESP software. Wavenumbers of 1800 to 2000 cm^{-1} and 1506 cm^{-1} were set as the baseline and the standard for qualitative analysis of the changes in the functional groups.

Cellulose Crystallinity

The specimen and control sample were made into small samples with dimensions of 10 mm × 10 mm × 3.2 mm. The relative crystallinities of the MDF surface or interior were measured using an X-ray diffractometer (D/MAX2200X Rigaku Corporation, Tokyo).

Morphology

The specimen and control samples were made into small samples with dimensions of 5 mm × 5 mm × 3.2 mm. The morphologies were determined using an environmental scanning electron microscope (QUANTA200, FEI, Shanghai). For the morphology scanning, the samples were prepared by two methods, cutting for cross-section and peeling for plane-section.

X-Ray Photoelectron Spectroscopy

The specimen and control sample were made into small samples with dimensions of 10 mm × 10 mm × 3.2 mm. X-ray photoelectron spectroscopy measurements were conducted on the surface/interior of the specimen or control sample using an X-ray photoelectron spectrometer (ESCALABMK, VG, England). In this study, changes in the MDF chemical structure were analyzed qualitatively by measuring the bond energy of the C element. The C1 signal peak at 285 eV, which was set as the energy standard, was characterized as C-C, C-H, or C = C bonds. Corresponding to C1, the C2 signal peak, with a chemical shift of 1.7 ± 0.2 eV, was characterized as C-O bonds. The C3 signal peak, with a chemical shift of 3.1 ± 0.2 eV, was characterized as C=O or O-C-O bonds. Finally, the C4 signal peak, with a chemical shift of 4.3 ± 0.2 eV, was characterized as O-C=O bonds.

RESULTS AND DISCUSSION

Mechanical Properties

The modulus of rupture (MOR), modulus of elasticity (MOE), and internal bonding strength (IB) of the specimen were apparently higher than those of the control sample, which are below the minimum requirements for the national standard (Table 1). One possible reason for this is that hydrogen bonding is the main bonding mechanism in the control sample. This needs to be further verified by other measuring techniques.

The 24 h thickness swelling (TS) of the control sample was four times higher than that of the specimen. Many hydrogen bonds and few chemical bonds were present on the surface of the control sample, while the hydrogen bonds were damaged, and the entry of water molecules was accelerated by the swelling of fibers during the dipping process. However, the MDF control sample could keep the basic form, which indicated that a certain amount of chemical bonds maintained their basic form inside the control sample.

Table 1. Mechanical Properties of the Specimen and Control Sample

Index		Mean	Variance	Variation coefficient	Minimum	Maximum
MOR	Specimen	34.42	0.490	2.03	33.15	35.17
(MPa)	Control sample	21.38	2.067	6.73	19.42	23.64
MOE	Specimen	2888.5	605.2	0.85	2842.0	2924.1
(MPa)	Control sample	2310.2	7644.9	3.78	2157.4	2452.0
IB (MPa)	Specimen	0.89	0.00045	2.39	0.86	0.92
	Control sample	0.25	0.00040	8.01	0.22	0.28
TS(%)	Specimen	24.63	1.311	4.65	22.63	25.70
	Control sample	110.91	51.15	6.45	101.83	122.34
Density	Specimen	0.805	0.0004	2.47	0.784	0.827
(g/cm$^{3)}$	Control sample	0.826	0.0001	1.35	0.817	0.842

FTIR Analysis

The FTIR spectra of the specimen and the control sample are shown in Fig. 1. It can be seen that the two samples had quite different characteristic absorption peaks. The absorption frequencies in the range of 3400 to 3200 cm^{-1} are the O-H stretching vibrations. The absorption peak at 3423 cm^{-1} for the control sample represents not only intermolecular O-H stretching vibrations, but also O(2)H…O(6) stretching vibrations inside the cellulose molecule. The absorption peak at 3423 cm^{-1} for the specimen also represents O(3)H…O(5) stretching vibrations inside the cellulose molecule, and the intensity of the absorption of the characteristic peak of the specimen was significantly reduced compared to that of the control sample. This indicates that the number of hydroxyls in the specimen was reduced. The reduction of O(2)H…O(6) hydrogen bond inside the cellulose molecule shows that the hydroxyl in C(3) and C(6) may react with lignin.

Absorption frequencies of 1738 to 1709 cm^{-1} represent C=O stretching vibrations for non-conjugated ketones, carbonyls, and esters. The relative intensity of the absorption peak of the specimen was greater than that of the control sample. The reason for this may be that the C=O of the control sample was primarily from the ester group, and such groups were formed by the esterification reaction between the carboxyls of lignin and the hydroxyls of cellulose. The C=O of the specimen was also primarily from the ester group, but the alcoholic hydroxyls of lignin were partially changed to carboxyls by the catalytic oxidation of the laccase mediator system. Consequently, the number of carboxyls and the content of C=O increased.

Absorption frequencies of 1690 to 1630 cm^{-1} represent the C=N stretching vibrations of imines. The characteristic absorption peak 1657 cm^{-1} was observed in the specimen, but not in the control sample. The reason for this is that the Schiff base reaction occurred between the aldehyde group of lignin and the amino group of chitosan. Aljawish *et al.* (2012) also observed a novel absorbance peak of Schiff base bond as a function of the bound covalently to the free amino of chitosan.

Absorption frequencies near 1630 cm^{-1} represent the C=C characteristic absorption peak. The characteristic absorption peaks for the specimen and control samples were located at 1620 and 1630 cm^{-1}, respectively. The absorption peak intensity of the treated specimen was significantly reduced compared to that of the control sample. The reason for this is that C=C was primarily from an aromatic ring, which can undergo a hydrogen atom rearrangement reaction with activation by the laccase-mediator system on the specimen. Consequently, the aromatic ring structure changed and the C=C number decreased.

Absorption frequencies of 1162 to 1125, 1120 to 1115, and 1060 to 1015 cm^{-1} represent C-O-C asymmetric valence vibrations, C-C or C-O stretching vibrations, and the C-O valence vibrations of C(3)-O(3)H, respectively. The wavenumbers of characteristic absorption peaks in these three absorption frequencies of the specimen were 1156, 1102, and 1027 cm^{-1}, respectively, which were increased from the 1160, 1114, and 1055 cm^{-1} of the control sample. This is because the content of aldehyde groups in the lignin in the specimen increased as a result of the catalytic oxidation of the laccase-mediator system. The polycondensation and coupling reaction were then enhanced, and the contents of C-C and C-O-C in the structure were increased.

The characteristic absorption peaks in the absorption frequency range of 800 to 750 cm^{-1} indicate the existence of three adjacent hydrogen atoms on the aromatic ring. There was a weak absorption peak at the frequency 780 cm^{-1} in the specimen spectra, but it was not observed in the specimen spectra. This indicates that a substitution reaction occurred on the aromatic ring. The bonds among the functional groups were closer and the formed network structure was more complex in the specimen.

Based on the infrared spectrum analysis, it can be seen that the self-adhesive reaction of the control sample was primarily by means of esterification between hydroxyls and carboxyls, hydrogen bonding between hydroxyls, and condensation between aldehyde groups and hydroxyls. Another study also verified the same bonding mechanism (Felby *et al*. 2004). The reaction depended on the numbers of the functional groups, which means that hydrogen bonding was the main reaction, followed by polycondensation, and then esterification. In addition to the former three reactions, the self-adhesive reaction of the specimen also contained a coupling reaction of free radicals, in which C-O-C was the primary chemical bond formed. It is difficult to determine which was the primary reaction between the coupling and the polycondensation reactions because the C-O-C chemical bond was produced in both reactions. In addition, the Schiff base reaction also occurred between aldehydes and amino groups. From the relative absorption peak intensity, it can be seen that coupling and polycondensation were the main reactions, followed by hydrogen bonding, the Schiff base reaction, and then esterification. The self-adhesive in MDF was very complex. In general, the new chemical bonds decreased the distance between the hydroxyl groups, making it easier to form hydrogen bonds to improve the mechanical strength of the MDF. Therefore, water can be prevented from penetrating into MDF.

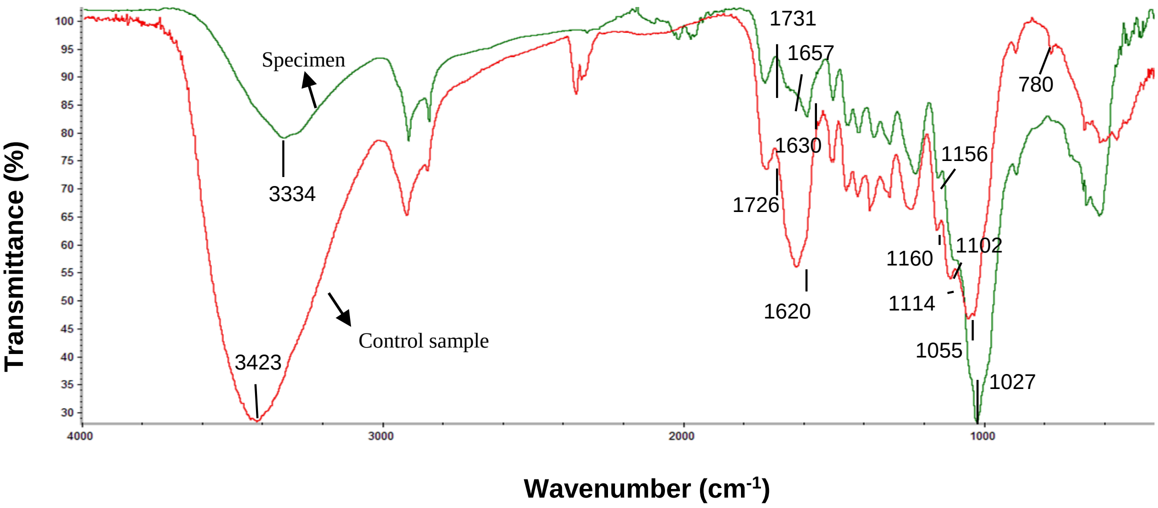

Fig. 1. FTIR of specimen and control sample

Relative Crystallinity Analysis

The relative crystallinity of the cellulose located in the specimen interior was calculated using the Turley formula with the maximum diffraction intensity (peak 002) and the minimum diffraction intensity (around $2\theta = 18°$) (Fig. 2).

Variation characteristics of relative crystallinity can be summarized based on two aspects. One is that the relative crystallinity of the surface was higher than that of the surface, because the cellulose molecular chains were rearranged, and some were formed in to new crystallization regions. In addition, the hemicelluloses and lignin on the upper and lower surfaces of MDF became degraded more than in the interior because the surface temperature was higher than the interior temperature, so the relative crystallinity of the surface increased.

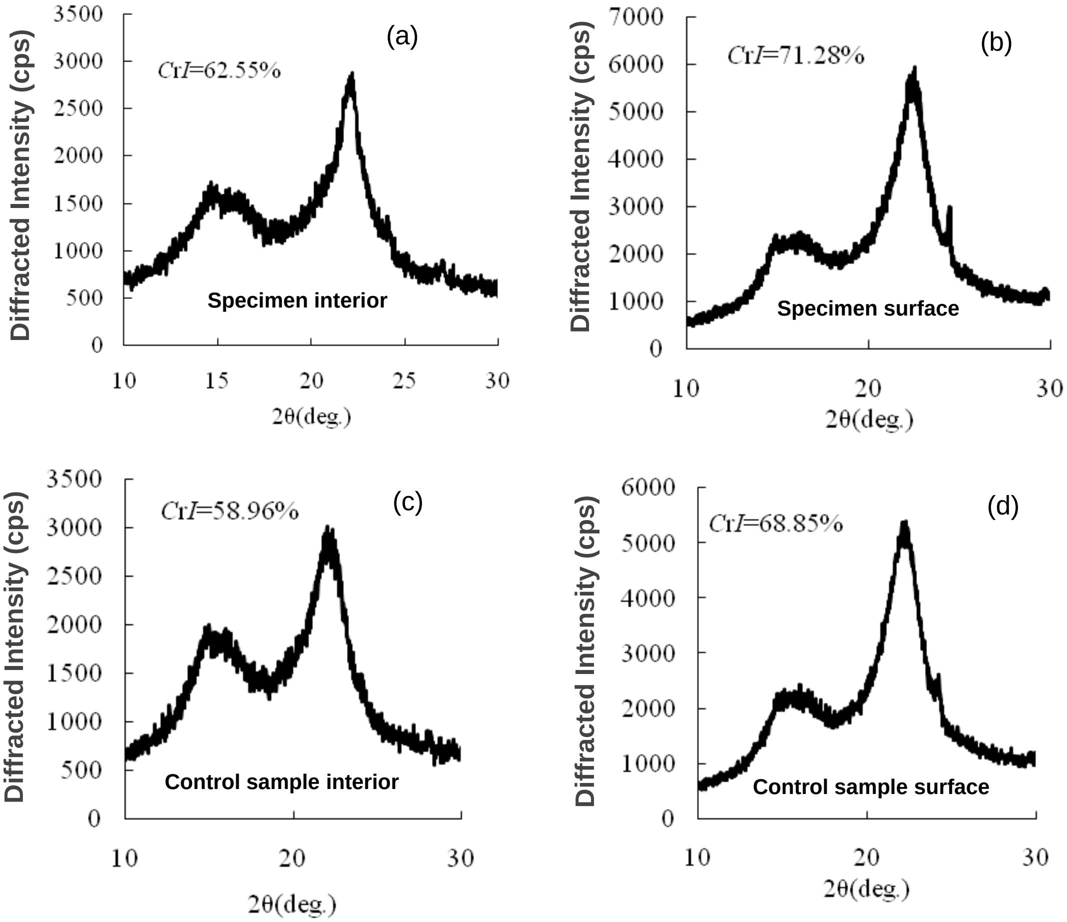

Fig. 2. Relative crystallinities of the MDF specimen/control sample: (a) specimen interior, (b) specimen surface, (c) control sample interior, and (d) control sample surface

Another aspect is that the relative crystallinity of the specimen was higher than that of the control sample because the hydroxyls of lignin were oxidized to aldehydes or ketones by the laccase-mediator system, thus reducing the possibility of hydrogen bonding between the hydroxyls of lignin and the hydroxyls of cellulose. Meanwhile, high pressure brought the hydroxyl groups of cellulose into closer proximity and increased the possibility of intramolecular bonding and intermolecular bonding of cellulose, thus increasing the crystallinity.

Morphology

The plane-section morphologies of the peeled specimen and control sample are shown in Figs. 3 and 5, from which it can be seen that fibers peeled from the interior of the control sample maintained their original morphology. The small folds were formed because of the broken hydrogen bonds. The wood fibers of the specimen broke and lost their original morphology under the action of external forces. This indicates that the forces between the fibers of the specimen were larger than those of the control sample.

The cross-section morphologies (Figs. 4 and 6) show that all the fibers from the specimen and the control sample were flattened. For the specimen, the fibers were stacked closely and the interfaces were difficult to distinguish, but some gaps were still observed, which were the flow channels for moisture. Both the size and number of gaps in the specimen were smaller than those in the control sample. The temperatures and pressures for preparing the specimen and the control sample were the same. As the temperature of the fiberboard dropped, the energy absorbed by hydrogen bonds was still lower than the energy needed to break the hydrogen bonds, so the hydroxyls on the wood fibers reacted with the surrounding hydroxyls to form the strong hydrogen bonds. However, in the cooling process, the flowing moisture inside the board broke some hydrogen bonds without breaking the chemical bonds. Based on the FTIR analysis, hydrogen bonding was the main bonding mechanism in the control sample; therefore, its porosity was greater than that of the specimen.

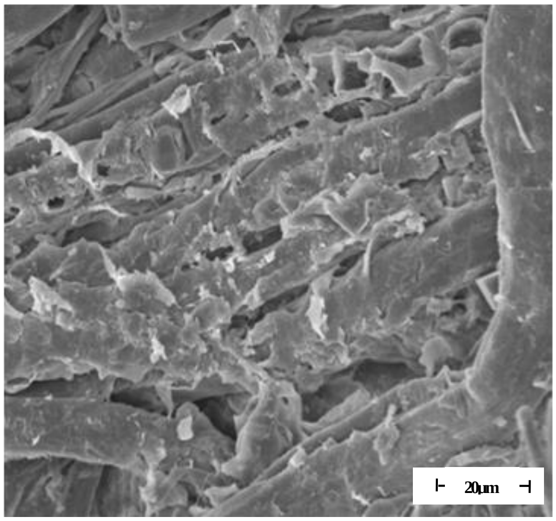

Fig. 3. The plane-section morphology of specimen

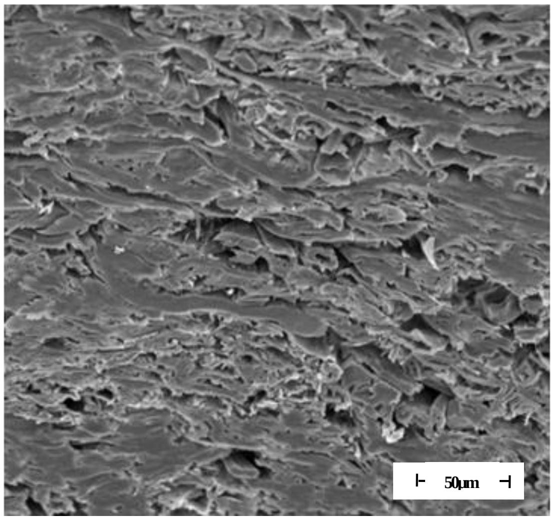

Fig. 4. The cross-section morphology of specimen

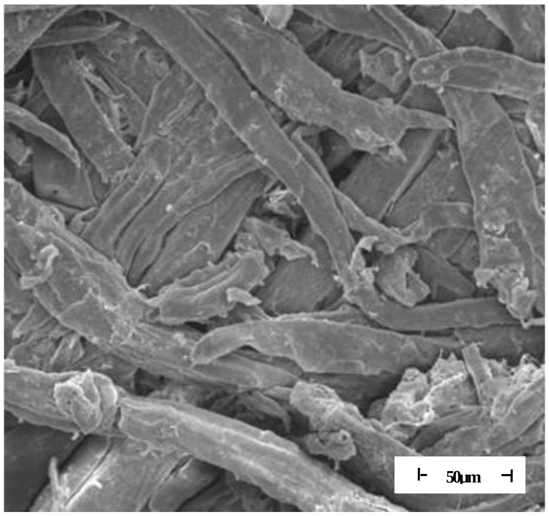

Fig. 5. The plane-section morphology of control sample

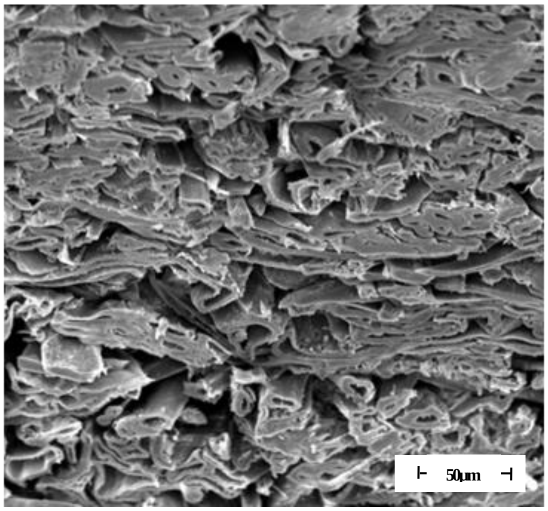

Fig. 6. The cross-section morphology of control sample

XPS

X-ray photoelectron spectra and results of data analysis are shown in Fig. 7 and Table 2, respectively. The ability of the laccase-mediator system to modify the chemical properties of wood fiber was characterized as the O/C ratio. The higher O/C ratio of the specimen surface than that of the control sample surface indicates cross-linking with lignin.

The reason for this may be that the laccase-mediator system degraded some lignin and lowered its degree of polymerization. Lignin was then easier to move to the MDF surface with the assistance of moisture during the hot-press process. The increase in lignin content resulted in a decrease in the surficial O/C ratio of the MDF. Additionally, alcoholic hydroxyls were catalytically oxidized to aldehyde groups by the laccase-mediator system. The polycondensation between the aldehyde groups and hydroxyls, which generated a water molecule, also further increased the surficial O/C ratio of the MDF. Two reasons account for the higher O/C ratio of the specimen interior than that of the control sample: one is that some lignin was transferred to the MDF surface; the other reason is the addition of chitosan. Table 2 shows that the difference between the superficial and the interior O/C ratios of the control sample was small, but it was large for the specimen. This is also because the laccase-mediator system degraded some lignin, making it easier for lignin to move to the MDF surface.

Table 2. Interior/Surface O/C Ratio of MDF Specimen and Control Sample

MDF		O/%	C/%	O/C/%
Interior	Control sample	19.36	75.51	25.64
	Specimen	21.46	72.27	29.69
Surface	Control sample	18.71	76.51	24.54
	Specimen	17.21	77.60	22.18

Further analysis was carried out on the C sub-peak spectra. The bond energies of C2, C3, and C4 increased, but C1 decreased for the specimen interior relative to the control sample. The reason for this is that, with the catalysis of the laccase-mediator system, alcoholic hydroxyls on the lignin were oxidized to aldehyde groups, which then proceeded to a condensation reaction with hydroxyls to increase the number of carbonyls; consequently, the bond energy of C2 increased. The increase of C3 was due to the increasing number of carbonyls with the oxidation of non-phenolic lignin promoted by the mediator. Additionally, the degraded lignin and the vanillin with low molecular weight entered into cellulose to form cross-linking polymerization with cellulose or adjacent cellulose, and the generated glycosidic bond also greatly increased the content of C3. The increase of C4 was due to the further oxidation of aldehydes promoted by the laccase-mediator system. C1 directly reflects the content of lignin, and the decrease of C1 suggests the movement of lignin to the MDF surface.

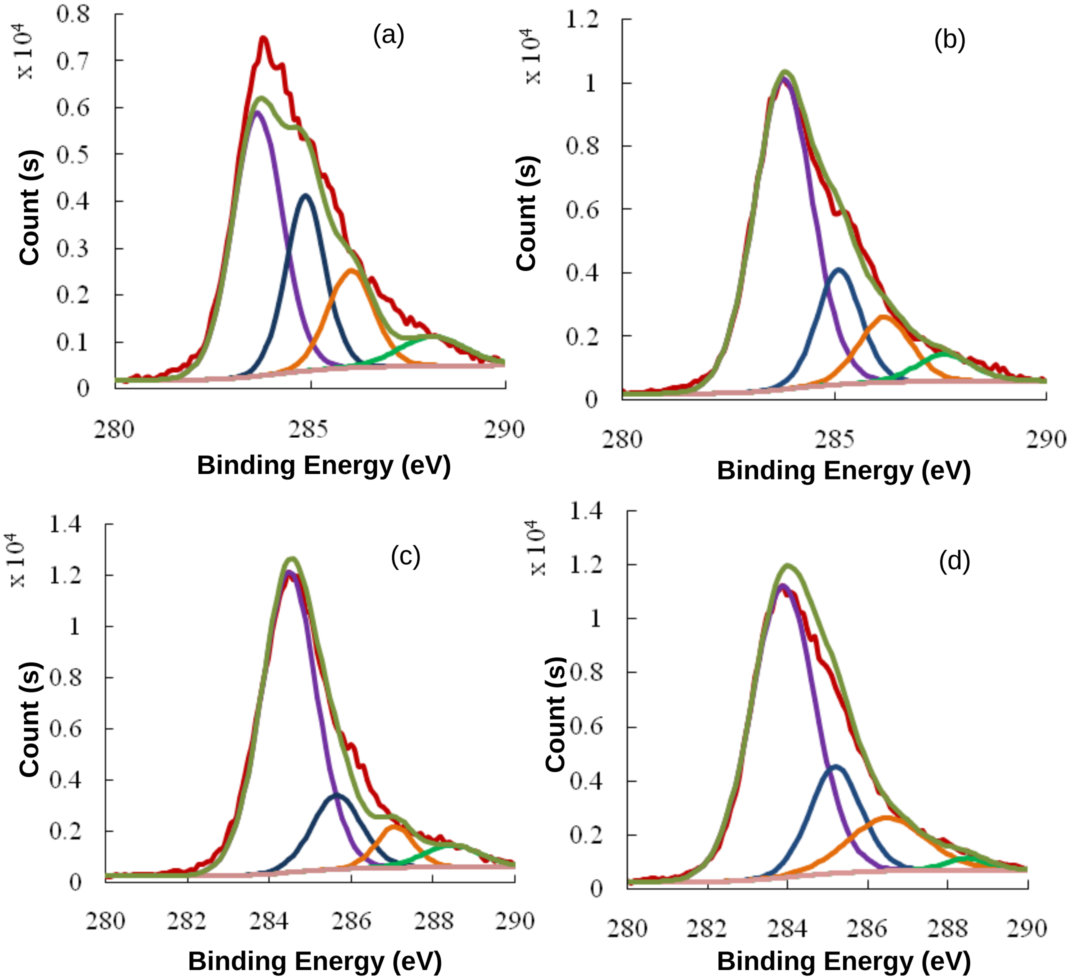

Fig. 7. XPS of the specimen and control sample: (a) specimen interior, (b) specimen surface, (c) control sample interior, and (d) control sample surface

Furthermore, the bond energies of C1 and C4 increased, but C2 and C3 decreased for the specimen surface relative to the control sample. The reason for this is that the degradation of lignin increased the content of superficial lignin and increased the C1 bond energy. The reduction of C2 was due to the esterification reaction between hydroxyls and carboxyl groups. Subsequently, the number of C-O bonds decreased, but the increase in ester bonds increased the C4 bond energy. The decrease of C3 resulted from the rapid pyrolysis of hemicellulose at high temperature, and the pyrolysis product increased the C4 bond energy.

CONCLUSIONS

1. The prepared MDF specimen in this study met the performance requirements of the national standard GB/T11718-2009.
2. The self-adhesive process was realized through esterification, hydrogen bonding, polycondensation, coupling, and Schiff base reaction, among which coupling and polycondensation were the main reactions.

3. During the reaction process, the new generated crystalline region in the cellulose enhanced the MDF mechanical strength. During the hot-press process, the degraded lignin moved to the MDF surface and increased the content of carboxyls, which resulted in the high content of aldehyde groups in the MDF interior. Consequently, the chemical bonds in the MDF interior and surface were different.

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REFERENCES CITED

Aljawish, A., Chevalot, I., Piffaut, B., Rondeau-Mouro, C., Girardin, M., Jasniewski, J., Scher, J., and Muniglia, L. (2012). "Functionalization of chitosan by laccase-catalyzed oxidation of ferulic acid and ethyl ferulate under heterogeneous reaction conditions," *Carbohyd. Polym*. 87(1), 537-544. DOI: 10.1016/j.carbpol.2011.08.016

Altaner, C., Knox, J. P., and Jarvis, M .C. (2007). "*In situ* detection of cell wall polysaccharides in sitka spruce (*Picea sitchensis* (bong.) carrier) wood tissue," *BioResources* 2(2), 284-295. DOI: 10.15376/biores.2.2.284-295

Azhar, S., Henriksson, G., Theliander, H., and Lindstrom, M. E. (2015). "Extraction of hemicelluloses from fiberized spruce wood," *Carbohyd. Polym*.117, 19-24. DOI: 10.1016/j.carbpol.2014.09.050

Baldrian, P. (2006). "Fungal laccases-occurrence and properties," *FEMS Microbiol. Rev.* 30(2), 215-242. DOI: 10.1111/j.1574-4976.2005.00010.x

Barsberg, S., and Thygesen, L. G. (1999). "Spectroscopic properties of oxidation species generated in the lignin of wood fibers by a laccase catalyzed treatment: Electronic hole state migration and stabilization in the lignin matrix," *BBA-Gen Subjects*. 1472(2), 625-642. DOI: 10.1016/S0304-4165(99)00192-0

Burton, S. G. (2003). "Laccases and phenol oxidases in organic synthesis – A review," *Curr. Org. Chem.* 7(13), 1317-1331. DOI: 10.2174/1385272033486477

Camarero, S., Ibarra, D., Martínez, M. J., and Martinez, A. T. (2005). "Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes," *Appl. Environ. Microbiol*. 71(4), 1775-1784. DOI: 10.1128/AEM.71.4.1775-1784.2005

Duan, X. F., Cao, Y. L., Cao, Y. J., Zhou, G. W., Chen, Y. S., Zhu, J. Q., and Zhao, B. L. (2007), "Changes of intensities of reactive oxygen species free radicals from laccase-treated woods," *Scientia Silvae Sinicae* 43(3), 134-136.

Felby, C., Thygesen, L. G., and Sanadi, A. (2004). "Native lignin for bonding of fiber boards-evaluation of bonding mechanisms in boards made from laccase-treated fibers of beech (*Fagus sylvatica*)," *Ind. Crop. Prod*. 20(2), 181-189. DOI:10.1016/j.indcrop.2004.04.020

Galli, C., and Gentili, P. (2004). "Chemical messengers: Mediated oxidations with the enzyme laccase," *J. Phys. Org. Chem.* 17(11), 973-977. DOI: 10.1002/poc.812

GB/T 11718-2009 (2009). "Medium density fiberboard," Standards Press of China, Beijing.

González-García, S., Feijoo, G., Heathcote, C., Kandelbauer, A., and Moreira, M. T. (2011). "Environmental assessment of green hardboard production coupled with a laccase activated system," *J. Clean. Prod.* 19(5), 445-453. DOI:10.1016/j.jclepro.2010.10.016

Kacik, F., Smira, P., Kacikova, D., Vel'kova, V., and Nasswettrova, A. (2015). "Chemical alterations of pine wood saccharides during heat sterilization," *Carbohyd. Polym.* 117, 681-686. DOI: 10.1016/j.carbpol.2014.10.065

Kalliola, A., Asikainen, M., Talja, R., and Tamminen, T. (2014). "Experiences of kraft lignin functionalization by enzymatic and chemical oxidation," *BioResources* 9(4), 7336-7351. DOI: 10.15376/biores.9.4.7336-7351

Kilpelainen, I., Xie, H., King, A., Granstrom, M., Heikkinen, S., and Argyropoulos, D. S. (2007). "Dissolution of wood in ionic liquids," *J. Agr. Food Chem.* 55(22), 9142-9148. DOI: 10.1021/jf071692e

Kurt, R., Cil, M., Aslan, K., and Cavus, V. (2011). "Effect of pressure duration on physical, mechanical, and combustibility characteristics of laminated veneer lumber (LVL) made with hybrid poplar clones," *BioResources* 6(4), 4886-4894. DOI: 10.15376/biores.6.4.4886-4894

Lahtinen, M., Kruus, K., and Heinonen, P. (2009). "On the reactions of two fungal laccases differing in their redox potential with lignin model compounds: Products and their rate of formation," *J. Agric. Food Chem.* 57(18), 8357-8365. DOI: 10.1021/jf901511k

Madhavi, V., and Lele, S. S. (2009). "Laccase: Properties and applications," *BioResources* 4(4), 1694-1717. DOI: 10.15376/biores.4.4.1694-1717

Nord, F. F., and Schubert, W. J. (1959). "Lignin and the formation of wood," *Experientia* 15(7), 245-253. DOI: 10.1007/BF02158066

Nousiainen, P., Maijala, P., Hatakka, A., Martinez, A. T., and Sipila, J. (2009). "Syringyl-type simple plant phenolics as mediating oxidants in laccase catalyzed degradation of lignocellulosic materials: Model compound studies," *Holzforschung* 63(6), 699-704. DOI: 10.1515/HF.2009.066

Simon, C., and Pizzi, A. (2003). "Tannins/melamine-urea-formaldehyde (MUF) resins substitution of chrome in leather and its characterization by thermomechanical analysis," *J. Appl. Polym. Sci.* 88(8), 1889-1903. DOI: 10.1002/app.12042

Witayakran, S., and Ragauskas, A. J. (2009). "Modification of high-lignin softwood kraft pulp with laccase and amino acids," *Enzyme Microb. Technol.* 44(3), 176-181. DOI: 10.1016/j.enzmictec.2008.10.011

Zhang, J. Z., Chen, H., Pizzi, A., Li, Y. H., Gao, Q., and Li, J. Z. (2014). "Characterization and application of urea-formaldehyde-furfural co-condensed resins as wood adhesives," *BioResources* 9(4), 6267-6276. DOI: 10.15376/biores.9.4.6267-6276

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