

# **ORIGINAL ARTICLE**

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Utilization of enzymatic hydrolysate from corn stover as a precursor to synthesize an eco-friendly adhesive for plywood II: investigation of appropriate manufacturing conditions, curing behavior, and adhesion mechanism



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## **Abstract**

In this study, further research on an enzymatic hydrolysate-ammonium dihydrogen phosphate (EHADP) adhesive was carried out. Appropriate hot pressing conditions were clarified by measuring the bond strength of three-ply plywood bonded with EHADP adhesive, and the results indicated that the appropriate fabricate conditions were 170 °C and 5 min. The value of wet shear strength fulfilled the requirements of China National Standard GB/T 9846–2015 when plywood was fabricated by the appropriate conditions. In the research of curing behavior, the insoluble mass proportion promoted significantly as heating temperature and time were  $\geq$  170 °C and 5 min. Furthermore, a pyrolysis gas chromatography/mass spectrometry analysis indicated that adding ammonium dihydrogen phosphate (ADP) catalyzed the conversion of monosaccharides in the EHADP adhesive. The adhesion mechanism of the EHADP adhesive was studied by Fourier transform-infrared spectroscopy analysis, and the chemical changes indicated that the adhesion mechanism was attributed to both mechanical and chemical bonding between the wood elements and the cured EHADP adhesive.

Keywords: EHADP adhesive, Plywood, Appropriate manufacture conditions, Curing behavior, Adhesion mechanism

#### Introduction

The development of renewable bio-based materials is a significant topic of concern with continuous deterioration in the global environment [1–6]. However, most of these advanced materials and technology are remain in the laboratory, and their applications require further research. In contrast, traditional wood-based materials are familiar in furniture, architecture, and in interior

decoration [7–12]. Wood-based materials are fabricated by bonding woody elements with formaldehyde-based or petroleum-derived resins [13, 14]. However, the formaldehyde emissions from these materials, particularly in indoor environments, pose potential health hazards, and the fossil fuel resources used in petroleum-derived resins are nonrenewable [15–17]. These issues have prompted the wood-based materials industry to exploit eco-friendly adhesives which formulated by renewable, non-toxic, and low-cost resources.

Recently, saccharides have been utilized for preparing eco-friendly wood adhesive, which exhibits excellent mechanical bonding properties and water resistance.

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The curing mechanism of saccharide-based adhesives is attributed to the saccharides converting to chemically active furan compounds during heating, and these compounds react with polyphenol compounds [18], organic acids [19], amino compounds [20], and ammonium salts [21] to form a stable cross-linked polymer. However, the price of purified saccharide is a disadvantage compared with synthetic adhesives, which limits current applications of a saccharide-based adhesive. Therefore, it is necessary to identify cheaper and more efficient saccharide resources as raw materials.

Enzymatic hydrolysates derived from the biological conversion of agricultural crop straw are an important feedstock for bioethanol fermentation [22-25], and it contains abundant saccharides from the hydrolysis of cellulose and hemicellulose [26, 27]. Based on the chemical composition of the enzymatic hydrolysate, a corn stover enzymatic hydrolysate was utilized and synthesized with ammonium dihydrogen phosphate (ADP) to prepare an enzymatic hydrolysate-ammonium dihydrogen phosphate (EHADP) adhesive for plywood in our previous research [28]. The optimal synthetic conditions, synthesis, and curing mechanism were investigated, and the results confirmed that the synthesized EHADP adhesive provided bond strength and water resistance by forming a cross-linked polymer after the heat treatment. In addition, a thermal analysis suggested that the polymerization of the EHADP adhesive can occur at a lower temperature. Therefore, in this study, we investigated the appropriate manufacturing conditions for plywood bonded by the EHADP adhesive synthesized under optimized conditions. Furthermore, the curing behavior and adhesion mechanism was also clarified.

## Materials and methods

#### Materials

Corn stover was obtained from an agricultural products company (Huaian, Jiangsu Province, China) and milled to a 20–80 mesh size before utilization. Poplar veneers were provided by a wood products company in Hebei Province, China. Ammonium dihydrogen phosphate (analytical-grade reagent) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The enzyme Cellic (CTec2 cellulase) with filter paper activity of 250.0 FPU/mL was provided by Novozymes NA (Franklinton, NC, USA).

## Preparation of the enzymatic hydrolysate from corn stover and synthesis of the enzymatic hydrolysate-ammonium dihydrogen phosphate adhesive

The detailed preparation process for the enzymatic hydrolysate (EH) followed our previous research [28]. In brief, the corn stover particles were pretreated in dilute

sulfuric acid, and the pretreated solid corn stover residues were enzymatically hydrolyzed by CTec2 cellulase (20 FPU/g glucan). Then, the EH was concentrated to 90 wt% solid content and used for synthesizing the adhesive.

The synthetic conditions for the EHADP adhesive followed our previous study [28], in which the mass proportion between the enzyme hydrolysate and ADP was 90/10, and the synthesis time and temperature were 100 °C and 1 h, respectively. The viscosity and pH values of the synthesized EHADP adhesive were 361.7 mPa·s and 2.8, as measured by a HAAKE rotational rheometer MA S60 (HAAKE CO., Karlsruhe, Germany) and Leici pH meter PHBJ-206 (Leici, Shanghai, China).

#### Manufacture of plywood and shear strength measurement

Three-ply plywood (300 mm  $\times$  300 mm) was fabricated from 1.5 mm thickness wood veneers and the EHADP adhesive was synthesized. To investigate the optimal manufacturing conditions, the hot pressing temperature and time were changed, and the detailed manufacturing conditions are shown in Table 1. The shear strength evaluation method was referred from the China National Standards GB/T 9846.7–2004 [29], in which the plywood was cut into 12 specimens (100 mm  $\times$  25 mm) ( Fig. 1[28]), and 6 were immersed in a 63  $\pm$  2 °C water bath for 3 h to measure wet shear strength and the other six samples were measured in a dry condition. The loading rate of tensile force was 1.0 mm/min, and the dry and wet shear strengths were calculated by the formula:

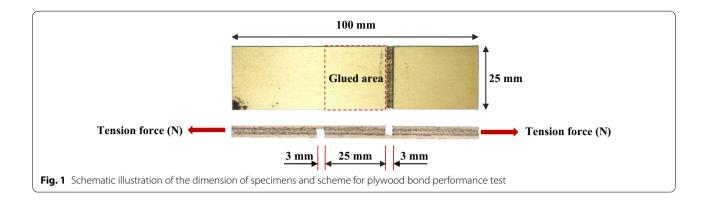
Shear strength = 
$$\frac{\text{Tension force (N)}}{\text{Glued area (mm}^2)}$$
. (1)

The average values of shear strength, standard deviations, and wood failure in each test were calculated, and multiple comparisons of one-way ANOVA analysis on the average values of shear strength derived from each

Table 1 Detailed information of manufacture conditions of the plywood bonded by EHADP adhesive

Groups	Hot pressing temperature (°C)	Hot pressing time (min)	Spread rate (g/m²)	Pressure (MPa)
Group 1	130	7	140	1
	150			
	170			
	190			
Group 2	170	3	140	1
		5		
		7		
		9		

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hot pressing conditions was carried out. A p value < 0.05 was considered significant.

#### Curing behavior of the EHADP adhesive

The curing behavior of the EHADP adhesive was investigated using the insoluble mass proportion test and a pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) analysis. First, the synthesized adhesive solution (EHAPD) and concentrated EH were lyophilized to prepare the uncured samples. The uncured EHADP adhesive was divided into two groups to investigate the effects of heating temperature and time on the insoluble mass proportion. The samples were heated at 130, 150, 170, and 190 °C for 7 min in group 1, and heated at 170 °C for 3, 5 7, and 9 min in the other group. The cured adhesives were dried in a vacuum oven at 60 °C for 15 h. Approximately, 2 g of the cured adhesive was boiled in ultrapure water for 4 h, and the wet insoluble mass was obtained after filtration. This experimental process was carried out five times, and the wet insoluble mass was vacuum dried at 60 °C for 15 h to obtain the final insoluble mass. The insoluble mass proportion was further calculated by Eq. (2), and the average values and standard deviations were calculated for further analysis.

Laboratories, Ltd., Fukushima, Japan), and the detailed information on setting the instrument parameters and temperature programming are referred from our previous study [30]. The results were analyzed using the NIST 08 mass spectra library, and the maximum similarity index (SI) values of the identified chemical compounds ≥ 85 were recorded.

#### Adhesion mechanism of the EHADP adhesive

The adhesion mechanism of EHADP was investigated by measuring the chemical changes on the bonded interface of the plywood by FT-IR (Vertex 80, Bruker, Bremen, Germany). The sampling method is shown in Fig. 2. The samples obtained from the bonding interface of the plywood (bonded by EHADP adhesive at various temperatures) were milled to a powder. The powder was boiled in ultrapure water for 4 h to remove soluble substances. After filtration, the wet insoluble substances were vacuum dried at 60 °C for 15 h, and the dried insoluble substances were used for the measurement. As a control, poplar and insoluble mass (derived from cured EHADP adhesive at various temperatures) were also prepared by the same procedure. The infrared spectra were obtained using the KBr disk method and recorded with 32 scans at

Insoluble mass proportion (%) = 
$$\frac{\text{Weight of dried insoluble mass (g)}}{\text{Weight of heated sample (g)}} \times 100\%$$
. (2)

The slope (k) between the adjacent temperature and time condition was calculated by fitting a linear function to exhibit the growth rate of the insoluble mass proportion. The abscissas values were set to 1 and 2 in each calculation for convenient comparison.

The chemical changes in the volatile compounds produced during the curing process were investigated by Py-GC/MS (GCMS-QP2010, Shimadzu Co., Ltd., Kyoto, Japan). A~1 mg aliquot of lyophilized uncured EH and EHADP samples were pyrolyzed at 170 °C for 60 s in a Multi-Shot pyrolyzer (EGA/PY-3030d, Frontier

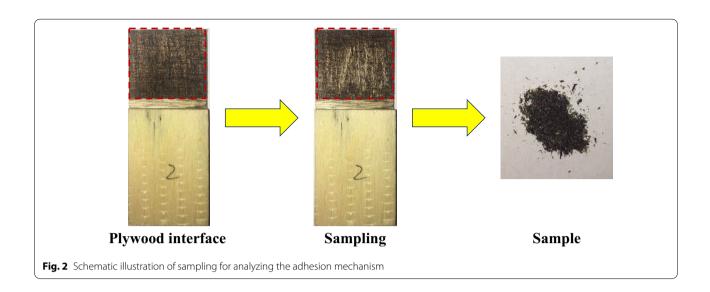
a resolution of  $4 \text{ cm}^{-1}$ .

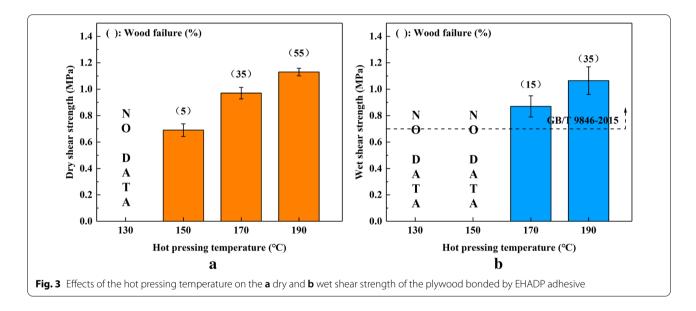
#### **Results and discussion**

### Effects of hot pressing conditions on bond performance

Plywood was manufactured under various hot pressing temperatures and times to investigate the relationship between manufacture conditions and bond ability. Figure 3 shows the shear strength of the plywood bonded with the EHADP adhesive at different hot pressing temperatures. A clear increasing trend in dry/wet shear strength and wood failure was observed with the addition

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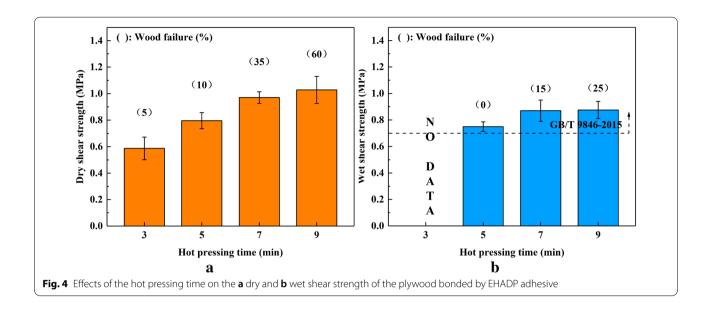


of hot pressing temperature, and the maximum values were obtained from boards hot pressed at 190 °C. However, the plywood exhibited zero bond strength at a fabricated temperature of 130 °C and weak water resistance at 150 °C. This result seems to inconformity to our previous research, in which the results of thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) indicated that the curing reaction of the EHAPD adhesive occurs at approximately 135 °C [28]. There is a possible reason for this phenomenon, which is due to the moisture movement from the veneer and EHADP adhesive during the hot pressing process, and this movement affected the actual temperature in the core layer of the board [31]. In addition, although the maximum value

was obtained from the board hot pressed at 190 °C, as the hot pressing temperature was  $\geq$  170 °C, the wet shear strength of the plywood fulfilled the requirements of China National Standard GB/T 9846–2015 ( $\geq$  0.7 MPa) [32]; therefore, the appropriate hot pressing temperature of the plywood bonded with EHADP adhesive was 170 °C.

The effects of hot pressing time was confirmed by fabricating the board at 170 °C for 3–9 min, and the results are shown in Fig. 4. A growth trend of shear strength and wood failure were obtained in the region of 3–9 min under dry conditions, implying that the bond ability of the EHADP adhesive was promoted by adding heating time. Plywood board hot pressed for 3 min was destroyed

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during the immersion treatment, suggesting that 3 min is insufficient to cure the EHADP adhesive. As the pressing time was prolonged from 5 to 9 min, the average wet shear strength and wood failure values were positively correlated with pressing time; however, analysis of variance indicated no significant difference between the board hot pressed for 7 or 9 min, suggesting that the bonding performance of the EHADP adhesive was maximum at 7 min. As the wet shear strength of the plywood bonded for  $\geq$  5 min achieved the requirement of China National Standard GB/T 9846–2015 ( $\geq$  0.7 MPa) [32], the appropriate hot pressing time was decided as 5 min.

Judging from the results of plywood bonded at different conditions, the appropriate manufacturing conditions were set to 170 °C and 5 min. When the plywood was fabricated under optimum conditions, wet shear strength was 0.75 MPa, which reached the requirement of the GB/T 9846–2015 standard [32].

## Curing behavior of the EHADP adhesive Insoluble mass proportion

The uncured adhesive was heated at various temperatures and times to confirm the effects of the curing conditions on the EHADP adhesive. The results of the insoluble mass proportion and the slope (k value) between adjacent conditions are shown in Fig. 5. The effect of heating temperature is shown in Fig. 5a. As heating temperature was increased, the insoluble mass proportion was promoted. When the uncured EHADP adhesive was heated at 130 and 150 °C, the insoluble mass proportion was maintained at a low level (0.3 and 6.9%, respectively) and the growth trend was gradual (k=6.6), indicating that the EHADP adhesive curing process was insufficient at

130–150 °C. However, as the temperature reached 170 °C, the amount of insoluble mass proportion increased substantially (58.8%, k=51.9), suggesting that most of the adhesive cured at this temperature. Furthermore, the insoluble mass proportion of the EHADP adhesive heated at 190 °C reached the maximum value (77.9%). The effect of heating temperature on the insoluble mass proportion illustrates that the weak water resistance of plywood fabricated at 130 and 150 °C was attributed to insufficient curing of the EHADP adhesive.

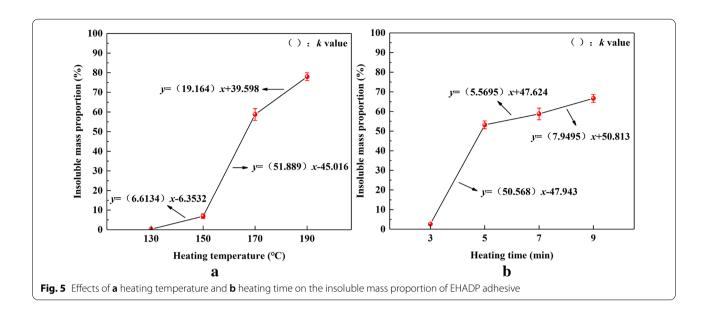
Regarding to effects of heating time (Fig. 5b), only 2.7% of the insoluble mass was obtained when the adhesive heated for 3 min. This result also explains why the plywood bonded at 170 °C for 3 min exhibited low water resistance (Fig. 4). As the heating time was increased to 5 min, a noticeable rise in the insoluble mass proportion was observed (53.2%, k=50.6), and the growth trend leveled off gradually by increasing the heating time to 7 and 9 min (k=5.6 and 7.9, respectively).

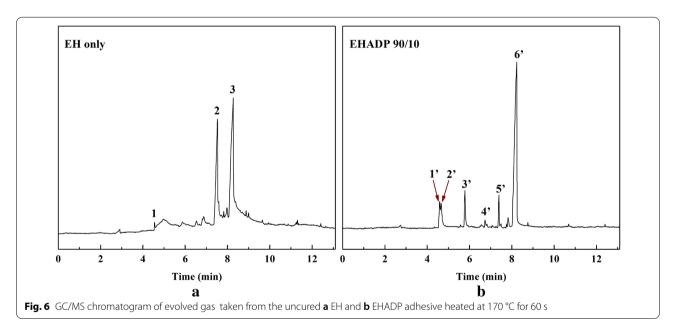
Based on these results, the insoluble mass proportion showed similar trend with the bond performance of the plywood, indicating that the curing efficiency of the EHADP adhesive was promoted as the heating temperature was increased to 170  $^{\circ}$ C and heating time was prolonged to 5 min.

## Py-GC/MS analysis

A Py-GC/MS analysis was carried out on the uncured EH and EHADP adhesive to inquiry the chemical changes in the curing treatment, and the chromatogram of evolved gas is shown in Fig. 6. The results of identified chemical compounds are shown in Table 2 and Fig. 7. Under the EH-only condition, the peaks

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located at 4.55, 7.53, and 8.27 min were identified as furfural, 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP), and 5-hydrxoymethylfurfural, respectively, which are typical pyrolysis products of saccharides [33–36]. Significant differences were observed on the chromatogram from the EHADP sample, and some new chemical compounds were identified during the pyrolysis process. Chemicals I, II, and III were furan compounds, indicating that adding ADP catalyzed conversion of the monosaccharides in the EHADP adhesive. In addition, the existence of heterocyclic compound IV was attributed to a reaction

between reducing sugars and ammonium salts [37, 38], which was evidence that ADP participates in the curing reaction.

#### Adhesive mechanism of the EHADP adhesive

The adhesive mechanism of the EHADP adhesive was confirmed by measuring the FT-IR spectra of the bond interface of the plywood fabricated at different pressing temperatures. To distinguish the effects of the chemical transformation from the curing of EHADP adhesive itself, the spectra of insoluble mass derived from heating

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Table 2 Identified chemical compounds in evolved gas derived from uncured EH and EHADP adhesive heated at 170  $^{\circ}$ C for 60 s

Samples	Peak number	RT (min)	SI	Compound	CAS	MW	Formula	Chemical structure number
EH only (100/0)	1	4.55	85	Furfural	98-01-1	96	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	А
	2	7.53	94	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	28,564-83-2	144	$C_6H_8O_4$	В
	3	8.27	94	5-Hydrxoymethylfurfural	67-47-0	126	$C_6H_6O_3$	C
EHADP (90/10)	1′	4.59	98	Furfural	98-01-1	96	$C_5H_4O_2$	Α
	2′	4.66	96	3-Furaldehyde	498-60-2	96	$C_5H_4O_2$	1
	3′	5.78	97	2-Furancarboxaldehyde, 5-methyl-	620-02-0	110	$C_6H_6O_2$	II
	4′	6.73	89 89	2,5-Furandicarboxaldehyde 2-methoxy-6-methylpyrazine	823–82-5 2882–21-5	124 124	$C_6H_4O_3$ $C_6H_8N_2O$	III IV
	5′	7.39	95	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	28,564-83-2	144	$C_6H_8O_4$	В
	6′	8.23	96	5-Hydrxoymethylfurfural	67-47-0	126	$C_6H_6O_3$	C

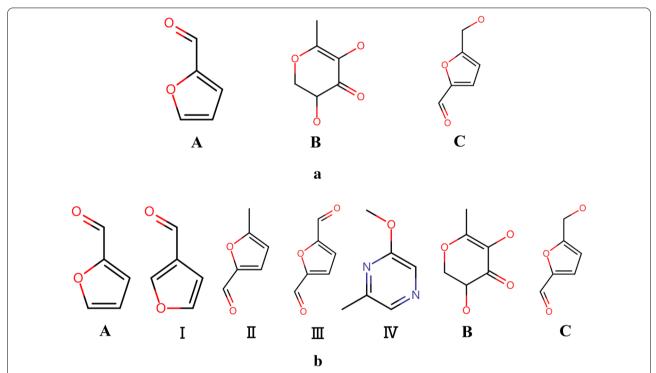


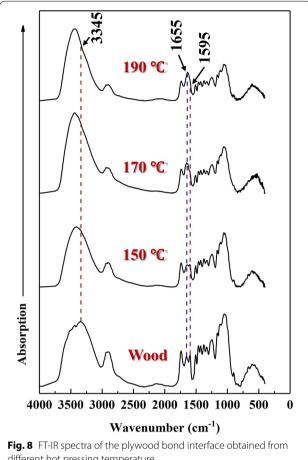
Fig. 7 Chemical structure of the identified compounds in the evolved gas derived from the uncured EH and EHADP adhesive heated at 170 °C for 60 s

the EHADP adhesive at different temperatures were also detected.

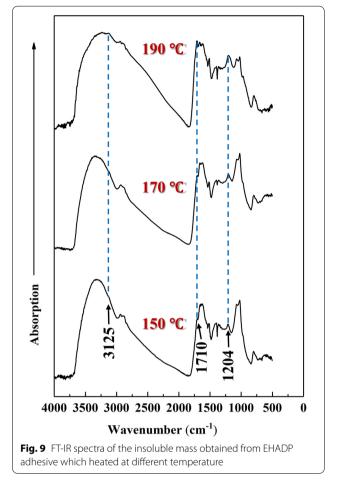
The chemical changes in the bonded interface are shown in Fig. 8. Two peaks located at 3345 and 1595 cm<sup>-1</sup> disappeared and one peak at 1655 cm<sup>-1</sup> was enhanced with the increase in hot pressing temperature compared with the wood sample. However, referring to the spectra of insoluble mass in Fig. 9, three peaks

located at 3125, 1710 and 1204 cm<sup>-1</sup> increased in signal intensity with increasing heating temperature, and these absorption bands were attributed to C–H stretching vibrations of the furan ring, a carbonyl group, and C–O–C stretching, respectively. The results of chemical changes in the insoluble mass suggest that the increase in heating temperature promoted the degree of cross linkage in the cured EHADP adhesive; therefore, the

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different hot pressing temperature



insoluble mass proportion was positively correlated with heating temperature. In addition, the absorption bands of the insoluble mass were not detected in the bond interface spectra, indicating that the decrease and increase in signal intensity in Fig. 8 were likely attributed to chemical changes in the wood element. Therefore, the peak located at 3345 cm<sup>-1</sup> is due to the intramolecular forces in cellulose [39], and 1595 cm<sup>-1</sup> is the COO- stretching bond of hemicellulose [39, 40]. The disappearance of these bands may be due to pyrolysis of cellulose and hemicellulose catalyzed by the acidity derived from the EHADP adhesive. The peak at 1655 cm<sup>-1</sup> is attributed to the conjugated carbonyl groups (C=O) [39, 40], and the increase in this signal may be due to a reaction between the wood and the EHADP adhesive and aryl ketones or furanone compounds formed on the bonding interface.

The results of chemical changes at the bond interface and the chemical analysis of the cured EHADP adhesive from our previous study implied that the adhesion mechanism of plywood bonded by the EHADP adhesive was as follows: polymerization of the EHADP adhesive formed a cross linkage that provided physical adhesion strength, and the EHADP adhesive also reacted with the wood element during the curing process, which formed carbonyl groups. Judging from the results of bond performance, the wood failure of the plywood bonded with EHADP adhesive was low, implying that the physical adhesion strength which obtained by the polymerization of EHADP adhesive is the primary contribution on the cohesion of adhesive.

## Conclusion

In this study, the appropriate manufacturing conditions, curing behavior, and adhesion mechanism were investigated. The appropriate hot pressing temperature and time of the plywood needed to bond with the EHADP adhesive were determined by comparing the shear strength and wood failure of board fabricated under various conditions, and the results showed that hot pressing temperature and time were positively correlated with the bond performance. Considering that the plywood bonded at 170 °C for 5 min

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achieved 0.75 MPa wet shear strength, which fulfilled the GB/T 9846-2015 standard, the appropriate manufacture conditions of the three-ply plywood bonded by EHADP adhesive were determined to be 170 °C for 5 min. The results of insoluble mass proportion implied that curing efficiency was promoted as the heating temperature and time≥170 °C and 5 min, and this phenomenon also explains the bond performance. In addition, the Py-GC/ MS analysis clarified the adding of ADP provided two roles during the curing process; it catalyzed the chemical conversion of monosaccharides in the EHADP adhesive and participated in the curing reaction. Furthermore, the adhesion mechanism of the EHADP adhesive was clarified by FT-IR analysis of the bond interface of plywood and the insoluble mass of the cured EHADP adhesive. The chemical changes suggested that both polymerization of the EHADP itself and the reaction between the wood elements and adhesive provided the plywood bond strength.

#### **Abbreviations**

EH: Enzymatic hydrolysate; ADP: Ammonium dihydrogen phosphate; EHADP: Enzymatic hydrolysate-ammonium dihydrogen phosphate; Py-GC/MS: Pyrolysis gas chromatography—mass spectrometry.

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#### Authors' contributions

Zhongyuan Zhao: proposed the concept, investigated curing behavior and curing mechanism, and writing the manuscript. Di Wu: investigated the results of bonding performance. Caoxing Huang: prepared the enzymatic hydrolysate and synthesized the adhesive. Min Zhang: review and editing the manuscript. Kenji Umemura: proposed the concept and guided the experiments. Qiang Yong: prosed the research plan, analyzed the curing behavior and adhesion mechanism, and reviewed the manuscript.

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## Availability of data and materials

There are data and materials obtained during research followed by testing, analyzing and discussing.

#### **Competing interests**

The authors declare that they have no competing interests.

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