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Research Article

Fully Biobased Thermosetting Adhesive from Enzymatic Saccharification Residue

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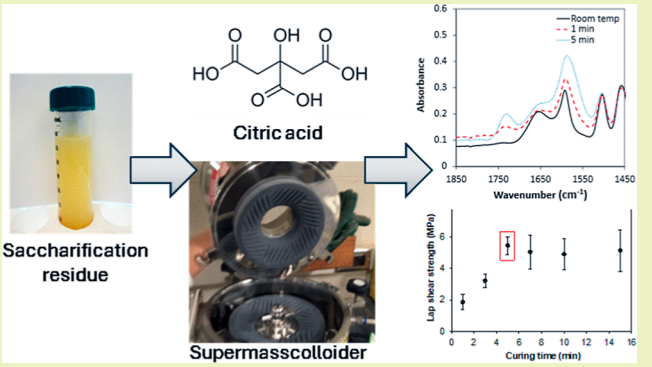
ABSTRACT: This study was aimed at using the solid residue of enzyme-based cellulosic sugar production (saccharification) to formulate a fully biobased heat-curable wood adhesive. The novelty of such utilization lies in three implemented strategies: (1) valorizing lignin in the hardwood saccharification residue without chemical prerefining, (2) increasing reactive surfaces by wet-grinding the lignin-rich residue, and (3) blending citric acid (biobased cross-linker) as an adhesive component to enhance bond performance of the residue. Single-lap shear specimens were prepared and tested. Results showed that saccharification improved the wood bonding ability of the resulting residue. Grinding the residue into smaller particles also led to stronger bonding. Without citric acid (only saccharification residue), the lap shear strength value attained 80% (or 6.25 MPa) of that of a commercial phenol-formaldehyde (PF) wood adhesive. With 33.3% w/w citric acid (66.7% w/w saccharification residue), the dry and wet bonding properties achieved values comparable to those of PF. Ester cross-linking was verified to account for such enhanced bonding. This formaldehyde-free, competitive adhesive product signifies waste valorization realized via an organic solvent-free process. Upon optimizing the grinding step, this adhesive could contribute to the viability of the cellulosic sugar-based biorefinery system, as a collateral benefit and a win−win strategy for utilizing plant biomass.



KEYWORDS: *cellulosic ethanol, ultrafine friction grinding, polycarboxylic acid, lignin-based thermosetting adhesive, saccharification byproducts*

INTRODUCTION

The valorization of residual biomass is an important task for a sustainable bioeconomy. One such residue, which potentially exists in large quantities, is the byproduct of cellulosic biorefineries, where the polysaccharide portions of lignocelluloses are broken down via enzymatic or acid hydrolysis to monomeric sugars.[1,2] This saccharification (sugar-liberating) process generates lignin-rich streams in the form of either (1) nonconverted solid residues after polysaccharides are extracted or (2) lignin (in the liquid phase) pre-extracted before polysaccharides conversion.[3] Regardless of the form of existence, which depends on the process design of a biorefinery, the amount of lignin generated is significant. For the U.S. alone, the national volume target of cellulosic biofuel in the year 2024 is 4.13 billion liters (or 1.09 billion ethanol-equivalent gallons).[4] Based on an ethanol yield of 355 L per dry metric ton of lignocellulosic biomass (low-case scenario in Langholtz et al., 2014)[5] and an approximate lignin content of 25%, an (annual) amount of 2.9 million metric tons of lignin is available from cellulosic sugar-based biorefinery. Noteworthily, this estimation has not considered the lignin byproduct in the case of cellulosic sugar-based (nonfuel) chemicals. Therefore, profitably valorizing such potentially abundant byproducts of saccharification would add value to biomass and generate additional revenue for the bioeconomy.

Enzymatic hydrolysis lignin is the primary byproduct of lignocellulose biomass saccharification as the enzymatic route is more common than acid hydrolysis.[6] This lignin is distinct and yet, in potential application areas, complementary to the lignin recovered from industrial pulping. Kraft lignin, the potentially largest source of lignin feedstock, is recovered from the widely used kraft pulping process.[7,8] During kraft cook, lignin is removed from the woody raw material to obtain cellulose pulp; the dissolved lignin can be precipitated from the spent pulping chemical (commonly called black liquor) through, for example, the commercialized LignoBoost process at a high yield, high purity, and low ash content.[9] Compared to kraft lignin, enzymatic hydrolysis lignin contains a higher amount of carbohydrate; it also retains more native β-O-4

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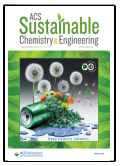

linkages (the most abundant bond type between lignin units) due to the milder biochemical process.[10] In contrast, kraft lignin has a higher content of recalcitrant C−C bonds due to the opportunities of condensation (repolymerization) reactions subsequent to the extensive breakage of β-O-4 linkages. As such, kraft lignin is less reactive than native lignin but more resistant to thermal degradation than enzymatic hydrolysis lignin.[10,11] For materials applications, it was commented that kraft lignin is suitable for carbonization to produce carbon fiber and activated carbon, while enzymatic hydrolysis lignin is suited for thermoplastic blending and expected to be more reactive in chemical transformation.[10,12]

Due to its polyphenolic structure, one long-pursued application area of lignin is its use as a phenol substitute in synthesizing phenol-formaldehyde (PF) adhesives. However, the resulting (plywood) shear bond strength was compromised if kraft lignin exceeding 30% was used for phenol substitution.[13] On the other hand, the residue from ethanol biorefinery (of corn stover, inferred from Zhang et al., 2016) in its unrefined form was demonstrated feasible in replacing 50 wt % of phenol in the synthesis of PF resin, providing adhesive bonding that satisfied the industrial requirements for exterior-grade plywood.[12,14] Furthermore, it was shown that the compromise in adhesive bond strength frequently associated with phenol substitution could be alleviated and a 100% phenol substitution could be achieved if lignin was pre-extracted from the bioethanol production residue (of corn stalk/stover).[15,16] For wood feedstock, Stücker et al. (2016) showed that enzymatic hydrolysis residues of hardwood could also be used for phenol substitution (at 20 wt %) in making lignin-based PF adhesives.[17] A phenol substitution limit of 40% could be achieved if the hardwood (oak) lignin in the bioethanol production residue was preisolated to enhance its reactivity.[18] In short, all these findings suggest the potential of utilizing enzymatic saccharification residues for thermosetting adhesives (lignin-based PF).

As a progression from lignin-PF adhesives, which are partially biobased (formaldehyde is typically fossil-sourced), it was envisioned in this study that fully biobased and formaldehyde-free formulations could be enabled. Although not specific to enzymatic hydrolysis residues, lignin-based adhesives that are formaldehyde-free have been proven possible with the use of curing/cross-linking agents. Some examples of such mediators are polyethylenimine, glutaraldehyde, hexamethylenetetramine, and furfural (which is bio-based).[19−24] One other group of cross-linkers is multifunctional carboxylic acids, which can react with hydroxyls of the host material to form ester linkages. A typical example of such compounds is citric acid. It has been successfully used as either a cross-linking agent for cellulosic materials such as cotton fabric and nanofiltration membranes or an adhesive (or bond promoter) for wood powder molded products and veneer-based panels.[25−28] Since citric acid can be derived from citrus fruits or through microbial production from sugar-based media, its adhesive use with lignin-rich, enzymatic hydrolysis residues would make it possible to produce a fully biobased thermosetting adhesive.

The objective of this study was to investigate the technical feasibility of a fully biobased, formaldehyde-free wood adhesive from the lignin-rich saccharification residue. Several strategies were employed to achieve the stated goal: (1) simplify the preparation process by utilizing the residue without preisolating or purifying for lignin, (2) circumvent the needs for chemical premodifications of lignin thereby also the use of organic solvents, (3) alleviate the reactivity issues by wet grinding to increase reactive surfaces, and (4) use citric acid as a cross-linking component to enable a fully biobased adhesive. To establish its robustness, the combined strategy was demonstrated on hardwood whose native lignin is conventionally known to be less reactive (fewer free ortho-positions in the phenolic structure) compared to grasses and softwood yet recently shown also or as effective for the synthesis of thermosetting adhesives.[29,30]

MATERIALS AND METHODS

Materials. The starting material used in this study was aspen (*Populus tremuloides*) wood particles (20-mesh) Wiley-milled from wood chips donated by the Cloquet mill (Minnesota) of the Sappi group. The cellulase cocktail used for saccharification was Celluclast 1.5L (Sigma-Aldrich; endoglucanase and exoglucanase from *Trichoderma reesei*) and Novozyme 188 (Sigma-Aldrich; β-glucosidase of *Aspergillus niger*). Citric acid and adipic acid (Figure S1) as esterifying compounds were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Sodium hydroxide solutions of 0.5 N (for hydroxyl group determination) and 0.1 N (for washing of cured samples prior to FTIR spectroscopy) were purchased from Fisher Scientific (Waltham, MA, USA). All chemicals were used without prior purification. The PF adhesive [StructurFast(TM) OS-51TT] used was kindly provided by Hexion Inc.

Preparation of Adhesive Feedstock. Aspen wood was alkaline-pretreated and enzymatically hydrolyzed to attain two levels of glucan-to-glucose conversion: 45 and 80% based on the glucan content of the pretreated sample. The biomass pretreatment approach was rationalized and reported in a separate publication, which addressed binderless films of the saccharification residue.[31] Further details of the procedure for pretreatment, saccharification, chemical composition, and hydroxyl content are outlined in the Supporting Information.

The saccharified samples in aqueous suspension (2 wt %) were ground using an ultrafine friction grinding machine "Supermasscolloider" (Masuko Sangyo Co., Ltd., Japan). The grinding protocol was published elsewhere.[31] In a nutshell, the grinding disks were first brought into slight contact with each other when rotating and then, at the time of slurry feed, closed in further (hence the negative clearance) for a distance ranging from −25 to −175 μm, to induce a range of grinding friction from low to high, respectively. Details of the grinding operation are provided in the Supporting Information.

Formulation and Testing of the Adhesive. The saccharification residue in an aqueous suspension was used as the adhesive for single-lap-joint assemblies of birch wood substrates. Each wood adherend strip was 40 mm in length and 8.5 mm in width. The loading of the saccharification residue was set at 11 mg (dry mass equivalent) for a lap joint area of 55 mm^2 (6.5 mm × 8.5 mm), giving an adhesive spread rate of 200 g/m^2. 200 g/m^2 is a common spread rate used in wood veneer lamination studies (such as Kurt and Cil, 2012).[32] To prepare for adhesive spreading, an aqueous suspension of the saccharification residue was brought to 30 wt % solid content by evaporating excess water in an oven at 55 °C. The 30 wt % solid content was optimized from preliminary trials based on the ease of adhesive spreading.

For formulations containing citric acid, the acid was first dissolved in an aqueous suspension of the ultrafine friction ground saccharification residue. Like the case of citric acid-free formulation, the solid content was adjusted to 30 wt % before use. The presence of the ground saccharification residue was maintained at 200 g/m^2 (11 mg) on the spread area, while the amount of citric acid was varied (Table S1). This approach was intended to keep a similar amount of the saccharified particles in the glue line but resulted in different spread rates of the total solids. To allow performance evaluations, a commercial PF resin (50 wt % solid content) was used as a reference adhesive for the lap joint bonding at both 200 g/m^2 and a higher

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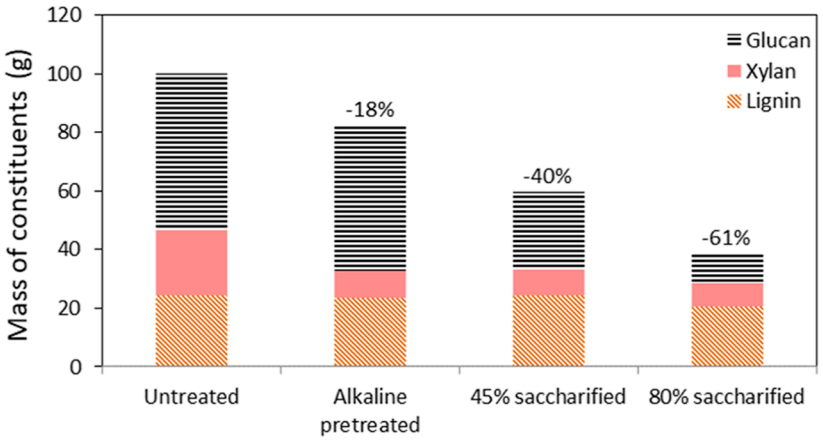

Figure 1. Relative presence of three major constituents of aspen wood after alkaline pretreatment and saccharification (note: the mass loss is relative to the starting 100 g of these three constituents combined).

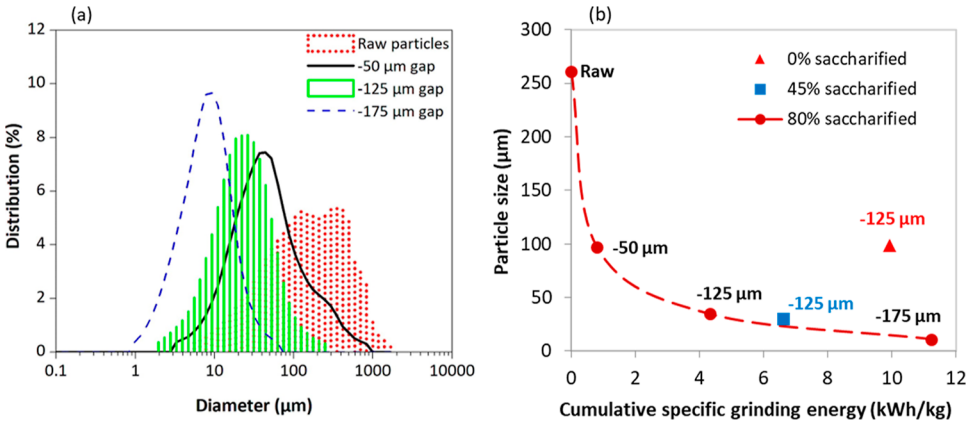

Figure 2. (a) Particle size distribution of saccharification residue ground at various disk clearance values (for 80% saccharified particles) and (b) effect of the grinding energy input on particle size. Note in (b) that the labels near the data points depict the smallest disk clearance in a series of successive grinding steps. The line between data points in the figure is merely for showing the data trend.

spread rate (found later to be 300 g/m^2) corresponding to the citric acid dosage that provided the strongest bonding.

To understand the role of citric acid (tricarboxylic) in promoting wet bond strength, adipic acid (Figure S1), a non-cross-linking bifunctional carboxylic acid, was tested as a substitute of citric acid in the formulation. The amount of saccharification residue was fixed at 11 mg per bond area. The dosage of adipic acid, in mmol/g of saccharification residue, was kept at the optimized dosage of citric acid (found later to be 2.6) or twice the dosage. The protocol for mixing, adjusting solid content, and adhesive spreading was the same as the case of citric acid.

All lap joint assemblies in this study were bonded at 180 °C, which is the typical curing temperature of the PF resin. The hot pressing was conducted at a pressure of 1.2 MPa and for a specified time duration between 1 and 15 min.

Characterization. The pretreated samples were analyzed for particle size by using Microtrac BLUEWAVE laser diffraction. The equipment determines particle sizes ranging from 0.1 to 2800 μm. To compensate for nonspherical materials (e.g., fibers), Microtrac BLUEWAVE is equipped with modified Mie calculations. The measurement protocol was detailed in a separate study.[33] The particle size was expressed as the mean diameter of the volume distribution, which can be interpreted as the diameter of a sphere having the same volume as the particle.

The lap joint assemblies were evaluated in tensile mode (5 mm/min loading rate) by using an Instron 5542 fitted with a 500 N load cell. The initial span between the grips was one inch. The lap joint shear strength was calculated as the maximum force required to shear apart a unit area of bonding. A total of 8−12 specimens were tested to allow calculation of the average for sample assessment. The lap joint assemblies were tested in either their dry or wet states. For shear strength in the dry state, the bonded specimens were preconditioned for 2 days (equilibrium) at 50 ± 2% relative humidity and 23 ± 1 °C in accordance with ASTM D2339-20.[34] For wet shear strength, the specimens were tested after 24 h of soaking in water (23 °C), adopting the conditions stated in ASTM D1037-12.[35]

To examine heat-induced (curing) reactions between citric acid and saccharification residue, the two adhesive components were mixed at the selected (33.3:66.7) weight ratio, placed under the fume hood for water evaporation targeting 30 wt % solid content, and then heated (cured) in an oven at 180 °C for a duration of 1−5 min. To represent the case without heat curing, the adhesive mix was merely air-dried under the fume hood. The reacted (or dried) mixtures were washed with NaOH (0.1 N) for 2 min to convert free carboxyl groups to carboxylate. The washed samples were then air-dried before being characterized using FTIR spectroscopy (Nicolet Series II Magna-IR System 750) at the attenuated total reflectance (ATR) mode. The infrared spectra were collected (32 scans) in the wavenumber range of 4000−600 cm^{-1} at a resolution of 4 cm^{-1}. The spectra obtained were corrected for relative band intensity distortion, band shift, and deviation from Beer's law using the ATR correction algorithm in OMNIC software (Thermo Fisher Scientific Inc.).[36] The corrected spectra were then analyzed for the chemical bond formation.

RESULTS AND DISCUSSION

Chemical Composition and Particle Size. Figure 1 illustrates the relative presence of glucan, xylan (the major

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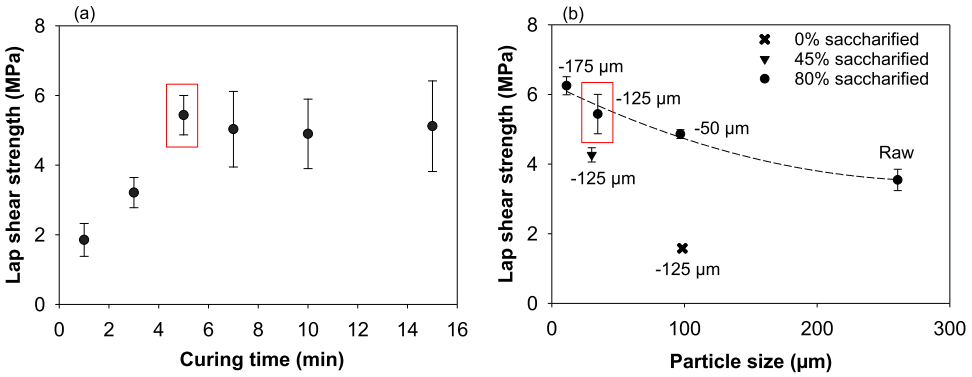

Figure 3. Shear strength of wood lap joints bonded (180 °C hot pressing) solely with saccharification residue (spread rate 200 g/m^2): effects of (a) hot-pressing time (80% saccharified sample; ground at −125 μm clearance) and (b) particle size and saccharification (5 min hot pressing). The boxed data points in both figures refer to the same sample (80% saccharified, −125 μm grinding, and 5 min curing).

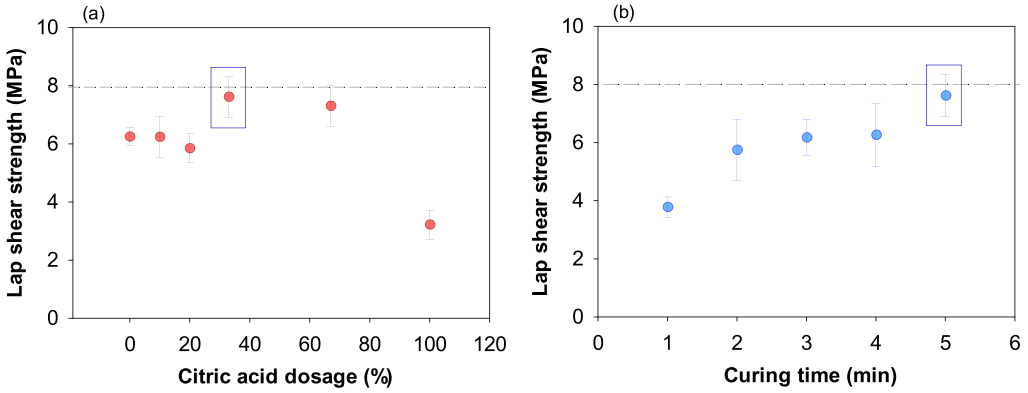

Figure 4. Lap shear strength of wood bonding with saccharification residue (−175 μm grinding) cured with citric acid (180 °C curing): (a) effect of citric acid mass fraction in the adhesive (5 min curing) and (b) influence of hot-pressing time (for curing adhesive containing 33.3 wt % citric acid and 66.7 wt % saccharification residue). The horizontal discontinuous line refers to the reference value measured from lap joints bonded with PF resin at a spread rate of 200 g/m^2. The boxed data points in both figures refer to the same sample (citric acid 33.3 wt % and 5 min curing).

hemicellulose of hardwood), and acid-insoluble lignin in the solid samples before and after saccharification. In essence, Figure 1 shows that alkaline pretreatment resulted in (partial) removal of the hemicellulose, as widely established,[37] such that the xylan mass fraction dropped 60% in the present study. Lignin removal, on the other hand, was not apparent owing to the mild NaOH treatment temperature of not higher than 100 °C.[1] After the pretreated sample was enzymatically hydrolyzed to liberate 45 and 80% of its glucan, the relative presence of lignin increased (from 29% in the pretreated sample) to 41 and 54%, respectively. Essentially, the saccharification process was harnessed to generate a lignin-rich byproduct substrate for the adhesive intended in this study.

Figure 2a shows the size reduction of saccharification residue upon grinding; the particle size shifted to a smaller mean value when the disk clearance was increasingly closed (friction increasingly applied). The size distribution became narrower when higher friction was exerted, indicating an increased uniformity in size. Solid residues of 45 and 80% saccharification could be ground into smaller and more uniform particles than the nonsaccharified biomass (Figure S2). More specifically (see Figure 2b), the 80% saccharified sample (initial size 260 μm) was ground into particles (11 μm) that were smaller than the nonsaccharified biomass (∼100 μm) at a similar energy input (of 10−11 kW h/kg). As such, saccharification increased the ease of the subsequent size reduction of the particles. This could be attributed to the (1) loosening of the cell wall structure, as inferred from the reported pore volume increase of wood after glucan removal[38] and (2) decreased presence of (fibrillar) cellulose making it easier for size reduction. Interestingly, particles of the 45% saccharified sample (ground at 6.6 kW h/kg) were similar in size to the 80% saccharified sample if the latter (based on the trend line) were ground at the same energy input. This implies the potential of controlling the particle size of the saccharification residue (45 or 80% saccharified) by adjusting the grinding energy input.

Wood Bonding with Saccharification Residue. For bonding studies, the hot-pressing (curing) time was first examined by using ground particles (−125 μm clearance) of the 80% saccharified sample. Figure 3a shows that the shear strength of the resulting lap joints increased from 1.8 MPa (1 min curing) to 3.2 MP at 3 min curing and reached a maximum of 5.4 MPa at 5 min. The wood bond strength remained similar at 5−15 min of hot pressing, suggesting that 5 min was an optimal curing time. In a related study, Kalami et al. (2017) synthesized lignin-formaldehyde resin utilizing the lignin byproduct of (corn stover) bioethanol production.[16] Their wood bonding exhibited a dry shear strength of 3.4 MPa (cured at 180 °C for 3−4 min), which was quite similar in value (3.2 MPa) compared to the lap joints (also cured at 180 °C) in the present study for 3 min hot pressing. In another study, a similar (plywood) lap shear strength value (3.5 MPa) was also reported for an adhesive spread rate of 200 g/m^2

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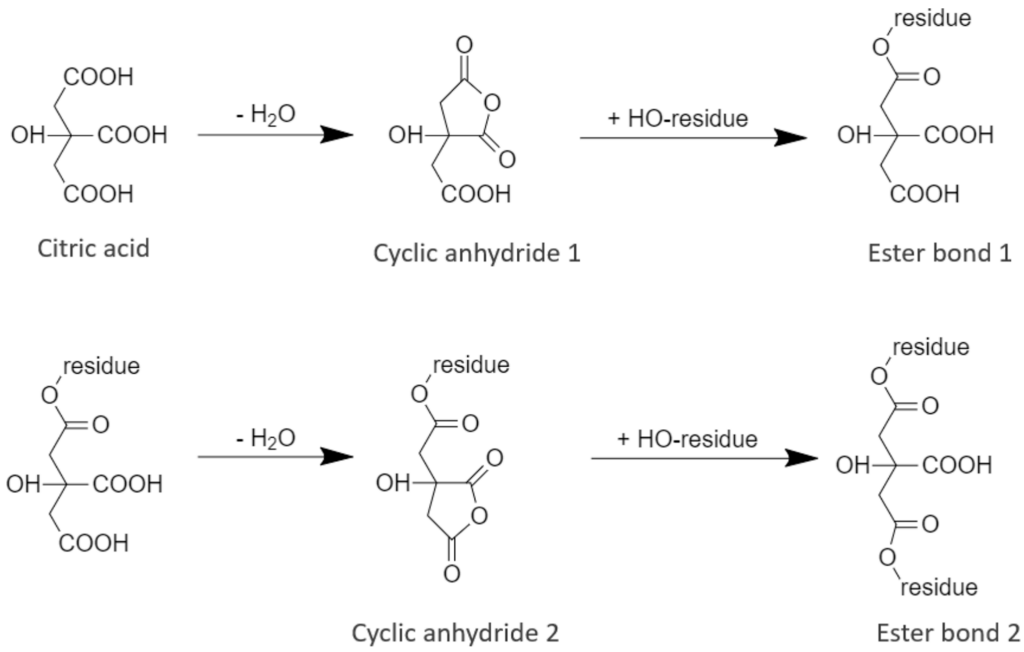

Figure 5. Proposed mechanism of esterification reaction between citric acid and saccharification residue. Note: when the mixture was cured between wood substrates, some of the ester linkages would be with wood to realize wood bonding.

(same as this study), but their adhesive was of 1:1 mix of lignin (recovered from kraft biorefinery) and Novolac PF resin.[39] Thus, the saccharification residue adhesive in the present study is competitive yet simpler to prepare, and it could attain an even stronger wood bonding (5.4 MPa) if the curing time was extended (from 3 to 5 min).

The effects of the particle size were subsequently examined (Figure 3b). For the 80% saccharified sample, the lap shear strength of the bonded joints (5 min curing) ranged from 3.6 MPa ("raw" for no grinding) to 6.3 MPa (ground at −175 μm clearance), with no noticeable wood failure, as the particle size decreased from 260 to 11 μm. This effect was ascribed to specific surface areas which were larger in smaller particles, hence more bonding sites were available. By comparing values at similar particle sizes (i.e., eliminating size effect), the influence of saccharification levels on the lap shear strength could be discerned (Figure 3b). At a particle size of 30−35 μm, the residue from 80% saccharification exhibited a significantly higher lap shear strength (5.4 MPa) than that obtained from 45% saccharification (4.3 MPa). Additionally, the 80% saccharified sample performed remarkably better than the nonsaccharified sample, with a lap shear strength of 4.9 MPa compared to 1.6 MPa, at a particle size of 97−98 μm (Figure 3b). The effect of saccharification is thus evidenced. This could be related to the higher presence of lignin and xylan, both of which are hydroxyl-containing and readily accessible (amorphous), in the solid residue of saccharification. Additionally, there could also be condensation of lignin moieties with furfural that resulted from the thermal breakdown of xylan during hot pressing, in which case, some extents of cross-linking would be resulted.[23,40]

Wood Bonding Enhanced by Citric Acid. With citric acid, it was anticipated that the lap shear strength (highest at 6.3 MPa) attained with the (80%) saccharification residue (−175 μm grinding) could be further enhanced. In this regard, Figure 4a shows that citric acid mass fractions up to 20 wt % were either ineffective or counterproductive. Esterification reactions, in general, involve carboxylic acids as acylating agents and a strong acid as the catalyst; however, the reactions (catalyzed) are often accomplished using carboxylic acid anhydrides or acid chlorides which are a more reactive acylant.[41] Without an added catalyst, self-catalyzed esterification of (cellulosic) hydroxyl groups by citric acid was proven feasible; the ester cross-linking was promoted by higher curing temperatures and citric acid doses.[42] It is postulated that with insufficient citric acid, the condition for self-catalyzed esterification in the present study was inadequate; so, esterification and cross-linking were deficient, and especially for 20 wt % citric acid, there was an appreciable amount of unreacted or free citric acid, which functioned as a plasticizer.[43] The plasticization effect was postulated to weaken the cross-link-deficient bond line, thereby possibly explaining the decreased lap shear strength (20 wt % citric acid) compared to the case without citric acid.

As the citric acid level was increased to 33.3 wt % (66.7 wt % saccharification residue), an increased lap shear strength (7.6 MPa) was observed (Figure 4a), suggesting that the esterification reaction was allowed to proceed to forming (ester) cross-links between the saccharification residue or/and wood adherend. No further improvement, however, was noted as the citric acid dosage was increased to 66.7 wt %. When wood substrates were bonded with citric acid alone (spread rate 200 g/m^2), the lap shear strength was only 3.2 MPa. Based on the tested formulations, 33.3 wt % citric acid was optimal. This dosage is within the published range of citric acid optimized at 20−30 wt % for molded products (hardwood powder) or particleboard (sorghum bagasse) and 75 wt % for plywood (sucrose made up the remaining 25 wt % of adhesive).[27,44,45]

Figure 4b shows that with 33.3 wt % citric acid, a curing time of 3 min (6.2 MPa) was sufficient to attain the lap shear strength value (6.3 MPa) of 5 min pressing in the absence of citric acid (see Figure 3b or 4a, first data point in both cases; spread rate 200 g/m^2). Noteworthily, the citric acid-enhanced

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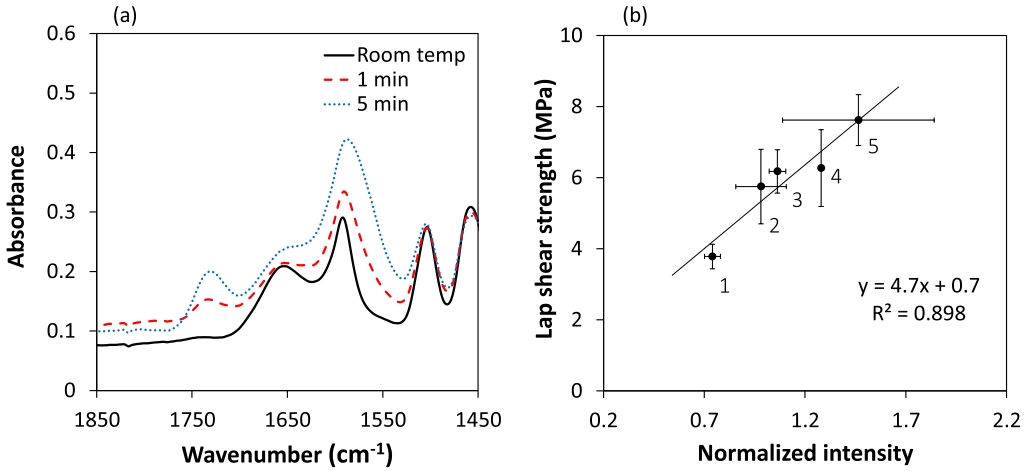

Figure 6. (a) FTIR spectra of adhesive solid: uncured (air-dried) versus 1 min and 5 min curing at 180 °C (all NaOH washed before spectrum collection) and (b) correlation between the normalized intensity of ester bands (1735 cm^{-1}/1505 cm^{-1}) and lap shear strength values (note: the numbers next to the data points are curing time in minutes).

bonding at 5 min curing time (7.6 MPa) was comparable to that of the PF resin (spread rate 200 g/m^2) cured at the same conditions (180 °C for 5 min) and tested in the present study (plotted as a horizontal discontinuous line in Figure 4a,b).

Esterification in Citric Acid-Cured Saccharification Residue. The proposed reaction between citric acid and the residue of saccharification was the formation of ester linkages between the two (Figure 5), similar to the esterification of cellulose with citric acid.[26] The esterification mechanism, as illustrated in Figure 5, dictates that two carboxyl groups (of citric acid) first combine to form a cyclic acid anhydride which then reacts with a hydroxyl group (of the host substrate), thereupon an ester linkage is formed, freeing up one carboxyl group.[25] This implies that free carboxyl functionalities (−COOH) are present, in addition to ester linkages (RCOOR′), in the esterified sample. The evolution of esters in the sample can be monitored using FTIR through their carbonyl (C═O) band between 1735 and 1750 cm^{-1}.[46] However, the carbonyl group of carboxylic acid is manifested in an adjacent band within the range of 1700−1730 cm^{-1}. To remove interference, it was essential to wash the esterified sample with sodium hydroxide (NaOH 0.1 N) before spectrum collection.[47]

Upon NaOH washing (followed by air drying), the 1725 cm^{-1} band of esterified samples shifted to a higher wavenumber (1735 cm^{-1}) with an accompanied decrease in intensity (Figure S3). This indicated the removal of unreacted citric acid and/or carboxyl group interference. The 1654 cm^{-1} band in the spectra was likely due to adsorbed water which was also reported for both wood (1650 cm^{-1}) and alkali-treated wood (1655−1658 cm^{-1}).[48,49] The peak at around 1590 cm^{-1} (Figure S3) is typically ascribed to the aromatic skeletal vibration of lignin.[50] However, an adjacent band was also expected at 1580 cm^{-1} when free carboxyl groups (of citric acid) were converted into carboxylates ($-COO^-$) upon NaOH washing.[51] Thus, in such cases, the 1590 cm^{-1} band was a combined contribution of lignin (in the saccharification residue) and carboxylates of the washed samples. Referring to Figure 6a, the bands at 1735 cm^{-1} correspond to the "unmasked" contribution of ester carbonyl groups (C═O stretching mode) after NaOH washing. The ester carbonyl band was insignificant when there was no curing (room temperature air drying), showing the absence of ester formation (Figure 6a). As curing was induced (heating at 180 °C) for a time duration from 1 to 5 min, the band intensity at 1735 cm^{-1} increased correspondingly, indicating an increase in ester linkages.

To quantify the relative amount of ester linkages, the aromatic skeletal vibration band of lignin at 1505 cm^{-1} was used as an internal reference.[52,53] Based on Ando and Umemura (2021)'s findings, and in the context of this study (hardwood), citric acid could react with cellulose (primary hydroxyl group) and xylan (secondary hydroxyl), while its reaction with lignin would be mainly via the primary hydroxyl groups of the β-O-4 substructure.[54] Hence, it is anticipated that lignin's aromatic ring structure would not be affected by esterification; this justifies the use of its 1505 cm^{-1} band for intensity normalization. When the values of these normalized intensities (1735 cm^{-1}/1505 cm^{-1}) were plotted against the lap shear strength (Figure 6b), it is evident that a higher bond strength is associated with a higher amount of ester bonds. So, the improved wood bonding was due to ester linkages enabled by citric acid.

Next, the relative importance of lignin, xylan, and (residual) glucan in the saccharification residue was evaluated for their contribution of hydroxyl groups, which are necessary for esterification with citric acid. In this study, the accessible hydroxyl content was determined (via acetylation followed by titration) as 4.71 mmol/g of saccharification residue, on average. Using the mass fraction data of xylan and glucan in the saccharification residue, and assuming a crystallinity of 70% (justification provided in Table S2) for cellulose, the predominant glucan, it can be estimated that xylan and glucan contributed a total of 3.13 mmol of hydroxyl groups per gram of saccharification residue. The lignin hydroxyl groups were thus estimated to be 1.58 mmol/g of residue. If the cellulose hydroxyl groups were fully accessible (i.e., no crystallinity), an additional amount of 2.3 mmol/g of hydroxyl groups would be accessible (an increase from 0.99 to 3.3 mmol/g), giving a total hydroxyl content of 7.0 mmol/gram (Table S2). The percent distribution of the hydroxyl groups would change from 21:45:34 to 47:30:23 for glucan, xylan, and lignin, respectively. This estimation, though preliminary, suffices to illustrate the significance of residual glucan and xylan with their hydroxyl

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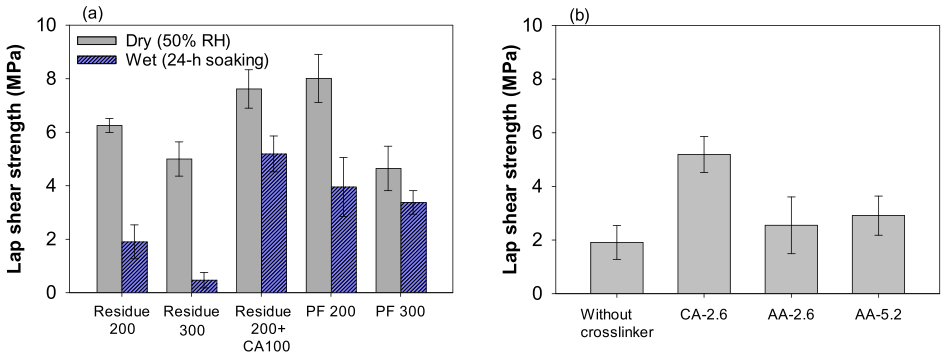

Figure 7. (a) Performance of wood lap joints bonded with or without citric acid (CA) and comparisons with PF resin. The numbers after the residue, PF, or citric acid refer to their respective spread rates in g/m^2 and (b) wet bond performance of saccharification residue cured with adipic acid (AA) in comparison with citric acid. The dosage (e.g., 2.6) of AA or CA was listed in mmol/g of the residue (80% saccharified) which was maintained at 11 mg. (Note: for both figures, the lap joints were hot-pressed at 180 °C for 5 min).

group contributions, although they were present in smaller portions in the saccharification residue. This lends merits to the proposed valorization strategy for saccharification residue without prerefining for its lignin. Based on estimations from this study, together with the published disruption of cellulose crystallinity by extensive grinding,[55] it can be inferred that one likely benefit of grinding was to expose the inaccessible hydroxyl groups for esterification. This hypothesis also corroborates the increased shear bond strength (without citric acid) as the grinding severity increased (Figure 3b). Lastly, further examination of the different types of hydroxyl groups within glucan, xylan, or lignin could be accomplished via nuclear magnetic resonance analyses to help discern their respective reactions with citric acid.[54] Such comprehensive analyses are beyond the scope of the present work and are preferably undertaken for a separate report.

Performance Assessment of Optimized Formulation and Role of Citric Acid in Wood Bonding. To further understand wood bonding enhancement by citric acid, values of lap shear strength were obtained after soaking the joint assemblies in water for 24 h. As previously mentioned, the amount of saccharification residue was maintained at 11 mg (200 g/m^2 spread rate) in wood bonding with or without citric acid. Therefore, the dosing of citric acid (at the highest performing loading of 33.3 wt %) would add extra solid (100 g/m^2) on the bond area, causing a higher spread rate (300 g/m^2) of the adhesive blend. For a fair evaluation, two additional sets of reference lap joints were prepared by increasing their spread rate to 300 g/m^2 with either the saccharification residue (no citric acid) or the PF resin.

Figure 7a shows that citric acid improved the wet performance of the bonded lap joints. Without citric acid, the average lap shear strength values of the saccharification residue upon 24 h of soaking dropped to 1.9 MPa (200 g/cm^2 spread rate) and 0.5 MPa (300 g/cm^2), signifying that the wood bonding was mostly via hydrogen bonding. In contrast, wood bonded with the citric acid-cured saccharification residue was 5.2 MPa in lap shear strength when wet, showing an approximate 70% bond strength retention (based on 7.6 MPa; dry). Interestingly, the bond strength of PF resin or the saccharification residue (no citric acid), tested either dry or wet, was higher in the case of a 200 g/m^2 spread rate than 300 g/m^2. A similar trend was reported with the dry shear strength of wood blocks bonded with phenol resorcinol-formaldehyde resin at a spread rate of 200 g/m^2 (8.75 MPa) versus 250 g/m^2 (7.43 MPa).[56] It is plausible that the typical hot-pressing time and temperature (also implemented in this study) for PF curing have been optimized for its common spread rate of 200 g/m^2, such that its bond line was more adequately cured, and so the resulted bonding was higher than the case of 300 g/m^2. In brief, when lap joints bonded with either 200 or 300 g/m^2 of PF were compared to the citric acid-enhanced bonding, the latter was found either stronger or comparable. Said differently, the enhanced bonding, at worst, was overall comparable (dry and wet strength inclusive) to PF bonding under the test conditions.

The citric acid-enhanced wet performance could be a result of either cross-linking (covalent bonding between molecules) or substitution of the hydroxyl groups of the saccharification residue and wood adherend (adjacent to the bond line); both cases improve water resistance. To ascertain the significance of cross-linking, an additional set of esterification studies was conducted using polycarboxylic acid (PCA) but without inducing cross-linking. As elaborated earlier (for Figure 5), two carboxyl groups (of a PCA molecule) are required to form a cyclic acid anhydride intermediate before an ester linkage can be realized. A bifunctional carboxylic acid, such as adipic acid (refer to Figure S1), could form an ester bond by reacting with a hydroxyl group of the host material, but there will only be one carboxyl group that remains, because of which, no further cyclic acid anhydride can be formed to afford a second ester linkage needed for cross-linking.[25] As such, adipic acid was examined to illustrate the case of esterification where cross-linking was absent.

Figure 7b illustrates the 33.3 wt % dosage of citric acid (5.5 mg for 11 mg saccharification residue) as 2.6 mmol for every gram of the saccharification residue used for bonding (55 mm^2 area). Adipic acid bonding was examined at the same dosage (2.6 mmol/g) or twice the dosage of citric acid. The 2× dosage was to double the number of molecules as an attempt to simulate the case of citric acid which (each molecule) could substitute two hydroxyl sites of the host material (compared to one hydroxyl substitution by adipic acid). Results showed that at either dosage, adipic acid did not markedly improve the wet bond strength of the saccharification residue (Figure 7b). Therefore, the hydroxyl group substitution (or hydrophobization) was not the main contributor to the observed improvement in wet bond strength. Conversely, chemical cross-linking, when enabled (the case of citric acid), improved the wet performance substantially. It was evidenced that citric

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acid functioned as a cross-linking adhesive component, thereby improving wet bond performance.

Sustainability Considerations. Having established the technical feasibility of the saccharification residue adhesive, it would be of value to obtain preliminary information about its sustainability performance. A full-scale sustainability study would involve using a life-cycle approach for ecological and socioeconomic evaluations. For the sake of simplicity, the global warming potential (GWP) metric was assessed focusing on the climate burden of the adhesive required for one square meter of wood joints (with resulting bond strength comparable to the case of PF resin). With the current study parameters (Table S3; see notes under the table), a higher climate burden is seen using the saccharification residue adhesive (1.40 kg of CO_2 eq/m^2 bond area) compared to PF resin (0.56 kg of CO_2 eq/m^2 bond area). The higher burden is primarily due to the ultrafine friction grinding step (0.93 kg of CO_2 eq/m^2 bond area), which is responsible for two-thirds of the climate burden of this new adhesive. Reducing the cumulative grinding energy from 11.2 to 0.8 kW h/kg would decrease the climate burden of the adhesive production (for 1 m^2 of bond area) to a level (0.54 kg CO_2 eq) similar to PF (0.56 kg CO_2 eq), assuming everything else is comparable. This is a large decrease but may be achievable using coarser particles ground at −50 μm clearance (see Figure 2b for their grinding energy). Future efforts to examine citric acid-enhanced bonding based on such particles might help incentivize this avenue of improvement. Additional efforts are also warranted in optimizing the citric acid dosage (or spread rate) to improve the sustainability metric of the adhesive. Lastly, there are more immeasurable benefits of the saccharification residue adhesive, which are beyond consideration in the analysis, such as being formaldehyde-free and utilizing saccharification byproducts. A full-scale sustainability study would provide a larger picture for more thorough assessments.

CONCLUSIONS

A fully biobased adhesive system was formulated using a lignin-rich saccharification residue of hardwood without having to isolate lignin. Enzymatic saccharification increased the ease of their subsequent grinding. Grinding the residue into smaller particles improved their bonding to the wood substrates. Thus, the employed strategy of increasing reactive surfaces for bonding was proven to be successful. When different saccharified samples were examined at similar particle sizes, higher saccharification levels were found favorable for achieving a higher wood bond strength. It follows that enzymatic saccharification, in the process of liberating fermentable sugars from woody biomass, also imparts desirable properties to the hydrolysis residue for adhesive applications. This has the implication of reducing the preparation cost of the intended adhesive coproduct.

By addition of citric acid, the wood bonding of the saccharification residue, tested either dry or wet, could be enhanced to levels comparable to those of a commercial PF resin. The improved performance, a result of ester cross-linking enabled by citric acid, signifies a competitive, fully biobased adhesive that is formaldehyde-free. Aqueous processing (safer solvent), mechanical activation (reduced chemical derivatives), and esterification cross-linking with citric acid (safer chemistry) are added features of the adhesive in adhering to the principles of green chemistry. The high inclusion rate of the residue (at 66.7 wt %) in the proposed adhesive also brings forth an effective means for managing the byproduct of enzymatic saccharification.

Overall, this study demonstrates a practical strategy for increasing the value of woody biomass saccharification residue. To improve sustainability performance, it would be logical to optimize citric acid-enhanced bonding using coarser particles (e.g., those ground at −50 μm clearance) for energy efficiency of adhesive production. Following that, full-scale life-cycle studies and technoeconomic assessments are recommended. Additional performance evaluations could also be conducted, as needed, to allow operational adjustment relevant to the process design (e.g., pretreatment type) and feedstock (e.g., biological origin of biomass) of a biorefinery of interest.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.3c07759.

> Experimental details of the pretreatment, saccharification, chemical composition and hydroxyl content analysis, and grinding; chemical structures of citric acid and adipic acid; particle size distribution of ground particles of different saccharification levels; FTIR spectra of washed and unwashed samples; summary table of adhesive system composition; estimated distribution of hydroxyl groups in saccharification residue; and crude estimation of GWP of the saccharification residue adhesive (PDF)

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The manuscript was written through contributions of all authors. All the authors have given approval to the final version of the manuscript.

Notes

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