Effects of Crosslinking on the Mechanical Properties and Biodegradability of Soybean Protein-Based Composites

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ABSTRACT: A green composite with good mechanical properties and acceptable biodegradability was developed using wood flour and soybean protein that was modified by thermal-caustic degradation and chemical crosslinking with glyoxal and polyisocyanate (PMDI). Fourier transform infrared (FTIR) spectroscopy and scanning electron microscope (SEM) in combination with the traditional evaluations were employed to investigate the structure, morphology, and properties of the crosslinked soybean protein and the crosslinking-modified wood/soybean protein composites to understand the effects of the crosslinker species on the mechanical properties, water resistance, and microbial biodegradation of soybean protein-wood flour composites. The results indicated that the chemical crosslinking modification could improve the mechanical properties and water resistance but decrease the biodegradability of the wood/protein composite to a certain extent. Both glyoxal and PMDI alone as crosslinkers could not perfectly modify the soybean protein because of the high reactivity of PMDI and low crosslinking reactivity of glyoxal. The incorporation of glyoxal with PMDI could result in the desired crosslinking efficiency and good interfacial adhesion by compromising the advantages and disadvantages of glyoxal or PMDI alone as crosslinkers, which balanced the performances of the wood flour/soybean protein composite. The preferable combination crosslinker was composed of 50 wt % glyoxal and 50 wt % PMDI. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41387.

INTRODUCTION

Composites manufactured with petroleum-based polymer matrices are widely used in different fields due to their low price, low density, excellent mechanical properties, and excellent chemical stability, which have brought great convenience to human life.[1] However, most of these currently used composites are nondegradable such that their disposal after their service life has become a serious challenge. The disposal method mainly includes incineration, landfill and recycling; however, all three of these methods have clear drawbacks, making it difficult to overcome the significant environmental threat to human survival.[2]

With decreasing reserves of nonrenewable fossil fuels and growing environmental concern, developing environmental friendly biodegradable polymers to partially substitute for synthetic polymers has attracted the attention of many researchers.[3] Natural or bio-based polymeric materials, such as starch, cellulose, lignin, chitin, or proteins could be ideal for biodegradable composites due to their advantages including their abundance (widely cultivated and high yield), renewability, ecological compatibility, ease of collection and processing, full biodegradability.[4]

Soybean is one of the world's most important agronomic crops and is cultivated throughout the world. Soybean protein is extracted from soybean meal that is a by-product of the manufacture of edible soybean oil and is mainly used as animal feeds; however, it is not fully utilized.[5] Native soybean protein has a compact globular structure resulting from weak intermolecular interactions including hydrogen bonds, electrostatic bonds, Van der Waals forces, disulfide bonds, and hydrophobic interactions. In addition, soybean protein has abundant hydrophilic groups such as amides, hydroxyls, and carbonyls exposed to the exterior of the globular structure, while the hydrophobic groups are buried inside. Therefore, native-soybean-protein-based plastics have inherent shortcomings such as poor water resistance, low mechanical strength, and high brittleness that greatly limited their industrial application.

Recently, many completely degradable composites have been prepared from renewable plant fiber (hemp, jute, ramie, sisal, kenaf, etc.)-reinforced bio-based polymers.[6,7] However, the fiber loading and the interfacial adhesion were not desirable, and the comprehensive properties were not satisfied; in addition, the cost was not acceptable for many common applications. With the rapid development of the wood processing industry, a fairly

large amount of wood scrap is produced from timber processing such as shavings and sawdust, which are generally abandoned or burned. To fully make use of the wood scrap with more value added, environmental friendly bio-composites may offer a solution by combining the modified soybean protein with the wood flour or wood fiber.[8] Compared with lignocellulosic fibers, the low-aspect-ratio wood flours as reinforcement of composite have some advantages such as low cost, ease to obtain and process from wood scrap, less challenging to obtain proper blend with polymer matrix, etc.[9]

Interfacial adhesion is known to have a significant effect on the properties of composites. It has been confirmed that the poor interfacial adhesion could be improved using compatibilizers or crosslinkers to form bridges of chemical bonds between the wood reinforcements and the polymer matrices.[9] Glyoxal,[10] glutaraldehyde,[11] and polyamidoamine-epichlorohydrin (PAE)[12] are the most commonly used crosslinking agents for soybean protein. A suitable and effective crosslinking agent can result in sufficient and even crosslinks between the molecules or chains of soybean protein through their reactions with functional groups of soybean protein. Glyoxal could react with various amino acids in protein, especially lysine and arginine. Polyisocyanate has very reactive isocyanate groups that can react not only with the active groups of proteins such as amino and hydroxyl but also with the hydroxyl groups of wood.[13] Thus, polyisocyanate has often been used to synthesize polyurethane (PU) resin as wood adhesive in recent decades due to its versatile application properties, high flexibility in formulation, good weathering resistance, and excellent adhesion properties resulting from the chemical linkages between PU and wood.[14,15]

Fully biodegradable green composites prepared with glyoxal and PMDI-modified soybean protein and wood flour have rarely been reported. The main goal of the present work was to investigate the effects of crosslinkers with various PMDI/glyoxal ratios on the mechanical properties, water absorption, and microbial degradability of these low-cost wood/soybean protein composites, by which balanced the biodegradability and mechanical properties suitable for more commercial applications.

EXPERIMENTAL

Materials
Soybean protein isolate (SPI) with a protein content of 93.1 wt % was provided by Harbin High Tech Soybean Food, China. Poplar wood flour with particle sizes between 40 and 60 meshes was supplied by the Bio-based Material Key Lab of Ministry of Education, Northeast Forestry University. The moisture content of the wood flours was ~8% (air dried) before use. Polymeric MDI Millionate® MR-200 with a NCO content of 31.2 wt % or NCO functionality of 2.8 was supplied by Nippon Polyurethane Industry, Japan. The other chemicals used in this study such as glyoxal (GO), sodium hydroxide, and formic acid were reagent grade and were purchased from local chemical companies.

Thermal-Caustic Degradation of Soybean Protein
In a reaction kettle, 352 g of water and 48 g of 50 wt % NaOH solution were charged. In total, 218 g of SPI was gradually introduced into the kettle after the NaOH solution was heated to ~70°C, and the mixture was kept at 70°C for 1 h. Finally,

the reaction products were cooled to room temperature, and the pH was adjusted to ~6.5 using formic acid, and then, the solution was stored at ~5°C.

Composite Compounding
Wood flour was blended with thermal-caustic degraded soybean protein (the mass ratio of the solid soybean protein over wood flour was 15 : 85) in a high-speed mixer (1500 rpm) for 5 min at room temperature. Then, the mixture was further mixed with the crosslinker (10% GO, 7.5% GO/2.5% PMDI, 5% GO/5% PMDI, 2.5% GO/7.5% PMDI, or 10% PMDI, based on the liquid soybean protein). The final mixture was transferred into a fluoride-plating metal mold with dimensions of 25×35 mm² followed by compression molding at 120°C and 7–8 MPa for 320 s. The obtained composite sheets from the above molding pressing had thicknesses of ~3.5 mm and were labeled as DW10G, DW3GP, DWGP, DWG3P, and DW10P, respectively, according to the components of crosslinkers used (where W, G, and P referred to wood flour, glyoxal, and PMDI, and the numbers between the letters corresponded to the relative mass ratio of the two crosslinkers). Two control panels were fabricated with the same compounding processing: one labeled as SW using wood flour and native soybean protein without any crosslinker, and the other one labeled as DW using wood flour and degraded soybean protein without any crosslinker.

The composites were moisture conditioned at 23°C in a 60% RH chamber for 3 weeks before determining all the properties.

CHARACTERIZATION

Solid Content, Viscosity, Molecular Weight, and Work Time of Degraded Soybean Protein
The degraded soybean protein solution was kept in a 25°C water bath for 1 h before testing its viscosity using a Brookfield DV-II+Pro viscometer. Approximately 1 g of protein sample (M1, accuracy to 0.001 g) was placed in a weighed crucible (M_2, accuracy to 0.001 g) and then kept in a 103°C oven until achieving constant weight (M_3, accuracy to 0.001 g) before determining the solid content as $(M_3 - M_2)/M_1 \times 100\%$.

The degraded soybean protein solution was diluted into thinner solutions with concentrations of 1 wt % to determine the molecular weight using an Agilent 1100 GPC equipped with two chromatographic columns in series, namely a 79911GF-083 (MW range: 100–30,000) and a 79911GF-084 (MW range: 10,000–200,000). Each GPC sample was diluted with water to a concentration of 0.5% by weight. The mobile phase was water with a flow rate of 1 mL/min. The pressure on the columns was 78 psi. A differential refractive index detector was employed.

The work life of the modified soybean protein solution was determined by observing the fluidity of the mixture of the protein solution with the crosslinker in a 25°C chamber. The amount of time from the moment of blending the soybean protein solution with the crosslinker until the mixture could not be evenly spread onto the wood surface with a bristle brush by hand was defined as the work life (also called pot life).

Density and Thickness Determination of Composite
Five rectangular specimens with dimensions of 60×15 mm² were cut from the composites to determine their density. The
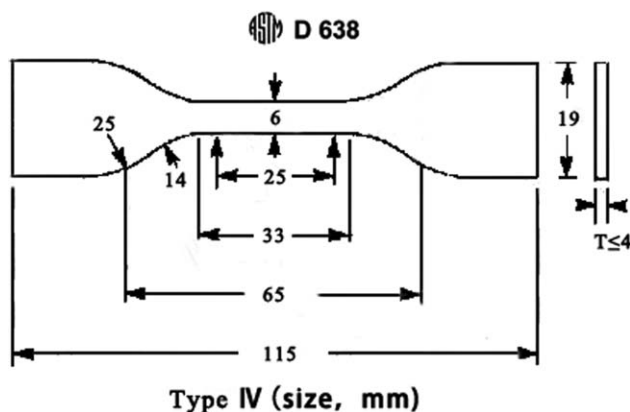
Figure 1. Dimensions of tensile test specimen.

average thickness (c, mm) from the two edges and the middle of each specimen, the length (a, mm), width (b, mm), and weight (W_1, g) of each specimen were measured according to ASTM D5947-03. The composite density (kg/m^3) was calculated using the formula $\rho = 10^6 \times W_1/(a \times b \times c)$, and the average value from five replicates was reported.

Tensile Properties of Composites
The tensile properties of the composites were characterized in accordance with ASTM D638-03 using a Universal Testing Machine (SANS, Shenzhen, China) with a strain rate of 5 mm/min at a gauge length of 50 mm. Type IV tensile specimens were cut from the composites with the dimensions shown in Figure 1.

Water Absorption and Water Resistance of Composite
The water absorption of the composites was evaluated according to ASTM D570-98. The specimens were dried in a 50°C oven for 24 h, then cooled in desiccators and weighed (W_2). The pre-conditioned specimens were immersed in distilled water in a 23°C thermostat water bath for 24 h. Then, the composites were removed from the water and placed on dry filter paper to remove the free water on the specimen surface and then immediately weighed again (W_3) to determine the mass loss. These wet specimens were again dried in a 50°C oven for 24 h and weighed again (W_4). The water absorption content (AC_{water}) was calculated using the equation $AC_{Water} = [(W_3 - W_4)/W_2] \times 100\%$. An average value from three replicates was reported.

The water resistance of the composite was determined by placing the Type IV tensile specimens into boiling water and then observing every 5 min whether the specimen withstood 1 h water boiling (recorded as "passed" if the narrowest neck of the specimen maintained integrity without disassembly or "failed" while indicating the boiling time from the moment the specimen was placed into the boiling water to when the narrowest neck of the specimen apparently disassembled).

FTIR Analysis
To investigate the chemical structures of the soybean protein before and after the crosslinking modifications, the degraded soybean protein solution was mixed with 10 wt % of modifier based on the liquid protein solution, then placed on Teflon film and cured at 120°C for 30 min. The dry cured sample was

mixed with KBr (mass ratio 1 : 120) and molded into a transparent FTIR disk before FTIR analysis using a Magna-IR 560 E.S.P FTIR spectrometer in the range from 4000 to 400 cm^{-1}.

Morphology of Crosslinked-Soybean Protein and Composites Determined by SEM Analysis
The fracture surfaces of some of the crosslinked-soybean protein samples in the FTIR analysis were examined by SEM. Some of the composite specimens before and after biodegradation were cut off immediately using a Feather Microtome Blade to obtain a smooth surface, which was also examined by SEM. The SEM samples were coated with ~10 nm of gold before observation with a QUANTA-200 SEM (FEI Co).

Biodegradation Test of Composites
A mineral salt medium was prepared that contained 0.7 g/L KH_2PO_4, 0.7 g/L K_2HPO_4, 0.7 g/L $MgSO_4 \cdot 7H_2O$, 1.0 g/L NH_4NO_3, 0.005 g/L NaCl, 0.002 g/L $FeSO_4 \cdot 7H_2O$, 0.002 g/L $ZnSO_4 \cdot 7H_2O$, 0.001 g/L $MnSO_4 \cdot H_2O$ and 15 g/L agar according to ASTM G21–2009, and the pH was adjusted to 6.5 using a 0.01 mol/L NaOH aqueous solution. The medium and specimen were sterilized via autoclaving at 121°C for 20 min. The culture dish was sterilized at 180°C for 3 h. Inoculation operations were performed on an asepsis table. The mineral salt medium was poured into each culture dish, cooling, and solidifying naturally.

Aspergillus niger was inoculated on the Czapek Dox Medium at 28°C for one week, and the medium was washed with distilled water three times, and the fluid mold was collected. The concentration of the spore suspension was measured using a hemocytometer and diluted to a concentration of 1.0×10^6 a/mL.

Five dried composite sheets (20 × 20 × 3.5 mm^3) were oven dried at 60°C for 24 h and then immediately weighed (W_5). The cooled sheets were sprayed with 0.2 mL of *Aspergillus niger* suspension before being immersed into the mineral salt medium in the culture dish. The culture dishes were placed into the incubator at 28°C for biodegradation. A total of 7 culture dishes for each composite were prepared, and one of them was acquired from the incubator every week to determine the mass loss caused by biodegradation. The composite sheets after biodegradation were again oven dried at 60°C for 24 h and weighed (W_6). The extent of biodegradation (R) in terms of mass loss was given by $R = (W_5 - W_6)/W_5 \times 100\%$, and the average value from five sheets was reported.

Statistical Analysis
The data in the current study were statistically evaluated using the statistical software package Minitab version 15. The data are reported as the mean value ± standard deviation of the replicates. A single factor analysis of variance was conducted to differentiate the significant differences among the mean values of the data according to the least significant difference criteria with a 95% confidence level ($P < 0.05$).

RESULTS AND DISCUSSION

Crosslinking Modifications of Degraded Soybean Proteins
Soybean proteins consist of two major fractions according to their sedimentation constants, 11S and 7S, with molecular weights
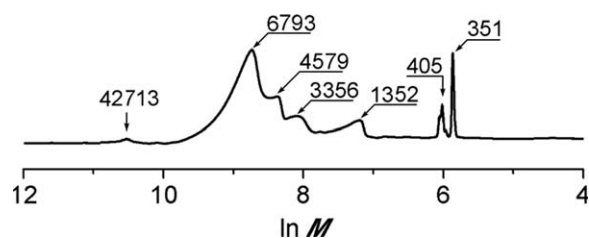
Figure 2. GPC spectrum of caustic-degraded soybean protein.

ranging from 15,0000 to 36,0000.[16] These large-molecular-weight polypeptide chains usually adopt regular structures such as α-helices or β-pleated sheets to form a compact globular structure of native soybean proteins. Thus, the majority of polar groups are embedded in the globular particles, which are unavailable to effectively absorb to and/or interact with the lignocellulosic substrate to form good interfacial adhesion. This property is undesired for application in wood composites such as plywood, particleboard, medium-density fiberboard, and oriented strand board.[17] Caustic degradation is an effective method used to unfold the globular protein.[18] After caustic degradation at above 70°C for 1 h in the presence of 6 wt % NaOH, soybean proteins not only completely unfolded their globular structures by destroying all the weak intermolecular interactions between the polypeptides but also degraded to form smaller polypeptide fragments via the hydrolysis of the peptide groups.[19] The molecular weight of the degraded protein was sharply reduced from hundreds of thousands to thousands, as illustrated by the GPC analysis in Figure 2. In addition, the degraded soybean protein was easy to dissolve in water to form a homogeneous solution with much lower viscosity (845 mPa s) but a higher solid content (~36.8%).

The composite DW compounded from the completely unfolded soybean protein after caustic degradation with a wood substrate could effectively improve the interfacial adhesion between the wood and protein to achieve better mechanical properties, as confirmed by the test results in Table I. The composite DW had a dry tensile strength that was 77.1% higher than that of the composite SW compounded from wood and native soybean protein. This improvement was attributed to the more effective interactions between the caustic-degraded protein and wood via hydrogen bonding and Van der Waals forces because all the buried polar and active groups in the globular soybean protein were sufficiently released after caustic degradation.

Table I. Tensile Properties and Water-Boiling Test of Soybean Protein-Wood Flour Composites

Composition ID	Tensile strength (MPa)	Water- boiling test
SW	6.12 ± 0.76 D	Failed (35 min)
DW	10.84 ± 0.53 C	Failed (2 min)
DW10G	18.34 ± 0.72 A	Passed
DW10P	15.96 ± 0.61 B	Passed

Values are reported as average value ± standard deviation. The standard deviations followed by different letters are significantly different at $p<0.05$.

However, both composites DW and SW exhibited very poor water resistance and completely disassembled after being kept in boiling water for ~2 min and 35 min, respectively, indicating that the interactions between wood and soybean protein only (whatever native one or caustic degraded) were weak. The poor water resistance of the soybean protein was attributed to the high hydrophilic polypeptide chains and the absence of chemical crosslinks between polypeptide chains. For the native soybean protein, its globular structure built up by weak intermolecular interactions is easily eroded and then destroyed by water or moisture. However, water cannot easily penetrate through globular particles because this structure is rather compact and most of the hydrophobic (apolar) groups of soybean protein embedded within the globular particles can further retard water penetration. Thus, the composite SW could withstand water boiling for 35 min. After thermal-caustic degradation, the globular structures of the native soybean proteins were completely destroyed, and the molecular weight of the long-chain polypeptide decreased remarkably to less than thousands because of the hydrolysis of the peptide. These changes led the degraded soybean proteins to exhibit very good solubility in water or worse water resistance though completely unfolded soybean protein, which could absorb to wood more effectively and form better interfacial adhesion. Therefore, the composite DW exhibited better mechanical properties but was easily eroded by water and therefore disassembled after 2 min of being immersed in boiling water. However, a good green composite compounded from wood and soybean protein should exhibit water resistance to some extent to withstand the ambient erosion from moisture and water such that the composites are able to maintain the necessary mechanical properties and dimensional stability during their serving cycle. On the basis of the above probing experiment, appropriate chemical crosslinking modification is expected to produce the necessary water resistance but acceptable biodegradability of the wood/soybean protein composite.

Glyoxal is often used as a crosslinking agent of soybean protein because it contains two active aldehyde groups that are able to crosslink soybean proteins via Maillard-type chemical reactions,[20] as illustrated in eq. 1 of Figure 3. The formation of the Schiff-base structure (─C═N─) from glyoxal-crosslinked soybean protein was confirmed by the FTIR at ~1590 cm^{-1}, which was attributed to the C═N stretching modes, and at ~1351 cm^{-1}, which was attributed to C─N bending deformation modes (Figure 4). The test results in Table I demonstrate that the composite DW10G compounded from wood flour and glyoxal-modified soybean protein (degraded) not only had a further increased tensile strength (as high as 18.34 MPa) but also exhibited better water resistance (withstanding 1 h of the water boiling) as compared with the composites with soybean protein without crosslinking modification (DW and SW), which was attributed to the formation of a three-dimensional network of soybean protein crosslinked by glyoxal.

Every PMDI molecule has 2.8 isocyano groups on average, which react not only with active groups such as ─NH$_2$ and ─OH in soybean protein but also with the ─OH of wood through a covalent bond, as demonstrated by eqs. 2 and 3 in
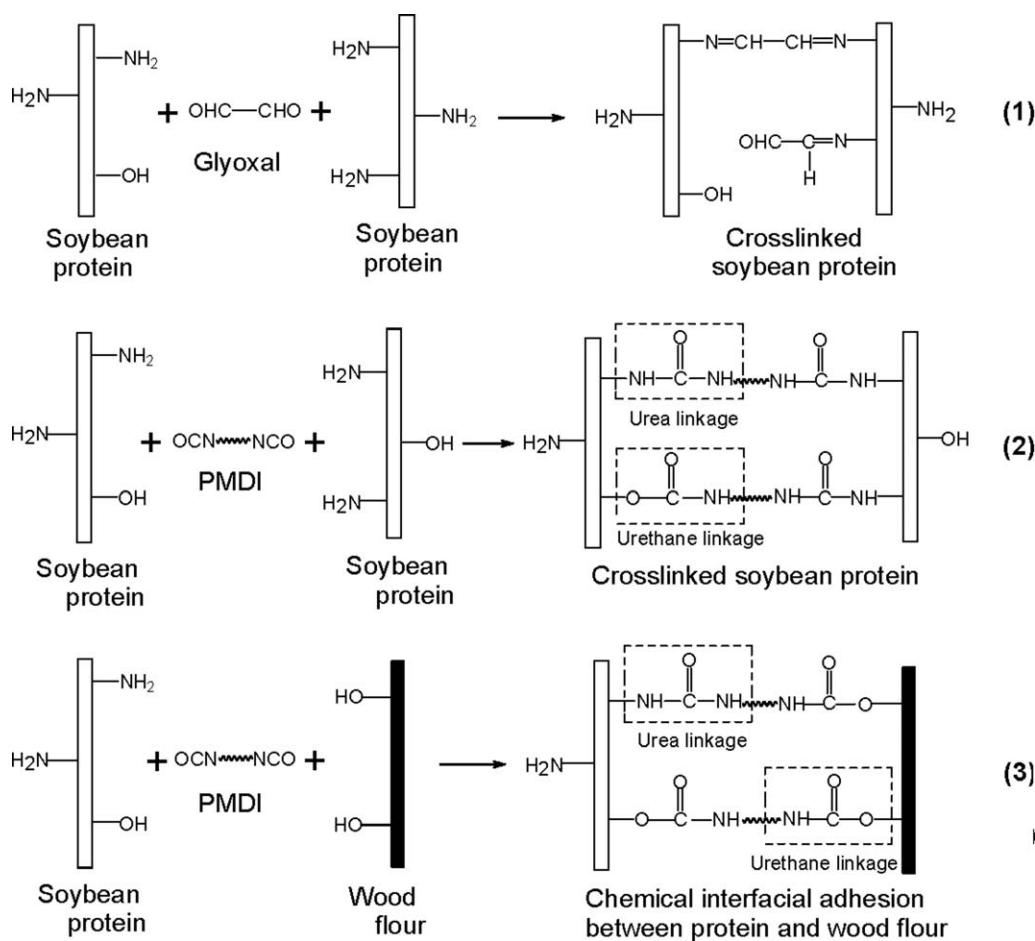
Figure 3. Schematic illustrations of the modifying mechanisms by glyoxal and PMDI.

Figure 3. The FTIR absorptions detected at $\sim$1590 cm^{-1} attributed to the C—N stretching modes and those detected at $\sim$1351 cm^{-1} attributed to C—N bending deformation modes (Figure 4) confirmed the formation of urea bridges and urethane

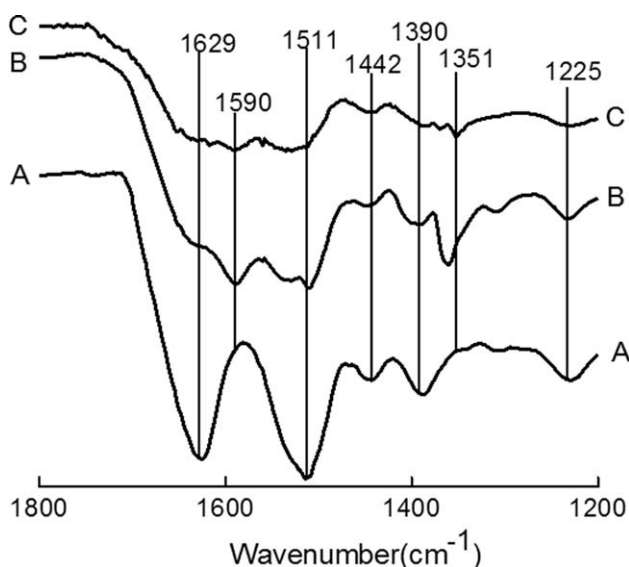**Figure 4.** (A) FTIR spectra of soybean protein and (B) that crosslinked by glyoxal and (C) PMDI.

bridges during the crosslinking reaction of soybean protein with PMDI. Thus, the tensile strength of the composite (DW10P) compounded from wood flour and PMDI-modified soybean protein increased up to 15.96 MPa and could withstand 1 h of the water boiling test. Additionally, the reactive isocyanate of PMDI could most likely react with hydroxyl groups of wood to form strong interfacial adhesion via chemical urethane bridges,[21] as illustrated by eq. 3 in Figure 3.

Theoretically, composite DW10P should exhibit better mechanical properties than DW10G because PMDI is inclined to form strong chemical bonds between soybean protein and wood. However, the former only exhibited a tensile strength of 15.96 MPa, which was 13% lower than the latter. This result was due to the fairly high reactivity of PMDI to the degraded soybean protein, which led to the formation of an uneven crosslinked structure. Because isocyante is more reactive to amino than the hydroxyl groups of protein,[13] the viscosity of the PMDI-modified soybean protein solution increased very rapidly when PMDI was introduced, as illustrated in Figure 5, and the crosslinking modification preferred to form urea bridges, as demonstrated in eq. 2 in Figure 3. Some particle-like aggregates began to form in the soybean protein solution after being mixed for $\sim$5 min, indicating that the insoluble polyurea were produced from the local crosslinking of polypeptide chains by PMDI. The insoluble resultants were
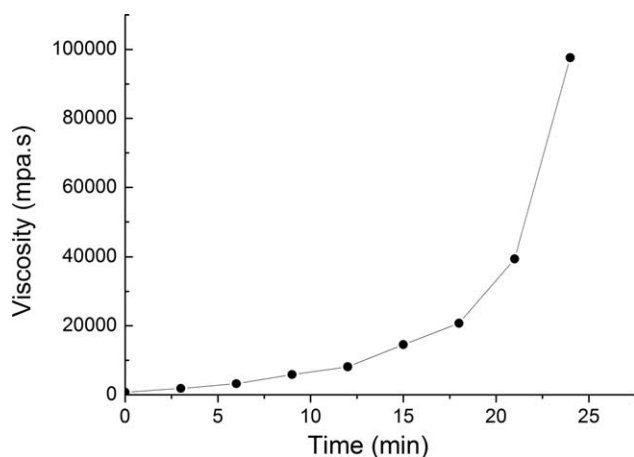
Figure 5. Viscosity increase of soybean protein-PMDI system as a function of time.

Figure 7. Work life of soybean protein solution modified by various crosslinkers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

separated from the protein solution and gradually aggregated to form heterogeneous structures with some pores. Many particle-like polyurea aggregates were apparently observed in the SEM micrograph of the cured PMDI-modified soybean protein solution [Figure 6(B)], resulting in a rather irregular fracture surface with some pores as compared with the smooth and compact one from the cured glyoxal-modified soybean protein [Figure 6(A)]. This structure was inclined to decrease the crack propagation energy when the composite was loaded because the stress would concentrate at the defects (both pole and polyurea aggregates) and accelerate the crack propagation, resulting in lower mechanical properties than that of the glyoxal-modified composite (Table I).

Additionally, with the rapid increase of viscosity and increasingly more particle-like aggregates formed, the PMDI-modified soybean protein solution declined to form undesired wood-protein interfacial adhesion for poor fluidity and wettability, which might lead to decreased mechanical properties. Therefore, the fabrication of the composite must be finished far before the PMDI-modified soybean protein solution achieved gelation. However, the PMDI-modified soybean protein solution had a short work life (also called pot life) of ∼25 min, which was insufficient for manufacturing the composite commercially.

While glyoxal or PMDI as a crosslinker alone could improve the mechanical properties and water resistance of the wood/soybean protein green composites, the above results indicated that gly-
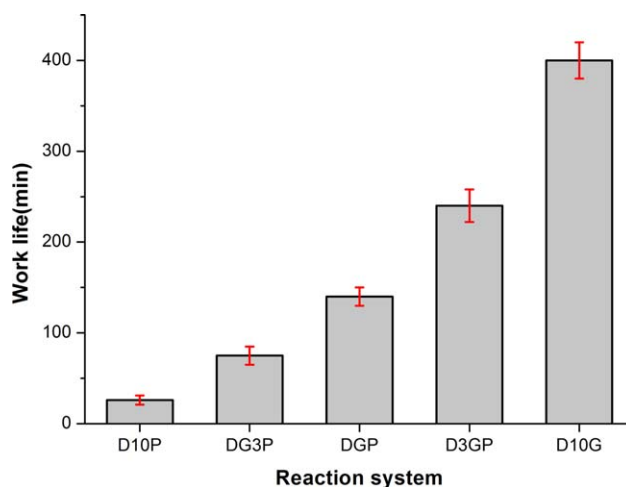
oxal or PMDI alone was not desired for the soybean protein due to their distinct disadvantages. Glyoxal has less crosslinking reactivity to soybean protein due to the steric hindrance between the two aldehyde groups and therefore gives the degraded soybean protein a longer work life (Figure 7) but less crosslinking efficiency. Compared with GO, PMDI has too high of crosslinking reactivity to form an uneven crosslinking structure and undesired work life.[22]

An ideal crosslinker should have both a good crosslinking efficiency and acceptable work life. Thus, the combination crosslinkers from glyoxal and PMDI were designed by varying their mole ratios in the current study, and their effects on the performances of the modified wood/soybean protein composite were investigated. A crosslinker formulation that was able to compromise the even crosslinking structure of low-reactivity glyoxal and the strong chemical interfacial adhesion of high-reactivity PMDI was expected to be optimized.

Mechanical Properties and Physical Properties of Composites
In the combination crosslinker composed of glyoxal and PMDI, low reactive glyoxal was able to dilute the concentration of high active PMDI and competed with PMDI to react with the protein. Thus, the mixture of soybean protein solution with the combination crosslinker had prolonged work lives, as illustrated
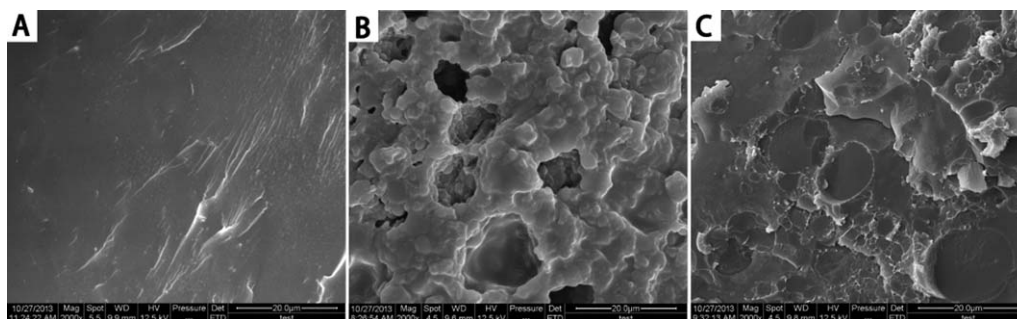

Figure 6. SEM micrographs of the fractured surface of the cured soybean protein solution crosslinked by (A) glyoxal, (B) PMDI, and (C) glyoxal/PMDI = 1.

Table II. Properties of Soybean Protein-Wood Flour Composites

ID	Density (kg/m^3)	Tensile strength (MPa)	Water absorption (%)	Water-boiling test
SW	967 ± 10.40A	6.12 ± 0.76 F	143.81 ± 2.60 A	Failed (35 min)
DW	853 ± 6.48DE	10.84 ± 0.53 E	96.81 ± 0.94 B	Failed (2 min)
DW10G	932 ± 8.50B	18.34 ± 0.72 C	61.54 ± 1.12 DE	Passed
DW3GP	908 ± 4.76C	22.32 ± 1.28B	59.02 ± 0.43 EF	Passed
DWGP	863 ± 4.64D	27.23 ± 1.14 A	56.99 ± 0.67 F	Passed
DWG3P	856 ± 6.18DE	21.74 ± 1.41B	62.97 ± 0.39 D	Passed
DW10P	844 ± 12.52E	15.96 ± 0.61D	66.52 ± 0.31 C	Passed

Values are reported as average value ± standard deviation. Standard deviations followed by different letters are significantly different at $p<0.05$.

in Figure 7. Upon increasing the glyoxal level, the work life clearly increased, and the formation of particle-like aggregates was also remarkably reduced, as illustrated in the SEM micrograph of the cured soybean protein modified by the combination crosslinker [Figure 6(C)], implying that a less uneven crosslinked structure was formed and the wettability of the soybean protein to wood was improved. Therefore, with the glyoxal level increased from 0 wt % (DW10P) to 25 wt % (DWG3P) and then to 50 wt % (DWGP), the resultant composites exhibited an ever-increasing tensile strength, as shown in Table II. However, glyoxal had poor crosslinking reactivity due to the steric hindrance from the two adjacent aldehyde groups, further increasing the glyoxal level in the combination crosslinkers from 50 wt % (DWGP) to 75 wt %(DW3GP) and then to 100 wt % (DW10G), leading to the gradual decrease of the tensile strength.

Theoretically, the higher PMDI level in the combination crosslinkers most likely led to a stronger and more compact composite because stronger chemical bonds were formed between the interfaces of the soybean protein and wood flour, as illustrated by eq. 3 in Figure 3. However, under the same mold pressing conditions, these composites assumed gradually deceased densities from 932 to 844 kg/m^3 with the continuously increasing PMDI level in the combination crosslinker from 0 to 100 wt %. Considering the nonlinear tensile change and the decreased density further confirmed that the addition of high-reactivity PMDI preferred to form an uneven crosslinking structure instead of the expected interfacial adhesion due to the quicker reaction of PMDI with the amino groups of the proteins.[23]

The mechanical properties of the composite DWGP (combination crosslinker with PMDI/glyoxal = 1) were much better than those of the composites DW10G (100% glyoxal as crosslinker) or DW10P (100% PMDI as crosslinker), indicating that a synergistic effect between PMDI and glyoxal existed by sufficiently retarding the rapid and uneven reaction of the protein with the high-reactivity PMDI but maximally increased the chemical interfacial adhesion between the wood and protein. Compared with the composite SW, the composite DWGP had a tensile strength improvement as high as 345%.

Water Absorption

The water absorption of the composite may induce irreversible mechanical and physicochemical changes in the polymers and composites, such as hydrolysis, chemical degradation, cracking,

and/or interfacial debonding,[24,25] especially in a green composite that compounded from the hydrophilic wood and soybean protein. In addition, the absorbed water may carry some fungi into the internal composite and initiate biodegradation across the composite instead of the common mode of compact materials that are biodegraded gradually from the surface into the core layer. Therefore, the water absorption generally led to a decrease of the mechanical properties but an improvement of the biodegradation of the composite. Because the water absorption of the composite is closely related to the interfacial bonding form,[26] the chemical crosslinking modification on the degraded soybean protein yielded chemical networks between protein chains and strong chemical bonds between the wood-protein interfaces, which prevented water from diffusing and absorbing freely and endowed the composites with acceptable water resistance (withstanding 1 h of water boiling). Thus, after the chemical crosslinking modification, the water absorptions of all the wood/soybean protein composites ranged from 56.99% to 66.52%, which were significantly lower than those of the composites SW and DW, as shown in Table II. The composite SW with native soybean protein had the maximal water absorption up to 143.81%. The composite DW using degraded soybean protein without crosslinking modification exhibited reduced water absorption (~96.81%). This comparison indicated that the completely unfolded globular soybean protein by caustic degradation released all the polar and apolar groups outside, in which the polar groups could effectively attach to wood to form a better protein-wood interface that reduced the water penetration rate and then the water absorption, while the abundant apolar groups increased the hydrophobicity of the soybean protein and thus helped the soybean protein to resist water absorbing to some extent.[27]

Biodegradation

Soybean protein is ready to be decayed by *Aspergillus niger*.[28] The biodegradation test results in Figure 8 indicate that both the composite and wood were gradually decayed by *niger* as the test period increased in terms of continuously increasing weight loss. Poplar wood (the control) could not be easily attacked by *niger* with a total weight loss of 2.08% after 7 weeks of degradation, while the composites had weight losses of ~8.63–9.62%. This comparison implied that the main weight loss should be attributed to the degradation of the soybean protein in the composites. The weight losses of the soybean protein in the composites were approximately estimated by dividing the total
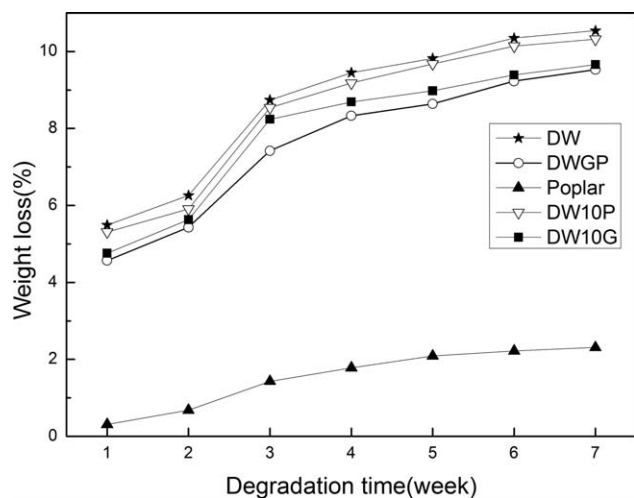
Figure 8. Weight losses of some composites and poplar wood control.

Figure 9. Weight losses of protein in some composites. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

weight loss of the composite after excluding the weight loss of the wood into the original weight of the protein in the composite, as shown in Figure 9.

Because the degraded soybean protein had very poor water resistance (as shown in Table I) for completely unfolded and remarkable decrease of molecular, it was ready to be swollen by water (Table II) such that *niger* could easily enter and attack the internal layer of the composite. Thus, the biodegradation half-life of the soybean protein was the shortest, being less than 4 weeks. The SEM micrographs in Figure 10 confirmed that the composite DW was obviously disintegrated due to significant biodegradation of the soybean protein for 3
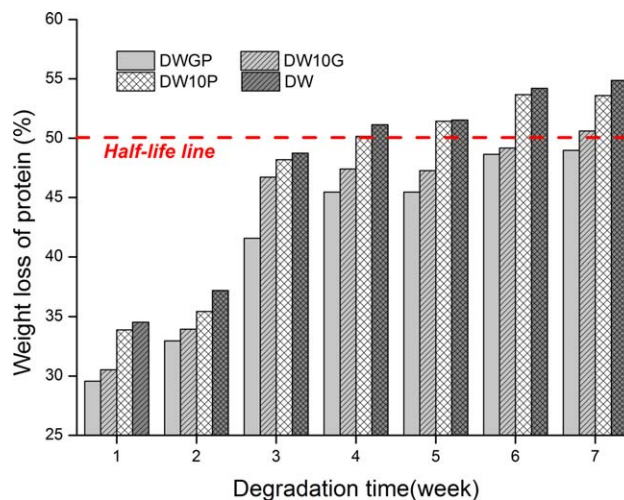
weeks and almost completely disintegrated when it was attacked by *niger* for 7 weeks. The weight loss of the soybean protein in the composite DW10P after being attacked by *niger* for 5 weeks was more than 50 wt %, while that of the composite DW10G required 7 weeks. This result indicated that the uneven crosslinking structure resulting from the high-reactivity PMDI was more easily attacked by microbes. The combination crosslinker of PMDI and glyoxal exhibited better biodegradation resistance than PMDI or glyoxal alone because their combination improved the crosslinking efficiency and wood-protein interface. In addition, the composite DWGP
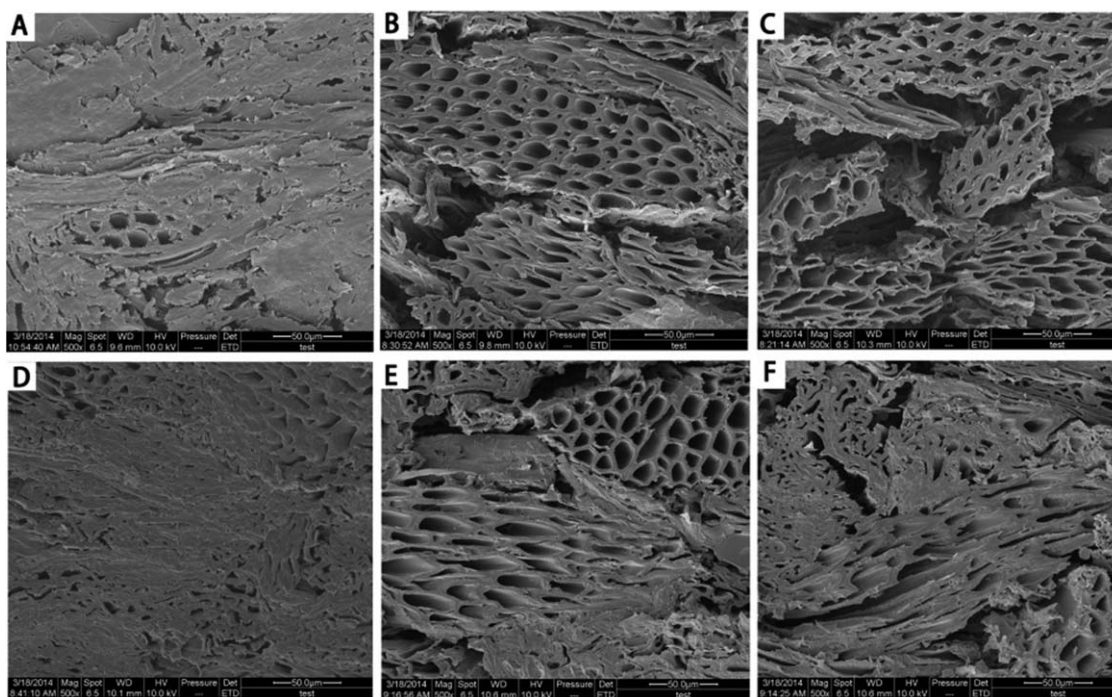

Figure 10. SEM photographs of the composites DW (A, B and C) and DWGP (D, E and F) after being biodegraded for various weeks (0, 3, and 7 weeks from left to right).

exhibited the best biodegradation resistance with the lowest weight loss of soybean protein (48.8 wt %) after the 7-week microbial attack. The SEM micrographs in Figure 10 demonstrate that most of the wood-protein interface was still well maintained after 7-week biodegradation, implying that this biodegradable composite had a longer service life. In terms of weight loss during the 7 weeks of *niger* attacks, the biodegradable order of the composite modified by various crosslinkers was as follows: DW (no crosslinker) > DW10P > DW10G ≈ DWG3P ≈ DW3GP > DWGP (the curves of DWG3P and DW3GP are not shown in Figure 8 to prevent overlapping).

Combining all the results above, it is feasible to balance the mechanical properties, water absorption and biodegradation of soybean protein-wood flour composites by properly incorporating glyoxal with PMDI as a combination crosslinker. This is according with the report that the improved mechanical properties of the soybean protein imply a higher degree of inter and intramolecular interactions including crosslinking networks, and thus, breaking down such composites requires harsher degrading conditions or longer times, which decrease the water absorption and biodegradation.[29] However, further research needs to be performed to correlate the mechanical properties changes with the biodegradation time, by which a balanced service life and biodegradation cycle for practical applications can be obtained. With the balanced mechanical properties, water absorption and biodegradation, soybean protein-wood flour composites have the potential for a wide range of applications such as food packaging, interior panels, electrical enclosures, and other areas to replace the widespread use of nondegradable plastics.

CONCLUSIONS

Waste wood flour and soybean protein obtained from renewable sources were used to fabricate biodegradable green composites. The composite compounded from wood flour and soybean protein without crosslinking modification exhibited good biodegradation but poor mechanical properties and water resistance. Chemical crosslinking modification by PMDI, glyoxal, and their mixture had significant effects on the mechanical properties, water absorption and biodegradability of the obtained composite. The incorporation of a proper combination of the crosslinker glyoxal with PAPI resulted in the desired crosslinking efficiency and good interfacial adhesion by compromising the advantages and disadvantages of the single crosslinker, which was able to balance the performances of the wood flour/soybean protein composite. Further research needs to be performed to correlate the mechanical properties changes with the biodegradation time for the practical applications of this green composite.

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