Production of Levulinic Acid from *Pennisetum alopecuroides* in the Presence of an Acid Catalyst

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The perennial grass *Pennisetum alopecuroides* was degraded using a conventional heating method with sulfuric acid. The effects of temperature (150 to 200 °C), reaction time (30 to 210 min), acid concentration (2% to 10%), and solid-liquid ratio (1:10 to 1:4) were optimized for *P. alopecuroides* hydrolysis. The production of levulinic acid was strongly affected by variations in these parameters. The optimum conditions with respect to reaction temperature, time, acid concentration, and solid-liquid ratio were 190 °C, 60 min, 8%, and 1:6, respectively. The maximum levulinic acid yield using the optimum conditions was 50.49%. The residues obtained from various temperatures were also intensively characterized using Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and thermogravimetric (TG) analyses. These results extend the current understanding of the bioconversion and utilization of renewable lignocellulosic biomass.

Keywords: Pennisetum alopecuroides; Lignocellulose; Hydrolysis; Levulinic acid; Acid catalyst

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INTRODUCTION

The rising costs of petroleum-based resources, along with the environmental threats associated with their use, have incentivized the search for sustainable alternative resources. Biomass, such as lignocellulosic materials, can appreciably contribute to the global economy of alternative energy production because of their abundant availability, low cost, need for their proper disposal, and suitability to yield value-added products (Alonso *et al*. 2010; Zhou *et al*. 2011; Ma *et al*. 2012). Therefore, finding ways to economically exploit the abundant wastes of this natural resource to produce valuable products, such as levulinic acid, is extremely appealing.

Levulinic acid is a short chain fatty acid with a ketone carbonyl and an acidic carboxyl group, and it is a strategic chemical that can be used directly in various applications or as a platform for other derived products (U. S. Dept. of Energy 2004; Bozell and Petersen 2010). Because of the imminent energy crisis and the increasing prices of comparable products, levulinic acid has the potential of being an important basic chemical material. It is used to produce a number of biochemicals including succinic acid, resins, polymers, herbicides, pharmaceuticals, flavoring agents, solvents, plasticizers, antifreeze agents, and biofuels (Rackemann and Doherty 2011). Levulinic acid is produced from lignocellulose biomass by dehydrating hexose (mainly glucose), which is present in significant amounts in the cellulose fraction of some agricultural residues and hardwoods.

Raw materials used for its production include starch (Cha and Hanna 2002), sorghum grain (Qi and Milford 2002), and agricultural wastes (Chang *et al*. 2007; Yan *et al*. 2008).

Levulinic acid is usually produced in a series of hydrolysis reaction steps followed by a dehydration step. These reactions are more efficient in an acidic medium where the degradation of polymeric pentosan molecules can occur relatively quickly during the hydrolysis step. The reaction pathway for the acid-catalyzed conversion of cellulose biomass to levulinic acid is shown in Fig. 1. Moreover, the levulinic acid yield and the reaction rate increase with increasing H^+ ion concentration at elevated temperatures. Therefore, the yield of levulinic acid is dependent upon the reaction temperature and acid concentration. Levulinic acid production by the hydrolysis of lignocellulosic waste materials with a mineral acid catalyst has been explored (Harris 1975). Other studies investigated levulinic acid production at higher temperatures (220 to 240 °C) (Fitzpatrick 1995) and in shorter reaction times (Zhang and Zhao 2010).

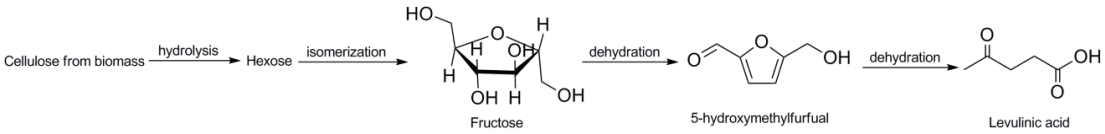

Fig. 1. Reaction pathway for the acid-catalyzed conversion of cellulosic biomass to levulinic acid

The perennial grass *Pennisetum alopecuroides* is a versatile and adaptable plant. It thrives in many weather conditions, lengths of growing seasons, soil types, and land conditions, and it can be cultivated to provide resources for biorefineries. Studies on levulinic acid production from *P. alopecuroides* are scarce. The technology most commonly used to convert lignocellulose to levulinic acid is acid hydrolysis. The acid concentration, solid to liquid ratio, operating temperature, and hydrolysis time play significant roles in determining both the yield and selective production of levulinic acid during acid hydrolysis.

This study investigated the production of levulinic acid from *P. alopecuroides* using a mineral acid-catalyzed hydrolysis process. The effect of various operating parameters, such as the reaction temperature, solid to liquid ratio, acid concentration, and reaction time, were investigated, and their optimum conditions to maximize levulinic acid yield were determined.

EXPERIMENTAL

Feedstock Sources

Pennisetum alopecuroides was obtained from the experimental farm of the South China Agricultural University (Guangzhou, P. R. China). The fresh, raw material was cut into small pieces, washed with distilled water, and dried in an oven at 80 °C for two days. The dried pieces were ground, sieved into a 40 to 60 mesh powder, and stored in the drier for later use.

The composition of the dried material was determined by the National Renewable Energy Laboratory (NREL) standard analytical procedure TP-510-42618 (2008). All reagents used were of analytical grade.

Methods

Hydrolysis process

The hydrolysis reaction was conducted in a 100-mL Teflon™-lined autoclave. Dry *P. alopecuroides* was placed in the reactor with 60 mL of sulfuric acid solution. The mixture was treated at the designated temperatures and times. Afterwards, the reactor was cooled to room temperature using flowing water. The mixture was centrifuged at 6000 rpm for 10 min. The precipitate was washed three times with distilled water and oven-dried at 80 °C. The supernatant was neutralized before the analysis of hydrolysis products. Experimental runs were performed at five different acid concentrations (2, 4, 6, 8, and 10 wt.%), five levels of solid-liquid weight ratios (1:4, 1:5, 1:6, 1:7.5, and 1:10 g/mL), six different temperatures (150, 160, 170, 180, 190, and 200 °C), and five different reaction times (30, 60, 90, 120, and 150 min).

Product analysis

The concentration of the hydrolysis products, such as monosaccharides (glucose, xylose, and arabinose) and volatile components (levulinic acid, 5-hydroxymethyl furfural, and furfural), was determined using high-performance liquid chromatography (HPLC). The hydrolysis solution was diluted, filtered, and injected into a SUGAR SH1011 column (6.5 × 300 mm, Shodex, Japan) operating on a 2695S Controller machine with an e2695 RID detector (Waters, USA). The operating conditions were as follows: reaction temperature, 50 °C; detector temperature, 50 °C; mobile phase, 0.005 M sulfuric acid; and flow rate, 0.50 mL/min.

The levulinic acid yield was calculated by Eq. 1,

$$\%Yield = \frac{Y_{act}}{Y_{th}} \times 100 \quad (1)$$

where Y_{act} is the experimentally determined yield (g) and Y_{th} is the maximum theoretical yield based on 100% conversion of hexose to levulinic acid (g), which was calculated by Eqs. 2 and 3,

$$Y_{act} = \mathrm{C} \times V \quad (2)$$

$$Y_{th} = \frac{Mwt_L}{Mwt_G} \times \frac{\%P}{100} \times W \quad (3)$$

where C is the levulinic acid concentration in g/L, V is the mixture volume in L, W is the mass of sample (g), $\%P$ is the glucan wt.% in raw material, and Mwt_F and Mwt_p are the molecular weights of levulinic acid and glucose, respectively.

The hydrolysis conversion ratio (HCR) of the raw material was calculated using the following equation,

$$\%HCR = \frac{m_i - m_r}{m_i} \times 100 \quad (4)$$

where m_i is the mass of initial material (g) and m_r is the residual mass after hydrolysis (g).

The levulinic acid selectivity was calculated by the Eq. 5:

$$\%Selectivity = \frac{Yield}{HCR} \times 100 \quad (5)$$

Characterization of residues

The residues were characterized by thermogravimetric (TG) analysis (NETZSCH STA449C, Germany, ramped from 313 K to 873 K at 10 K/min) Fourier transform infrared spectroscopy (FTIR) (Perkin–Elmer, USA, Spectra GX spectrometer, KBr pellets), and scanning electron microscopy (SEM) (SEI Qanta 200HV SEM apparatus, Japan, coated with gold).

RESULTS AND DISCUSSION

Chemical Composition of Raw Material

The chemical composition (w/w) of the raw material was determined according to a standard analytical procedure (NREL 2008). It was composed of cellulose (39.14%), hemicellulose (15.89%), acid-insoluble lignin (22.04%), ash (1.65%), and other components including water and extractives (21.28%).

Levulinic Acid Production

Pennisetum alopecuroides can be hydrolyzed with various acids, and the acid concentration, reaction temperature, reaction time, and solid to liquid ratio influence the levulinic acid yield. Figure 2 shows the HPLC analysis of the main hydrolysis products. Monosaccharides including glucose, xylose, and arabinose were detected with retention times of 15.30, 15.86, and 17.22 min, respectively. Levulinic acid (LA) was detected at 23.73 min. 5-hydroxymethyl furfural (5-HMF) and furfural (FF) were eluted at 42.43 and 63.37 min, respectively. The eluates at 21.09 and 22.51 min might be formic acid and acetic acid, respectively.

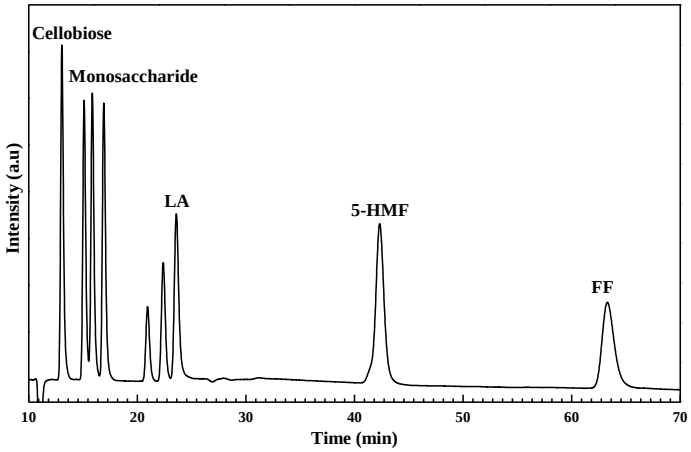

Fig. 2. HPLC chromatogram of *Pennisetum alopecuroides* hydrolysis products

Effect of the Acid Concentration

Theoretically, high acid concentration can shorten the time needed for *P. alopecuroides* hydrolysis, but the reagent costs and acid corrosiveness increase in association with the acid concentration. Generally, the acid concentration is not higher than 10%. The effect of acid concentration (2, 4, 6, 8, and 10 wt.%) on the levulinic acid yield was tested in hydrolysis reactions carried out at 180 °C and a solid-liquid ratio of 1:10 for 60 min (Fig. 3).

The levulinic acid yield was much lower in 2, 4, and 6 wt.% acid reactions than in the 8 wt.% reaction, which produced the maximum levulinic acid yield of 48.77%. The slight decrease in levulinic acid yield in the 10 wt.% acid reaction indicated that beyond certain sulfuric acid concentrations, the rate of levulinic acid loss to decomposition products became higher than its rate of production.

The mechanism of mineral acid catalysts during biomass hydrolysis has been explained previously (Horvat *et al*. 1985; Wang *et al*. 2014). Briefly, hydrogen protons penetrate the crystal cellulose and break the 1,4-glycosidic bonds between cellulose macromolecules. The cellulose crystal structure is then converted into a stable form, and hydrolyzed monosaccharides are converted into 5-hydroxylmethyl furfural. Finally, 5-hydroxylmethyl furfural is open looped with the formation of levulinic acid and formic acid. Therefore, increased sulfuric acid concentration is predicted to accelerate the conversion of biomass from *P. alopecuroides*.

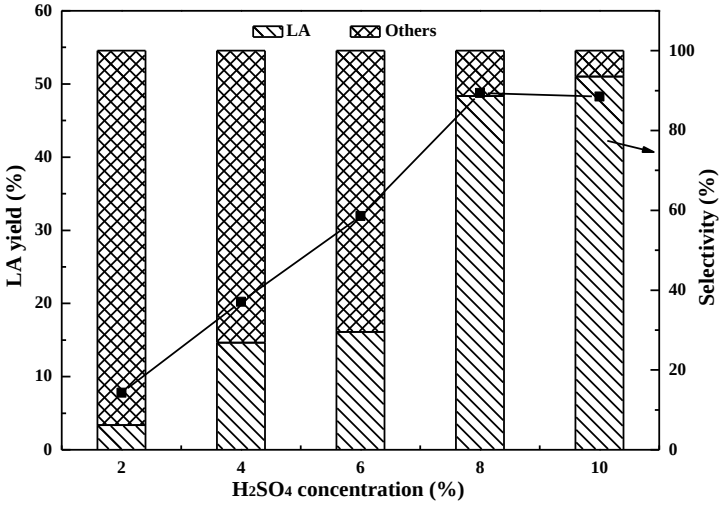

Fig. 3. The effect of H_2SO_4 concentration on the yield and selectivity of LA. Conditions: solid-liquid to ratio of 1:6; temperature of 180 °C; time of 60 min

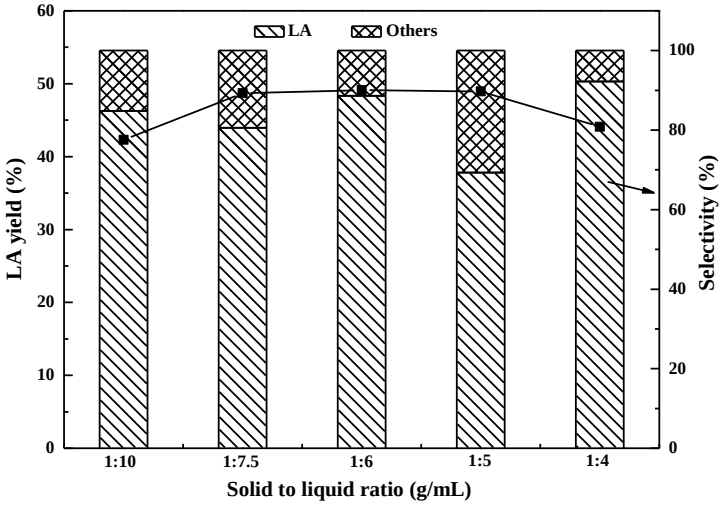

Fig. 4. The effect of the solid-liquid ratio on the yield and selectivity of LA. Conditions: 8% H_2SO_4; temperature of 180 °C; time of 60 min

Effect of the Solid-Liquid Ratio

Increasing the solid-liquid ratio up to 1:6 resulted in an increased levulinic acid yield (Fig. 4). However, the levulinic acid yield decreased when the solid-liquid ratio was greater than 1:6. The maximum levulinic acid yield obtained at the ratio of 1:6 (49.13%) decreased to 44.11% when the solid-liquid ratio was 1:4. This result was attributed to the earlier onset and faster rate of levulinic acid decomposition. However, the observed effect of the solid to liquid ratio resulted from pentosan acting as the limiting component in the solid waste material. Conversely, when the solid to liquid ratio got higher, the hydrolyzing acid was the limiting component (*e.g.*, a dilute acid solution), and the opposite effect would be expected. Additionally, lignocellulosic particles mixed with the acidic solution would be difficult for a well stir and prevented the internal penetration of the acid to cellulosic fiber due to the high solid to liquid ratios resulted in the swelling and agglomeration of lignocellulosic particles. However, high solid to liquid ratios reduce the consumption of acid and increase the concentration of sugar.

Effect of the Temperature

Temperature is an important factor in chemical reactions, especially for the hydrothermal depolymerization of biomass. The polymeric cellulose is insoluble in water at the temperature lower than 320 °C and 25 MPa due to the inter- and intra-molecular hydrogen-bonding structure (Deguchi *et al.* 2006); therefore, high temperature was expected to promote lignocellulose hydrolysis. Levulinic acid yield was measured in six different hydrolysis reaction temperatures from 150 to 200 °C with 8% acid and a 1:6 solid-liquid ratio (Fig. 5). The levulinic acid concentration increased sharply at 190 °C (50.49% yield). Beyond this temperature, the yield rose only slightly to a maximum value of 51.48%. This result indicated that higher temperatures favored both higher levulinic acid yield and faster formation kinetics, up to the maximum achieved yield. Therefore, the optimum reaction temperature was set at 190 °C.

An increase in the reaction temperature decreased the hydrogen ions released from sulfuric acid, which was completely dissociated at high temperatures because of the decreased dielectric constant of water. Thus, the higher temperatures (>190 °C) and acid concentration (>8%) had less effects on the product yield and reaction rate, inversely, resulting in the growing energy and sulfuric acid consumption.

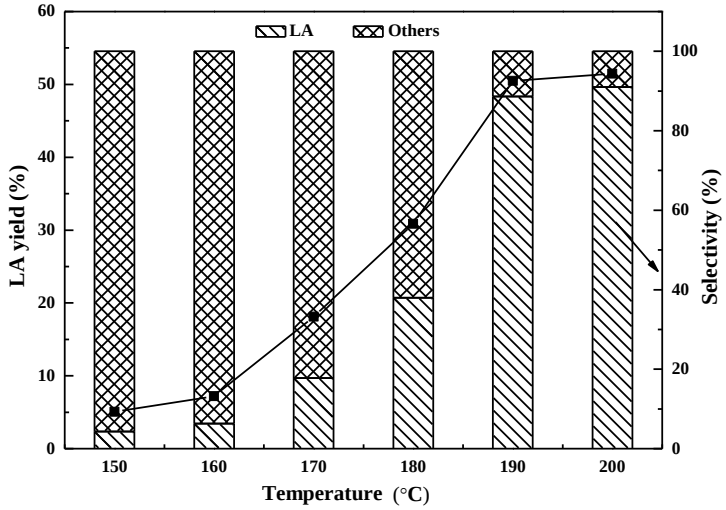

Fig. 5. The effect of the temperature on the yield and selectivity of LA. Conditions: solid-liquid ratio 1:6; 8% H_2SO_4; time of 60 min

Effect of the Reaction Time

To investigate the effect of hydrolysis time on levulinic acid yield, six different hydrolysis reaction times from 30 to 150 min were selected, while using 8% acid and 1:6 solid-liquid ratio at 190 °C (Fig. 6). Hydrolysis time exhibited a considerable effect on levulinic acid yield. As the reaction time increased from 30 to 60 min, the yield increased lineally from 22.33% to 50.49%. However, further increases in the reaction time did not notably improve the levulinic acid yield. Thus, the 60-min reaction time was sufficient for the hydrolysis of *P. alopecuroides* to levulinic acid. During this process, cellulose in raw material was first converted into water-soluble oligosaccharides; then, the oligosaccharides were degraded into hexose and the hexose further converted into LA and others in the acidic condition. With the increase of temperature, the yield of LA was decreased due to the formation of oligomers.

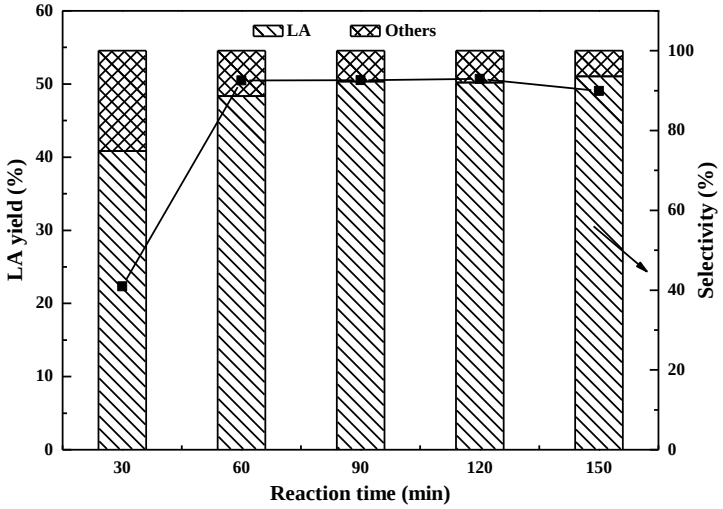

Fig. 6. The effect of the reaction time on the yield and selectivity of LA. Conditions: solid-liquid ratio 1:6; 8% H_2SO_4; temperature of 190 °C

Table 1. Component Analysis of Hydrothermal Depolymerization Residues Based on Temperature

Temperature (°C)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
Raw	39.14	15.89	22.04	1.65
150	17.22	6.99	72.02	3.77
160	10.30	3.56	83.39	2.74
170	9.59	2.12	85.26	3.04
180	7.25	0	89.57	3.18
190	0.24	0	95.72	4.04
200	0.21	0	96.40	3.39

Condition: solid-liquid ratio 1:6; 8% H_2SO_4; time of 60 min

Characterization of the Samples

Chemical composition of residues treated at various temperatures

Temperature had a considerable effect on *P. alopecuroides* hydrolysis (Fig. 5). The components of the original and residues treated at different temperatures were determined using an analytical method (NREL 2008). The chemical composition of the samples noticeably changed with the increasing reaction temperature (Table 1). Cellulose, hemicelluloses, and lignin contents in raw *P. alopecuroides* were 39.14%, 15.89%, and 22.04%, respectively. After treatment with dilute acid, the components were notably

changed. Cellulose decreased with increasing temperature to its lowest content of 0.21% at 200 °C. The hemicellulose content decreased with increasing temperature and was completely removed at 180 °C. Hemicellulose is an amorphous structure that is easily hydrolyzed by acid catalysts. Lignin, an aromatic polymer, generally cannot be degraded by acid catalysts or dissolved in water. Accordingly, the residue lignin content increased when reaction temperature increased from 150 to 200 °C, with values of 72.02% and 96.40%, respectively. Interestingly, cellulose decreased from 150 to 190 °C, and this result was attributed to the special structure of cellulose. The peripherally semi-crystalline cellulose fiber was first hydrolyzed at a lower temperature, leaving cellulosic crystal rods with strong hydrogen bonds; crystal cellulose was nearly decomposed when the temperature was increased (Himmel 2009). In sum, the cellulose and hemicellulose content gradually increased with decreasing temperature. The reaction rate was similar to the result of levulinic acid yield obtained from various temperatures.

Scanning electron microscopy

To further investigate the characteristics of residues hydrolyzed at various temperatures, SEM was used to determine morphological changes (Fig. 7). An untreated vascular bundle with a diameter of approximately 100 microns had a tight and smooth surface (Fig. 7a). After chemical treatment at 150 °C, the bundle became loose, and the fibers were partially separated, with the main cell framework collapsing. This observation correlated the chemical analysis showing the removal of hemicellulose. However, the main skeleton of the *P. alopecuroides* was visible (Fig. 7b). Higher temperature resulted in considerable modifications to the cell wall structure; for example, *P. alopecuroides* cell walls were completely destroyed after hydrolysis at 180 °C (Fig. 7e).

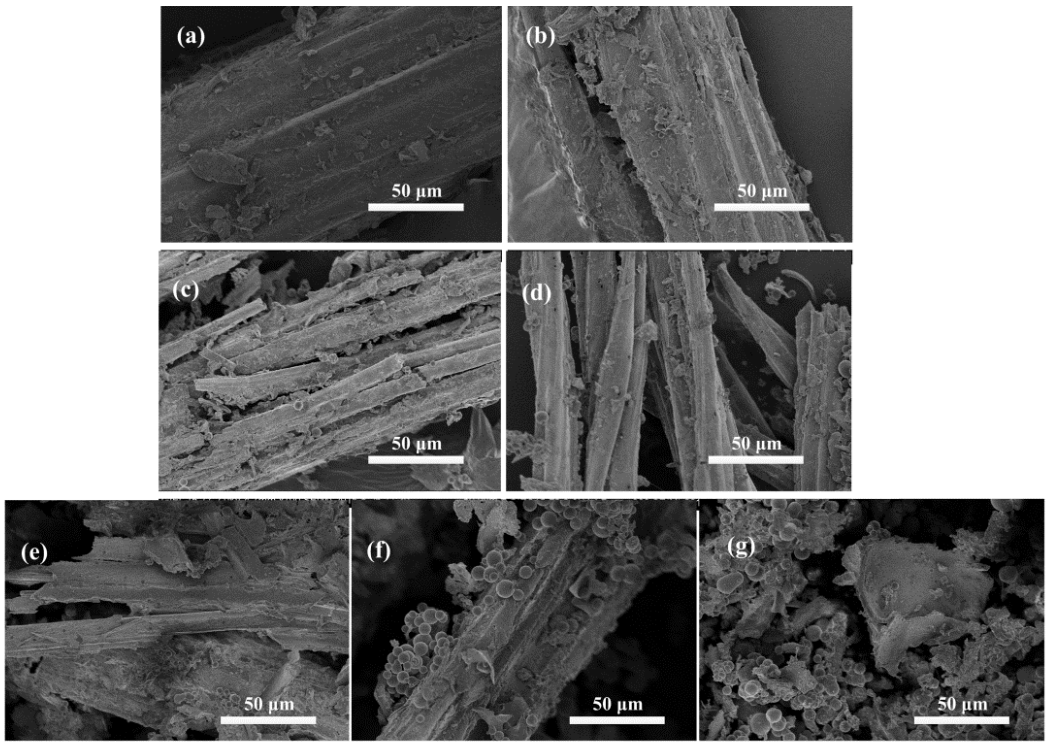

Fig. 7. SEM images of raw and treated *P. alopecuroides.* (a): raw *P. alopecuroides*, (b) to (g): residual *P. alopecuroides* treated from temperature of 150 to 200 °C. Condition: solid-liquid ratio 1:6; 8% H_2SO_4; time of 60 min

Generally, the lignin-hemicellulose and lignin-cellulose links were broken above 180 °C, removing hemicellulose and cellulose into the liquid phase (Table 1). However, lignin was not dissolved in water, and it easily aggregated (Fig. 7f, g). The residues treated at 190 and 200 °C contained 95.72% and 96.40% lignin, respectively. Accordingly, the rising temperature increased the accessibility of the cellulose, which enhanced cellulose and hemicellulose hydrolysis and enhanced the levulinic acid yield.

Fourier transform-infrared spectroscopy

Fourier transform-infrared spectroscopy was used to determine the constituent and chemical structure changes caused by different pretreatment methods (Fig. 8). The absorption peaks were assigned as previously described (Yuan *et al*. 2015). The band at 3352 cm^{-1} was attributed to –OH stretching vibrations. Peaks at 1255 and 1731 cm^{-1} were assigned to xylan in hemicellulose. Absorption peaks at 3362, 2900, 1365, and 1143 cm^{-1} were attributed to cellulose. Lignin showed the characteristic absorption bands at 2900, 1600 to 1500, 1423, 1314, and 830 to 750 cm^{-1}.

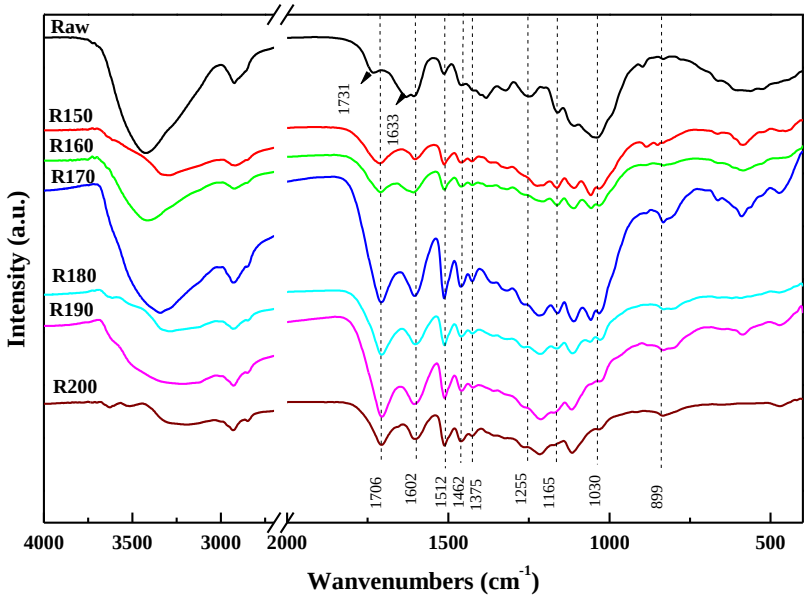

Fig. 8. Dynamics changes in the FT-IR spectra of the raw and residual *P. alopecuroides* treated at various temperatures. R150 to R200: residues obtained from temperatures of 150 to 200 °C

The spectrum of the raw material contained the characteristic absorption peaks of cellulose, hemicelluloses, and lignin. The absorption peaks at 1255 and 1731 cm^{-1}, which represent the C-O and C=O stretching vibrations of the acetyl ester unit of hemicellulose, decreased gradually with increased temperature and completely disappeared at 180 ºC, implying efficient hemicellulose degradation. This result confirmed the composition analysis (Table 1), which showed that hemicellulose was degraded at 150 ºC and completely removed at 180 ºC. The bands at 1602, 1512, and 1462 cm^{-1}, which represented the C=C stretching from the aromatic ring, increased with the elevated temperature, demonstrating that the remaining *P. alopecuroides* was mainly composed of lignin. In contrast, the decreased absorption at 3390, 2915, 1725, and 1030 cm^{-1} was the characteristic infrared absorption of the cellulose. This result indicated efficient hydrolysis, as shown in Fig. 5, and further confirmed that higher temperature enhanced the hydrolysis of the substrate.

Thermogravimetric analysis

Thermogravimetric analysis is a convenient method to assess the physical and chemical properties of biological macromolecules. In the absence of oxygen and at a specific heating rate, the change of the sample mass was monitored against time or temperature. Important parameters that determine the quality and yield of raw and residual *P. alopecuroides* were heating rate, final temperature, holding time at the final temperature, and the nature and physical properties of raw materials (Bansal *et al*. 1988). Most thermal decomposition of lignocellulosic materials occurs between 200 and 400 °C. Lignin is the first component to decompose at a low temperature (120 to 200 °C) and continues decomposition to approximately 900 °C. Hemicellulose is a light fraction component that also decomposes at low temperatures (160 to 360 °C). Cellulose is the last component to start to decompose (240 to 390 °C) (Luangkiattikhun *et al*. 2008).

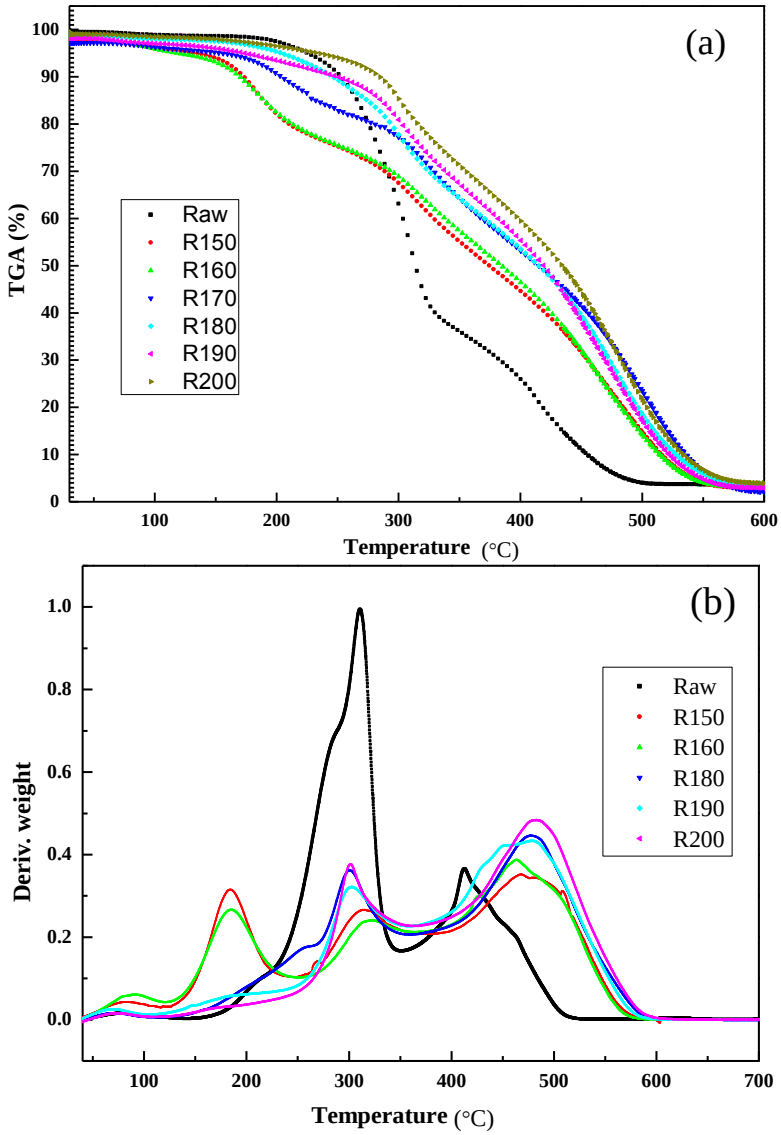

Fig. 9. TG analysis of the feedstock and residues at a heating rate of 20 °C/min. Raw, untreated raw material; R150 to R200, residues treated at 150 to 200 °C

The thermal stability of the residues increased with increasing hydrothermal reaction temperature, exhibiting particular improvement at 190 °C (Fig. 9a). Combined with the analysis of the components (Table 1), this data showed that the main components of the residues were lignin. When the hydrolysis temperature increased, the G-type lignin content increased. The raw material was quickly degraded, which induced poor stability at lower temperatures because of the easy devolatilization of the S-type lignin and condensation of the G-type lignin. Materials was calcined at 550 °C and left a remainder of Ca^{2+}, Mg^{2+}, and other inorganic salts.

Derivative weight analysis showed similar results (Fig. 9b.). Residue heated at 200 °C achieved the maximum decomposition rate at a higher temperature, while residues heated at 150 °C reached the maximum decomposition rate at a lower temperature. Therefore, residues obtained from the heating temperatures of 190 °C and 200 °C contained more stable lignin than those obtained from the 150 °C and 160 °C temperature treatments. This result indicated that the lignin structure was flexible at lower temperatures and compact at higher temperatures. Moreover, the residue coking phenomenon took precedence when the reaction temperature was increased, suggesting that the polymer of the residue was condensed at the high temperature. Because the reaction absorbed a lot of heat, the exothermic peak in the DTA curve was lower than that in the residues treated at the lower temperatures.

CONCLUSIONS

1. *P. alopecuroides* biomass was degraded under conventional heating conditions using sulfuric acid. The optimal temperature, reaction time, acid concentration, and solid-liquid ratio were 190 °C, 60 min, 8%, and 1:6, respectively.
2. Reaction temperature exerted the greatest influence on *P. alopecuroides* hydrolysis. As the reaction temperature increased, the chemical composition of the sample was modified. Hemicellulose was removed at 180 °C. Approximately 0.21% and 96% of cellulose and lignin, respectively, were retained in after treatment at 190 °C.
3. SEM showed changes in the morphology of the residues after different temperature treatments. Higher temperatures increased the accessibility of cellulose and enhanced the hydrolysis of cellulose and hemicellulose, which improved levulinic acid yield. FTIR spectra further confirmed that the temperature exhibited a considerable enhancement effect on the hydrolysis of the feedstock.
4. Thermogravimetric analysis indicated that the thermal stability of the residues increased with increases in the hydrothermal reaction temperature. Poor stability of the residues at lower temperatures was attributed to devolatilization of S-type lignin and easy condensation of G-type lignin. This research has provided an effective way to realize the goal of the utilization of biomass.

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