

Article

Two-Step Hydrothermal Pretreatments for Co-Producing Xylooligosaccharides and Humic-like Acid from Vinegar Residue

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Abstract: This study proposes an efficient strategy for co-producing high-value-added xylooligosaccharides (XOS) and humic-like acid (HLA) from vinegar residue based on two-step hydrothermal pretreatments. During the first-step hydrothermal pretreatment (170 °C, 50 min), 29.1% of XOS (X₂-X₆) was obtained. The XOS yield was further improved to 36.2% with endoxylanase hydrolysis, thereby increasing the value of (X₂-X₄)/XOS from 0.8 to 1.0. Subsequently, the second-step hydrothermal pretreatment was investigated to produce HLA from the solid residue of the first-step hydrothermal pretreatment. The highest HLA yield was 15.3% in the presence of 0.6 mol/L of KOH at 210 °C for 13 h. In addition, 31.7% of hydrochar by-product was obtained. The mass balance results showed that 1000 g of vinegar residue produced 67.9 g of XOS, 91.6 g of HLA, and 189.5 g of hydrochar. Therefore, this study provides a promising pathway for comprehensive use of lignocellulosic biomass in producing XOS and HLA.

Keywords: lignocellulosic biomass; pretreatment; autohydrolysis; humic acid; endoxylanase hydrolysis



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1. Introduction

Vinegar residue is a by-product of the solid-state fermentation of vinegar performed using glutinous rice, wheat bran, and rice as the raw materials. It is an abundant and cheap lignocellulosic resource. China's vinegar industry produces about 3 million tons of vinegar residue every year [1]. Vinegar residue has a certain degree of acidity and corrosiveness, and direct landfilling can cause soil and water pollution. At present, vinegar residue is mainly used to produce methane, ethanol, xylose, feed, and cultivation substrate, as well as for the repeated brewing of vinegar [2]. However, these processes often produce other by-products, which can cause environmental pollution and resource waste. For example, Liu et al. [2] used simultaneous saccharification and fermentation (SSF) technology to produce ethanol and xylose from vinegar residue; however, the residue slurry (mainly containing sugars, lignin, and yeast) of the SSF produced after solid-liquid separation may pollute the environment or cause resource waste if it is not effectively utilized. Therefore, the development of other high-value products with no solid residue produced for utilizing vinegar residue is critical. Vinegar residue is acidic and has a recalcitrant lignocellulosic structure mainly composed of cellulose, hemicellulose, and lignin [3]. These inherent characteristics limit the effective reuse of vinegar residue. Hydrothermal pretreatment (HP), also known as liquid hot water pretreatment or autohydrolysis, is considered an effective method for lignocellulose treatment due to its excellent ability to destroy lignin and hemicellulose and expose cellulose [4]. Pinales-Márquez et al. [5] reported that HP

facilitated the production of high-value-added products such as xylooligosaccharides (XOS) from hemicellulose, thus improving the circular bio-economy.

HP is a less polluting pretreatment method [6]. Water in HP can autoionize to produce H^+ at high temperatures. Thus, catalysis of the breakage of glycosidic bonds leads to depolymerization of the xylan skeleton and removal of acetyl groups from the main chain of xylan, forming an acidic hydrothermal environment, which further promotes the removal of xylan from the structure of lignocellulose [7]. However, this pretreatment method often fails to deliver satisfactory XOS output [8]. Acid-assisted (organic acid or inorganic acid) HP can improve the XOS yield because a large amount of H^+ can accelerate the degradation of xylan [9]. Zhu et al. [1] used a biphasic system consisting of 0.5% maleic acid and 60% phenoxyethanol to hydrolyze vinegar residue at 140 °C for 1 h and obtained a 47.3 wt % yield of XOS (X_2 - X_6). To produce more XOS with a low degree of polymerization (DP), endoxylanase hydrolysis is often arranged after HP or acid-assisted HP [10]. Yang et al. [9] treated delignified poplar with lactic acid (pH 2.5) at 170 °C for 30 min and then hydrolyzed it with endoxylanase; as a result, the yield of XOS (X_2 - X_6) increased by 16.9 wt % compared to that without endoxylanase.

Humic acid (HA) is a condensed carbon structure with rich oxygen-containing functional groups such as carboxyl, phenolic hydroxyl, quinone, and methoxy groups that is an important organic component of the soil [11]. It plays a crucial role in preserving water in soil, providing necessary nutrients and trace elements for the soil and plants; in addition, it plays a key role in the global carbon cycle [12]. The natural formation of HA usually takes a long time [13]. However, alkaline HP can accelerate the conversion of lignocellulosic biomass to HA [11], and this HA is called humic-like acid (HLA). The structure and characteristics of HLA have been proven to be similar to those of HA [14]. At the same time, hydrochar, a by-product that can be used as biofuel, is produced in this alkaline HP process [15]. Shao et al. [16] reacted corn stalk in NaOH solution (pH 10) at 180 °C for 4 h; they extracted 19.6 wt % of HLA and obtained 50.7 wt % of hydrochar.

In this study, vinegar residue was first pretreated using HP to prepare XOS (X_2 - X_6), and the effect of the HP temperature on the XOS yield was investigated. Then, the XOS autohydrolysate was hydrolyzed with endoxylanase to improve the yield of XOS, and the proportion of (X_2 - X_4) in XOS (X_2 - X_6) was also studied. The solid residue of vinegar residue obtained after the first-step HP (Stage 1) was used as a raw material to prepare HLA in the second-step HP assisted by KOH (Stage 2). The effects of the KOH concentration, HP temperature, and time on the HLA yield were investigated. The surface structure and elemental composition of the HLA were analyzed using a scanning electron microscope (SEM) along with energy-dispersive X-ray spectroscopy (EDS), Fourier transform infrared spectrometry (FT-IR), X-ray photoelectron spectroscopy (XPS), and an elemental analyzer (EA), and the content of the total acidic functional groups (TAG) in the HLA was determined. In addition, the influence of the KOH concentration, HP temperature, and time on the output of hydrochar in Stage 2 was also discussed, and its higher heating value (HHV), elemental analysis, and chemical composition were measured. The purpose of this study was to co-produce XOS and HLA using two-step HPs of vinegar residue to provide a new strategy for the utilization of lignocellulosic biomass.

2. Materials and Methods

2.1. Materials

Fresh vinegar residue (unprocessed wet solid residue after vinegar extraction) was kindly provided by Jiangsu University of Science and Technology (Zhenjiang, Jiangsu, China). It was ground with a high consistency refiner (Jilin Paper Mill Machinery Manufacturing Branch, Jilin, China) at a disc spacing of 0.2 mm, and the obtained vinegar residue was used as the starting material for this study. The contents of glucan, xylan, arabinan, and lignin in the vinegar residue were 25.4%, 18.8%, 7.9%, and 27.8%, respectively. Endoxylanase with 11.0 IU/mL activity was provided by the Jiangsu Kangwei Biotechnology Co., Ltd. (Yancheng, Jiangsu, China). Standard samples of xylobiose (X_2), xylotriose (X_3),

xylotetraose (X_4), xylopentaose (X_5), and xylohexaose (X_6) were purchased from Megazyme (Wicklow, Ireland). Commercial humic acid (CHA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals such as potassium hydroxide (KOH) and hydrochloric acid (HCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. First-Step Hydrothermal Pretreatment

The first-step HP (Stage 1) of the vinegar residue was performed in a 100 mL stainless-steel reactor with Teflon lining (Yuhua Instruments Co., Ltd., Shanghai, China) at a solid-to-liquid ratio of 1:10 (w/v). The reactor was placed in a thermostatic oil bath (HH-SA, Changzhou Jintan Liangyou Instrument Co., Ltd., Changzhou, Jiangsu, China). The temperature was increased from 50 °C to the set temperature (150, 160, 170, or 180 °C) and then held at that temperature for 50 min [17]. After cooling, the sample was filtered using a circulating water vacuum pump (SHZ-D, Gongyi Yuhua Instrument Co., Ltd., Gongyi, Henan, China) to achieve solid-liquid separation. The liquid phase (XOS autohydrolysate) was collected to analyze the monosaccharides, the XOS, and their degradation products; it was then subjected to hydrolysis with endoxylanase to obtain the low DP XOS, while the solid residue was thoroughly washed with distilled water for further use. The XOS yield was calculated as the percentage of the content of XOS (g) in the autohydrolysate or enzyme hydrolysate to the content of the initial xylan (g) in the substrate (Equation (1)). $(X_2-X_4)/XOS$ was calculated as the ratio of the content of (X_2-X_4) (g) to the content of $XOS(X_2-X_6)$ (g) in the autohydrolysate or enzyme hydrolysate (Equation (2)).

$$\text{XOS yield (wt \%)} = \frac{\text{XOS } (X_2 - X_6) \text{ content in hydrolysate (g)}}{\text{Xylan content in raw vinegar residue (g)}} \times 100 \quad (1)$$

$$(X_2 - X_4)/XOS = \frac{(X_2 - X_4) \text{ content in hydrolysate (g)}}{XOS(X_2 - X_6) \text{ content in hydrolysate (g)}} \quad (2)$$

2.3. Second-Step Hydrothermal Pretreatment

The solid residue from Stage 1 was subjected to the second-step HP (Stage 2). Approximately 3.0 g of the solid residue and a certain volume of different concentrations of KOH (0.3, 0.4, 0.5, 0.6, or 0.7 mol/L) were added to a 50 mL stainless-steel reaction vessel with para-polyphenol lining to maintain the total liquid volume at 30 mL and then mixed well. The reactor was placed in an electric blast drying oven (101-1B, Super Instrument Manufacturing Co., Ltd., Shaoxing, Zhejiang, China), and the reaction was performed at different temperatures (190, 200, 210, or 220 °C) for various times (11, 12, 13, 14, 15, or 16 h).

After cooling, the samples were centrifuged at 8000 rpm for 5 min. The solid residue (the so-called hydrochar) was thoroughly washed and then freeze-dried in a freeze dryer (FDU-2110, EYELA, Tokyo, Japan) to a constant weight, while the liquid fraction was acidified with 1 mol/L HCl to pH 2.0 according to the standard method recommended by the International Humic Substances Society (IHSS) [18]. The sample was centrifuged at 8000 rpm for 5 min, and the obtained precipitate (namely HLA) was washed twice with water and then freeze-dried for further characterization. The HLA yield was calculated as the percentage of the content of produced HLA (g) to the content of solid residue (g) added to the reaction (Equation (3)).

$$\text{HLA yield (wt \%)} = \frac{\text{HLA content (g)}}{\text{Solid residue added (g)}} \quad (3)$$

2.4. Endoxylanase Hydrolysis

Endoxylanase-assisted hydrolysis of the XOS autohydrolysate from Stage 1 was performed according to the method of Zhu et al. [17]. The reaction system was 20 mL, the reaction temperature was 50 °C, and the xylanase addition was 1 IU/mL. The reaction was carried out in a 1 mol/L sodium citrate buffer system (pH 4.8) at 150 rpm for 12 h. During

the course of endoxylanase hydrolysis, aliquots were withdrawn at 0, 4, 8, and 12 h. The samples were placed in a boiling water bath for 5 min. After centrifugation, the obtained supernatant was used to analyze the amount of XOS and xylose. All aforementioned experiments were performed in duplicate to ensure the reproducibility of the experimental results, and the data are presented in the form of the mean and standard deviation.

2.5. Analytical Methods

The chemical compositions of vinegar residue and pretreated vinegar residue, the concentration of fermentable sugars (glucose and xylose), and the concentration and yield of XOS were determined according to a previously reported method [17]. SEM-EDS was used for analyzing the surface structure morphology and elemental compositions; EA was used for the analysis of N, C, H, S, and O; FT-IR and XPS were used for analyzing changes in the chemical bond between the functional groups; and the titration method was used for analyzing the content of TAG according to the method of Wang et al. [14]. HHV was determined using a calorific value meter (JZLR-9000A, Changyao Technology Co., Ltd., Shanghai, China) according to the method of Fakudze et al. [19].

3. Results and Discussion

3.1. Xylooligosaccharides Produced from the First-Step Hydrothermal Pretreatment

Because XOS of DP 2-4 was reported to promote the growth of *Bifidobacterium adolescentum*, the $(X_2-X_4)/XOS$ was important [20]. The effects of different HP temperatures (150, 160, 170, or 180 °C) on the XOS (X_2-X_6) yield and $(X_2-X_4)/XOS$ from vinegar residue are shown in Figure 1. The yield of XOS (X_2-X_6) was increased from 8.5% to 29.1% (5.5 g/L) when the temperature was increased from 150 to 170 °C. This suggested that increasing the temperature accelerated the conversion of xylan to XOS, thereby increasing the yield of XOS. However, when the temperature was further increased to 180 °C, the yield of XOS decreased to 21.6%. This indicated that 170 °C was the optimal HP temperature for XOS production. High temperature negatively affects XOS preparation because it may result in excessive degradation of XOS [21]. As the temperature was increased from 150 to 180 °C, the value of $(X_2-X_4)/XOS$ increased slightly. This indicated that the XOS released by the HP of the vinegar residue was mainly composed of X_2 , X_3 , and X_4 and that temperature negligibly affected the distribution of XOS.

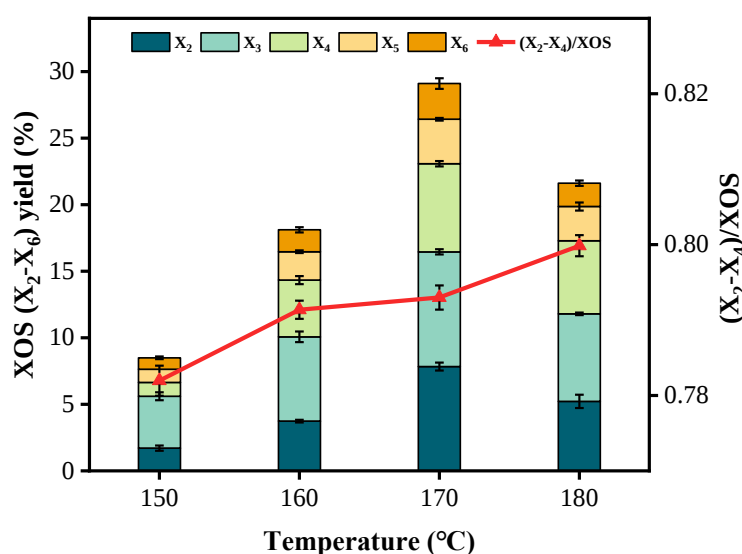


Figure 1. Effects of different hydrothermal temperatures on the XOS (X_2-X_6) yield and $(X_2-X_4)/XOS$ from vinegar residue.

In addition, the HP of the thoroughly washed vinegar residue was also performed at the optimal temperature (170 °C) of the unwashed vinegar residue. The yield of the XOS and the value of $(X_2-X_4)/XOS$ in the XOS autohydrolysate from the washed vinegar residue were 25.4% and 0.8, respectively, which were lower than those in the XOS autohydrolysate from the unwashed vinegar residue. This indicated that the HP of the unwashed vinegar residue was effective in producing XOS.

The pH of the initial reaction system of the unwashed vinegar residue was 3.84 because of the presence of some organic acids (acetic acid, propionic acid, and lactic acid) (see the Supplementary Materials Table S1, Figures S1–S3), which rendered the solution acidic with a large amount of H^+ , accelerating the dissolution and hydrolysis of xylan to XOS, thereby improving the yield of XOS [9,22]. Therefore, vinegar residue without washing could not only reduce the process water and wastewater treatment but also could improve the yield of XOS because of the presence of residual organic acids in the vinegar residue.

Zhu et al. [17] autohydrolyzed poplar at 170 °C for 50 min and obtained 24.7% XOS (X_2-X_6); the value of $(X_2-X_4)/XOS$ was 0.6. In this study, 29.1% and 25.4% XOS yields were obtained and nearly 0.8 of $(X_2-X_4)/XOS$ was obtained from the autohydrolysates of the washed and unwashed vinegar residue at 170 °C for 50 min, respectively, which were higher than the experimental results for poplar [17]. Thus, vinegar residue can be potentially used to prepare XOS.

The contents of glucan, xylan, and lignin in the unwashed vinegar residue were 25.4%, 18.8%, and 27.8%, respectively (Table 1). The vinegar residue was subjected to HP at different temperatures (150, 160, 170, or 180 °C) for 50 min. As the HP temperature was increased from 150 to 180 °C, the xylan content decreased from 17.7% to 6.1% and the xylan removal rate increased from 26.0% to 84.0%, which occurred because of the promotion of the degradation of xylan to XOS and xylose due to the presence of H^+ in the autohydrolysate [22]. This indicated that increasing the temperature was beneficial to the removal of xylan from the vinegar residue under experimental conditions [23]. Furthermore, the removal of xylan from vinegar residue resulted in increases in the contents of glucan and lignin from 25.4% and 27.8% to 36.8% and 39.8%, respectively, which corresponded to the glucan removal of 29.1% and the lignin removal of 29.8%. This indicated that an excessively high reaction temperature will lead to a loss in glucan and lignin. Therefore, according the XOS yield (Figure 1) and the chemical composition of the vinegar residue (Table 1), 170 °C was selected as the optimal HP temperature for Stage 1.

Table 1. Chemical compositions of vinegar residue after the first-step hydrothermal pretreatment at different temperatures (150–180 °C) for 50 min.

Temperature (°C)	Solid Yield (wt %)	Glucan (wt %)	Xylan (wt %)	Arabinan (wt %)	Lignin (wt %)	Removal (wt %)		
						Glucan	Xylan	Lignin
Raw Material	-	25.4 ± 0.4	18.8 ± 0.5	7.9 ± 0.3	27.8 ± 0.1	-	-	-
150	78.6 ± 0.5	29.9 ± 0.5	17.7 ± 0.6	1.7 ± 0.1	30.9 ± 0.4	7.5 ± 0.2	26.0 ± 0.1	12.5 ± 0.4
160	68.1 ± 0.1	34.4 ± 0.1	11.9 ± 0.6	0.0 ± 0.0	35.4 ± 0.8	7.9 ± 0.1	56.9 ± 0.4	13.2 ± 0.4
170	59.8 ± 0.2	36.6 ± 0.5	8.8 ± 0.2	0.0 ± 0.0	36.0 ± 0.1	13.8 ± 0.2	72.0 ± 0.9	22.4 ± 0.5
180	49.0 ± 0.4	36.8 ± 0.2	6.1 ± 0.3	0.0 ± 0.0	39.8 ± 0.3	29.1 ± 0.9	84.0 ± 0.7	29.8 ± 0.9

3.2. Endoxylanase-Assisted Hydrolysis of the Xylooligosaccharides Autohydrolysate

The XOS autohydrolysate of the vinegar residue contained some XOS with DP > 6, quantification of which was not possible because of the lack of standards (Figure S1). A study showed that XOS with a high DP could be efficiently converted by endoxylanase to XOS with a low DP [17]. Therefore, to improve XOS production with a low DP, endoxylanase (1.0 IU/mL) was used to hydrolyze the XOS autohydrolysate for 12 h (Figure 2). The results showed that the yield of XOS was 13.0% when the XOS autohydrolysate obtained from Stage 1 at 150 °C was hydrolyzed with endoxylanase for 4 h, and it decreased to 12.2% when the hydrolysis time was extended to 8 h (Figure 2A). This was because the XOS was

partially hydrolyzed into monosaccharides by endoxylanase for a long time, which reduced the yield of XOS. In addition, all of the XOS autohydrolysates obtained from Stage 1 at 160, 170, and 180 °C showed the highest yields of XOS at 8 h of endoxylanase hydrolysis (23.4%, 36.2% (6.8 g/L), and 32.7%, respectively), which indicated that 8 h was the best reaction time for endoxylanase hydrolysis under the experimental conditions (Figure 2B–D). Furthermore, consistent with the conclusion in Section 3.1, setting the temperature of Stage 1 at 170 °C was more favorable for XOS production, and the XOS yield at 170 °C after endoxylanase hydrolysis was enhanced by 7.1%. The $(X_2-X_4)/XOS$ ratios of the four XOS autohydrolysates increased with the endoxylanase hydrolysis time. When endoxylanase was used for hydrolysis for 8 h, the ratios obtained were 0.9, 0.9, 1.0, and 1.0, respectively. This was significant compared to that obtained from the XOS autohydrolysates, which were all increased by about 0.2 before endoxylanase hydrolysis. This suggested that the XOS with a low DP obtained using endoxylanase hydrolysis exerted a good probiotic effect [24].

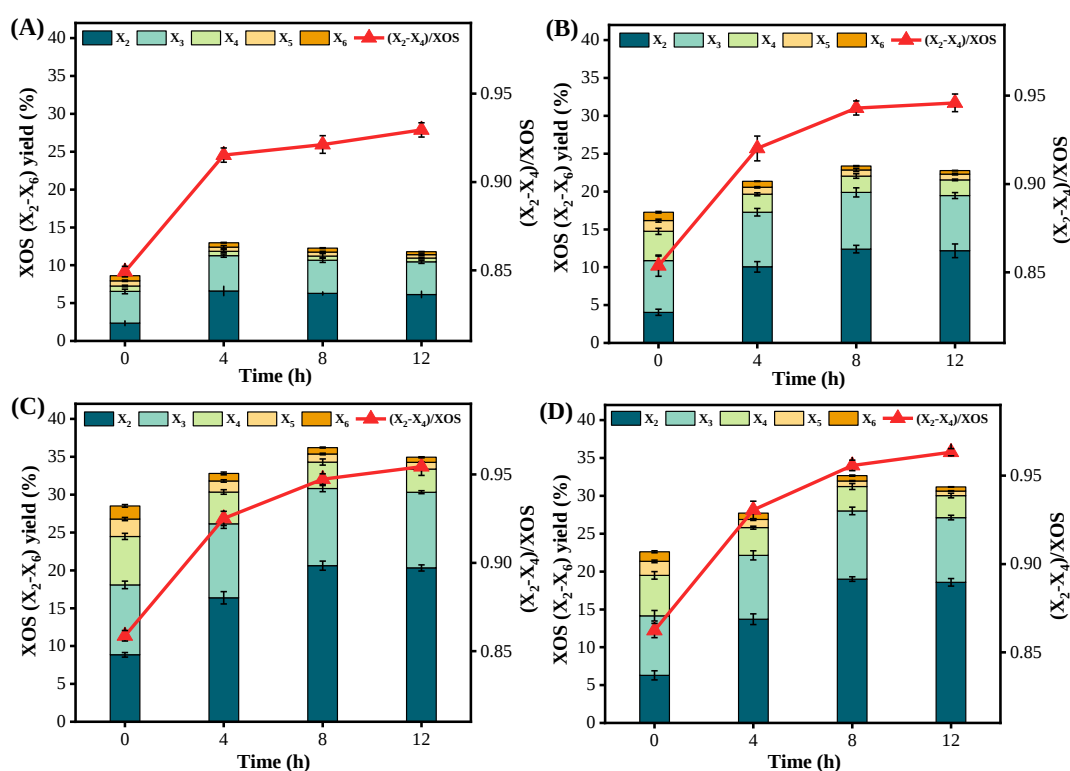


Figure 2. Effect of enzymatic hydrolysis time on XOS yield and $(X_2-X_4)/XOS$ during the endoxylanase hydrolysis of the XOS autohydrolysate obtained at different hydrothermal temperatures: (A) 150 °C; (B) 160 °C; (C) 170 °C; (D) 180 °C.

3.3. Humic-like Acid Prepared from the Second-Step Hydrothermal Pretreatment

Based on the conclusions drawn in Section 3.1, the solid residue obtained from the Stage 1 of the vinegar residue at 170 °C was selected for the second-step alkaline HP to produce HLA and hydrochar (Figure 3). The solid residue was subjected to alkaline HP at 200 °C for 12 h using different concentrations of KOH solutions (Figure 3A). The HLA yield increased from 9.2% to 12.2% when the KOH concentration was increased from 0.3 to 0.6 mol/L. This was because the alkaline solution could break the linkage between cellulose and hemicellulose as well as lignin [25,26], and the increase in KOH concentration promoted the hydrolysis of polysaccharides and lignin, thereby providing more precursors for HLA production. However, when the KOH concentration was further increased to 0.7 mol/L, the HLA yield decreased to 11.1%. This showed that a high alkali concentration was harmful to HLA production. The results indicated that 0.6 mol/L KOH was optimal for HLA production. The mechanism of HLA preparation is that in high-temperature alkaline

solution, water-soluble lignin is degraded into phenols, and carbohydrates will be partially degraded into monosaccharides or converted into organic acids via a “peeling” reaction. Some monosaccharides will dehydrate to form furfural or hydroxymethylfurfural. These degradation products will be re-polymerized into HLA [27]. Organic acids produced in the solution also neutralized the alkali [28]; therefore, the pH of the solution was reduced from 13.69–13.96 to 8.43–9.97.

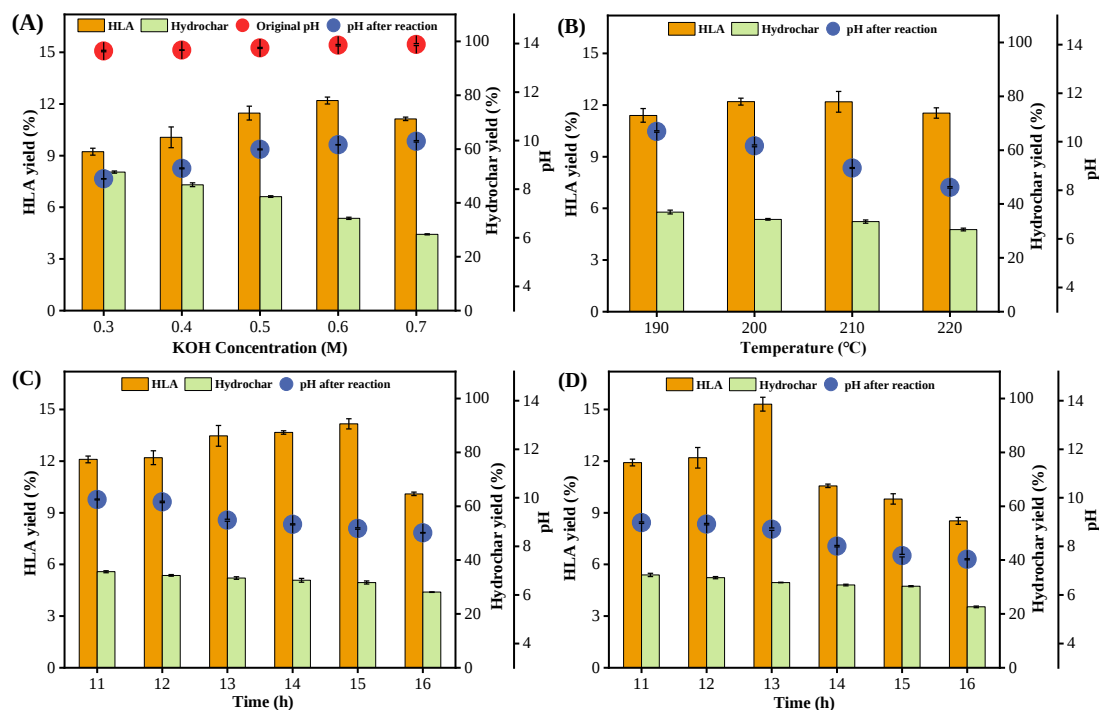


Figure 3. The yields of HLA and hydrochar obtained from the vinegar residue after the second-step hydrothermal pretreatment and the pH of the solution before and after the reaction: (A) 0.3–0.7 mol/L KOH, 12 h, 200 °C; (B) 0.6 mol/L KOH, 12 h, 190–220 °C; (C) 0.6 mol/L KOH, 11–16 h, 200 °C; (D) 0.6 mol/L KOH, 11–16 h, 210 °C.

The solid residue was hydrolyzed with 0.6 mol/L of KOH for 12 h at different temperatures (190, 200, 210, or 220 °C) (Figure 3B). The yield of HLA was 11.4% when the temperature was 190 °C. This indicated that only some of the polysaccharides and lignin were degraded and dispersed in the aqueous phase at 190 °C, while more remained insoluble in the solid phase [27]. The highest yields of 12.2% were obtained for HLA at both 200 and 210 °C. However, after further increasing the temperature to 220 °C, the yield of HLA decreased to 11.5%. This was because at 220 °C, dehydration and condensation between the generated HLA resulted in the formation of hydrochar, which reverted back to the solid phase [29].

As similar HLA yields were obtained when 0.6 mol/L of KOH was used to hydrolyze the solid residue for 12 h at 200 and 210 °C, the HP of the solid residue with 0.6 mol/L KOH at 200 and 210 °C were performed simultaneously for 11–16 h (Figure 3C,D). At 200 and 210 °C, the HLA yields tended to increase and then decrease with an increase in the hydrothermal time. This was because most of the cellulose, hemicellulose, and lignin had not yet been degraded when the HP time was insufficient, while excessive time would lead to excessive degradation of lignin to monophenols, which increased the difficulty in conversion to HLA [30]. At 210 °C for 13 h, the highest HLA yield of 15.3% was obtained. This indicated that a temperature of 210 °C, a KOH concentration of 0.6 mol/L, and a time of 13 h were optimal for the preparation of HLA. Furthermore, an appropriate increase in the HP temperature could reduce the time required to produce HLA. The increase

in temperature and time also promoted the production of organic acids. The pH of the solution after 13 h of HP at 210 °C was 0.37 lower than that at 200 °C but 1.24 higher than that of 16 h of HP at 210 °C.

The by-product hydrochar could be generated during alkaline HP. The existing insoluble lignin was directly isomerized into hydrochar, and the monosaccharides, furfural, hydroxymethylfurfural, and phenols dissolved in the aqueous phase also were re-polymerized to form hydrochar [31]. As shown in Figure 3, the increase in KOH concentration and temperature along with the extension of time led to a reduction in the hydrochar yield. For example, under the condition that resulted in the highest yield of HLA, the yield of hydrochar was 31.7%, which was 1.6% less than the yield of hydrochar produced under the conditions of 0.6 mol/L of KOH, 200 °C, and 13 h. This was because decarboxylation became stronger, resulting in the tendency to produce more gas- and liquid-phase products when the solid residue was incubated at a high temperature and in a highly alkaline environment for a long time [31,32].

3.4. Characterization of Humic-like Acid

SEM was used for characterizing the microscopic morphology of the CHA and HLA 210-11/13/15 obtained via two-step HPs of vinegar residue. Structural and morphological differences were observed between the CHA and HLAs. The surface of the CHA was relatively flat and consisted of irregularly shaped blocks put together in an orderly manner. The surface of the HLAs had many spherical bumps and a more compact structure than that of the CHA (Figure 4). The elemental distribution of the sample surface was obtained using EDS. C, O, Na, Al, and Cl were the major elements of the CHA (with O content being the highest), and some minor elements were Fe, K, S, and Mg. The HLAs had the highest content of C and the second highest content of O. The content of C in the HLAs increased with the extension of the alkaline hydrothermal pretreatment time, while the content of O showed the opposite trend. In addition, the reduction and removal of some trace elements such as Na, Fe, P, and Ca became evident upon observing the surface elemental composition of the different HLAs. According to the results of Yang et al. [27], the tulip tree was hydrolyzed with 0.5 mol/L of KOH at 200 °C for 24 h, and the collected supernatant was acidified with 6 mol/L of HCl to pH 3 to prepare HLA. The SEM revealed that the spherical bulges on the surface of the HLA were closely connected, which was similar to the surface morphology of the HLAs prepared in this study.

The atomic ratios of H/C and O/C were important indicators of the degree of aromatic condensation of humic acids [26], and changes in their ratios could reflect the degree of dehydration and decarboxylation reactions [16]. Table 2 shows the elemental analysis results for the CHA and HLAs. The H/C ratios of the three HLAs were similar: all were higher than 1.0 and higher than that of the CHA, which indicated the presence of an aromatic skeleton in the chemical structure of the HLAs [27] and that the aromatic contents were similar and all lower than that of CHA. The significantly lower O/C ratio of the HLAs compared to that of the CHA indicated that the decarboxylation reaction during the preparation of the HLAs was stronger than that of the CHA. The lower O/C ratios also indicated the lower content of oxygen-containing functional groups in the HLAs. A strengthening oxidant such as H₂O₂ could be added to the supernatant after the alkaline HP, or O₂ could be injected into the hydrothermal process so as to introduce carboxyl groups into the HLA and improve the O/C ratio [33]. Compared with CHA, HLAs have higher N content, indicating that HLAs have a higher electron transfer ability [14]. The decrease in S content indicated that the increase in HP time in Stage 2 weakened the hydrolysis reaction of sulfur-containing compounds in organic matter and that less S was stabilized in the HLAs [34].

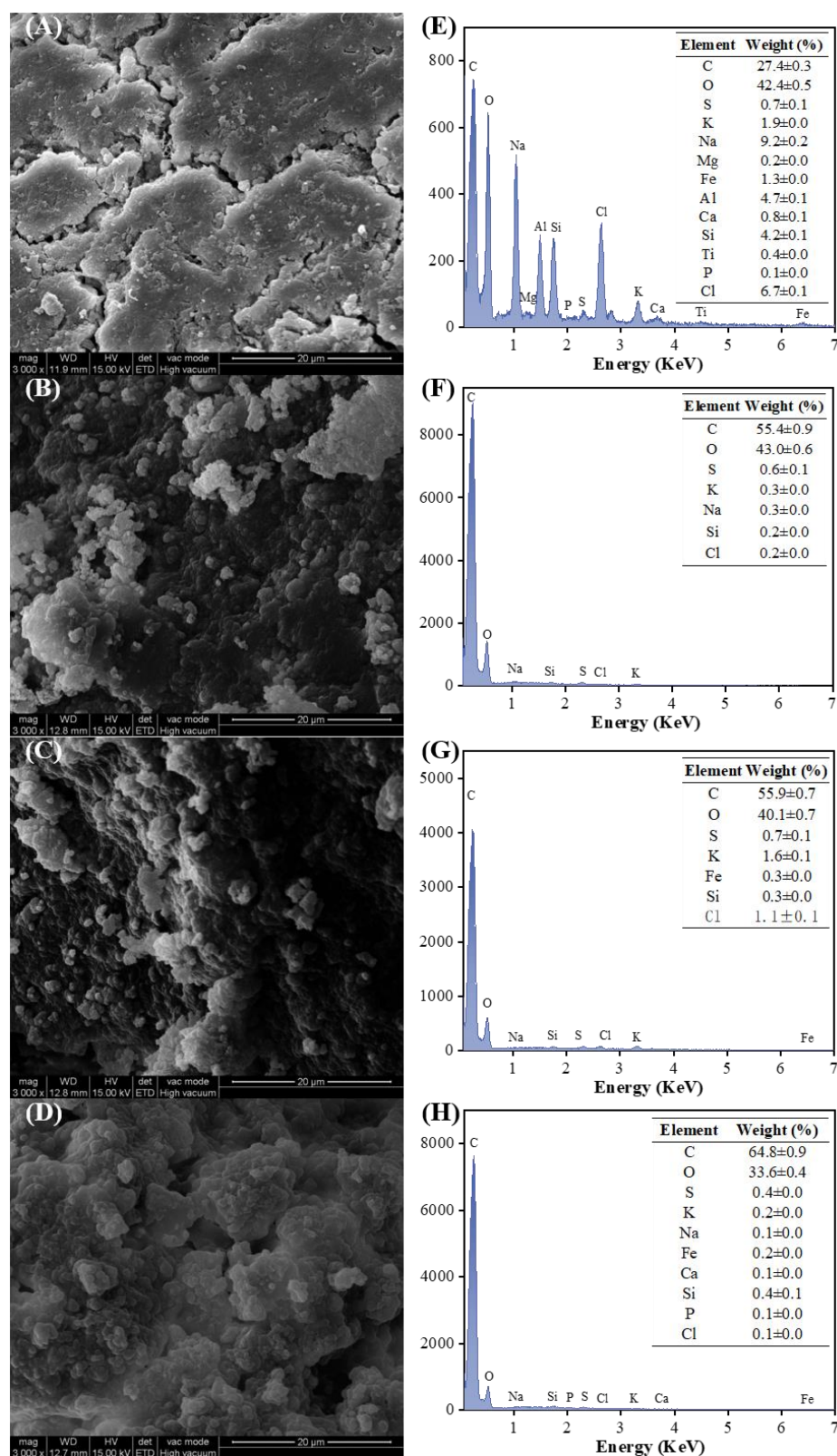


Figure 4. SEM images for CHA (A) and HLA 210-11/13/15 (B–D); EDS results for CHA (E) and HLA 210-11/13/15 (F–H). Abbreviations: CHA, commercial humic acid; HLA 210-11/13/15, humic-like acid (HLA) produced from the supernatant of the second-step hydrothermal pretreatment (0.6 mol/L of KOH at 210 °C for 11, 13, and 15 h, respectively) after acidification (using 1 mol/L of HCl to pH 2) and precipitation.

Table 2. Elemental analysis of CHA and HLA 210-11/13/15.

Projects	C (wt %)	H (wt %)	O (wt %)	N (wt %)	S (wt %)	O/C	H/C
CHA	41.8 ± 0.4	3.4 ± 0.2	24.9 ± 0.5	1.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	1.0 ± 0.1
HLA 210-11	70.7 ± 0.6	7.5 ± 0.2	18.8 ± 0.6	1.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	1.3 ± 0.1
HLA 210-13	71.20.7	7.6 ± 0.4	18.6 ± 0.2	1.5 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	1.3 ± 0.0
HLA 210-15	72.30.5	8.0 ± 0.3	17.2 ± 0.4	1.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.0	1.3 ± 0.1

Abbreviations: CHA, commercial humic acid; HLA 210-11/13/15, humic-like acid (HLA) produced from the supernatant of the second-step hydrothermal pretreatment (0.6 mol/L of KOH at 210 °C for 11, 13, and 15 h, respectively) after acidification (using 1 mol/L of HCl to pH 2) and precipitation.

The chemical functional groups in the CHA and HLAs were determined using FT-IR spectroscopy. The spectra of the three HLAs were similar, although they differed somewhat from that of the CHA (Figure S2). The transmission peaks of the HLAs and CHA at 3300–3500 cm^{−1} were attributed to the tensile vibration of the OH group of phenol, indicating that phenolic substances were involved in the synthesis of the HLAs and CHA. The absorption peak at 1510–1630 cm^{−1} caused by the C=C stretching vibration in the benzene ring also confirmed the presence of aromatic structures in the HLAs and CHA, although the content of aromatic structures in the HLAs was slightly lower than that in the CHA because the peak strength of the HLAs was slightly weaker than that of the CHA. The broad absorption peaks at 2800–3000 cm^{−1} and 1400–1480 cm^{−1} of the HLAs were attributed to the tensile and deformation vibrations of aliphatic –CH and –CH₂; however, the two peaks in the CHA were very weak, indicating that there were more aliphatic groups in the HLAs than in the CHA. The absorption peak of HLAs at 1690–1720 cm^{−1} was caused by the tensile vibration of C=O in the carboxyl group [35]. C–O stretching and –OH deformation vibration in the carboxyl group also caused the absorption peak of the HLAs at 1210–1240 cm^{−1}. Both the peaks for the HLAs were stronger than those for the CHA, indicating that the content of carboxylic acid in the HLAs was higher than that in the CHA. The HLAs showed a weak peak at 1010–1040 cm^{−1}, which indicated that the carbohydrate content in the HLAs was less than that in the CHA because this peak arose from the stretching vibration of C–O in carbohydrates [36].

To obtain more information regarding surface structure, the CHA and HLAs were analyzed using XPS (Table 3). According to the results of [26], the C 1s spectrum can be divided into three peaks after deconvolution, namely C1 (284 eV), C2 (286 eV), and C3 (288 eV). Most of the CHA and HLAs were in the C1 state, and the relative content of the HLAs even exceeded 50% (Figure S3). This was because of the presence of aromatic structures in the CHA and HLAs, which contributed to the high C1 content. In addition, the HLAs contained many aliphatic compounds, resulting in more C1 in the HLAs than in the CHA. The relative content of C2 and C3 in the HLAs was less than that in the CHA, and the relative content of C3 was half of that of the CHA. As C2 and C3 content mainly arise from carbohydrates [37], this indicated that the carbohydrate content of the HLAs was not as much as that in the CHA. The O/C ratios of the three HLAs were similar, and all were lower than that of the CHA. This may have been caused by the higher content of fatty acids and hydrocarbons in the HLAs than in the CHA as well as the lower content of carbohydrates and lignin. According to the results of Chundawat et al. [38], the O/C ratios of fatty acids and hydrocarbons were lower than those of carbohydrates and lignin.

The acidity of HA is mainly due to the presence of phenolic hydroxyl and carboxyl groups, and its TAG content was generally 1 mmol/g to more than 14 mmol/g [39]. Figure 5 shows the results of a TAG analysis (including phenolic hydroxyl and carboxyl) of the CHA and HLAs. The TAG content of the CHA was about 3.1 mmol/g, which was the lowest. The TAG content of the three HLAs differed by about 0.2 mmol/g, and the TAG content of HLA 210-13 was the highest (approximately 4.0 mmol/g). This may have been caused by the presence of a large number of carboxyl groups in the HLA. The TAG content in the HA correlated positively with its biological activity, which indicated that the HLAs possessed higher biological activity than the CHA.

Table 3. XPS analysis of CHA and HLA 210-11/13/15.

Projects	^a C1 (%)	^b C2 (%)	^c C3 (%)	O/C
CHA	39.0 ± 0.2	43.6 ± 0.6	17.4 ± 0.3	0.26 ± 0.1
HLA 210-11	57.1 ± 0.7	35.5 ± 0.6	7.4 ± 0.4	0.18 ± 0.1
HLA 210-13	52.0 ± 0.6	38.2 ± 0.4	9.8 ± 0.5	0.18 ± 0.0
HLA 210-15	51.4 ± 0.6	42.0 ± 0.5	6.6 ± 0.2	0.16 ± 0.1

Abbreviations: CHA, commercial humic acid; HLA 210-11/13/15, humic-like acid (HLA) produced from the supernatant of the second-step hydrothermal pretreatment (0.6 mol/L of KOH at 210 °C for 11, 13, and 15 h, respectively) after acidification (using 1 mol/L of HCl to pH2) and precipitation. ^a C1 corresponds to C-C or C-H; ^b C2 corresponds to C-O; ^c C3 corresponds to C=O or O-C-O.

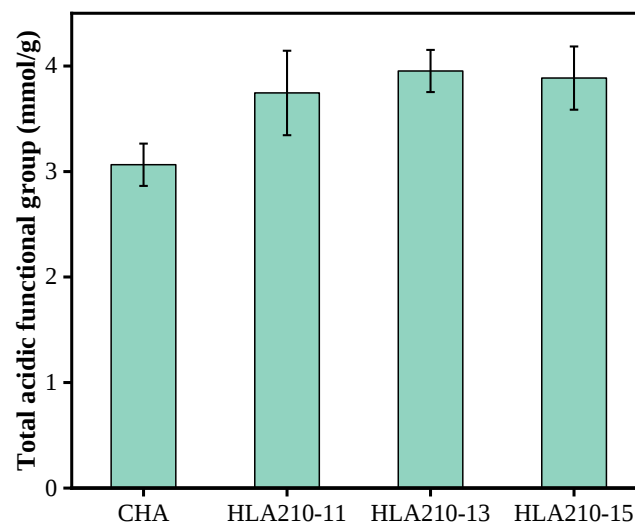


Figure 5. Total acidic functional groups (TAG) of CHA and HLA 210-11/13/15. Abbreviations: CHA, commercial humic acid; HLA 210-11/13/15, humic-like acid (HLA) produced from the supernatant of the second-step hydrothermal pretreatment (0.6 mol/L of KOH at 210 °C for 11, 13, and 15 h, respectively) after acidification (using 1 mol/L of HCl to pH 2) and precipitation.

3.5. Characterization of Hydrochar from the Second-Step Hydrothermal Pretreatment

The HHV is an important characteristic of biomass or biofuels [40]. The HHV of the vinegar residue and hydrochar obtained from Stage 2 were measured using a calorific value meter (Table 4). The HHV of the hydrochar was 2.5 MJ/kg lower than that of the vinegar residue. Analysis of their chemical composition revealed that the glucan content of the hydrochar was 17.7% higher than that of the vinegar residue, although the xylan and lignin content were 11.4% and 13.3% lower than that of the vinegar residue, respectively. According to the results of Matuana et al. [41], the HHVs of glucan, xylan, and lignin were about 17.4, 17.5, and 25.6 MJ/kg, respectively. Therefore, the massive loss in lignin and xylan caused the loss in the HHV of the hydrochar; in addition, it led to a reduction in its C, H, and N content and an increase in its O content.

Table 4. Higher heating value (HHV), elemental analysis, and chemical composition of vinegar residue and Hydrochar 210-13.

Item	Properties	Vinegar Residue	Hydrochar 210-13
Higher heating value	HHV (MJ/kg)	18.6 ± 0.8	16.1 ± 0.4
Elemental analysis	Carbon (wt %)	45.1 ± 0.4	41.2 ± 0.6
	Oxygen (wt %)	46.3 ± 0.5	52.3 ± 0.7
	Hydrogen (wt %)	6.6 ± 0.1	6.2 ± 0.3
	Nitrogen (wt %)	1.7 ± 0.1	0.3 ± 0.1

Table 4. Cont.

Item	Properties	Vinegar Residue	Hydrochar 210-13
Chemical composition	Glucan (wt %)	25.4 ± 0.4	43.1 ± 0.2
	Xylan (wt %)	18.8 ± 0.5	7.4 ± 0.5
	Lignin (wt %)	27.8 ± 0.1	14.5 ± 0.3

Abbreviations: Hydrochar 210-13, hydrochar prepared from the solid residue of the second-step hydrothermal pretreatment (0.6 mol/L of KOH at 210 °C for 13 h) after solid–liquid separation.

Hydrochar 210-13 corresponded to hydrochar prepared by using the solid residue of the first-step hydrothermal pretreatment at 170 °C as a raw material and the second-step hydrothermal pretreatment at 210 °C with 0.6 mol/L of KOH for 13 h. This experiment was performed in two replicates.

3.6. Mass Balance

The mass balance of the integrated process of co-producing XOS, HLA, and hydrochar from vinegar residue is shown in Figure 6: 1000 g of vinegar residue contained 254.4 g of glucan, 187.6 g of xylan, and 277.6 g of lignin. In total, 54.5 g of XOS (X₂–X₆) and 24.1 g of xylose were obtained after HP at 170 °C for 50 min. Approximately 67.9 g of XOS was released by hydrolyzing the XOS autohydrolysate with 1 IU/mL of endoxylanase for 8 h. After the first-step HP, 598.4 g of solid residue was recovered, including 219.0 g of glucan, 52.7 g of xylan, and 215.4 g of lignin. The solid residue was further pretreated with 0.6 mol/L of KOH at 210 °C for 13 h to obtain 189.5 g of hydrochar (including 81.7 g of glucan, 14.0 g of xylan, and 27.5 g of lignin). The collected supernatant was acidified with 1 mol/L of HCl to pH 2.0, and 91.6 g of HLA was obtained. It was obvious that the first-step HP of vinegar residue assisted by endoxylanase was beneficial to the production of XOS, and the generated solid residue was also efficiently utilized in the second-step HP assisted by KOH. Overall, the strategy of two-step HPs of vinegar residue to prepare XOS and HLA efficiently utilized its lignocellulosic components and provided a theoretical basis for the utilization of other biomasses rich in lignocellulosic components.

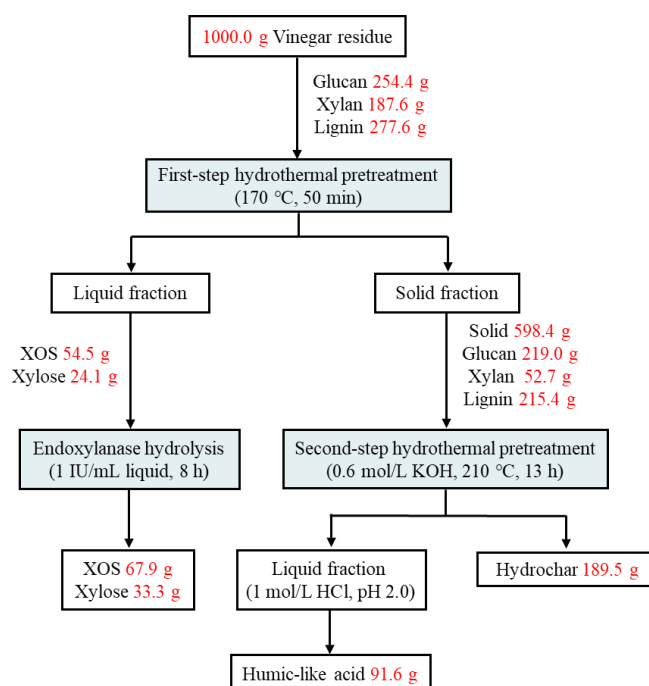


Figure 6. Mass balance of combined process of producing XOS, HLA, and hydrochar from vinegar residue.

4. Conclusions

This study demonstrated a two-step hydrothermal pretreatment (HP) process for the co-producing of XOS and HLA from vinegar residue. The endoxylanase-assisted first-step HP improved the XOS yield from 29.1% to 36.2% and the $(X_2-X_4)/XOS$ ratio from 0.8 to 1.0. The KOH-assisted second-step HP of the solid residue from the first-step HP was used for producing HLA. The optimal alkaline HP conditions were as follows: 0.6 mol/L of KOH, 210 °C, and 13 h. After alkaline HP, an HLA yield of 15.3% and a by-product hydrochar yield of 31.7% were obtained. Thus, this study presents a potential strategy for the valorization of vinegar residue.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/xxx/s1>, Figure S1: HPAEC chromatogram of (A) the XOS autohydrolysate from vinegar residue at 170 °C for 50 min and (B) the XOS obtained by endoxylanase hydrolysis autohydrolysate of vinegar residue at 170 °C; Figure S2: FT-IR spectra of CHA and HLA 210-11/13/15; Figure S3: High-resolution C 1s XPS spectra of CHA (A) and HLA 210-11/13/15 (B–D); Table S1: Organic acids composition analysis in the unwashed vinegar residue.

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