Liquid Hot Water Pretreatment of Wheat Straw for Full Carbohydrates Biorefinery

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Liquid hot water (LHW) and alkali-promoted LHW pretreatments of wheat straw were comparatively studied at temperatures from 100 °C to 180 °C to investigate their ethanol production and pentose recovery. An amount of 4.52 g/L ethanol was obtained by fermentation from the synergistic substrate treated with LHW under optimal temperature (140 ºC) and enzymatic hydrolysis (EH). Under these conditions, the recovery rate of pentose was 48.8% and 58.1% for xylose and arabinose, respectively. After the pretreatment and bioconversion processes, 20.3% cellulose, 10.5% xylan, and 19.5% lignin remained solid. The alkali promoter introduced into LHW enhanced the bioconversion efficiency of the substrate, which resulted in 5.82 g/L ethanol, and 57.5% xylose and 59.0% arabinose recovery, respectively. The results from this study contributed an effective manner for co-production of ethanol and pentose, enlarging the utilization efficiency of carbohydrates.

Keywords: Wheat straw; Liquid hot water; Bioconversion; Ethanol; Xylose

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INTRODUCTION

The search for sustainable energy is of great importance due to the current dwindling of nonrenewable energy resources and their unabated negative environmental impacts. The depletion of fossil resources has resuscitated the development biomass as a fuel source. The main components in biomass can be separated and converted into fuels, power, heat, and value-added chemicals by a biorefinery process (Negro *et al*. 2017). The main advantage of biorefinery processing is that the material is converted into multiple products, thus maximizing the value derived from the biomass feedstock.

Bioethanol from various lignocellulosic materials is considered a clean and renewable energy source. However, a major problem in the transition from petroleum-based feedstocks to biorenewable resources is the effective liberation of sugars from lignocellulosic materials and their fermentation to ethanol (Paulova *et al.* 2015). Lignocellulosic materials consist mainly of cellulose, hemicelluloses, and lignin. The lignin content, cellulose inaccessibility, and fibre strength are factors that contribute to the recalcitrance of lignocellulosic materials (Mosier *et al.* 2005). In achieving good yields of sugars and ethanol, the lignocellulosic biomaterials should be converted to a substrate that can be easily hydrolysed by commercial cellulolytic enzymes or enzyme-producing microorganisms (Agbor *et al.* 2011).

Pretreatment is one of the key steps of the conversion procedure in the production of cellulosic ethanol. Liquid hot water (LHW) pretreatment is an effective configuration

for removing lignin and hemicelluloses, as well as rendering cellulose accessible (Mussatto 2016). The use of water in the liquid state avoids the formation of fermentation inhibitors that occur at elevated temperatures (Yang and Wyman 2004). This also eliminates the need for a final washing or neutralization step. The low cost of water is also an advantage for large-scale application (Agbor *et al.* 2011). However, at high temperature, the hot water cleaves the acetyl linkages, thus liberating acids that may facilitate the degradation of hemicelluloses into inhibitors such as furfural and 5-hydroxymethyl furfural (Palmqvist and Hahn-Hägerdal 2000; Moiser *et al.* 2005). Kohlmann *et al.* (1995) stated that the subsequent degradation of products from carbohydrates can be minimized by maintaining the pH between 4 and 7. Under these conditions, alkali can be added to hot water to neutralize the acid produced during the LHW treatment. The acetyl and other uronic acid substitutions on hemicelluloses are also removed by alkali. In addition, the alkaline hot water causes the biomass to swell, disrupts the lignin structures, and breaks the linkages between lignin and other carbohydrate fractions, making the carbohydrates in the hetero-matrix more accessible (Chandra *et al.* 2007).

Wheat straw is regarded as a waste material with wide availability and a low price. Also, it does not compete with food. In this study, the effects of the LHW and alkali-catalyzed LHW on the bioconversion of wheat straw were comparatively studied. Enzymatic hydrolysis and fermentation were conducted semi-simultaneously under each optimal condition. The microorganism (yeast) used for ethanol production in this study preferred hexose over pentose. Thus, glucose in the hydrolysate of EH was fermented to ethanol, and the pentose (xylose and arabinose) was released for further utilization. The conversion of pentose into furfural will be conducted in further research. The present work envisions two concurrent channels for hexose and pentose utilization, thus yielding maximum efficiency and matching the concept of biorefinery.

EXPERIMENTAL

Materials

Wheat straw was obtained from the Hebei Province, China and ground to 0.3 mm to 0.45 mm. The powder obtained was dewaxed with ethanol/toluene (1:2, v/v) for 8 h. The main chemical components of the dewaxed wheat straw (DWS) were identified as 41.8% cellulose, 25.5% xylan, and 22.1% lignin (including 20.2% acid insoluble lignin and 1.9% acid soluble lignin). The standard deviation was < 2%.

Pretreatment

The pretreatments were performed in an autoclave. One gram of sample was immersed in 20 mL water and 20 mL 0.2% NaOH aqueous solution, respectively. The atutoclaves were held in an oil batch at 100 °C, 120 °C, 140 °C, 160 °C, and 180 °C, respectively, and the residence time was fixed at 2 h. At the end of each incubation, the autoclave was air-cooled to room temperature. The whole pretreatment slurry was subjected to enzymatic hydrolysis (EH) and further fermentation. All the experiments were performed in duplicate, and average values and corresponding derivations are illustrated in results.

Enzymatic hydrolysis and fermentation

After pretreatment, the whole slurry was cooled to room temperature and transferred to a 50 mL flask. The slurry with alkali-promoted LHW was adjusted to pH 5.5 with an acetic acid. Digestibility was determined with enzymatic cocktails of 20 FPU (filter paper unit) per g of glucan plus 30 IU xylanase per g of xylan (kindly supplied by Shanghai Youtell Biochemical Co., Ltd., Shanghai, China), based on the chemical composition of the dewaxed material. The EH was performed at 50 °C in an air bath shaking incubator at 150 rpm for 24 h. The hydrolysates were sampled periodically and analyzed by a high-performance anion exchange chromatography (HPAEC) system (Dionex, ICS 3000, Sunnyvale, CA, USA), as described by Yang *et al*. (2011).

The experimental scheme is shown in Fig. 1.

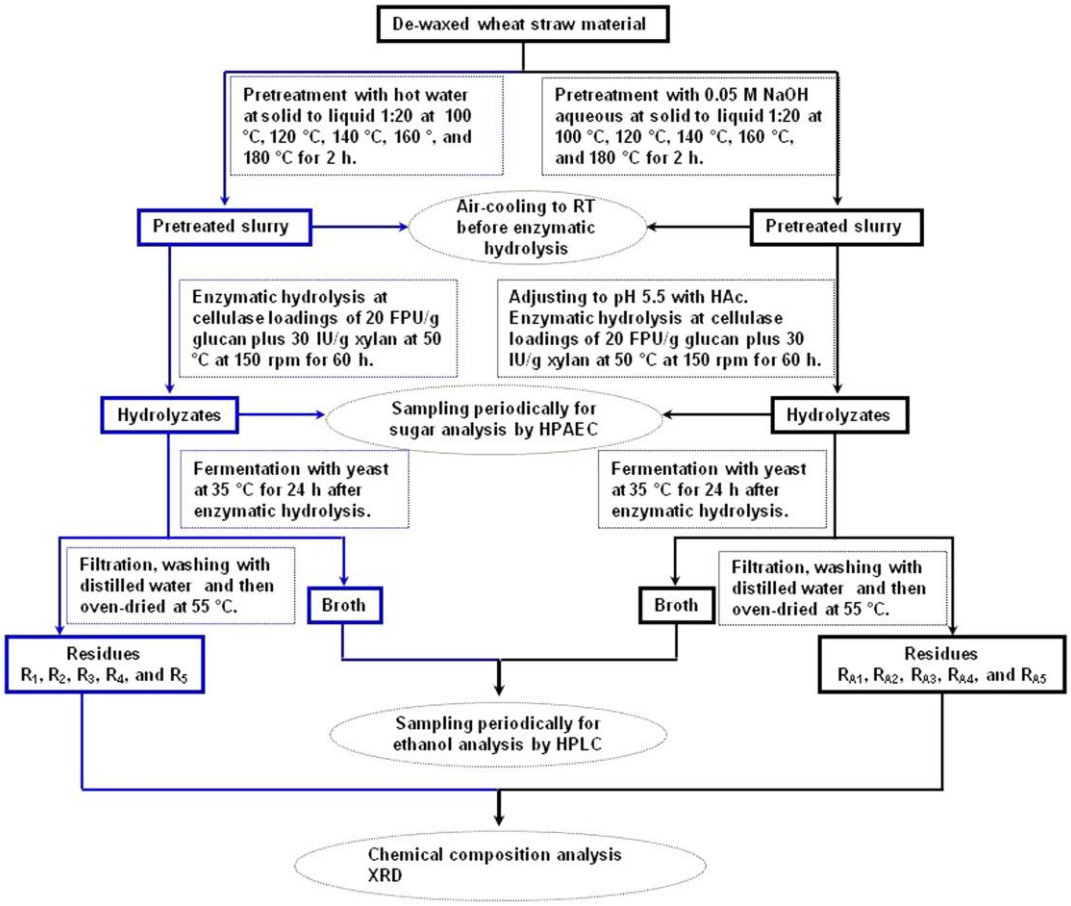

Fig. 1. Schematic of experimental methods

Before fermentation, the yeast (*Saccharomyces cerevisiae*, kindly supplied by Shanghai Youtell Biochemical Co., Ltd., Shanghai, China) was cultivated at a concentration of 30 g/L in 2% glucose aqueous solution containing 10 g/L yeast extract and 20 g/L peptone at 35 °C for 1.5 h. At the end of EH, 1.5 mL of the yeast culture was added to each flask for fermentation at 35 °C. The broth was sampled periodically to measure the concentration of ethanol with an HPLC system (Agilent 1200 series, Agilent Technologies, Palo Alto, CA, USA) equipped with a refractive index detector. The separation was performed on an Aminex column HPX-87H ion exclusion column (300

mm × 7.8 mm, Bio-Rad Laboratories, Hercules, CA, USA) (Yang *et al.* 2011). All of the operations were performed in triplicate, and the average values and corresponding derivations were given. After fermentation, the residues were collected by filtration, thoroughly washed, and then oven-dried at 55 °C. Residues from the synergistic treatments of LHW and bioconversion process were labeled as R_1, R_2, R_3, R_4, and R_5, respectively. The remaining substrates after alkaline LHW pretreatment, EH, and fermentation were labeled as R_{A1}, R_{A2}, R_{A3}, R_{A4}, and R_{A5}, respectively. All the fermentation processes were performed in duplicate, and average values and corresponding derivations are illustrated in results.

Methods

Chemical composition

The contents of carbohydrates and lignin in residues were calculated based on the weight of the starting materials (1 g). The measurement for carbohydrates and lignin detection was conducted according to the Klason method (KCL 1982; Dence 1992). The analysis of hydrolysates was conducted according to the method used by Yang *et al.* (2011).

X-ray powder diffraction

X-ray powder diffraction patterns of the residues were obtained using an XRD-6000 instrument (Shimadzu, Kyoto, Japan) with a Ni-filtered Cu Kα radiation (λ = 1.54 Å) at 40 kV and 30 mA. The X-ray diffractograms were recorded with the reflection method at a scanning speed of 2°/min in the diffraction angle 2θ, with a range of 5° to 35°. The crystallinity index (%) was determined according to the method described by Segal *et al.* (1959), using Eq. 1,

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

where I_{002} is the intensity of maximum diffraction of the crystalline region at 2θ = 22.5°, and I_{am} is the intensity of diffraction attributed to the amorphous region at approximately 2θ = 18°.

RESULTS AND DISCUSSION

The composition of the feedstock and its transformation after the pretreatment are some of the critical parameters for measuring the bioconversion efficiency. Figure 2 shows the recovery of cellulose, xylan, acid-soluble lignin, and acid-insoluble lignin of the residues. The decrease of xylan in the residues indicated that the dissolving of hemicellulose fractions occurred during LHW pretreatment, which was confirmed by the increased hemicellulosic content of the hydrolysates (Fig. 3). The undetectable amount of arabinan in the residues and hydrolysates indicated that the arabinose was further degraded. Yu *et al.* (2015) reported that arabinose was less stable than xylose under LHW conditions. However, the effect of temperature on the content of residual lignin was negligible. This finding was consistent with early literature, in indicating that lignin was more resistant to the pretreatment process than carbohydrates. The deconstruction and recondensation of the associated lignin during LHW treatment led to the rearrangement

of lignin on the surface of the lignocellulosic feedstock (Obama *et al.* 2012). The lignin may have acted as an obstacle for the adsorption of enzymes onto the carbohydrates. As pretreatment temperature increased, the carbohydrates further degraded, and the remaining content in the residues decreased. Thus, the ratio of lignin to carbohydrates increased with the pretreatment temperature.

In lignocellulosic materials, cellulose is considered a crystalline component, whereas hemicellulosic and lignin fractions are considered amorphous. The crystallinity degrees of the residues are shown in Table 1. The crystalline indexes (CrI) of the residues increased with the pretreatment temperature. This phenomenon was attributed to the removal of hemicelluloses and re-localization of lignin (Xiao *et al.* 2011; Obama *et al.* 2012).

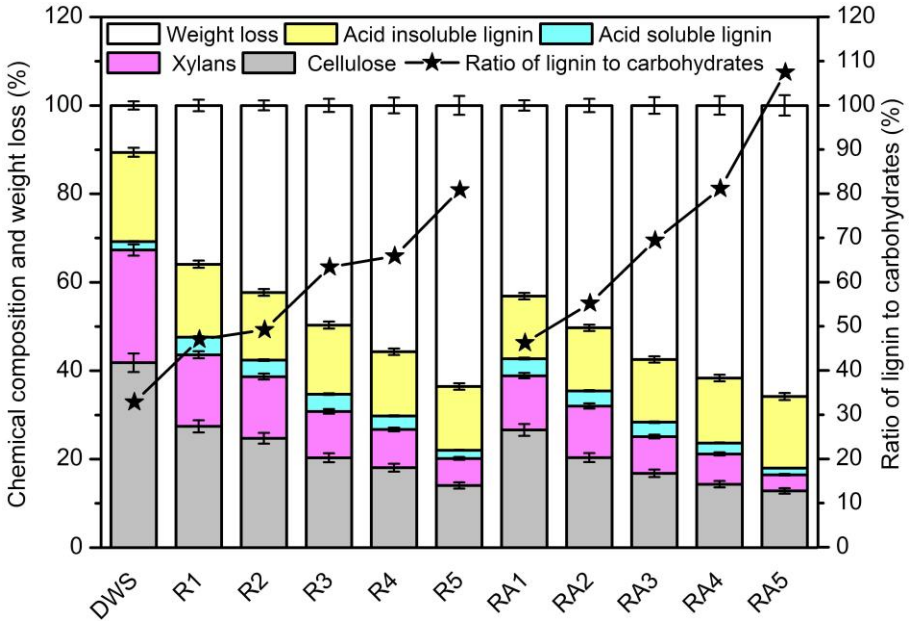

Fig. 2. Chemical composition of residues and ratio of remaining lignin to carbohydrates

The decrement of CrI was observed in samples R5 and RA5, which were mainly caused by the degradation of crystalline cellulose. Compared to the residues from the LHW pretreatment, the cellulosic fractions obtained from the alkali catalyzed LHW pretreatment had a little higher CrI values. The partial dissolution of the amorphous cellulosic fraction may have contributed to this result. In addition, the relatively higher content of cellulose in the residue was also associated with higher CrI of the residues (Fig. 2). The increase in CrI was in agreement with the results reported by Barman *et al.* (2012), in which the CrI of wheat straw increased from 53.3% to 60.3% after 1.5% NaOH pretreatment (Barman *et al.* 2012)

Table 1. The Crystalline Index (CrI) of Residues Obtained from Enzymatic Hydrolysis and Yeast Fermentation

	DWS[a]	R_1[b]	R_2	R_3	R_4	R_5	R_{A1}	R_{A2}	R_{A3}	R_{A4}	R_{A5}
CrI (%)	59.0	56.7	58.6	60.4	62.3	57.4	56.6	59.3	60.3	63.1	58.8

[a] Dewaxed wheat straw was labeled as DWS); [b] Related to Fig. 1

Effect of Pretreatment on the Bioconversion of Wheat Straw

The LHW pretreatment was expected to disrupt the carbohydrate-lignin complex, allowing the hydrolytic enzymes to gain access to the carbohydrates. The effect of LHW on the bioconversion of wheat straw was investigated by EH and yeast fermentation. Xylanase was supplemented to boost cellulose saccharification. The digestion of hemicelluloses may have enlarged the contact area between cellulose and the enzyme. In addition, the inhibitory effect was also reduced as the xylan and xylan-oligomers degraded to xylose (Qing and Wyman 2011). The efficiency of the LHW pretreatment on the bio-digestion of wheat straw is shown in Fig. 3.

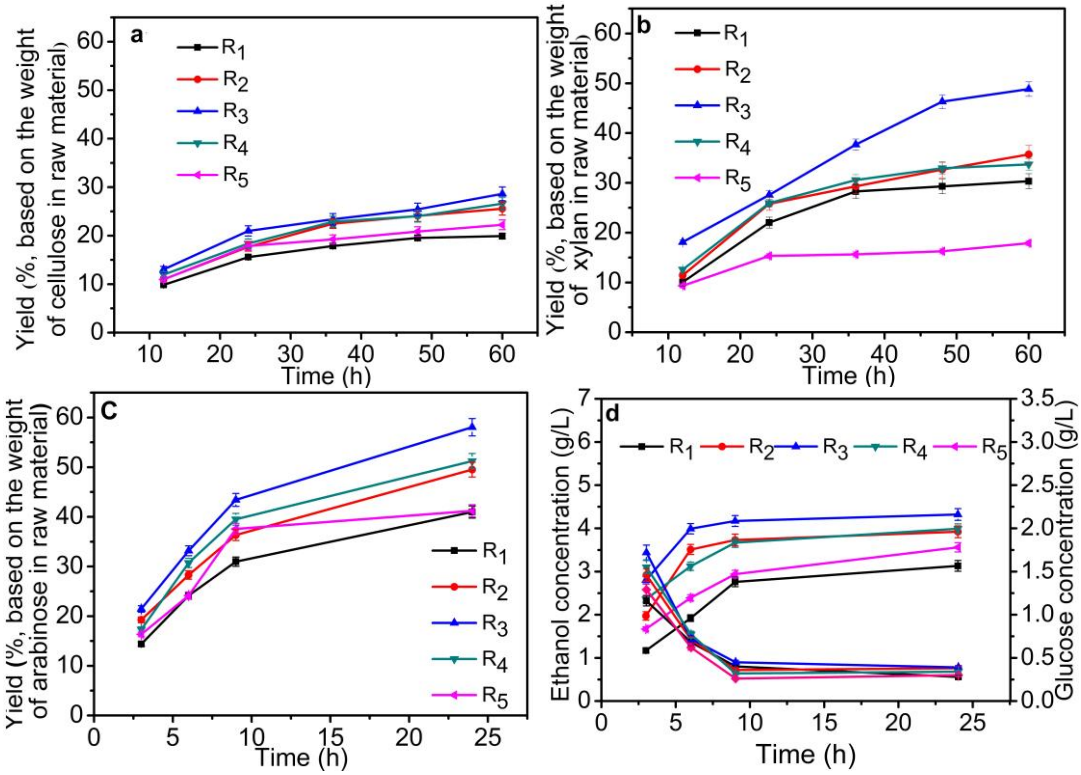

Fig. 3. Bioconversion of wheat straw with synergism of LHW treatment, enzymatic hydrolysis, and fermentation: a) glucose; b) xylose; c) arabinose; and d) ethanol

The results of EH indicated that 140 ºC was the most favorable temperature for the LHW pretreatment. After pretreatment and enzymatic hydrolysis, the yields of glucose, xylose, and arabinose were 28.6% (5.98 g/L), 48.8% (5.23 g/L), and 58.1% (1.19 g/L), respectively. The glucose in the broth was further fermented to ethanol by yeast. The concentration of glucose decreased with the accumulation of ethanol. At the end of fermentation, the maximum ethanol concentration was yielded as 4.52 g/L, with about 0.28 g/L -0.39 g/L glucose remained in the broth. However, as the pretreatment temperature was further gradually increased to 180 °C, a decreased ethanol yield was obtained from the EH and fermentation of the cellulosic residues. This phenomenon is probably related to the level of inhibitors, such as acetic acids, furfural, and 5-HMF, in the pretreatment slurry, which negatively affect the activity of the enzymes (Michelin and Teixeira 2016). Imman *et al.* (2014) suggested that the concentration of furans increased

markedly as the temperature increased from 140 °C to 160 °C. Moreover, the degradation products from hemicelluloses and lignin in the LHW pretreatment slurry could form pellets and attach to the surface of the feedstock. These pellets were steric hindrances for the accessibility of enzymes (Wang *et al.* 2015; Yu *et al*. 2015). In addition, as temperature increased, the ratio of lignin to carbohydrates in the residues increased. The lignin from the LHW pretreatment was found to limit sugar release during EH by the non-effective adsorption of enzymes (Sun *et al.* 2016). The high lignin content in solid form could contribute to the low bioconversion of substrate.

In contrast to the LHW's absence of a promoter, alkali was found to be efficient for enhancing the yield of glucose, xylose, and arabinose *via* EH with concomitant improvement of ethanol production from fermentation (Figs. 4 and 5). With the synergistic treatments of the alkali promoted LHW treatment and EH, the yields of glucose, xylose, and arabinose were 35.8% (7.47 g/L), 57.5% (6.15 g/L), and 59.0% (1.21 g/L), respectively. The subsequent fermentation yielded 5.82 g/L ethanol with around 0.63 g/L glucose in the broth. At the end of fermentation, the residual glucose was 0.44 g/L -0.63 g/L. This phenomenon was attributed to the fragmentation and dissolution of lignin and hemicelluloses in the aqueous alkaline, which was in accordance with the low remaining xylose and lignin content in the residues from the synergism of the alkali-catalyst LHW treatment and EH (Fig. 2). Imman *et al.* (2015) also reported that the addition of alkali to LHW led to a higher total sugar yield from EH as compared to the respective LHW pretreatment. The discrepancy of arabinose release during EH of substrates from LHW and alkaline-LHW was negligible and relative to the degradation of arabinose during pretreatments.

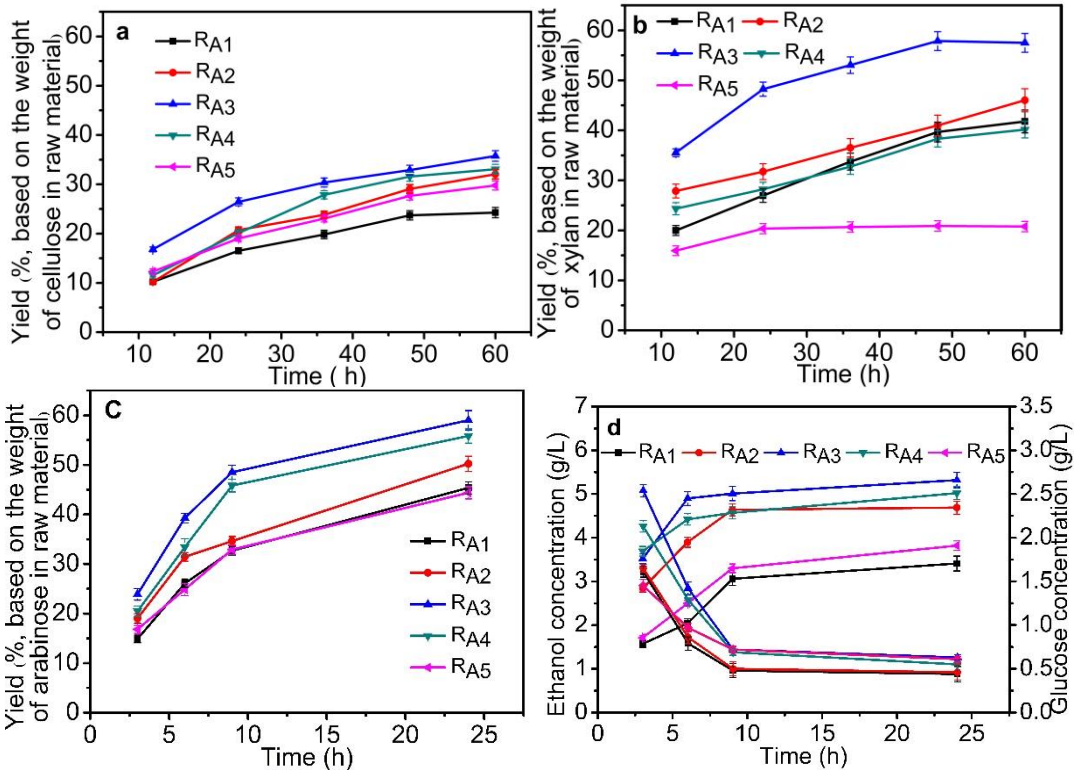

Fig. 4. Bioconversion of wheat straw with synergism of alkali promoted LHW treatment, enzymatic hydrolysis, and fermentation: a) glucose; b) xylose; c) arabinose; and d) ethanol

The effects of LHW and alkali-promoted LHW at different temperatures were comparably studied, and the results are shown in Fig. 5. For all of the temperatures examined, alkali had no obvious effect on the yield of arabinose due to the instablility of arabinose. The beneficial effect of alkali on the yield of xylose was found to decrease with increased treatment temperature, which was consistant with the results reported by Imman *et al.* (2014). This result might be attributed to the fact that alkali neutralized the H+ organized from the ionization of water and performed deacetylation (Li *et al.* 2014). These reactions resulted in a decrease of catalysts for the hemicelluloses solution during alkali-LHW. The enhancement of the glucose yield during EH and ethanol *via* fermentation exhibited a similar trend. However, the effect of alkali on ethanol yield decreased as the pretreatment temperature increased to 180 °C. It was speculated that a considerable amount of carbohydrates and lignin was degraded at high temperatures that corresponded to a high level of inhibitors for microorganism in the slurry.

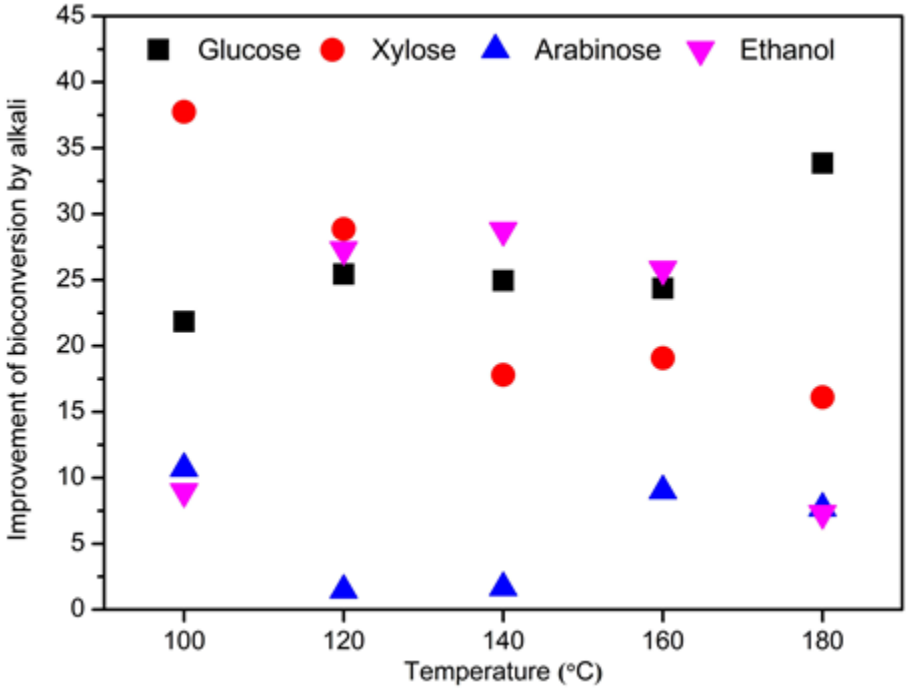

Fig. 5. The enhancement of bioconversion of wheat straw with alkali-promoted LHW as compared to LHW

CONCLUSIONS

1. Optimal temperature for LHW pretreatment of wheat straw was 140 °C, which resulted in a maximum bioconversion of de-waxed wheat straw by EH and yeast fermentation.
2. The 0.2% NaOH aqueous solution improved the efficiency of the LHW pretreatment. The maximum enhancement of alkali for ethanol production, xylose, and arabinose recovery were 28.8%, 37.8%, and 9.0%, respectively.

3. Alkali-promoted LHW was more suitable than LHW for the improvement of wheat straw bioconversion.

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