Effect of Low Temperatures and Residence Times of Pretreatment on Glucan Reactivity of Sodium Hydroxide-Pretreated Rice Straw

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Received: 24 March 2017, Revised: 30 October 2017, Accepted: 26 November 2017

Abstract

Alkaline pretreatment of lignocellulosic biomass is an approach to enhance the susceptibility of the biomass that is subsequently converted into fermentable sugars. The efficacy of the sodium hydroxide pretreatment of rice straw RD41 was evaluated in terms of total solid removal, lignin removal, glucan recovery, and glucan conversion yields. The pretreatment conditions were 50, 60, 70, 80, and 100 °C, and each temperature kept for 1 to 5 h. The effect of pretreatment temperatures was more pronounced than that of the pretreatment times. The elevated temperatures caused higher total solid removal and lignin removal. The highest total solid removal (52.5 to 55.8 %) was found in the pretreatment at 100 °C. At this temperature, the highest lignin removal (~87 %) could be obtained regardless of the residence times of the pretreatment. Most of the glucan (~80 to 100 %) was preserved in the pretreated rice straw. Lower temperatures (50 and 60 °C) favored higher glucan preservation (> 90 %) in the pretreated solids. Glucan conversion of the 3 h pretreatment time samples of each pretreatment temperature revealed that more than 80 % of glucan conversion could be accounted for in samples pretreated at 70 to 100 °C within 24 h of saccharification. The lower temperatures required a prolonged pretreatment time to reach a higher glucan conversion (~90 %), as found in the 50 °C, 5 h pretreated rice straw. The optimal conditions of this simple method are economically feasible, and can be applied to testing the reactivity of herbaceous lignocellulose in future research.

Keywords: Rice straw, lignocellulosic material, sodium hydroxide pretreatment, glucan conversion, glucan reactivity

Introduction

Rice is a major crop in Thailand, and is cultivated throughout the country [1]. As a result, an abundant amount of straw is left as an agricultural waste in fields after harvest. Most of the straw is burnt and is integrated into the soil to prepare the field for cultivation in the next crop cycle [2]. However, straw combustion generates air pollution by emitting carbon dioxide, carbon monoxide, and methane [2]. Utilization of rice straw as a raw material for the production of valuable biobased chemicals is a promising approach to solve the above problem. These biobased chemicals, such as sugars, can be used as intermediates for the production of other valuable chemicals by chemical and biological processes.

Rice straw is a lignocellulosic material composed of 3 major constituents: cellulose (or glucan) (35 - 40 %, w/w), hemicellulose (25 - 30 %, w/w), and lignin (10 - 15 %, w/w) [3]. The feasibility of fermentable sugar production of lignocellulosic material has been studied intensively. Lignocellulose is a non-food feedstock that is abundant and sustainable. Most lignocellulose is derived from agricultural wastes. The scope of the research in this area involved biomass pretreatment to increase enzyme
accessibility for sugar production, optimal conditions for enzymatic saccharification, and fermentation, to produce ethanol. Agricultural residues that have been investigated include wheat straw [4-8], corn stover [9-12], sorghum straw [13], barley straw [14], and rice straw [15-18].

Although lignocellulosic materials are suitable to serve as raw materials for the production of fermentable sugars, the recalcitrance of plant cell walls impedes the conversion of carbohydrate polymers into monosaccharides [19]. Lignin and hemicelluloses are the major barriers to cellulase accessibility. Cellulases catalyze the hydrolysis of cellulose to produce glucose [20]. Thus, pretreatment is an essential method to reduce the recalcitrance of plant cell walls. Pretreatments help break up the structure of biomass, remove lignin and hemicelluloses, and decrease cellulose crystallinity. All these make the biomass more susceptible to the hydrolytic enzymes used in a bioconversion process [21].

There are several applicable pretreatment methods, including physical, chemical, and physicochemical pretreatments [19]. In terms of alkaline pretreatment, the advantages include delignification and removal of hemicelluloses. In addition, cellulose becomes more amorphous, due to the deconstruction of hydrogen bonding among cellulose microfibrils, leading to the swelling of biomass; thus, the inner surface increases. Furthermore, most of the cellulose is retained in the solid phase, and can be hydrolyzed in the enzymatic saccharification procedure. Unlike acid pretreatment, alkaline pretreatment can be conducted at low temperatures, such as room temperature, and at low pressure [10,19]. Several kinds of alkali have been investigated for pretreatment of lignocellulosic biomass, including alkali with hydroxide ions, e.g., sodium, calcium, ammonium, and potassium [4,22,23]. Among these, sodium hydroxide has been studied the most for its efficacy in pretreatment, since it is completely water-soluble, and the effectiveness of sugar yields following the pretreatment is satisfied [19,21]. In addition, sodium hydroxide pretreatment is suitable for herbaceous crops and agricultural residues, because these types of biomass contain low lignin content compared to softwood [24,25].

Up to now, no research has attempted to study the conversion of Thai rice straw into fermentable sugars in terms of accessing the reactivity of glucan resulting from low-temperature pretreatments. Most studies have aimed to obtain maximum yields of fermentable sugars or ethanol production instead [26]. The simplicity of the pretreatment method proposed in this study makes application in testing the reactivity of lignocellulosic biomass feasible. This study follows the concept of biobased economy, described by FitzPatrick et al. [27], in order to utilize agricultural waste, i.e., rice straw, and to produce valuable by-products (fermentable sugars) from the waste. The aim of this research is to study the efficacy of sodium hydroxide pretreatment of the rice straw in terms of accessing the reactivity of glucan of the biomass pretreated at various conditions, with a function of residence times at low pretreatment temperatures.

Materials and methods

Feedstock preparation
Rice straw (Oryza sativa RD41) was harvested at a local farm in Nakhon Pathom province, Thailand. Mature above-ground biomass was obtained after removing the reproductive part and air-dried before cutting and milling. The biomass was cut to approximately 2.5 cm and milled using a blender. The milled straw was sieved to pass a 20-mesh screen. The feedstock sample was stored in an air-tight jar with a screw cap and kept at room temperature before use.

Reagents
All chemicals used in this study were reagent grades purchased from Sigma-Aldrich (Sigma Chemical Co., USA), UNIVAR (Ajax Finechem, Australia), LABCHEM (Ajax Finechem, Australia), and EMSURE (Merck Millipore, Germany).

Chemical composition of rice straw
All analytical methods for determination of rice straw composition were done following the Laboratory Analytical Procedures (LAP) prepared by National Renewable Energy Laboratory (NREL, Golden, Colorado, USA). Moisture and ash content of the native straw were determined by following the
methods described by Sluiter et al. [28] and Sluiter et al. [29], respectively. Determination of structural carbohydrates and lignin was performed with extractive-free biomass. Extractive-free rice straw was prepared by exhaust extraction of rice straw with distilled water for 24 h, followed by exhaust extraction with 95% ethanol for 24 h using a Soxhlet apparatus [30]. Water and ethanol extracts were dried using a rotary evaporator, followed by drying in a vacuum oven to obtain the amounts of extractive. The extractive-free biomass was air-dried before being subjected to 2-step acid hydrolysis for determination of structural carbohydrates and lignin [31]. Neutralized hydrolyzate was subjected to sugar analysis by HPLC method. Acid-insoluble lignin was measured by measuring the absorbance at 320 nm with a spectrophotometer. Acid-insoluble lignin was determined by a gravimetric method. All experiments were done in triplicate.

**Sodium hydroxide pretreatment**

Sodium hydroxide pretreatment was carried out to primarily evaluate the efficiency of pretreatment conditions. Since the majority of plant cell wall constituents that are removed by sodium hydroxide pretreatment are lignin and other substances besides cellulose, total solid removal, lignin removal, and cellulose recovery were used to primarily evaluate the efficiency of the pretreatment conditions in this study.

To investigate the effect of residence times and temperatures on total solid removal, lignin removal, and glucan recovery, 5% (w/v) sodium hydroxide (NaOH) was used to pretreat the rice straw at various conditions, i.e., 50, 60, 70, 80, and 100°C each, for 1, 2, 3, 4, and 5 h, respectively. This pretreatment procedure was adapted from Sophonputtanaphoca [8]. Pretreatment in each condition was carried out in a 20-mL scintillation vial with a plastic-lining screw cap. Approximately 0.3 g dry weight rice straw was added into the scintillation vial, and 10 mL of 5% NaOH was added before closing the screw cap. All samples were then incubated in water baths at predetermined temperatures and residence times, as described above. After the time elapsed, the individual pretreated slurry was washed to neutral and filtered using a gouch crucible. Washing was performed by 25 mL distilled water, followed by 10 mL 0.01 mM HCl, and then rinsed with 25 mL distilled water. Pretreated rice straw was dried in a vacuum oven at 40 °C to obtain a constant weight. Total solid recovery and total solid removal of all pretreatment conditions were calculated. The dried pretreated solids were stored in desiccators containing a desiccant for further compositional analysis (glucan content and lignin content), as described by Sluiter et al. [31]. Neutralized hydrolyzate was filtered through a 0.45-µm syringe filter and kept frozen prior to being subjected to a sugar analysis by glucose oxidase/peroxidase assay, as described below. All experiments were done in triplicate.

**Fermentable sugar production**

A selected residence time for each pretreatment temperature that gave high total solid removal, high lignin removal, and high glucan recovery was chosen for determination of optimal conditions for fermentable sugar production. Washed pretreated solid (without further drying) of each selected pretreatment condition was used for enzymatic hydrolysis in the fermentable sugar production process.

The enzymatic hydrolysis of the pretreated biomass was carried out by the method described by Sophonputtanaphoca [8]. Washed pretreated solid of the selected pretreatment condition was added into a 20-mL scintillation vial with a plastic-lining screw cap. A 5-mL aliquot of 0.1 M sodium citrate buffer, pH 4.8, was added into the vial, followed by the addition of 100 µL 2% (w/v) sodium azide as an antimicrobial reagent. Distilled water was then added into the vial to bring the total weight of the mixture to approximately 10 g. The actual weight of the mixture, weighing to the nearest 0.1 mg, was recorded. Enzymatic hydrolysis was initiated by adding a cellulase preparation (Accellerase 1500, Genencor, USA) in the ratio of 30 FPU/g glucan and adding a xylanase preparation (Accellerase XY, Genencor, USA) in the ratio of 2,750 U/g glucan. For zero-hour samples, denatured enzymes were prepared by boiling both cellulase and xylanase preparations in the buffer at 100 °C for 5 min. All reaction mixtures, except for the zero-hour samples, were placed on a rotator residing in an incubator at 50 °C for 24 h. The sample collections were performed at 0 and 24 h. Approximately 1-mL mixture was taken and filtered through a 0.45-µm syringe filter. All filtrates were collected and stored in microcentrifuge tubes before being kept...
in a freezer (−20 °C) for further sugar analysis to determine glucan conversion. All experiments were done in triplicate.

**Sugar analysis**

Glucose analysis by glucose oxidase/peroxidase (GOPOD) assay was adapted from Sophonputtanaphoca [8] and was employed to determine glucan content in the pretreated rice straw and glucan conversion after enzymatic hydrolysis of the pretreated rice straw. A 20-µL filtrate was put in a 96-well microplate, followed by adding 200-µL GOPOD reagent (GOPOD assay kit, Megazyme, Ireland). Glucose solutions with the concentrations of 0.1 mg/mL to 0.8 mg/mL were used as standard. The reaction mixture was then incubated in a microplate reader at 40 °C for 20 min. Absorbance of each sample was measured using a microplate reader at a wavelength of 510 nm. The amount of glucose detected in the 24-h sample was subtracted by the glucose content presented in the zero-hour sample. To convert glucose into its polymeric form (cellulose or glucan), a correction factor of 0.9 was applied. Glucan conversion was calculated by:

\[
\text{%Glucan conversion} = \frac{\text{Digested glucan, g}}{\text{Added glucan, g}} \times 100
\]  

(1)

where “digested glucan” refers to the amount of glucan that was converted into glucose, and “added glucan” refers to the amount of glucan preserved in the pretreated solid that was initially added to the reaction mixture for enzymatic hydrolysis.

All experiments were done in triplicate.

**HPLC analysis**

The hydrolyzate samples obtained from the determination of structural carbohydrates in the extractives-free biomass were filtered through 0.2-µm syringe filters prior to HPLC analysis. An Alltech HPLC system was equipped with an evaporative light scattering detector (ELSD) (Alltech, Model 200ES), a column heater (Alltech, Model 630), and a pump (Alltech, Model 626). A Rezex RPM-Monosaccharide Pb²⁺ column (300×7.8 mm, Phenomenex, USA) equipped with a guard column (Phenomenex, USA) was used to separate the monosaccharides in the samples. The separation conditions were as follows: 50 µL sample injection, HPLC-grade water as the mobile phase, 0.6 mL/min flow rate, 60 °C column temperature, and 25 to 30 min retention time.

**Statistical analysis**

Statistical analysis of the data, including averages and standard deviations (SD), were performed using Excel (Microsoft; Redmond, WA). One-way ANOVA with least significant difference (LSD) (with 95 % confidence), performed by SPSS version 11.5, was rendered to compare differences of the data.

**Results and discussion**

**Chemical composition of rice straw**

The chemical composition of the rice straw (RD41) is presented in Table 1. The moisture content in the rice straw sample was 6.68 %. Moisture content below 10 % is critical for compositional analysis used in this study. Higher moisture content in biomass samples will alter the acid concentration in the hydrolysis steps. This will result in a low bias in carbohydrate content, due to incomplete hydrolysis of polymeric sugars to monomeric sugars [32]. Likewise, high moisture content of biomass samples can affect the pretreatment by diluting the alkaline concentration in the same manner. Glucan or cellulose and hemicellulose (xylan and galactan) content were comparable to the composition of Thai rice straw reported previously [33]. However, the lignin and ash content of the rice straw investigated in this study was lower than that reported in the literature [33].
Table 1 Chemical composition of rice straw RD41.

<table>
<thead>
<tr>
<th>Component</th>
<th>% (w/w) of Dry Biomassa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucan</td>
<td>36.72±0.13</td>
</tr>
<tr>
<td>Xylan</td>
<td>23.47±1.47</td>
</tr>
<tr>
<td>Galactan</td>
<td>7.15±0.42</td>
</tr>
<tr>
<td>Acid-soluble lignin (ASL)</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>Acid-insoluble lignin (AIL)</td>
<td>11.07±0.01</td>
</tr>
<tr>
<td>Water extractives</td>
<td>15.49±0.58</td>
</tr>
<tr>
<td>Ethanol extractives</td>
<td>3.75±0.73</td>
</tr>
<tr>
<td>Ash</td>
<td>11.16±0.13</td>
</tr>
</tbody>
</table>

aNThe data is represented as average ± SD

Sodium hydroxide pretreatment
Total solid removal

A comparison of the effect of the sodium hydroxide pretreatment, in terms of residence times and temperatures on the total solid removal of the rice straw RD41, is presented in Figure 1. The effect of pretreatment temperatures was more pronounced than that of the residence times. The elevated temperatures caused higher total solid removal compared to the lower temperatures, as detected at all residence times. Thus, the highest total solid removals (52.5 to 55.8 %) were found in the pretreatment at 100 °C. In terms of the residence times (1 to 5 h) of the pretreatment, the longer pretreatment times trended to give higher total solid removal within the same pretreatment temperature. The effect of residence times on total solid removal varied depending on pretreatment temperatures. In comparison with the 1-h pretreatment, all total solid removal values of the 5-h pretreatment were significantly higher than that of the 1-h samples. The trend of this effect corresponded with the results reported by Sophonputtanaphoca [8], where the sodium hydroxide (NaOH) pretreatment was applied to wheat straw samples at 50 °C for 1 to 5 h.

Figure 1 Comparison of amounts of total solid removal (w/w of dry biomass) by sodium hydroxide pretreatment of rice straw RD41 at different residence times and temperatures. Error bars represent ±1 SD.
Lignin removal

Table 2 shows the amounts of acid-insoluble lignin (AIL), acid-soluble lignin (ASL), and total lignin determined in the pretreated rice straw obtained from all pretreatment conditions. The effect of the sodium hydroxide pretreatment on the level of lignin removal is shown in Figure 2. The amounts of lignin in this study referred to the combination of AIL and ASL. The intensity of the delignification corresponded with the level of total solid removal, as described above. The elevated temperatures and prolonged pretreatment periods caused greater amounts of lignin removed from the biomass. The highest lignin removal with the approximate amount of 87% was detected at the pretreatment condition of 100 °C, regardless of the residence times of the pretreatment, while the lowest lignin removal, with amounts no higher than 67%, was found at the pretreatment condition of 50 °C, regardless of the residence time of the pretreatment (Figure 2). McIntosh and Vancov [4] reported that temperature was the main factor in delignification of wheat straw by NaOH pretreatment. The optimal lignin removal occurred at temperatures higher than 60 °C, when each residence time was considered. In terms of effect of residence times on the intense of lignin loss, most of the distinct effects of all temperatures tested were revealed at 3 h and thereafter. One work reported that NaOH pretreatment induced significant amounts of lignin degradation during 24 h pretreatment of corn stover at room temperature [34]. Alkaline pretreatment causes substantial lignin solubilization when applied to lignocellulosic materials [35]. Gupta and Lee [22] revealed that pretreatment of switchgrass with 5% NaOH at 85 °C for 24 h removed more than 75% of lignin from biomass. The other study from Sophonputtanaphoca [8] showed that approximately 60% AIL in wheat straw was removed with 5% sodium hydroxide pretreatment at 50°C for 5 h. The delignification resulting from alkaline pretreatment is believed to be the mechanism of solvation and saponification of intermolecular ester bonds between lignin, hemicellulose, and cellulose [21,36].

Figure 2 Comparison of amounts of lignin removal (w/w of dry biomass) by sodium hydroxide pretreatment of rice straw RD41 at different residence times and temperatures. Error bars represent ±1 SD.
Table 2 Mass percent of acid-insoluble lignin (AIL), acid-soluble lignin (ASL), and total lignin based on original biomass retained in pretreated rice straw at various conditions. The data is represented as average ± SD.

<table>
<thead>
<tr>
<th>Pretreatment condition</th>
<th>%AIL</th>
<th>%ASL</th>
<th>%Total Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>Residence time (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>1</td>
<td>4.19±0.44</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.08±0.19</td>
<td>0.18±0.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.92±0.76</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.81±1.04</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.58±0.55</td>
<td>0.15±0.00</td>
</tr>
<tr>
<td>60</td>
<td>1</td>
<td>4.22±0.21</td>
<td>0.15±0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.89±0.06</td>
<td>0.16±0.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.22±0.15</td>
<td>0.16±0.00</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.77±0.22</td>
<td>0.16±0.00</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.78±0.49</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td>70</td>
<td>1</td>
<td>2.69±0.35</td>
<td>0.15±0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.12±0.17</td>
<td>0.15±0.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.06±0.08</td>
<td>0.15±0.00</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.65±0.25</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.46±0.09</td>
<td>0.13±0.00</td>
</tr>
<tr>
<td>80</td>
<td>1</td>
<td>1.93±0.05</td>
<td>0.13±0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.91±0.07</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.64±0.15</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.55±0.07</td>
<td>0.12±0.00</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.55±0.08</td>
<td>0.12±0.00</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>1.35±0.13</td>
<td>0.13±0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.34±0.18</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.33±0.21</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.34±0.03</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.33±0.05</td>
<td>0.12±0.00</td>
</tr>
</tbody>
</table>

Glucan recovery
Most of the glucan (~80 to 100 %) was preserved in the pretreated solids, as presented in Figure 3. The lower temperatures, i.e., 50 and 60 °C, resulted in higher glucan preservation (> 90 %) in the pretreated solids. More severity in pretreatment conditions, such as high temperature and high alkalinity, causes more carbohydrate losses when a lignocellulosic material is pretreated in such conditions [8]. One work reported that 98.5 % of glucan was preserved in the solid after pretreatment with 5 % sodium hydroxide at 85 °C for 24 h. The glucan preservation in the pretreated solid is due to low reactivity of cellulose with alkali and high crystallinity of cellulose [22]. Unlike lignin, alkaline pretreatment has little effect on removal of cellulose [32].
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Figure 3 Comparison of amounts of glucan recovery (w/w of dry biomass) by sodium hydroxide pretreatment of rice straw RD41 at different residence times and temperatures. Error bars represent ±1 SD.

**Fermentable sugar production**

Considering all factors determined in this study (total solid removal, lignin removal, and glucan recovery), a selected residence time, *i.e.*, 3 h of each pretreatment temperature was chosen to further study fermentable sugar production by enzymatic saccharification. The 3 h residence time was selected because the effect of pretreatment temperatures was more pronounced compared to the effect of residence times determined by all of the 3 factors. The 3 h residence time was relatively in the middle of the residence times tested in this study. Table 3 shows the glucan conversions of pretreated solids obtained from different pretreatment temperatures for 3 h. The conversions were achieved by cellulase and xylanase preparations. More than 60 % of glucan conversion at each temperature was achieved within 24 h of incubation. The glucan conversion yields of all pretreatment temperatures, except at 50 °C, were statistically comparable. Only the yield obtained from the conversion of the biomass pretreated at 50 °C was significantly lower than others. The glucan conversion yield of untreated rice straw was ~19 % (data not shown). This suggests that the accessibility of glucan to the cellulase and xylanase increased as a result of the alkaline pretreatment. In addition, the 24 h of incubation in the saccharification period was appropriate to see the different changes in the glucan conversions of treated and untreated biomass. Thus, the 24 h of incubation was selected to assess the glucan reactivity of rice straw.

Lignin content in the pretreated solids had a relationship with the glucan conversion. The lower the lignin content retained in the pretreated solid, the higher the glucan conversion detected. At the temperatures of 70 to 100 °C, the levels of lignin removal after the pretreatment for 3 h were higher than 80 %, and the glucan conversions after 24 h of incubation were higher than 80 %. On the contrary, at the lower pretreatment temperatures, *i.e.*, 50 and 60 °C, the glucan conversions were lower than 80 % after 24 h of incubation. Yan *et al.* [37] found that there was a positive correlation between cellulose conversion and lignin removal for fermented sweet sorghum bagasse after pretreatment with NaOH with the loading of NaOH lower than 20 %. The removal of lignin results from the solvation and saponification of intermolecular ester bonds cross-linking xylan, lignin, and other hemicelluloses. In addition, the solvation and saponification cause the removal of those cross-links and increase the porosity of the lignocellulosic materials. These also enhance the accessibility of hemicellulose, as well as cellulose, to enzymes [21].

In addition to the pretreatment conditions and glucan conversion presented in Table 3, another attempt of glucan conversion was carried out, with the rice straw pretreated at 50 °C for 5 h in order to investigate the effect of low pretreatment temperature and long residence pretreatment time. Interestingly,
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91.09±3.28 % glucan conversion can be reached after 24 h of saccharification. This suggests that longer pretreatment time is required when the pretreatment is conducted at low temperatures, in order to achieve high glucan conversion.

Table 3 Digestibility of glucan after 24 h of saccharification of pretreated rice straw RD41 at different pretreatment temperatures for 3 h. The data is represented average ± SD.

<table>
<thead>
<tr>
<th>Pretreatment temperature (°C)</th>
<th>%Glucan conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>68.41±4.66</td>
</tr>
<tr>
<td>60</td>
<td>78.85±4.71</td>
</tr>
<tr>
<td>70</td>
<td>82.70±2.96</td>
</tr>
<tr>
<td>80</td>
<td>81.42±4.93</td>
</tr>
<tr>
<td>100</td>
<td>84.33±7.21</td>
</tr>
</tbody>
</table>

Note: Enzyme loading: 30 FPU/g glucan (Cellulase) + 2,750 U/g glucan (Xylanase)

Conclusions

The glucan reactivity of sodium hydroxide-pretreated rice straw was primarily evaluated in terms of total solid removal, lignin removal, and glucan recovery. The pretreatment temperatures had a pronounced effect on those 3 factors compared to the pretreatment times. The levels of delignification were enhanced by the elevated pretreatment temperatures and were proportional to the total solid removal. The 3 h residence time of the pretreatment of each pretreatment temperature was selected to be used as a raw material for further fermentable sugar production. The high glucan conversion yields were achieved within 24 h of saccharification. Glucan conversion yields with higher than 80 % conversion were detected when the rice straw samples were pretreated at 70 to 100 °C. However, lower pretreatment temperatures (50 and 60 °C) gave lower glucan conversion yields (< 80 %). Surprisingly, approximately 90 % glucan conversion could be attained with the 50 °C, 5 h pretreated rice straw.

Acknowledgements

This research was funded by the Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Thailand (grant number 369/2557). The authors also would like to thank Dr. Michael Penner, Department of Food Science and Technology, Oregon State University, USA, for generously providing the sugar standards and enzyme preparations used in this study.

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