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Pretreatment of rice straw by hot-compressed water for enzymatic saccharification

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Abstract−The primary objective of this work was to measure the maximum amount of glucose that can be produced from Thai rice straw using hot-compressed water (HCW)-pretreatment before enzymatic saccharification. The optimal HCW-pretreatment temperature and time were found to be 180 °C/2 MPa for 20-30 min. However, the concentrations of the yeast inhibitors were strongly dependent on the HCW-pretreatment temperature and time. At temperatures over 180 °C/2 MPa or for more than 30 min at 180 °C/2 MPa in the HCW-pretreatment the combined concentration of these two inhibitors (Furfural and 5-Hydroxymethylfurfural) increased exponentially, while the glucose levels were near the maximal asymptote. At the more optimal HCW-pretreatment condition of 180 °C/2 MPa for 20 min, 25±3 kg of glucose could be produced from a 100 kg of rice straw, which is potentially economically competitive with other sources.

Keywords: Rice Straw, Pretreatment, Hot-compressed Water, Glucose, Saccharification

INTRODUCTION

Rice straw is a major agricultural residue and a potential lignocellulosic substrate for monosaccharide production in many countries that has largely been overlooked in the past. For instance, rice straw is not only produced at approximately 6 million tons (1 ton= 1,000 kg) per year in Thailand, but it also has a higher cellulose and lower lignin content than other feedstocks, such as rice hulls [1], cassava stem [2], and sawdust [3]. Rice straw is traditionally removed from the field in Thailand by open-field burning for new plantations, which results in localized pollution and global greenhouse gas emissions. The conversion of rice straw to bioethanol would not only reduce the gasoline consumption, but also reduce the carbon footprint (greenhouse gas production) from open-field burning [4].

Biofuels production from lignocellulosic feedstock can be briefly divided into the four steps: pretreatment, enzymatic saccharification, fermentation and distillation. The examples of biofuels are obtained from fermentation including bioethanol, biogas (bio-methane), bio-hydrogen and acetone-butanol-ethanol (ABE). The pretreatment step is critical in determining the final glucose concentration obtained in the enzymatic saccharification step, and often dominates the cost of the overall conversion process. A good pretreatment can lead to a lower required enzyme dose (one of the expensive components) and allow a high solid loading, which leads to a higher yield and lower heat consumption with an improved economic efficiency and reduced environmental cost.

The primary aim of the pretreatment step is to convert the native lignocellulosic feedstock to a form that can subsequently be easily accessed and degraded by enzymes to produce fermentable mono- or di-saccharides in the second stage. Various chemical pretreatment methods have been reported, such as alkaline treatment, ionic liquid, ammonia fiber explosion, lime pretreatment and dilute acid pretreatment [5,6]. Nevertheless, those chemical pretreatment methods reduce the environmentally friendly stance of the enzymatic saccharification and fermentation steps because of the use of strong and high concentration chemicals and solvents. Additionally, the subsequent stages of ethanol and butanol production from lignocellulosic feedstock have been reviewed elsewhere [7,8], and are not the aim of this report.

One promising pretreatment method for lignocellulosic feedstock that avoids such chemical agents is the use of hot-compressed water (HCW). The HCW-pretreatment has gained attention as environmentally friendly because it is chemical-free. For the HCW-pretreatment of lignocellulosic feedstock for sugar production, the fundamental characteristics of the process, such as the optimal operating parameters and the details on sugar production and fermentation-inhibitor formation during process, have been reported for several different feedstocks [9-11]. Moreover, the HCW-pretreatment could be coupled with biological pretreatment technologies such as delignification of biomasses by fungi [12]. However, the different types of feedstock, which have different structures and compo-

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sitions, gave different results and characteristics of pretreatment.

Our primary objective was to investigate the effect of the HCW-pretreatment temperature on the glucose yield (as a % by dry weight (dw) of cellulose in the feedstock) from the enzymatic saccharification of pretreated Thai rice straw. The temperature has been reported to be the most important parameter in determining the pretreatment efficiency [13], while the pressure, particle size, and pretreatment time were held constant. For the rice straw in this study, the optimal temperature was evaluated in terms of the highest glucose yield (% dw) obtained from the subsequent standard enzymatic saccharification. Rice farming in Thailand is generally cultivated at least twice a year and up to four times a year in the well irrigated areas, and so it is possible that these rice straw samples from Thailand might be easier to hydrolyze than full year plantation rice straw.

MATERIALS AND METHODS

1. Rice Straw

The rice straw sample (*Oryza sativa* L.), of approximately six months post-germination, was obtained from a local farmer in the Pathumthani province, Thailand, within the first time of harvesting. It was air dried, milled and sieved to a particle size of 0.85-1.00 mm. At ambient temperature, the rice straw sample had 8% (w/w) moisture content. Note that the testing method TAPPI standard (264 om-97) is suitable for woody biomass. Due to the high amount of inorganic compounds in non-woody biomass, a novel analytical method has been proposed. The dry rice straw was analyzed by the novel method applicable for non-woody biomass [14] and found to consist of (w/w, dw) 34.19% cellulose, 32.15% hemicellulose, 12.68% lignin, 12.02% ash and 8.96% extractives components. Compared to the Japanese rice straw which was analyzed by the same method, the amount of lignin in Thai rice straw was slightly lower than that of Japanese rice straw (20.20%).

2. Hot-compressed Water (HCW)-pretreatment

The HCW-pretreatment was carried out in a 250-mL 316 stainless steel reactor equipped with a turbine stirrer, internal cooler and a Parr model 4842 temperature and tachometer controller. The temperature inside the reactor was measured in real-time during each experiment by a k-type thermocouple and Macromedia AUTOWARE software®. The reaction vessel was charged with 10 g of rice straw and 100 g of deionized water to give approximately 10% (w/v) rice straw suspension in water. Because the properties of HCW are assigned by temperature, the pretreatment temperature was investigated before the pretreatment time. The initial pressure, reaction time and stirring speed were fixed at 2 MPa in N_2, 20 min and 500 rpm, respectively, while the temperature was varied between trials over the range of 120-200 °C. The pretreatment time was estimated from previous reports that used rice straw as a substrate and HCW as the pretreatment method [15]. The reactor was heated electrically at a rate of 5 °C/min to the reaction temperature. At the end of the reaction, the reactor was quenched in a water bath, cooling the mixture to approximately 50 °C within 10 min. The solid and liquid fractions were then separated by vacuum filtration and weighed with an analytical balance. Approximately 25 g of the wet solid fraction was then transferred to the enzymatic saccharification step, while the remaining solid was weighed, dried at 110 °C overnight and reweighed to measure the moisture content. After the HCW-pretreatment temperature was screened, the effects of the pretreatment time were investigated under the same conditions as above plus the optimal temperature, except varying the reaction time over the range of 5-60 min.

3. Enzymatic Saccharification

Analytical grade sodium hydroxide and citric acid, used to prepare the 5.0 mM citrate buffer (pH 4.8), were supplied by Merck. Commercial cellulase was provided by the PTT Research and Technology Institute. Note that the composition of the cellulase is confidential to PTT Public Co. Ltd., but it contains β-glucosidase and cellobiohydrolases. This commercial cellulase has activity of 40 FPU/mL. The enzymatic saccharification was performed in a 250-mL Erlenmeyer flask using 25 g of wet pretreated rice straw suspended in 50 mL of 5.0 mM citrate buffer (pH 4.8), to give a 10% (w/v, dw) solid suspension. The wet solid sample was partially separated for further analysis (See Section 5). Samples were shaken (500 rpm) in a water bath at 50 °C for 24 h. To obtain the maximum possible level of glucose production (saccharification efficiency), the amount of cellulase and saccharification time were at an excess in all batches, with a substrate to enzyme mass ratio of more than 1 : 1 [16]. The actual optimization of the enzymatic saccharification stage was not performed in this study, but will be in future studies. The glucose yield (% dw) was then calculated using Eq. (1),

$$\%\text{Glucose yield} = \left(\frac{\text{Produced glucose wt (g)}}{\text{Dry cellulose wt (g)}}\right) \times 100 \quad (1)$$

The amount (weight) of the produced glucose was determined by HPLC (Section 4). The glucose yield (%) was assumed to represent the maximum amount of glucose that can produced from the rice straw by the combined HCW-pretreatment and subsequent enzymatic saccharification. The moisture content of the remaining solid residue was then determined to calculate the overall mass balance (RESULTS AND DISSCUSTION, Section 3).

4. Liquid Products Analysis

The liquid products obtained from the HCW-pretreatment and subsequent enzymatic saccharification steps were analyzed by HPLC. Acetonitrile (HPLC grade) was obtained from Fisher and analytical grade glucose, xylose, furfural and 5-hydroxymethyl furfural (HMF) were purchased from Merck. The glucose and xylose content in each liquid fraction was analyzed using a Shimadzu, LC-10ADvp HPLC with a GL Science Inertsil-NH_2 column (4.6×250 mm) equipped with a refractive index detector. The mobile phase was 1 : 4 (v/v) ratio of water: acetonitrile at a flow rate of 1.0 mL/min. The amount of furfural and HMF in the liquid fraction was determined using a Varian 400 HPLC with a Restek Pinnacle II C18 column and UV detector at wavelength of 284 nm [17]. The mobile phase was a 4 : 1 (v/v) ratio of water: acetonitrile at a flow rate of 0.8 mL/min.

5. Solid Products Analysis

Pretreated rice straw and the enzymatic saccharified solids obtained from the different HCW-pretreatment temperatures or times were analyzed by scanning electron microscopy (SEM), using a JEOL Ltd. Model JSM-5410LV microscope, to observe the changes in morphology. The moisture content of all pretreated and saccharified products was measured in order to calculate the overall yield and

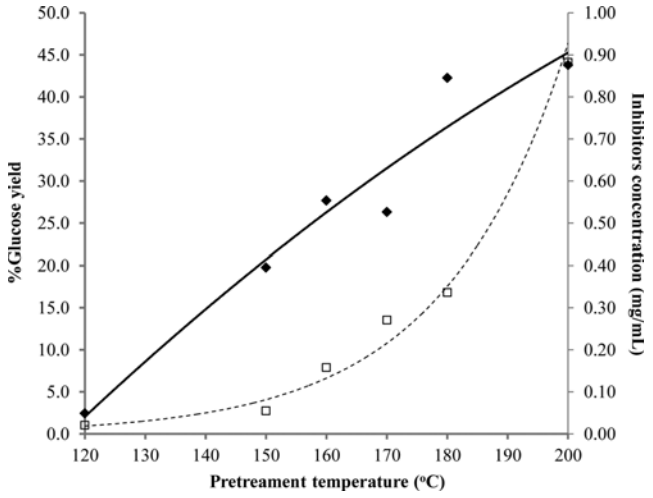

Fig. 1. The (solid line and filled diamond) glucose yield (% (w/w, dw)) and (dashed line and open square) combined concentration of furfural and HMF as a function of the HCW-pretreatment temperature at an initial pressure of 2 MPa N_2, reaction time of 15 min and stirring speed of 500 rpm. Data are shown as the mean±1 SD, and are derived from 3 independent repeats.

overall mass balance. To examine the moisture content, a known weight of moist sample was dried in an oven at 105 °C for 24 h. The dried sample was then cooled in a desiccator, weighed and the moisture content was calculated from weight loss divided by the weight of the moist sample.

RESULTS AND DISCUSSION

1. Optimal HCW-pretreatment Temperature

The glucose yield (% dw) and the concentration of furfural and HMF in the enzymatic hydrolysates of the different HCW-pretreated rice straw samples are illustrated in Fig. 1. It is clear that the glucose yield sequentially obtained in the enzyme saccharification stage increased with the HCW-pretreatment temperature over the tested range (120-200 °C), which will be due to the destruction of the rice straw structure by HCW. When the untreated rice straw was saccharified it provided a glucose yield of only 0.64% (dw), whereas the maximal glucose yield obtained, after a 200 °C HCW-pretreatment, was some 70-fold higher at 43.8% (dw). However, the glucose yield obtained after HCW-pretreatment at 200 °C was not significantly greater than that at 180 °C (42.27%, 1.09-fold lower), but the furfural and HMF concentration after a 200 °C pretreatment was higher than that at 180 °C. Furfural and HMF are known inhibitors of yeast fermentation of simple sugars to ethanol, especially over a combined concentration of 1.0 mg/mL [18]. Accordingly, the optimum HCW-pretreatment temperature in this work was selected as 180 °C to avoid the excessive formation of the fermentation inhibitors furfural and HMF.

To explain the effects of HCW-pretreatment of rice straw, the SEM images of untreated and HCW-pretreated rice straw samples were examined (Fig. 2). The longitudinal surface of the rice straw was destroyed after 15 min treatment by HCW-pretreatment and moreso with the increasing pretreatment temperature. Cellulose (dense white spots in the SEM images) was scattered on the 180 °C HCW-pretreated sample. Based on the ionization constant (pK_w) of saturated water, at 150 °C and 200 °C the pH values of water was 5.31 and 5.27, respectively [19]. Under an acidic condition, HCW penetrates into the rice straw, hydrolyzes hemicellulose and generates some organic acids. The surface visually erupted from the inside and was not eroded from outside, because the organic acids from hemicellulose were produced and released from inside the rice straw particles [15].

Representative SEM images of the enzymatic saccharified solid (rice straw remnants) revealed that the morphology of the HCW-pretreated samples changed during the subsequent enzymatic saccharification (Fig. 3). The cracked or opened surfaces of the rice straw were largely destroyed by the cellulose digestion. In addition, the inner surface of the rice straw HCW-pretreated at 150 to 160 °C was clearly digested in the subsequent enzymatic saccharification stage. The outer surface of the 170 °C HCW-pretreated samples, especially around the cracked stoma, was markedly damaged. The porosity of the inner surface was clearly observed in the 180 °C HCW-pretreated samples, which corresponds to the higher glucose yields obtained from these samples.

The analysis of the liquid fraction obtained from the HCW-pretreatment of rice straw at 120-180 °C did not reveal any significant concentration of monosaccharides (all were <1.0 mg/mL), such as glucose or xylose, but these were formed in the subsequent saccharification stage. In agreement with these results is that analysis of the solutes in the liquid fraction obtained from HCW-pretreated rice straw at 140-240 °C was previously reported to mainly be xylan and other oligosaccharides [15], whereas the detected concentration of free monosaccharides was below 0.2 mg/mL. To ensure the presence of xylan in hydrolysate, the HCW-pretreated liquid obtained from 180 °C was refluxed with 2% H_2SO_4 for 60 min and then subjected to analysis of monosaccharide. It was found that the xylose concentration in hydrolysate was approximately 5.4 mg/mL. In addition, formation of furfural and HMF was reported to be at approximately 3.35 mg/mL in the liquid fraction from the 200 °C HCW-pretreated Japanese rice straw [15], compared to a combined concentration of furfural and HMF in the liquid fraction of the 200 °C HCW-pretreated rice straw of over 4.00 mg/mL in this study. Almost all monosaccharides, mainly xylose, in the liquid fraction of the 200 °C HCW-pretreated rice straw converted to furfural and HMF.

2. Effects of the HCW-pretreatment Time

The effects of the HCW-pretreatment time were investigated over the range of 5-60 min at a constant 180 °C, under N_2 initial pressure of 2 MPa and stirring speed of 500 rpm. The glucose yield (% dw) and fermentation inhibitor concentration (as the sum of furfural and HMF concentrations) in the saccharified liquid as function of the HCW-pretreatment time are shown in Fig. 4. Increasing the pretreatment time simultaneously enhanced both the glucose yield and the concentration of inhibitors. However, the glucose yield rose less dramatically with increasing pretreatment times above 30-40 min as it approached the maximal asymptote, whereas the formation of the inhibitors (furfural and HMF) exponentially increased with increasing pretreatment times over 30 min. The opti-

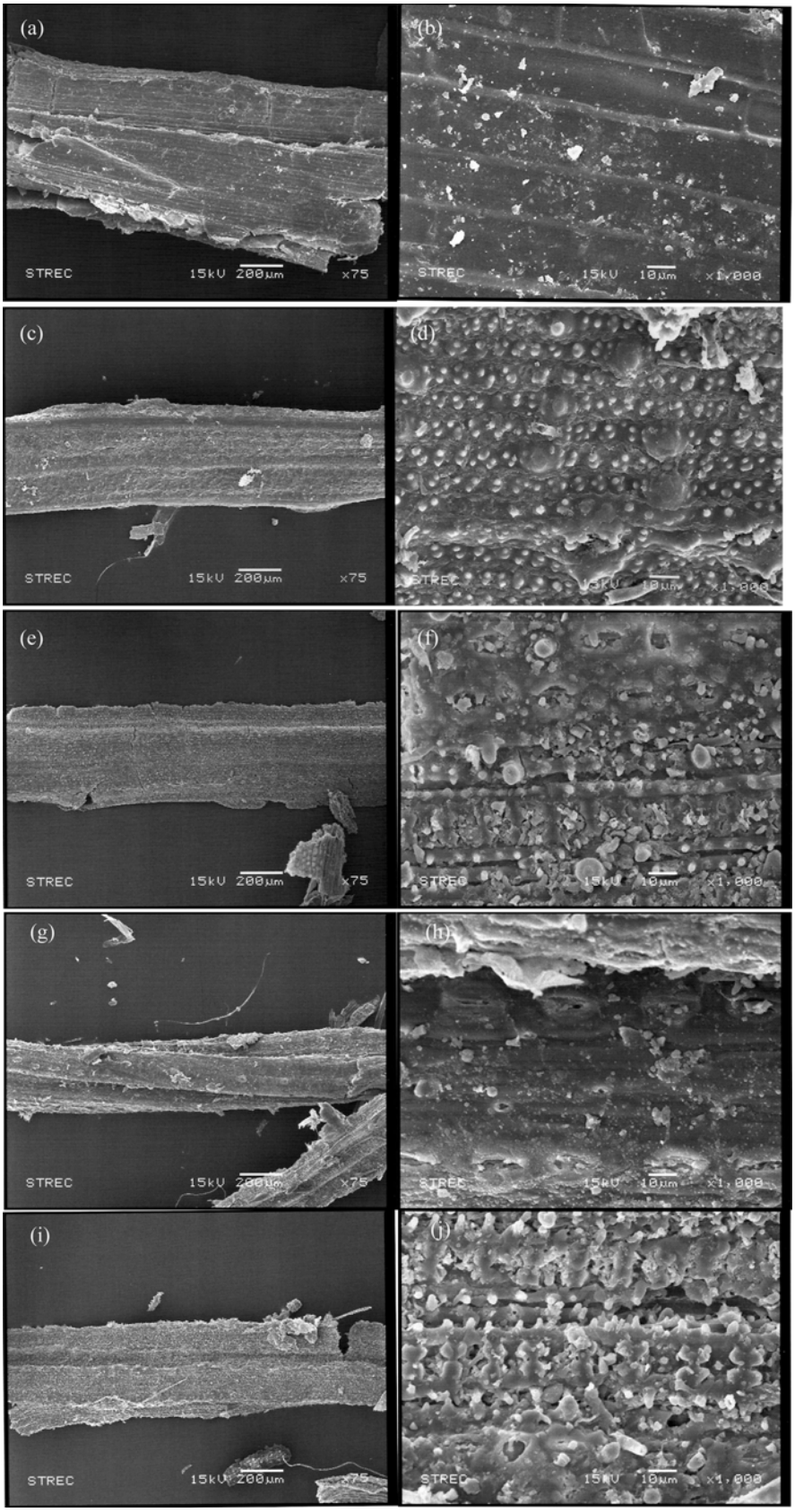

Fig. 2. SEM micrographs of rice straw (a), (b) without treatment, and (c)-(j) following HCW-treatment for 15 min at (c), (d) 150 °C, (e), (f) 160 °C, (g), (h) 170 °C and (h), (i) 180 °C. Images are at (a), (c), (d), (f), (g), (h) 75× or (b), (d), (f), (g), (i) 1000× magnification and are representative of those seen in at least 5 fields of view per sample and 3 independent samples.

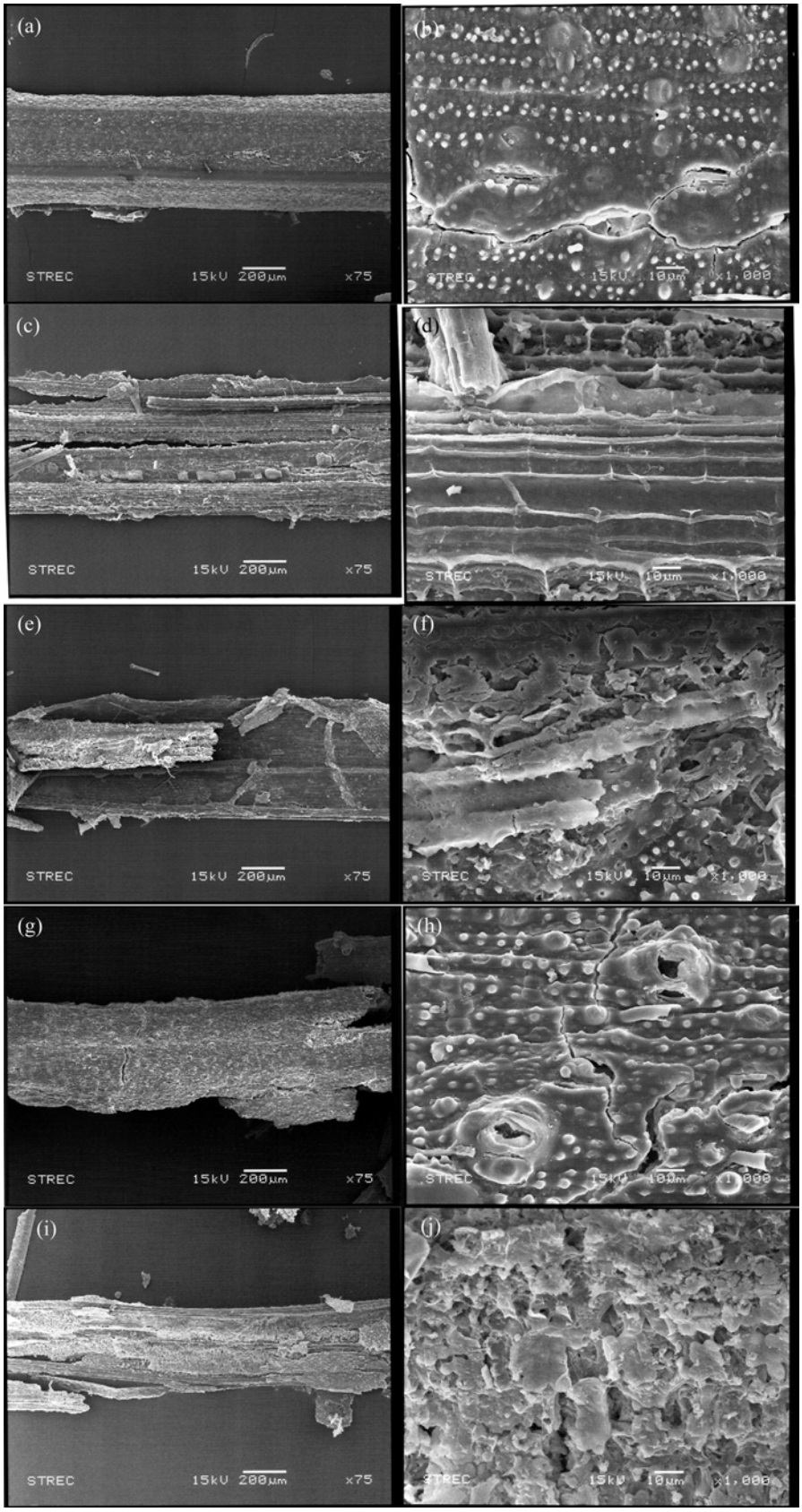

Fig. 3. SEM micrographs of enzymatic saccharified rice straw (a), (b) without pretreatment, and (c)-(j) following HCW-pretreatment for 15 min at (c), (d) 150 °C, (e), (f) 160 °C, (g), (h) 170 °C and (h), (i) 180 °C. Images are at (a), (c), (d), (f), (g), (h) 75× or (b), (d), (f), (g), (i) 1000× magnification and are representative of those seen in at least 5 fields of view per sample and 3 independent samples.

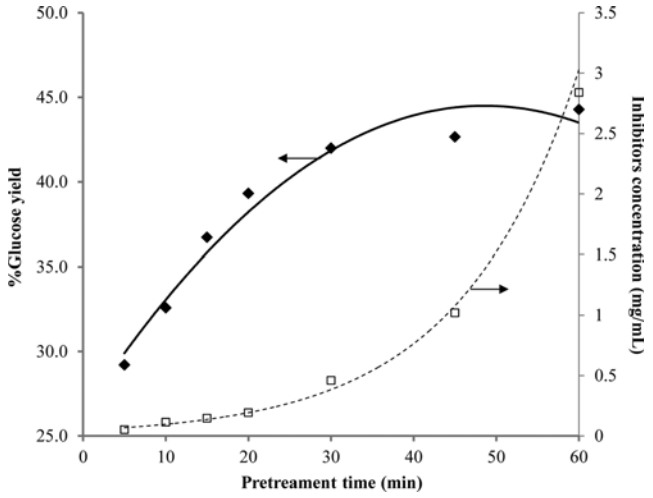

Fig. 4. The (solid line, filled diamond symbol) glucose yield (% (w/w, dw)) and (dashed line, open square symbol) combined concentration of furfural plus HMF (mg/mL), in the enzymatic saccharified liquid of 180 °C HCW-pretreated rice straw for various pretreatment times. Data are shown as the mean±1 SD, and are derived from 3 independent repeats.

mal HCW-pretreatment time was 30 min, giving a glucose yield and combined inhibitor concentration of 41.9±3.4% and 0.458±0.091 mg/mL, respectively. However, if a significantly lower inhibitor concentration was required, such as for using a sensitive yeast for fermentation, then using a 20 min pretreatment time could reduce the inhibitor concentration 2.4-fold (to 0.192±0.03 mg/mL) without compromising the glucose yield much (1.1-fold loss to 39.31±3.3%).

3. Mass Balance

To reveal the feasibility of glucose production from Thai rice straw, the overall mass balance of glucose production through the HCW-pretreatment and subsequent enzymatic saccharification process was evaluated (Fig. 5). The wet solid obtained from HCW-pretreatment at 180 °C for 20 min was approximately 35 g. The weight buffer was recalculated based on the assumption that all HCW-pretreated solid was subject into enzymatic saccharification process (no wet solid split off for further analysis). In the pretreatment step, the resultant liquid contained a glucose concentration of less than 1.0 mg/mL (and so less than 100 mg total glucose). Additional studies on this stream are essentially required to discover the value-added products for glucose production from rice straw. Following enzymatic saccharification, the liquid contained 2.45±0.35 g of glucose at a concentration of 35±5 mg/mL, giving a glucose yield of 42±5% from the saccharification step. Since this was derived from 100 g of rice straw, then this process is able to synthesize 245±35 kg of glucose from a ton of rice straw. At this condition, the enzymatic hydrolysis of cellulose was 62.10%.

The chemical composition of HCW-pretreated solid at 180 °C

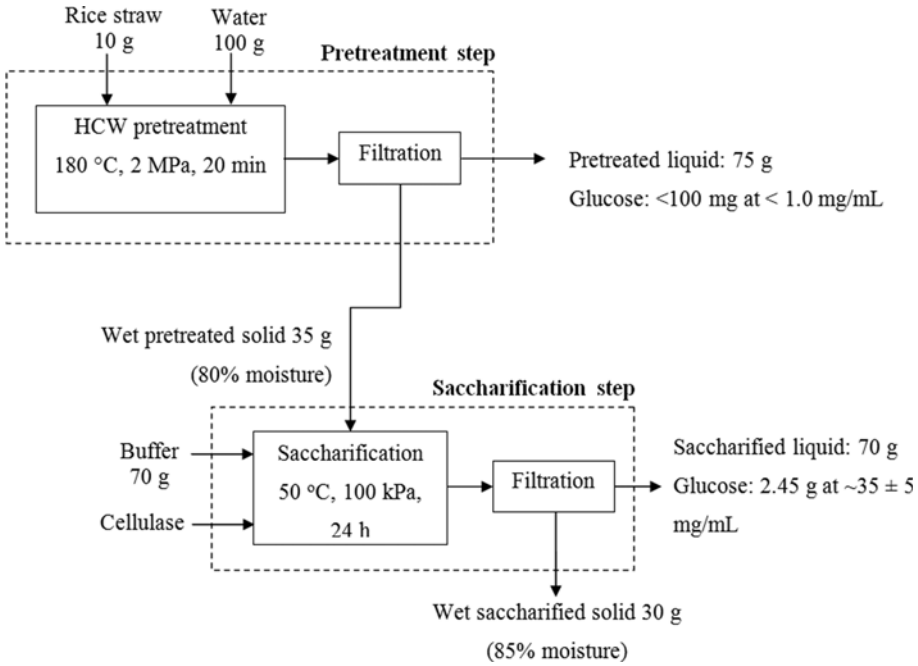

Fig. 5. The overall mass balance of glucose production from rice straw by HCW-pretreatment and enzymatic saccharification.

Table 1. The chemical composition of Thai rice straw, HCW-pretreated sample at 180 °C and the saccharified product

Sample	%Cellulose	%Hemicellulose	%Ash	%Lignin
Rice straw	34.19	32.15	12.02	12.68
HCW-pretreated sample	40.62	22.34	18.10	10.93
Enzymatic saccharified sample	25.44	22.44	28.75	16.86

and saccharified product are illustrated in Table 1. It is clear that hemicellulose partially removed from rice straw after HCW-pretreatment. For lignin, small amount of acid-soluble lignin could be eliminated from the rice straw by HCW-pretreatment. The %Cellulose in HCW-pretreated was enhanced due to the hemicellulose and lignin removal. The remarkable increase in ash content (increase by 16.7% (w/w, dw) or 1.5-fold) is because hemicellulose and cellulose were fractionated during the HCW pretreatment and enzymatic saccharification stages, respectively. Whereas, the lignin content (%) remained essentially constant (increased by 4.2% (w/w, dw)) because the lignin was only slightly removed beyond the pretreatment step only. The remaining cellulose in the wet saccharified solid could be crystalline cellulose, which cannot be enzymatic hydrolyzed.

CONCLUSION

Evaluation of the HCW-pretreatment of Thai rice straw in a batch reactor demonstrated that HCW-pretreatment at 180 °C/2 MPa for 20 min was suitable as a pretreatment for subsequent enzymatic saccharification. The maximum glucose concentration of the saccharified product from the HCW-pretreated sample (35±5 mg/mL) was approximately 57-fold more concentrated than in the no-pretreatment sample (0.7±0.5 mg/mL). At this optimal HCW-pretreatment condition, the amount of inhibitors (furfural plus HMF) in the resultant saccharified liquid was below the acceptable maximum level for yeast fermentation. The results obtained in this work are useful for developing scale-up or continuous pretreatment processes.

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