Effects of Chemical and Thermal Pretreatments on the Enzymatic Saccharification of Rice Straw for Sugars Production

Nurul Atika Mohamad Remli,a Umi Kalsom Md Shah,a,⁎ Rosfarizan Mohamad,a,b and Suraini Abd-Aziz a

The effects of alkaline pretreatment with NaOH, KOH, Ca(OH)2, and NaOCl at varying temperatures and concentrations on the production of sugars, changes in the morphological structure, and the chemical composition of rice straw were evaluated. Enzymatic saccharification of 2% (w/v) KOH-treated rice straw with autoclaving at 121 °C, 15 psi, 20 min, gave a maximum yield of 59.90 g/L of reducing sugars, which was slightly higher than that of NaOH (55.48 g/L) with the same conditions. Chemical composition analysis of the rice straw showed that the cellulose content was increased to 71% and 66% after pretreatments with NaOH and KOH, respectively. Fourier Transform Infrared (FTIR) spectroscopy revealed that solubilization and removal of the lignin component also took place. The scanning electron microscope (SEM) analysis showed a marked change in the morphological structure of the treated rice straw compared to the untreated rice straw. These results suggested that pretreatment of rice straw with either 2% (w/v) NaOH or KOH at high temperature could be a promising pretreatment method for sugars production.

Keywords: Lignocellulosic biomass; Rice straw; Alkaline pretreatment

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INTRODUCTION

The rising prices of crude oil and the continuous depletion of existing fossil fuel reserves, combined with concerns over global climate change, have created the need for alternative biofuels to replace fossil fuels. Among the alternative fuels, biobutanol is considered as the most potential biofuel as it has many chemical and physical features that are particularly attractive for application as a liquid fuel (Gupta and Tuohy 2013). In order to produce relatively cheap biobutanol, use of more economical substrates must be identified and evaluated. In this case, lignocellulose has been identified as the most suitable candidate for biobutanol production since it is abundant and not fully exploited (Qureshi and Ezeji 2008).

Rice straw is a particularly attractive lignocellulosic material for biofuel production since it is one of the most abundant renewable resources. In Malaysia, it was estimated that about 1.3 million tonnes of rice straw is produced annually from the 684000 hectares of rice fields (MOA 2004). The open field burning of rice straw remains as the conventional and current disposal technique used by most farmers.
Large amounts of rice straw produced are disposed of by burning, which is an environmental hazard, and can cause severe impacts on human health (Gadde et al. 2009; Nori et al. 2008). Hence, alternative uses of rice straw have been suggested, and one of them is utilizing the rice straw as a substrate for alternative biofuels (Binod et al. 2010).

Bioconversion of rice straw into biobutanol is a multi-step process consisting of pretreatment, enzymatic hydrolysis, and fermentation (Zhang and Cai 2008; Lee et al. 2008). To initiate the production of biobutanol from cellulosic biomass, bioconversion of the cellulosic components into fermentable sugars is necessary (Kumar et al. 2008). The key obstacle for fermentable sugars production is the recalcitrant nature of the raw biomass, and therefore pretreatment is particularly crucial to alter the cellulosic biomass by physical, thermal, or chemical means to facilitate rapid and efficient enzymatic hydrolysis of carbohydrates to fermentable sugars (Chang and Holtzapple 2000).

Despite being considered as a crucial step for the conversion of biomass to liquid fuels, biomass pretreatment is one of the main economic costs in the process. In fact, it has been described as the second most expensive unit cost in the conversion of lignocellulose to ethanol based on enzymatic hydrolysis, preceded only by feedstock cost. Therefore, developing a cost-effective and efficient biomass pretreatment technology is the most critical need for lignocellulosic biofuels (Yang and Wyman 2008). The common methods currently used or in development consist of alkali hydrolysis, dilute acid hydrolysis, uncatalysed steam explosion, acid-activated steam explosion, liquid hot water, ammonia fiber explosion, ozonolysis, and CO₂ explosion (Ruiz et al. 2008; Garlock et al. 2012; Govunami et al. 2013).

Among these methods, steam explosion is one of the best and efficient pretreatment methods, as it uses both chemical and physical techniques in order to break the structure of the lignocellulosic material (Cara et al. 2008; Taherzadeh and Karimi 2008). However, this method is unreliable and not cost-effective for softwood material. Furthermore, steam explosion generates some toxic derivatives that are unfavorable for the subsequent hydrolysis and fermentation steps.

Alkaline pretreatment procedures have the advantages of utilizing lower temperatures and pressures compared to other lignin pretreatment technologies (Mosier et al. 2005). This pretreatment process is also well suited for softwood material such as rice straw, as this material has lower lignin content than hardwood (Sanchez and Cardona 2008). Compared with acid or oxidative reagents, alkaline pretreatment is the most efficient method to break the ester linkages between the polysaccharides and the lignin (Sun et al. 2000). In addition, dilute acid pretreatment leads to corrosion problems and produces toxic intermediates at higher temperatures, resulting in low sugar yield (Zhao et al. 2007). The goals of this study were to assess the effects of alkaline pretreatment on the lignocellulosic structure and chemical composition of rice straw in the enhancement of sugar production by enzymatic hydrolysis of alkaline-treated rice straw.

**EXPERIMENTAL**

**Biomass Collection and Preparation**

Rice straw was collected from Sekinchan paddy field (Selangor, Malaysia). It was sundried and ground to 2 mm using a hammer mill grinder. Ground rice straw was stored in a cold room at 4 °C until use.
Alkaline Pretreatment

In the alkaline pretreatment method, the rice straw was soaked in 2% (w/v) alkaline solutions at different temperatures. The ratio of the liquid and solid was maintained as 10:1. The chemicals used were sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide (Ca(OH)₂), and sodium hypochlorite (NaOCl). The treated rice straw was extensively washed with tap water to remove impurities. It was then neutralized with 1 N HCl, washed with distilled water, and dried at 60 °C for 24 h. The lignocellulose components (cellulose, hemicellulose, lignin, and ash) were analyzed using acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) according to the method of Goering and Van Soest (1970).

Different temperatures

The effects of thermal pretreatments on the production of reducing sugars were studied by comparing rice straw fibers, which had been thermally treated at different temperatures. The temperatures used for the pretreatment process were 30 °C, 100 °C, and 121 °C.

Different alkali concentrations

Various alkali concentrations were used during pretreatment, which were 0.5, 1.0, 2.0, and 5.0% (w/v). The temperature used was the optimum condition obtained from a previous experiment.

Enzymatic Saccharification

Saccharification was carried out with commercial cellulase, Celluclast 1.5 L (10 FPU/g biomass) in 50 mM of citrate buffer (pH 4.8) with 5% (w/v) of dried rice straw. The reaction mixture was then incubated at 50 °C, 150 rpm for 72 h. Samples were withdrawn at the beginning of the reaction and after every 24 h. The samples were centrifuged at 10,000 rpm for 10 min and the supernatant was taken to check the obtained reducing sugars concentration. The reducing sugars produced were determined using the dinitrosalicylic acid (DNS) method.

Different enzyme loading

The effects of using a combination of cellulase and β-glucosidase on the performance of saccharification were investigated. Ten FPU of cellulase (Celluclast 1.5 L) were mixed with 50 IU of β-glucosidase (Novozyme 188, Denmark). Saccharification with only 10 PFU cellulase and without the addition of β-glucosidase was also performed for control experiments.

Characterization of the Rice Straw Fiber

Fourier Transform Infrared (FTIR) analysis

The spectra of the untreated and treated rice straw were analyzed using an FTIR spectrophotometer (Perkin Elmer). The FTIR spectra were recorded in a range of 280 to 4000 cm⁻¹ at a resolution of 4 cm⁻¹ resolution with 32 scans.
Scanning electron microscopy

Scanning electron microscopy (Hitachi S-3400N) was used to examine the surface morphology of the raw and treated rice straw. Dried samples were coated with gold and observed using an applied tension of 5 kV.

RESULTS AND DISCUSSION

Effects of Alkaline Pretreatments at Various Temperatures on Reducing Sugar Production

Pretreatment is a crucial process step for the conversion of lignocellulosic biomass into fermentable sugars. An efficient pretreatment method is needed so that subsequent enzymatic hydrolysis gives maximal sugar productivity, while at the same time avoiding the degradation or loss of carbohydrate. Initially, the effects of different types of alkaline pretreatments (NaOH, KOH, Ca(OH)₂, and NaOCl) at different temperatures (30 °C, 100 °C, and 121 °C) were conducted to determine which alkaline pretreatment condition gave the highest sugar production after enzymatic hydrolysis. Table 1 shows the concentration of total sugars in each pretreatment obtained from enzymatic hydrolysis of untreated and alkaline-treated rice straw under different temperatures.

Generally, alkaline pretreatment with different alkaline solutions was found to improve the enzymatic hydrolysis and to result in a higher yield of reducing sugar compared to untreated rice straw. The highest yield of reducing sugar was observed when the rice straw was subjected to 2% (w/v) KOH followed by heat pretreatment at 121 °C, 15 psi where the total reducing sugar obtained was 59.90±0.52 g/L with conversion rate of 69.61±0.60%. The conversion rate was calculated by dividing the concentration of the reducing sugar obtained with the potential sugar in the rice straw multiplied by 100. It was noted that alkaline pretreatment with KOH followed by thermal treatment at 121 °C (15 psi) increased 93% to the total reducing sugar obtained when compared to the untreated rice straw. On the other hand, the yield of total reducing sugar obtained from rice straw pretreated with NaOH at 121 °C, 15 psi was only slightly lower than the KOH-treated rice straw (55.48±0.19 g/L). These results were in good agreement with the finding of Singh and Trivedi (2013), who reported that a maximum production of glucose (58%) was obtained when bagasse was pretreated with 3% (w/v) NaOH followed by autoclaving at 121 °C for 30 min. The percentage of glucose obtained was only 4% higher than the percentage of glucose obtained when bagasse was pretreated with 3% (w/v) KOH (54%) prior to enzymatic hydrolysis.

The experiment showed that the alkaline pretreatment of the rice straw with 2% (w/v) NaOH or KOH in combination with thermal pretreatment caused a considerable increase of the total reducing sugars after enzymatic hydrolysis in comparison with Ca(OH)₂ or NaOCl. This is due to the pretreatment of rice straw with alkali causing swelling of the rice straw particles and increasing the internal surface area. This in turn decreased the degree of polymerization and cellulose crystallinity (Agbor et al. 2011). Alkaline pretreatment also resulted in lignin removal, rendering cellulose in the rice straw more accessible while avoiding the formation of acetyl and other uronic acid substitutions on hemicellulose that lessen the accessibility of the enzymes to the cellulose surface (Galbe and Zacchi 2007). The heat treatment applied further expanded the cellulose and
increased the water holding capacity of the rice straw, leading to further swelling of the rice straw. Hence, the increase in the porosity of the rice straw structure may permit greater enzyme accessibility into the internal structure of the rice straw thereby increasing the yield of the reducing sugar obtained (Zhang and Cai 2008; Sun and Cheng 2002). In this study, it was observed that alkaline pretreatment with NaOCl was the least efficient method of converting the lignocellulosic biomass into reducing sugars since the sugars production was not greatly improved when applying this alkaline pretreatment method.

Table 1. Total Reducing Sugar from Saccharification of Rice Straw Pretreated with 2% (w/v) Alkaline Solutions at Three Different Temperatures with an Enzyme loading of 10 FPU of Cellulase, pH 4.8 at 50 °C

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Conversion (%)</th>
<th>Total reducing sugar (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>5.12±0.32</td>
<td>3.18±0.21</td>
</tr>
<tr>
<td>Alkaline pretreatment at 30 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% NaOH</td>
<td>46.48±0.52</td>
<td>40.40±0.45</td>
</tr>
<tr>
<td>2% KOH</td>
<td>42.99±0.60</td>
<td>37.00±0.51</td>
</tr>
<tr>
<td>2% Ca(OH)₂</td>
<td>45.83±0.36</td>
<td>29.17±0.23</td>
</tr>
<tr>
<td>2% NaOCl</td>
<td>15.96±0.12</td>
<td>12.64±0.09</td>
</tr>
<tr>
<td>Alkaline pretreatment at 100 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% NaOH</td>
<td>56.97±1.57</td>
<td>49.52±1.36</td>
</tr>
<tr>
<td>2% KOH</td>
<td>55.04±0.54</td>
<td>47.36±0.47</td>
</tr>
<tr>
<td>2% Ca(OH)₂</td>
<td>63.21±0.22</td>
<td>40.23±0.14</td>
</tr>
<tr>
<td>2% NaOCl</td>
<td>24.72±1.12</td>
<td>19.57±0.89</td>
</tr>
<tr>
<td>Alkaline pretreatment at 121 °C, 15 psi (autoclaving)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% NaOH</td>
<td>63.83±0.21</td>
<td>55.48±0.19</td>
</tr>
<tr>
<td>2% KOH</td>
<td>69.61±0.60</td>
<td>59.90±0.52</td>
</tr>
<tr>
<td>2% Ca(OH)₂</td>
<td>61.34±2.91</td>
<td>41.46±1.85</td>
</tr>
<tr>
<td>2% NaOCl</td>
<td>26.65±2.55</td>
<td>21.10±2.02</td>
</tr>
</tbody>
</table>

Effects of Different Alkaline Concentrations on Reducing Sugar Production

In a preliminary experiment, the combination of alkali (2%) and thermal pretreatment (121 °C, 15 psi) generally resulted in higher sugar production after enzymatic saccharification. Therefore, an experiment to investigate whether increasing the concentration of alkali would increase the sugar production was also performed in this study. Initially, the effects of different concentrations (2 and 5%) of alkaline pretreatments on the enzymatic saccharification of rice straw were studied, with the results obtained shown in Table 2. As expected, the sugar yield was greater with higher alkaline concentration (5%). The yield of total reducing sugar obtained by alkali pretreatment with 5% (w/v) KOH was found to be 64.32±0.23 g/L, which was higher than pretreatment with NaOH (60.89±0.78 g/L) or with Ca(OH)₂ (43.69±0.52 g/L). However, the sugar yield between the two concentration tested did not show much
difference for all types of alkaline solution, except for NaOCl. The conversion rate of rice straw pretreated with 5% (w/v) KOH was only 3% higher than the conversion rate when rice straw was pretreated with 2% (w/v) KOH. An increase in conversion rate of only 6% was seen when the NaOH concentration was increased from 2 to 5%.

As KOH and NaOH-treated rice straw exhibited higher reducing sugar compared to other alkaline-treated rice straw, the optimum alkaline concentrations of these two alkaline solutions were further investigated. As suggested by Zhang et al. (2010), lower ranges (1 to 2%) of NaOH and other bases are recommended since the chemicals are expensive and the recovery is complex.

**Table 2. Total Reducing Sugar Production after Saccharification of Rice Straw Pretreated with 2% and 5% (w/v) of Alkaline Solutions at 121°C, 15 psi with an Enzyme Loading of 10 FPU of Cellulase, pH 4.8 at 50 °C**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Conversion (%)</th>
<th>Total reducing sugar (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOH</td>
<td>64.22±0.75</td>
<td>55.82±0.66</td>
</tr>
<tr>
<td>KOH</td>
<td>71.67±0.59</td>
<td>61.67±0.51</td>
</tr>
<tr>
<td>Ca(OH)₂</td>
<td>59.37±2.12</td>
<td>37.79±1.35</td>
</tr>
<tr>
<td>NaOCl</td>
<td>29.72±1.41</td>
<td>23.53±1.12</td>
</tr>
<tr>
<td>5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOH</td>
<td>70.05±0.90</td>
<td>60.89±0.78</td>
</tr>
<tr>
<td>KOH</td>
<td>74.74±0.26</td>
<td>64.32±0.23</td>
</tr>
<tr>
<td>Ca(OH)₂</td>
<td>68.64±0.82</td>
<td>43.69±0.52</td>
</tr>
<tr>
<td>NaOCl</td>
<td>57.05±0.73</td>
<td>45.17±0.58</td>
</tr>
</tbody>
</table>

The effects of KOH and NaOH concentrations (0.5 to 5%, w/v) on the pretreatment of rice straw at 121°C, 15 psi for 15 min are presented in Fig. 1. There was an increase of sugar production by increasing the alkaline concentration from 0.5% (w/v) to 5% (w/v).

**Fig. 1.** The effects of different NaOH and KOH concentrations ranging from 0.5 to 5.0% (w/v) on the performance of saccharification of rice straw using 10 FPU of Celluclast 1.5L, at pH 4.8 and 50 °C
The total reducing sugar obtained using 0.5% (w/v) NaOH and KOH were 31.64 g/L and 25.17 g/L, respectively. The reducing sugar production obtained using NaOH and KOH were increased to 55.82 g/L and 61.67 g/L, respectively, when the concentration of alkali was increased to 2%. However, it was found that the yield of reducing sugar produced did not significantly increase when 5% (w/v) alkali concentration was used throughout the pretreatment process. This finding is in agreement with the study done by Haque et al. (2012), who suggested that dilute NaOH treatment (2%) of lignocellulosic materials would be suitable to enhance the sugar production. Many other researchers (Sahare et al. 2012; Xiao et al. 2001) have also recommended mild alkaline pretreatment as an effective option for the pretreatment of lignocellulosic materials. Based on this result, it was decided that a 2% (w/v) alkaline concentration should be used at 121 °C, 15 psi for pretreating rice straw.

Characterization of Untreated and Treated Rice Straw

Chemical compositions of untreated and alkaline-treated rice straw

The main components of untreated and rice straw pretreated with various alkaline chemicals are presented in Table 3. Overall, alkaline pretreatments changed the main components in the rice straw, which resulted in different percentages of chemical components. Generally, rice straw pretreated with either NaOH or KOH contained higher amounts of cellulose content and lower amounts of lignin and hemicelluloses compared to untreated rice straw. These results indicated that alkaline pretreatment with KOH or NaOH could enhance the solubilization of hemicelluloses and structural modification of lignin, which would further increase the cellulose content (Ibrahim et al. 2011; Sills and Gossett 2011).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Lignin (%)</th>
<th>Ash (%)</th>
<th>Others (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>37.82±0.45</td>
<td>24.22±0.58</td>
<td>12.72±0.13</td>
<td>14.48±0.23</td>
<td>10.76±0.25</td>
</tr>
<tr>
<td>2.0% NaOH</td>
<td>70.93±0.86</td>
<td>15.99±1.92</td>
<td>2.72±0.88</td>
<td>2.85±0.65</td>
<td>7.23±0.49</td>
</tr>
<tr>
<td>2.0% KOH</td>
<td>66.22±2.84</td>
<td>19.83±1.15</td>
<td>2.89±0.18</td>
<td>3.67±0.07</td>
<td>7.38±1.88</td>
</tr>
<tr>
<td>2.0% Ca(OH)2</td>
<td>56.60±0.83</td>
<td>7.24±0.16</td>
<td>8.31±0.30</td>
<td>15.45±0.10</td>
<td>12.36±0.59</td>
</tr>
<tr>
<td>2.0% NaOCl</td>
<td>52.97±1.26</td>
<td>23.36±1.31</td>
<td>7.78±1.43</td>
<td>10.33±0.17</td>
<td>5.56±0.53</td>
</tr>
</tbody>
</table>

As is shown in Table 3, NaOH-treated rice straw had increased cellulose content by 87.54%, decreased lignin by 78.62%, and decreased ash by 80.32%, which seemed more effective than KOH. The increase of the cellulose content was possibly due to the removal of a large portion of the non-sugar components (lignin and ash) during pretreatment (Silverstein et al. 2007). As the cellulose microfibrils are well protected by lignin, the removal of lignin will promote the enzymatic hydrolysis of the lignocellulosic biomass. However, lignin reduction alone may not be an appropriate indicator for overall pretreatment effectiveness (Sharma 2012).

The reduction of the ashes content in NaOH-treated rice straw can be related to the removal of silica from the rice straw. Silica gives essential growth and mechanical
strength to the plant system (Neethirajan et al. 2009). Removal of silica may be very effective in increasing the accessibility of enzymes onto the surface of rice straw, leading to higher rate of enzymatic hydrolysis. As reported by Sun et al. (2000), silica was successfully removed (62.2%) from the rice straw when 1% of NaOH (55 °C, 2 h) was used to pretreat the rice straw.

**FTIR spectra and SEM analysis**

Besides the composition of lignin and hemicellulose in lignocellulose, the chemical bonds present and the morphology of the rice straw are also possible factors to influence enzymatic hydrolysis. Therefore, FTIR and SEM were applied to determine the chemical and morphological changes taking place in the rice straw after being pretreated with alkaline solutions followed by thermal pretreatment. The chemical bonds of the rice straw fibers were investigated by FTIR spectroscopy in the region of 750 to 4000 cm⁻¹, and are presented in Fig. 2.

![FTIR spectra and SEM analysis](image)

**Fig. 2.** Chemical changes in rice straw determined by FTIR wavelength range: 750 to 4000 (cm⁻¹)

As can be seen in Fig. 2, FTIR spectroscopy analysis showed obvious changes in the functional groups of lignin and cellulose after pretreatment. The peak of O-H stretching between 3000 and 3600 cm⁻¹ and the peak of –CH₂ stretching near 2900 cm⁻¹ can be monitored to distinguish features of cellulose after pretreatment (Sun et al. 2007). Based on the FTIR spectroscopy analysis, spectra of rice straw for untreated and alkaline pretreated rice straw showed the same profile. However, rice straw treated with NaOH exhibited the weakest absorbance at 3333 cm⁻¹, which might have resulted from the degradation of cellulose (Oh et al. 2005). The decreased band intensity after NaOH pretreatment is similarly reported in Rahnama et al. (2013) and He et al. (2008). In addition, the reduction in the amount of methyl and methylene of cellulose after pretreatment could be seen by changes in the absorbance band at 2902 cm⁻¹ (Schwanninger et al. 2004). The band at 1636 cm⁻¹ was generally diminished after alkaline pretreatment, suggesting that the alkaline pretreatment leads to removal of lignin in rice straw. Meanwhile, the absorbance band near 1210 cm⁻¹, which is related to C-O stretching band of ether linkage, was significantly decreased after pretreatment by NaOH compared to...
untreated rice straw. This indicated that alkaline pretreatment with NaOH cause the delignification effect to the rice straw (Goshadrou et al. 2011). Peaks at 2860, 1420, and 1430 cm\(^{-1}\) could be attributed to the C-H deformation within the methoxyl groups of lignin (Guo et al. 2008). After alkaline pretreatment, the adsorption peaks of these bands were decreased, suggesting that the acid-soluble lignin had been released. Moreover, the adsorption peaks for the aromatic hydroxyl groups which could be observed at 1375 and 1330 cm\(^{-1}\) reduced in the pretreated rice straw (Hsu et al., 2010). These bands might be due to the cleavage of ether bonds within the lignin. Other changes include the reduction of \textit{=}C-H bending vibration at 837 cm\(^{-1}\), signifying a lignin degradation from the aromatic hydrogen bond (Bahrin et al. 2012).

Morphological changes on the cellullosic surface of untreated and treated rice straw were observed using SEM (Fig. 3). The SEM images revealed that pretreatment induced morphological changes in the biomass. When compared with untreated rice straw, which was covered with a clear and smooth surface (Fig. 3A), the surface of the NaOH-treated rice straw was rugged and rough (Fig. 3B). The structure of KOH-treated rice was damaged and looked soft (Fig. 3C). When the rice straw was pretreated with Ca(OH)\(_2\), the surface area of the rice straw became more porous (Fig. 3D). These findings indicate that alkaline pretreatment promoted the removal of external fibers. The removal of external fibers will lead to an increase in the external surface area and the porosity of the rice straw, thus facilitating enzymatic hydrolysis. However, it can be seen clearly in Fig. 3E that the NaOCl-treated rice straw was completely different in morphology as compared with untreated and pretreatment with other alkaline solutions. The surface area of NaOCl-treated rice straw had many micropores. Considering the results of SEM and enzymatic saccharification, it is interesting to note that the total reducing sugar obtained after treatment with NaOCl was lower in contrast with NaOH and KOH pretreatment (Table 1). Even the rice straw NaOCl-treated rice straw contained many micropores compared to others. This means that, for NaOH and KOH, the treatment depolymerized and degraded the hemicelluloses while most of the structure of the cellulose was preserved. In contrast, pretreatment with NaOCl caused the cellulose structure to depolymerize and release of glucose. The release of glucose from the rice straw during pretreatment with NaOCl may have resulted in lower amounts of cellulose remaining in the pretreated solid residue, and thus decrease the glucose available for the enzymatic saccharification process (Hsu et al. 2010).

**CONCLUSIONS**

1. The alkaline pretreatment methods that resulted in lower lignin contents in the pretreated rice straw generally led to a higher enzymatic saccharification rate due to the higher enzyme accessibility to the surface of the rice straw.

2. Alkaline pretreatments with 2\% (w/v) KOH and NaOH followed by thermal pretreatment at 121 °C and 15 psi resulted in 58.5 to 64.5\% higher conversion rates of reducing sugars production than untreated rice straw, showing that alkaline pretreatments were effective for production of sugars from rice straw.

3. FTIR and SEM investigations showed that alkaline pretreatments caused chemical and morphological changes in the rice straw. The peaks of the cellulose and lignin
materials were decreased after alkaline pretreatment, indicating that some cellulose and lignin was degraded.

**Fig. 3.** Scanning electron microscope images of rice straw (3000×). (A) Untreated rice straw; (B) pretreatment with NaOH; (C) pretreatment with KOH; (D) pretreatment with Ca(OH)$_2$; and (E) pretreatment with NaOCl.
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