Pretreatment of Corn Stover Using Organosolv with Hydrogen Peroxide for Effective Enzymatic Saccharification

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Abstract: In this study, the chemical pretreatment method using ethanol organosolv with hydrogen peroxide was investigated to improve enzymatic saccharification of corn stover. The pretreatment method using ethanol with hydrogen peroxide in a flow-through reaction was proposed to lower the reaction severity such as the pretreatment temperature. With the same reaction time, the pretreatment process using organosolv (30 wt.% ethanol) containing 1 wt.% hydrogen peroxide at 150 °C resulted in a similar conversion yield as the result of the alkali pretreatment method using 15 wt.% aqueous ammonia at 170 °C. When corn stover was pretreated with 30 wt.% ethanol solution containing 5 wt.% hydrogen peroxide, a glucose conversion yield of 69.7 wt.% and glucose production of 23.8 g were achieved.

Keywords: biomass; pretreatment; corn stover; ethanol; organosolv; hydrogen peroxide

1. Introduction

Due to its high carbohydrate content, lignocellulosic biomass can be a good source of fermentable sugar for biofuel production [1]. In the bioconversion process of lignocellulosic biomass into biofuel such as fuel ethanol, pretreatment is essential and commonly performed to crack the lignin structure and loosen the crystalline structure of cellulose. Lignocellulosic biomass must be transformed to render the cellulose more accessible to enzymes that convert it into fermentable sugars. These sugars can be used as sources in the bioconversion process for the production of biofuels and chemicals. Various pretreatment processes have shown great potential for improving the efficiency of biomass utilization and lowering the processing costs of conversion processes [2,3]. Among various renewable cellulosic materials, corn stover is one of the most abundant, inexpensive and readily available resources. However, using conventional processing techniques with these carbohydrate sources for glucose and ethanol production has been shown to have low efficiency. The low cellulose-to-glucose conversion efficiency for lignocellulosic biomass is the result of some primary factors: the close physical and chemical association between carbohydrates and lignin and the crystallinity of the cellulose polymer itself [4]. Crystallinity is an important factor to consider in biomass pretreatment. It is known that chemical pretreatments modify the cellulose structure and crystallinity of lignocellulosic biomass, e.g., cellulose is modified to cellulose III during ammonia pretreatment, whereas organosolv pretreatment generally does not significantly modify the cellulose structure, but it depolymerizes the cellulose and removes amorphous lignin and hemicellulose, resulting in the change of crystallinity [5]. The modification of these structures is the main factor affecting the economics of the bioethanol production process, and much research has been carried out worldwide to develop the processes [1,6].
Biomass recalcitrance is an important feature of lignocellulosic biomass that hinders the enzyme reaction for the cellulose hydrolysis [6]; e.g., three main constituents in lignocellulosic biomass such as cellulose, hemicellulose and lignin and the nature of their intra- and inter-molecular interactions have been known to contribute to biomass recalcitrant. Among them, hemicellulose has covalent linkages with lignin forming lignin carbohydrate complexes and association with cellulose, which play an important role in hindering enzymatic hydrolysis of cellulose [6]. Modification of biomass structure and chemical composition through chemical pretreatment can be a major factor affecting the enzymatic saccharification of cellulose, and there have been many research efforts to develop an effective pretreatment method [1,7]. The organosolv pretreatment method is similar to the organosolv pulping process, but the extent of lignin removal in organosolv pretreatment is not as high as the organosolv pulping process. In organosolv pretreatment, ethanol is frequently used as the catalytic solvent for biomass pretreatment. Ethanol pretreatment is known as an effective pretreatment method of delignification and improves enzymatic hydrolysis. As the lignin is removed, typically, increasing enzymatic hydrolysis is observed [8,9]. Ethanol used in organosolv pretreatment can be easily recovered by the conventional distillation process and can be recycled. After pretreatment, ethanol recovery in the organosolv pretreatment process can separate lignin and carbohydrates, both of which are potential chemical feedstocks [10,11]. Mixed ethanol and hydrogen peroxide solutions have been used as a pretreatment for reducing wastewater production and improving recyclability. Ethanol is easily recyclable and has significant economic advantages [12,13]. Hydrogen peroxide has been used as an environmentally-friendly delignifying reagent, which enhances oxidative depolymerization of the lignin polymer in lignocellulosic biomass. Because hydrogen peroxide is widely used in the biomass pretreatment and bleaching process of the pulp and paper industry [14,15], it was anticipated that the addition of hydrogen peroxide to pretreatment using ethanol in this study would not only improve the pretreatment efficiency, but also reduce the reaction severity (temperature and time).

Conventional batch reactions such as the soaking process can only be operated at low temperatures due to the decomposition of carbohydrates in high temperature reactions, and thus, the reaction rate and efficiency are inevitably low. Furthermore, in the batch reaction mode, both pretreatment reagent and biomass remain together in the reactor during the reaction. Therefore, the solubilized components can undergo a side reaction at high temperatures; e.g., repolymerization and condensation, representing a large drawback. On the contrary, the flow-through process can effectively reduce the side reactions of released components during the pretreatment reaction, because the solubilized components can be removed as soon as they are released and collected in a reservoir tank [16]. In addition, the flow-through process requires shorter reaction times than that of the batch process.

In this study, the pretreatment process for corn stover by using an ethanol and hydrogen peroxide mixture was investigated and compared to the other pretreatment method such as aqueous ammonia pretreatment at mild temperatures. The organosolv pretreatment process was evaluated by comparing the reaction time and temperature and changing the concentration of hydrogen peroxide added to the ethanol solution.

2. Materials and Methods

2.1. Materials

Corn used in this study (Zea mays var. saccharata) was planted and harvested in East China in September 2015. The cellulosic residues of corn, i.e., corn stover (stalks and leaves), were collected and provided by the CJ Cheiljedang Corporation (Seoul, Korea). Corn stover was ground, passed through a sieve (50–100 mesh), dried and stored until it was used. The chemical composition of corn stover was analyzed to contain 34.2 wt.% cellulose, 22.4 wt.% hemicellulose and 13.3 wt.% acid-insoluble lignin (AIL). Ethanol (Duksan, CAS No. 64-17-5), ammonium hydroxide (Duksan, CAS No. 1336-21-6) and hydrogen peroxide (Duksan, CAS No. 7722-84-1) were purchased from Duksan Pure Chemicals Co. Ltd. (Ansan, Gyeonggi-do, Korea). Cellulase, Cellic® CTec2 (batch number: VCP10006, Novozymes
Inc., Bagsvaerd, Denmark), was used for enzymatic hydrolysis, and its activity was 60 FPU (filter paper units)/mL. The activity of the enzyme was measured following the standard laboratory analytical procedure (LAP) of the National Renewable Energy Laboratory (NREL; Golden, CO, USA).

2.2. Flow-Through Pretreatment

Pretreatment of biomass was carried out using a flow-through column reactor system. The chemical pretreatment of biomass was tested under various reaction conditions (pretreatment temperature, pretreatment time and chemical concentration). The pretreatment reactor system included a chemical reagent solution container, a high-pressure pump, a temperature-programmable gas chromatography oven, a stainless steel (SS-316) tubular reactor with a total volume of 85.5 cm$^3$ (i.e., 2.8 cm internal diameter × 13.9 cm length,) and a liquid sample collecting tank. The reactor system was operated in flow-through mode, wherein the chemical reagent solution was reacted with the packed biomass in the tubular reactor. The reactor system was under pressure by a nitrogen gas cylinder at 2.3 MPa to prevent flash evaporation. For the pretreatment experiment in this system, 20 g (dry basis) of biomass were placed in the reactor. The pretreatment reaction started by raising the reactor temperature using a convection oven. Preheating of the reactor was conducted for 15 min until the reactor temperature reached the target temperatures. The reaction time began once the internal temperature of the reactor reached the targeted temperature, and all pretreatment experiments were conducted in duplicate. The flow rate for flow-through reaction was 3.3 mL/min [17,18]. When the pretreatment reaction was completed, the pump was turned off, and the liquid sample in the liquid collecting reservoir was recovered. Then, the reactor was cooled in the air until it reached ambient temperature. The pretreated solid samples were recovered, washed and dried in an oven at mild temperature (45 °C) prior to storage.

2.3. Ammonia Soaking Pretreatment

The corn stover was pretreated by the ammonia soaking method. The pretreatment conditions were a 48-h pretreatment time at a liquid/solid ratio of 10 and at 60 °C. After pretreatment, the treated corn stover was recovered by filtration and washed with clean water (deionized water) until the wash water reached pH 7.0. The solid samples were dried in a drying oven at 45 °C prior to storage [18].

2.4. Enzymatic Digestibility Test

The pretreated corn stover was subjected to the enzymatic digestibility test using 0.1 M citrate buffer solution (pH 4.8) in 250-mL Erlenmeyer flasks. The enzymatic digestibility test conditions were 2.0 wt.% solid concentration, 50 °C, 150 rpm, 72 h and enzyme loading of 40 FPU/g of cellulose. The enzymatic digestibility of sugar was calculated as follows [19,20]:

Glucan digestibility (\%) = \( \frac{\text{Amount of released glucose} (g) \times 0.9}{\text{Total initial glucan} (g) \text{ in the reactor}} \times 100 \) (1)

XMG digestibility (\%) = \( \frac{\text{Amount of released xmg} (g) \times 0.88}{\text{Total initial XMG} (g) \text{ in the reactor}} \times 100 \) (2)

where 0.9 and 0.88 are the factors for conversion of glucose to equivalents of glucan and xmg (xylose + mannose + galactose) to equivalents of XMG (xylan + mannan + galactan), respectively.

2.5. Analytical Methods

The contents of sugars and acid-insoluble lignin (AIL) were analyzed following the standard LAP of NREL [21]. The chemical compositions of the solid samples obtained from pretreatment and the liquid sample from enzymatic digestion were determined using high-performance liquid chromatography (HPLC) and the Bio-Rad Aminex HPX-87H column (Bio-Rad Inc., Hercules, CA, USA; Cat. No. 1250098), as well as a refractive index (RI) detector (Model No. Waters 410, Waters Co., Milford, MA, USA). Sulfuric acid (H$_2$SO$_4$, 5.0 mM), as the mobile phase, was pumped into the HPLC
at a 0.6-mL/min flow rate and 60 °C column temperature. To prepare the liquid samples for the HPLC system, particles in the liquid samples were precipitated using a centrifuge for 10 min at 15,000 rpm and then filtered using 0.2-µm nylon syringe filters.

3. Results and Discussion

3.1. Pretreatment of Corn Stover

To make cellulose more accessible by enzymatic hydrolysis for effective production of fermentable sugars [22,23], pretreatments of corn stover using aqueous ammonia and organosolv were compared and evaluated. The aqueous ammonia pretreatment was carried out for comparison with the organosolv pretreatment. The aqueous ammonia pretreatments were conducted at the preselected conditions (Table 1) in the previous studies using two different reaction modes including batch-type soaking and flow-through processes [17,18]. According to Table 1, the solids remaining after aqueous ammonia pretreatment were 69.9 wt.% for the soaking method at moderate temperature (60 °C) and 44.9 wt.% for the flow-through process at high temperature (170 °C). In the organosolv pretreatment using 30 wt.% ethanol without hydrogen peroxide, the remaining solids were 62.9 wt.%. Organosolv pretreatment (30% ethanol with 1 wt.% H₂O₂, 5 wt.% and 10 wt.% hydrogen peroxide) resulted in remaining solids of 52.8 wt.%, 39.3 wt.% and 30.8 wt.% respectively. As the addition of hydrogen peroxide increased, the remaining solids decreased by 16–51 wt.% compared with the organosolv pretreatment without hydrogen peroxide. It was confirmed that the remaining solids decreased with increasing hydrogen peroxide concentration in the organosolv pretreatment process. When the concentration of hydrogen peroxide was 5 wt.% or more, despite the relatively low reaction temperature, remaining solids were reduced compared to those of aqueous ammonia pretreatment in flow-through mode. However, the remaining solids alone cannot be used for the evaluation of the pretreatment effect.

<table>
<thead>
<tr>
<th>Pretreatment Conditions</th>
<th>Remaining Solids (wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solutions</td>
<td>Process Type</td>
</tr>
<tr>
<td>30 wt.% ethanol only</td>
<td>Flow-through</td>
</tr>
<tr>
<td>30 wt.% ethanol with 1 wt.% H₂O₂</td>
<td>Flow-through</td>
</tr>
<tr>
<td>30 wt.% ethanol with 5 wt.% H₂O₂</td>
<td>Flow-through</td>
</tr>
<tr>
<td>30 wt.% ethanol with 10 wt.% H₂O₂</td>
<td>Flow-through</td>
</tr>
<tr>
<td>15 wt.% aqueous ammonia</td>
<td>Soaking</td>
</tr>
<tr>
<td>15 wt.% aqueous ammonia</td>
<td>Flow-through</td>
</tr>
</tbody>
</table>

Therefore, the chemical composition changes of the pretreated solids and untreated corn stover are presented in Tables 2 and 3. We examined the compositional changes such as glucan, XMG (sum of xylan, mannan and galactan) and lignin contents in the treated solid and compositions of the recovered liquid. As the concentration of hydrogen peroxide increased, the recovery yield of XMG decreased by 3.1 wt.% at 10 wt.% hydrogen peroxide, and the delignification was 90.3 wt.% (Table 2). However, the glucan content slightly decreased, but it was not greater than other component changes. A significant reduction of the solid remaining in the pretreated corn stover was presumed to be due to decreased XMG and lignin content. Upon pretreatment with aqueous ammonia and ethanol under similar conditions, the recovery yields of glucan from pretreated corn stover were 70.8 wt.% and 83.3 wt.%, respectively, for the flow-through process (Tables 2 and 3). The XMG recovery yield of the aqueous ammonia pretreatment was 48 wt.% lower than that of the ethanol process. In addition, delignification trends were similar in both processes ranging from 45.6–50.1 wt.%. As the concentration of hydrogen peroxide in the ethanol solution increased, delignification increased to 90.3% at 10 wt.% hydrogen peroxide, and the recovery yield of glucan was significantly reduced to 73.5 wt.%. The addition of 10 wt.% hydrogen peroxide to the organosolv pretreatment resulted in a 44 wt.% increase in delignification, even though its pretreatment temperature was not higher (150 °C).
than that (170 °C) of the aqueous ammonia process in flow-through mode and 170–190 °C of our previous reports [12,13]. The aqueous ammonia soaking process at 60 °C for 48 h, which is much lower than the temperature of the flow-through process (170 °C), resulted in near complete glucan recovery of 96.9 wt.%. According to the previous study, the typical temperature range of pretreatment using aqueous ammonia in flow-through mode was 170–210 °C [15–17]. However, as the reaction time increased to 48 times, the operating cost can be greatly increased if a large-scale plant were built. It was concluded that the ethanol pretreatment with hydrogen peroxide removed lignin significantly, even at relatively low temperatures.

Table 2. Contents and variation rate of major components before and after organosolv pretreatment, XMG (xylan + mannan + galactan).

<table>
<thead>
<tr>
<th>Pretreatment Conditions</th>
<th>Glucan</th>
<th>XMG</th>
<th>Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contents (wt.%)</td>
<td>Recovery Yield (wt.%)</td>
<td>Contents (wt.%)</td>
</tr>
<tr>
<td>Untreated corn stover</td>
<td>34.2</td>
<td>-</td>
<td>22.4</td>
</tr>
<tr>
<td>Flow-through 150 °C/60 min</td>
<td>28.5</td>
<td>83.3</td>
<td>17.4</td>
</tr>
<tr>
<td>30 wt.% EtOH</td>
<td>28.1</td>
<td>82.1</td>
<td>13.4</td>
</tr>
<tr>
<td>30 wt.% EtOH with 1 wt.% H₂O₂</td>
<td>27.1</td>
<td>79.2</td>
<td>6.5</td>
</tr>
<tr>
<td>30 wt.% EtOH with 5 wt.% H₂O₂</td>
<td>25.1</td>
<td>73.5</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Table 3. Contents and variation rate of major components before and after aqueous ammonia pretreatment.

<table>
<thead>
<tr>
<th>Pretreatment Conditions</th>
<th>Glucan</th>
<th>XMG</th>
<th>Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contents (wt.%)</td>
<td>Recovery Yield (wt.%)</td>
<td>Contents (wt.%)</td>
</tr>
<tr>
<td>Untreated corn stover</td>
<td>34.2</td>
<td>-</td>
<td>22.4</td>
</tr>
<tr>
<td>Soaking 60 °C 48 h</td>
<td>33.1</td>
<td>96.9</td>
<td>19.0</td>
</tr>
<tr>
<td>Flow-through 170 °C 60 min</td>
<td>24.2</td>
<td>70.8</td>
<td>9.0</td>
</tr>
</tbody>
</table>

3.2. Enzymatic Digestibility of Pretreated Corn Stover

Pretreatment of the corn stover under various condition was performed to examine subsequent enzymatic hydrolysis. For the enzymatic digestibility in Table 4, total produced glucose at 72 h of enzymatic hydrolysis (at 50 °C) was used to measure the enzymatic digestibility for glucan. Table 4 shows that the enzyme digestibility of the untreated corn stover was 32.8%, whereas that of the corn stover pretreated with 30 wt.% ethanol was increased to 51.5%; despite the pretreatment, the enzymatic digestibility only increased to a small extent. However, the aqueous ammonia pretreatment in two different modes, soaking and flow-through processes, showed an enzymatic digestibility of 69.6% and 74.5%, respectively. Pretreatment using aqueous ammonia was determined to be more effective than ethanol pretreatment in increasing enzyme digestibility of the corn stover. However, because the recovery and reuse of aqueous ammonia is rather difficult, organosolv, which can be recovered and reused, pretreatment remains desirable compared to ammonia pretreatment. In order to enhance the enzymatic digestibility of the organosolv-treated sample, 1–10 wt.% of hydrogen peroxide was added to the ethanol for more effective pretreatment. As a result of pretreatment using ethanol and hydrogen peroxide, the enzymatic digestibility of treated corn stover was increased to 91.0% for glucan with pretreatment using ethanol +10 wt.% hydrogen peroxide, which was also much higher than the 69–76% of the previous organosolv pretreatment study [12,13]. As the hydrogen peroxide concentration increased, the enzymatic digestibility greatly increased compared to that of the corn stover pretreated without hydrogen peroxide. The ethanol + hydrogen peroxide pretreatment in the flow-through reactor showed a much higher enzymatic digestibility even when performed at a lower temperature than that of the aqueous ammonia process.
Table 4. Enzymatic digestibility of pretreated corn stover by organosolv.

<table>
<thead>
<tr>
<th>Pretreatment Conditions</th>
<th>Solutions</th>
<th>Process Type</th>
<th>Temperature</th>
<th>Reaction Time</th>
<th>Glucan</th>
<th>XMG/Xylan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated corn stover</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32.8</td>
<td>14.8</td>
</tr>
<tr>
<td>30 wt.% EtOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>51.5</td>
<td>28.8</td>
</tr>
<tr>
<td>30 wt.% EtOH with 1 wt.% H₂O₂</td>
<td>Flow-through</td>
<td>150 °C</td>
<td>60 min</td>
<td>63.5</td>
<td>39.9</td>
<td></td>
</tr>
<tr>
<td>30 wt.% EtOH with 5 wt.% H₂O₂</td>
<td>Flow-through</td>
<td>150 °C</td>
<td>60 min</td>
<td>87.9</td>
<td>59.6</td>
<td></td>
</tr>
<tr>
<td>30 wt.% EtOH with 10 wt.% H₂O₂</td>
<td>Flow-through</td>
<td>150 °C</td>
<td>60 min</td>
<td>91.0</td>
<td>67.8</td>
<td></td>
</tr>
<tr>
<td>15 wt.% aqueous ammonia</td>
<td>Soaking</td>
<td>60 °C</td>
<td>48 h</td>
<td>69.6</td>
<td>56.5</td>
<td></td>
</tr>
<tr>
<td>15 wt.% aqueous ammonia</td>
<td>Flow-through</td>
<td>170 °C</td>
<td>60 min</td>
<td>74.5</td>
<td>77.6</td>
<td></td>
</tr>
</tbody>
</table>

3.3. Mass Balance of Organosolv Pretreatment

The conversion yield of glucose in the pretreated corn stover was expressed through mass balance after completion of enzymatic hydrolysis from the initial biomass. Figure 1 shows the mass balance of the aqueous ammonia pretreatment processes in two different modes. The pretreatment results were compared between the soaking and flow-through processes. The pretreated corn stover from the initial biomass exhibited a conversion yield and overall production of glucose of 67.4 wt.% and 23.0 g, respectively, in the soaking process and 52.7 wt.% and 18.0 g, respectively, for the flow-through process. After ammonia soaking pretreatment, the amount of residual glucan was 27 wt.% higher than that of the biomass pretreated with the flow-through process. Thus, the conversion yield and glucose concentration of the corn stover pretreated with the soaking process were higher than those of the flow-through process. However, because the reaction time of the ammonia soaking process is considerably longer than that of the flow-through process, a long pretreatment time and associated low economic efficiency can present problems when the ammonia soaking method is scaled up.

Figure 1. Mass balance of the pretreatment process using aqueous ammonia in two different modes. Pretreatment conditions: (a) soaking process, 60 °C temperature, 48 h reaction time; (b) flow-through process, 170 °C temperature, 60 min reaction time, 3.3 mL/min flow rate. Enzymatic hydrolysis test conditions: 2.0 wt.% substrate concentration, 72 h, 40 FPU/g of cellulose, 0.05 M citrate buffer, pH 4.8, 50 °C and 150 rpm.

Figure 2 presents the mass balance of the pretreatment process using ethanol and hydrogen peroxide. The glucose production and conversion yield of the corn stover pretreated using 30 wt.% ethanol were 14.7 g and 42.9 wt.%, respectively. The pretreatment using the organosolv process alone resulted in relatively lower conversion yield, which was even lower than that of aqueous
ammonia pretreatment in Figure 1, while the pretreatment using organosolv and hydrogen peroxide showed a higher conversion yield at the same reaction conditions of the pretreatment using organosolv alone. Again, the addition of hydrogen peroxide to enhance the pretreatment effect showed that glucose production and conversion yield were increased compared with pretreatment by ethanol alone. The result of the flow-through pretreatment process with organosolv containing 1 wt.% hydrogen peroxide in 30 wt.% ethanol at 150 °C exhibited similar results as the flow-through pretreatment process with 15% aqueous ammonia at 170 °C for the same reaction time. In addition, the corn stover pretreated with 30 wt.% ethanol containing 5 wt.% hydrogen peroxide exhibited a glucose conversion yield of 69.7 wt.% and produced 23.8 g of glucose. In the bioconversion of corn stover to biofuel, it was speculated that the pretreatment using ethanol and hydrogen peroxide may lower the reaction temperatures and reduce the reaction times. It also should be noted that the pretreatment process using ethanol has advantages because ethanol is a non-toxic chemical and easy to recover and reuse (recyclability) compared to ammonia.

Figure 2. Mass balance of the pretreatment process using organosolv alone and organosolv + hydrogen peroxide. Note: Pretreatment conditions; flow-through mode, 150 °C temperature, 60 min reaction time, 3.3 mL/min flow rate. Enzymatic hydrolysis test conditions: 2.0 wt.% substrate concentration, 72 h hydrolysis time, 40 filter paper units (FPU)/g of cellulose, 0.05 M citrate buffer, pH 4.8, 50 °C and 150 rpm.

4. Conclusions

In this study, the possibility of a pretreatment method using ethanol and hydrogen peroxide was investigated for fermentable sugar (monomeric sugar) production.

The flow-through pretreatment process with organosolv containing 1 wt.% hydrogen peroxide in 30 wt.% ethanol at 150 °C showed similar results as the flow-through pretreatment process with 15 wt.% aqueous ammonia at 170 °C for the same reaction time. In addition, corn stover pretreated with 30 wt.% ethanol containing 5 wt.% hydrogen peroxide resulted in the highest conversion yield (69.7 wt.%) and
produced 23.8 g of glucose out of 100 g (dry basis) corn stover. Ethanol in the organosolv pretreatment process is easier to recover and reuse than aqueous ammonia; therefore, it was concluded that the use of ethanol with hydrogen peroxide can be more appropriate for pretreatment of corn stover in a large-scale plant. It was observed that the organosolv process using ethanol with hydrogen peroxide resulted in a higher conversion yield and higher production of glucose at lower reaction temperatures than the ammonia pretreatment process.

Author Contributions: Y.C.P. conducted all experiments, summarized the data, and drafted a manuscript. T.H.K. and J.S.K. designed overall study and experiments, interpreted the results, wrote a manuscript, and finalized the manuscript. T.H.K. and J.S.K. equally contributed. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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