Comparison of Hydrolysis Conditions to Recover Reducing Sugar from Various Lignocellulosic Materials

Somkid Deejing* and Wuttichai Ketkorn
Department of Biology, Faculty of Science, Maejo University, Chiang Mai, 50290, Thailand.
*Author for correspondence; e-mail: somkid_d@mju.ac.th

ABSTRACT
Lignocellulosic materials such as rice straw, corn hull, corn stover and vetiver grass leaves were pretreated with 2.0 M NaOH and hydrolyzed with 1.0, 5.0 and 10.0% (v/v) sulfuric acid at 100, 111 and 121°C for 15 and 30 min. Among the test conditions, the maximum reducing sugar concentration from various hydrolysis condition obtained by hydrolysis with 1.0% (v/v) sulfuric acid at 111°C for 30 min for all materials. In this study, vetiver grass leaves was selected. The optimal condition of pretreatment and hydrolysis of vetiver grass leaves were investigated. It was found that the pretreatment with 1.0 M NaOH and hydrolysis with 2.0% (v/v) sulfuric acid at 111°C for 30 min showed high reducing sugar concentration.

Keywords: hydrolysis, lignocellulose, ethanol.

1. INTRODUCTION
Lignocellulosic materials are the most abundant organic compounds in nature and represent an important resource for producing valuable products. Thailand is an agricultural country, a lot of agricultural wastes are available in every year. Agricultural residues such as rice straw, corn hull, corn stover, bagasse and vetiver grass contain lignocellulose as the major component. Bioethanol can be produced from lignocellulosic materials using pretreatment followed by hydrolysis and fermentation [1-4]. Pretreatment is one of the most important steps in the process and high cost in the production of ethanol from lignocellulosic materials. The most common chemical pretreatment methods used for cellulosic feedstocks are dilute acid, alkaline, organic solvent, ammonia, sulfur dioxide of other chemicals to make the biomass more digestible by enzymes. Lignocellulosic materials requires a particular combination of the pretreatment methods to optimize the yields of the feedstock, minimize the degradation of substrate, and maximize the sugar yield. One of the most thoroughly investigated methods is steam pretreatment using an acid catalyst [5]. Steam pretreatment of corn stover at 190°C for 5 min using SO₃ as acid catalyst has been shown to give high sugar yields (almost 90% overall glucose yield and almost 80% overall xylose yield) after 72 h enzymatic hydrolysis [6]. Alkali pretreatment refers to the
application of alkaline solutions to remove lignin and various uronic acid substations on hemicellulose that lower the accessibility of enzyme to the hemicellulose and cellulose [7]. Generally alkaline pretreatment is more effective on agricultural residues and herbaceous crops than wood materials. There are three major hydrolysis processes for agricultural and wastes to produce a variety of sugars capable of making ethanol: dilute acid, concentrated acid, and enzymatic hydrolysis [8]. The main advantage of the low concentration acid in the hydrolysis process is that acid recovery may not be required and there will be no significant losses of acid. The diluted acid hydrolysis process uses high temperature (160°C) and pressure (10 atm). The acid concentration in the dilute acid hydrolysis process is in the range of 2-5% [9].

The objective of this study was to compare the pretreatment and hydrolysis condition giving high reducing sugar concentration from various lignocellulosic materials.

2. MATERIALS AND METHODS
2.1 The Hydrolysis Condition of Various Lignocellulosic Materials
Rice straw, corn hull, corn stover and vetiver grass leaves were air dried for 1 week and used as raw materials. The materials were pretreated with 2.0 M NaOH for 24 hours at ambient temperature and then washed with distilled water and dried overnight at 80°C. Three gram of pretreated materials were hydrolyzed with 20 ml of 1.0, 5.0 and 10.0% (v/v) sulfuric acid under autoclaving condition at 100, 111 and 121°C for 15 and 30 min. The hydrolysis was performed in triplicate at each condition. The supernatant was determined for total reducing sugar concentration by using glucose as standard follow Somogyi method [10]. The lignocellulosic materials which giving high reducing sugar concentration was selected.

2.2 Optimization of Pretreatment of Selected Lignocellulosic Materials with Sodium Hydroxide
The material giving high reducing sugar concentration was pretreated with 1.0, 2.0 and 4.0 M NaOH at ambient temperature for 24 hours. After pretreatment, the raw material was washed by distilled water and dried at 80°C. The pretreated material was hydrolysed according to the optimal condition. The hydrolysate was centrifuged at 6,000 rpm for 15 min. The supernatant was determined the total reducing sugar concentrations by Somogyi method. The treatment was performed in triplicate at each condition.

2.3 Optimization of Hydrolysis of Selected Lignocellulosic Materials with Sulfuric Acid
After the pretreatment of selected material under optimal sodium hydroxide concentration, the samples was hydrolysed with 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% (v/v) sulfuric acid at 111°C for 30 min. After that the hydrolysate solution was centrifuged at 6,000 rpm for 15 min. The total reducing sugar concentration in liquid fraction was determined follow Somogyi method. The hydrolysis was performed in triplicate at each condition.

3. RESULTS AND DISCUSSION
3.1 The Hydrolysis Condition of Various Lignocellulosic Materials
Rice straw which pretreated with 2.0 M NaOH and hydrolysed with 0, 1.0, 5.0 and 10.0% sulfuric acid at 100, 111 and 121°C for 15 and 30 min were investigated. The results of pretreatment and hydrolysis for 15 min were shown in Figure 1a. The hydrolysis at 100°C obtained reducing sugar 0, 1,341, 511 and 0 µg/ml, respectively. The reducing
sugar concentration after hydrolysis at 111°C were 0, 1,233, 468 and 0 μg/ml, respectively. The hydrolysis at 121°C the reducing sugar concentration were 0, 1,307, 179 and 0 μg/ml, respectively. The results of hydrolysis of rice straw with 0, 1.0, 5.0 and 10.0% at 100, 111 and 121°C for 30 min were shown in Figure 1b. The reducing sugar concentration after hydrolysis at 100°C were 0, 1,639, 308 and 307 μg/ml, respectively. After hydrolysis at 111°C obtained the reducing sugar concentration 0, 1,658, 326 and 0 μg/ml, respectively. The hydrolysis at 121°C obtained reducing sugar 0, 1,424, 1,002 and 52 μg/ml, respectively.

In this study, the results showed maximum reducing sugar concentration of rice straw at 1,658 μg/ml after pretreatment with 2.0 M NaOH and hydrolyzed with 0, 1.0, 5.0 and 10.0% (v/v) at 100, 111 and 121°C for a) 15 min and b) 30 min ( □ 0% H₂SO₄ ( □ 1.0% H₂SO₄ ( □ 5.0% H₂SO₄ ( □ 10.0% H₂SO₄.

Figure 1. Reducing sugar concentration of rice straw that pretreated with 2.0 M NaOH and hydrolyzed with 0, 1.0, 5.0 and 10.0% (v/v) at 100, 111 and 121°C for a) 15 min and b) 30 min ( □ 0% H₂SO₄ ( □ 1.0% H₂SO₄ ( □ 5.0% H₂SO₄ ( □ 10.0% H₂SO₄.

HCl and 0.5-1% H₃PO₃). After 3 h retention time, rice straw pentosans converted to a solution of monosaccharides, suitable for fermentation. Roberto et al. [14] studied the effects of H₂SO₄ concentration and retention time on the production of sugars and the by-products from rice straw at relative low temperature (121°C) and long time (10-30 min) in a 350 L batch reactor. The optimum acid concentration of 1% and retention time of 27 min was found to attain high yield of xylose (77%). Acid and water impregnation followed by steam explosion of barley straw was the best pretreatment in terms of resulting glucose concentration in liquid hydrolysate [15]. The highest yield of xylose (indicating efficient hydrolysis of hemicellulose) from Bolivian straw were found at a temperature of 190°C, and reaction time of 5-10 min, whereas considerably higher temperature...
(230°C) were needed for hydrolysis of cellulose [16]. The treatment of rice straw with sulfuric acid increase digestibility 57.0% [17].

The corn hull was pretreated with 2.0 M NaOH and hydrolyzed with 0, 1.0, 5.0 and 10.0% (v/v) sulfuric acid at 100, 111 and 121°C for 15 and 30 min. The results of pretreatment and hydrolysis for 15 min were shown in Figure 2a. The reducing sugar concentration after hydrolysis at 100°C were 42, 1,238, 180 and 0 μg/ml, respectively. The hydrolysis at 111°C the reducing sugar concentration were 50, 1,320, 112 and 0 μg/ml, respectively. After hydrolysis at 121°C the reducing sugar concentration obtained 0, 1,307, 179 and 0 μg/ml, respectively. The hydrolysis of corn hull with 0, 1.0, 5.0 and 10.0% at 100, 111 and 121°C for 30 min were shown in Figure 2b. The reducing sugar concentration after hydrolysis at 100°C were 22, 1,656, 258 and 0 μg/ml, respectively. After hydrolysis at 111°C the reducing sugar obtained 50, 1,674, 53 and 0 μg/ml, respectively. The reducing sugar concentration after hydrolysis at 121°C were 3, 1,579, 1,121 and 108 μg/ml, respectively. In this study, the results showed maximum reducing sugar concentration of corn hull 1,674 μg/ml after pretreatment with 2.0 M NaOH and hydrolysis with 1.0% (v/v) sulfuric acid at 111°C for 30 min. Saha and Bothast [18] found that the dilute sulfuric acid (0.5–1.0% (v/v) pretreatment of corn fiber at 121°C obtained the monomeric sugar yield 85–100% of the theoretical yield.

![Figure 2](image-url)

Figure 2. Reducing sugar concentration of corn hull that pretreated with 2.0 M NaOH and hydrolyzed with 0, 1.0, 5.0 and 10.0% (v/v) sulfuric acid at 100, 111 and 121°C for a) 15 min and b) 30 min (□) 0% H₂SO₄ (□) 1.0% H₂SO₄ (□) 5.0% H₂SO₄ (□) 10.0% H₂SO₄.

The pretreatment of corn stover with 2.0 M NaOH and hydrolysis with 0, 1.0, 5.0 and 10.0% (v/v) sulfuric acid at 100, 111 and 121°C for 15 and 30 min were studied. The results in Figure 3a showed reducing sugar concentration after hydrolysis under for 15 min. The hydrolysis at 100°C obtained the reducing sugar concentration 78, 1,605, 1,123 and 0 μg/ml, respectively. The reducing sugar concentration after hydrolysis at 111°C were 89, 1,409, 1,246 and 0 μg/ml, respectively. After hydrolysis at 121°C obtained reducing sugar 12, 1,673, 1,513 and 327 μg/ml, respectively. The hydrolysis with 0, 1.0, 5.0 and 10.0% (v/v) sulfuric acid at 100, 111 and 121°C for 30 min were examined. The results
were shown in Figure 3b. The reducing sugar concentration after hydrolysis at 100°C were 81, 1,711, 1,318 and 0 μg/ml, respectively. The hydrolysis at 111°C obtained reducing sugar concentration 70, 1,785, 1,367 and 15 μg/ml, respectively. The hydrolysis at 121°C the reducing sugar concentration were 1, 1,672, 1,599 and 591 μg/ml, respectively. In this study, the results showed maximum reducing sugar concentration of corn stover 1,785 μg/ml after pretreatment with 2.0 M NaOH and hydrolysis with 1% (v/v) sulfuric acid at 111°C for 30 min. Wyman et al. [19] reported an increase of the overall sugar yield from 56.8% (when using water pretreated corn stover) up to 93% by diluted sulfuric acid pretreatment. This improvement was attributed to the increase of xylan solubilization much faster than xylose degradation, due to acid action. After hydrolysis of corn stover with diluted sulfuric acid, glucose yield are maximized at short time (6 sec) and high temperature (240°C) [20]. The glucose yield (96-104%) was obtained from acid catalysed steam pretreated corn stover and xylose yields increase the 70-74% yield when pretreatment severity was reduced by using autocatalysis instead of acid-catalysed steam pretreatment, xylose yields were increased to 80-86%. Partial delignification of pretreated material was also evaluated as a way to increase the overall sugar yield. The overall glucose yield increased slightly due to delignification but the overall xylose yield decreased due to hemicellulose loss in the delignification step [6]. The maximum sugar recovery by water extraction of corn stover is produced by a synergistic effect treatment of 190°C for 5 min and an acid loading of 1.5% (w/w) at these conditions 25% of the sugars in the feedstock can be recovered as monomers or oligomers. The acid has greatly improved the sugar solubility at 180°C the sugar recovery has been only 1.5% without acid, while by using 3 (%w/v) of acid the sugar recovery has increased to 16.8%. Also the cellulose digestibility has been improved by the acid pre-impregnation, after 48 h of digestion the yield of glucose reached 93% of the theoretical by using the substrate that was pre-impregnated with 3% w/v of acid and treated at 190°C. The high acid loading has also been required to achieve the best recovery of glucose (85% of the initial glucan) as sum of water extraction and 48 h hydrolysis [21].

**Figure 3.** Reducing sugar concentration of corn stover that pretreated with 2.0 M NaOH and hydrolyzed with 0, 1.0, 5.0 and 10.0% (v/v) sulfuric acid at 100, 111 and 121°C for a) 15 min and b) 30 min (●) 0% H₂SO₄ (◆) 1.0% H₂SO₄ (□) 5.0% H₂SO₄ (●) 10.0% H₂SO₄.
The pretreatment of vetiver grass leaves with 2.0 M NaOH and hydrolysis with 0, 1.0, 5.0 and 10.0% (v/v) sulfuric acid and at 100, 111 and 121°C for 15 and 30 min were studied. The results of pretreatment and hydrolysis of vetiver grass leaves for 15 min were shown in Figure 4a. The reducing sugar concentration after hydrolysis at 100°C were 0, 1,591, 865 and 512 mg/ml, respectively. After hydrolysis at 111°C the reducing sugar concentration were 0, 1,476, 864 and 0 μg/ml, respectively. The hydrolysis at 121°C obtained reducing sugar concentration 0, 1,609, 1,524 and 181 μg/ml, respectively. For the hydrolysis of vetiver grass leaves with 0, 1.0, 5.0 and 10.0% (v/v) sulfuric acid at 100, 111 and 121°C for 30 min were examined. The results showed in Figure 4b. The reducing sugar concentration after hydrolysis at 100°C were 0, 1,715, 1,038 and 0 μg/ml, respectively. After hydrolysis at 111°C obtained 0, 1,774, 755 and 19 μg/ml, respectively. The reducing sugar concentration after hydrolysis at 121°C were 0, 1,621, 1,647 and 151 μg/ml, respectively. The results showed maximum reducing sugar concentration of vetiver grass leaves 1,774 μg/ml after pretreatment with 2.0 M NaOH and hydrolysis with 1.0% (v/v) sulfuric acid at 111°C for 30 min.

Figure 4. Reducing sugar concentration of vetiver grass leaves that pretreated with 2.0 M NaOH and hydrolyzed with 0, 1.0, 5.0 and 10.0% (v/v) sulfuric acid at 100, 111 and 121°C for a) 15 min and b) 30 min. 0% H₂SO₄, 1.0% H₂SO₄, 5.0% H₂SO₄, 10.0% H₂SO₄.

In this study, rice straw, corn hull, corn stover and vetiver grass leaves were pretreated with 2.0 M NaOH and hydrolysed with 0, 1.0, 5.0 and 10.0% sulfuric acid for 15 min and 30 min. The results of these condition showed low reducing sugar concentration at high concentration of sulfuric acid hydrolysis for every materials and every condition. Because of at high concentration of acid might the cause of the reducing sugar degradation [22]. The soft hydrolysis conditions led to a sugar-rich prehydrolysate. When using harsh pretreatment conditions, sugar recovery in liquids decreased. The optimization of pretreatment should be the maximization of the overall sugar yield, taking into account all fermentable sugars. The overall sugar yields obtained at mild pretreatment temperature 170-190°C increased with acid concentration (for acid concentrations up to 1%). By contrast, high pretreatment temperatures and elevated acid concentrations produced a decrease in overall sugar yield because cellulose recovery in the pretreated solids came down and sugars content in the prehydrolysates decreased as a result of degradation processes. The maximum
overall sugar yield of olive tree (36.3 g sugar/100 g raw material) was obtained at 180°C and 1% sulfuric acid concentration, representing 75% of all sugars in the olive tree biomass (48.7 g/100 g) [23]. Steam explosion of lignocellulosic materials can be related to the significant increases of the crystallinity index values of cellulose [24].

For this study, the maximum reducing sugar concentration of rice straw, corn hull, corn stover and vetiver grass leaves were 1,658, 1,674, 1,785 and 1,774 μg/ml, respectively. In this study, vetiver grass leaves were selected as raw material for ethanol production.

3.2 Optimization of Pretreatment Condition of Vetiver Grass Leaves with Sodium Hydroxide

The vetiver grass leaves was pretreated with 1.0, 2.0 and 4.0 M NaOH and hydrolysed with 1.0% (v/v) sulfuric acid at 111°C for 30 min. The results in Figure 5 showed that the optimal concentration of sodium hydroxide for pretreatment of vetiver grass leaves at 1.0 M NaOH. Gould [25] reported the extraction condition of hemicelluloses from the vetiver grass with a maximum yield of 35% with 4 M NaOH at ambient temperature for 8 h. The treatment of dewaxed maize stems, rye straw and rice straw with 1 M NaOH at 30°C for 18 h resulted in a dissolution of 78.0, 68.8 and 82.1% of the original lignin, and 72.1, 72.6 and 84.6% of the original hemicelluloses, respectively [26]. The optimal condition for the pretreatment of casava rhizome was 2 M NaOH [27]. The pretreatment of rice bran with 5 N NaOH for 10 min could increase 20% of sugar [28]. The pretreatment of cotton stalk and agricultural straw with 2% NaOH 90 min, 121°C/15psi resulted in the highest level of delignification 66% and 75.09-84.52%, respectively [29, 30] but increase 54.83% cellulose and decreased hemicellulose 61.07% and lignin 36.24% [31]. The pretreatment of corn stover with 10% NaOH for 1 hour in the autoclave reduced 95% lignin content. An increase in the concentration of sodium hydroxide significantly improved delignification at all combinations of temperature and time [32]. The main effect of sodium hydroxide pretreatment on lignocellulosic biomass is delignification by breaking the ester bonds cross-linking lignin and xylan, thus increasing the porosity of biomass [4,29,33]. Alkali pretreatment also caused glucan solubilization [29]. Neely [34] present that effective of alkaline pretreatment depends on the lignin content of the materials [34].
3.3 Hydrolysis of Vetiver Grass Leaves with Sulfuric Acid

The vetiver grass leaves were pretreated with 1 M NaOH and hydrolyzed with 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% sulfuric acid at 111°C for 30 min. The maximum reducing sugar concentration was obtained after hydrolysis of vetiver grass leaves with 2% sulfuric acid at 111°C for 30 min. The results showed in Figure 6. In this study, dilute acid (2% sulfuric acid) showed highest reducing sugar concentration. Hinman et al. [22] reported that dilute acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. The dilute sulfuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis [32,35,36]. Recently developed diluted acid hydrolysis processes use less severe conditions and achieve high xylan to xylose conversion yields. Achieving high xylan to xylose conversion yields is necessary to achieve favorable overall process economics because xylan accounts for up to a third of the total carbohydrate in many lignocellulosic materials. Sun and Cheng [3] reported the glucose concentration in the prehydrolyzate of rye straw was not significantly influenced by the sulfuric acid concentration and residence time, but it increased in the prehydrolyzate of bermudagrass with the increase of pretreatment severity. The xylose concentration in the filtrates increased with the increase sulfuric acid concentration and residence time.

The effect of combined heat treatment and acid hydrolysis at various concentrations on lignocellulotic biomass was reported. At high concentration of sulfuric acid (1.0-5.0 M H₂SO₄), hydrolysis the casava grate waste was achieved but with excessive carring or dehydration reaction. At lower acid concentrations, hydrolysis of casava grate waste biomass was also achieved with 0.3-0.5 M H₂SO₄ while partial hydrolysis was obtained below 0.3 M H₂SO₄ (the lowest acid concentration that hydrolysed casava grate waste biomass) at 120°C and 1 atm pressure for 30 min. High acid concentration is therefore not required for casava grate waste biomass hydrolysis [37]. The dilute sulfuric acid (0.5–1.0% (v/v) pretreatment of corn fiber at 121°C found that the monomeric sugar yield was 85–100% of the theoretical yield [18].

![Figure 6](image-url)

**Figure 6.** Reducing sugar concentration of vetiver grass leaves after pretreated with 1.0 M NaOH and hydrolyzed with 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% (v/v) sulfuric acid at 111°C for 30 min.
In this study, we decided to select the vetiver grass leaves as raw material for ethanol production. The reason of our decision, because of the vetiver was normal practice of growing for soil and water conservation. The vetiver need to cut down the leaves every few months to encourage tiller growth and to reduce the danger of fire in the dry season. For the value added of vetiver grass leaves, we tried to promote the growing of vetiver for soil and water conservation for solving the problem of soil destruction to the farmer. His Majesty the King of Thailand has repeatedly summoned that uses other than ones just mention, as well as the utilization of harvested material, should not be overemphasized to nullify the main uses of vetiver. There is no question that vetiver has a considerable ecological benefit in soil and water conservation as well as environmental protection. Its hidden economic value of and inexpensive means of stabilizing backslopes and sideslopes of the highway, railroad, as well as earthen dams is also increasingly being recognized. Increasing awareness of the ecological potential of vetiver in controlling pollution, and in protecting our environment such as in wastewater treatment, heavy metal contamination etc. are likely to be envisaged in the near future. The vetiver grass leaves could be applied as raw materials for ethanol production.

4. CONCLUSION

Rice straw, corn hull, corn stover and vetiver grass leaves were pretreated with 2.0 M NaOH and hydrolyzed with 1.0, 5.0 and 10.0% (v/v) sulfuric acid at 100, 111 and 121°C for 15 and 30 min. High reducing sugar concentration was obtained by hydrolysis with 1.0% (v/v) sulfuric acid at 111°C for 30 min in all of materials. The vetiver grass leaves was selected. The optimal condition of pretreatment and hydrolysis of vetiver grass leaves was 1.0 M NaOH and 2.0(1/0)% (v/v) sulfuric acid at 111°C for 30 min.

REFERENCES


