[Regular Paper]

Improvement of Sugar Yield from Alkaline Pretreated Herbaceous and Woody Lignocellulosic Biomass through Visible Light Illumination in the Presence of Silicon

Hiroshi TABATA†1)*, Ken TSUTSUMI†1), Yoich MATSUSHITA†1), Aya NISHIWAKI†2), Sachio HAYASHI†1), Kihachiro OGAWA†1), and Kenji TABATA†1)

†1) Dept. of Applied Chemistry, Faculty of Engineering, University of Miyazaki, Gakuen-Kibanadai, Miyazaki 890-2192, JAPAN
†2) Kibana Agricultural Science Station, Faculty of Agriculture, University of Miyazaki, Gakuen-Kibanadai, Miyazaki 890-2192, JAPAN

(Received December 28, 2010)

For the sustainable development of our society, much attention has been paid to biorefinery, where renewable lignocellulosic biomass is converted valuable chemicals and/or fuels. Alkakaline pretreatment before the enzymatic saccharification of the lignocellulosic biomass has been examined for a long time but further improvement is required to obtain a high yield of sugars. We have tried to improve the sugar yield using two different type lignocelluloses such as herbaceous napiergrass leaves and woody kudzu stalks. The trials to improve the sugar yields from the alkaline pretreated these lignocelluloses have been examined by supplementary subjecting to visible light illumination in the presence of Si. The yield of reducing sugars from herbaceous napiergrass leaves was improved from 60.3 to 86.7% by using the complementary illumination with Si after the enzymatic saccharification for 24 h. In the case of kudzu stalks, that was improved from 57.4 to 88.6% with the same treatment.

Keywords

Napiergrass, Kudzu, Silicon, Alkaline treatment, Visible light illumination, Enzymatic saccharification

1. Introduction

The use of fossil fuels is facing several difficulties, such as decreasing reserves, increasing prices and greenhouse gas emissions. The interest in renewable sources of energy, therefore, is rising. Biomass is the most abundant renewable resources on earth, and thus ethanol and/or valuable chemicals production from lignocellulosic biomass resources has attracted increasing worldwide interest1,2. The importance on the utilization of lignocellulose is to obtain a high yield of sugars by the saccharification of the biomass.

Lignocellulosic biomass includes plant cell walls, which contain primary cellulose, hemicellulose and lignin. Due to the organization and interaction between these polymeric structures, plant cell walls naturally resist the saccharification of the contained cellulose and hemicellulose. Among the different technologies available for the saccharification of lignocelluloses, conversion processes based on the use of cellulolytic enzymes seem to be the most promising for a large scale application3. The goal of enzymatic hydrolysis is to depolymerize the polysaccharides in the water insoluble solid fraction. There are, however, several problems with this approach. Most importantly, the high cost of cellulase enzyme production hinders the application of these enzymes to bioethanol manufacturing5. Pretreatment of the lignocelluloses before the enzymatic hydrolysis is therefore necessary for obtaining a high yield of sugars while at the same time decreasing the usage of the cellulase enzyme. In the pretreatment step, the structures of the lignocellulosic biomass are altered to make the holocelluloses (cellulose + hemicellulose) more accessible to the enzyme3.

Alkaline pretreatment processes utilize lower temperatures and pressures compared to other pretreatment technologies. Alkaline pretreatment specifically targets hemicellulose acetyl groups and lignin-carbohydrate ester linkages. These reactions help solubilize and extract lignin from the biomass and make easy to contact between the enzyme and holocelluloses. Cell wall and ultrastructural modifications are still required for most alkaline pretreatments, however, in order to enhance the saccharification of lignocelluloses5.

Photocatalytic degradation of lignin on TiO₂ has been reported5,6. Machard et al. conducted the experiments of degradation of lignin in alkaline aqueous ethanol solution with oxygen bubbling in the presence of TiO₂ under UV irradiation. Their results showed that lignin

* To whom correspondence should be addressed.
* E-mail: ng2803u@student.miyazaki-u.ac.jp
was depolymerized successively and aromatic ring was opened, which produced oxygenated compounds such as carboxylate and aldehyde.

Herbaceous lignocellulosic napiergrass (*Pennisetum purpureum Schumach*) grows rapidly, i.e., high leaf expansion and rapid leaf production have been known to occur in a tall canopy. Napiergrass can exhibit high dry matter productivity in warm regions. As a result, napiergrass is considered a promising feedstock for biofuel production\(^{5,6}\). Kudzu (*Pueraria lobata*) is a common in Asia and an invasive vine. It forms a dense stand of vines with growth rates that can exceed 2 m per week, and then the vine lignifies to hard wood in a few years\(^{9}\). Woody lignocellulosic kudzu stalks also are suggested to have a potential to supplement existing bioethanol feedstocks. Bioconversion of alkaline pretreated napiergrass into sugar and ethanol was previously examined using conventional cellulase preparations and genetically modified cellulase by one of our authors\(^{10}\).

This paper describes the trials to increase the sugar yield by subjecting the alkaline pretreated herbaceous napiergrass and woody kudzu stalks to the visible light illumination to explore the feasibility of solar light utilization.

2. Experimental Section

2.1. Materials

Napiergrass leaves harvested from the farm in our university were dried at 353 K in an oven for 48 h and then made into a fine powder by the use of a laboratory blender. The powder was sieved at 180-212 μm. The ligneous stalks of cultivated kudzu which were kindly supplied from Nara Agriculture Center were dried out at 353 K in an oven for 48 h, and milled into 212-300 μm.

Moisture analysis was conducted with an aquameter (M25-70, Shiro Co., Ltd.). Cellulose content was determined using the method of Crampton and Maynard\(^{11}\). Hemicelluloses and lignin were determined using the methods described by Goering and VanSoest\(^{12}\). The weight of ash was measured from the weight difference before and after calcination at 1123 K for 2 h.

2.2. Procedure

Napiergrass powder (10 g) was soaked in a 100 mL of 1.0 wt% NaOH solution and then stirred at 600 rpm at 373 K for 1 h. The alkaline pretreated fiber was separated from the alkaline solution in a centrifuge at 3000 rpm for 2 min, and the residue was neutralized with dilute acetic acid solution and then rinsed several times with deionized water to remove the salts. The rinsed residue was dried at 343 K for 1 h, and the cake of dried material crushed into a powder in a blender. The milled kudzu powder (10 g) also was soaked in the NaOH solution (1.0 wt%) at 373 K for 1 h. Neutralized and rinsed fibers were dried out, and the cake was crushed with the blender.

The complementary treatment for the dried residuals was carried out under visible light illumination using a Xe lamp (300 W, LX-300F, PE ILS) through a UV cutoff filter. The temperature of the reactor was controlled at 298 K with a chiller.

Si powder (60 mesh; Aldrich Chemical Co.) was used as a photocatalyst. A mixture of Si powder (0.4 g), deionized water (35 mL) and the alkaline pretreated napiergrass powder (1.5 g) in the vessel was degassed several times, and then argon gas (200 Torr, 1 Torr = 133.322 Pa) was introduced into the system. In the case of kudzu stalks, the mixture of Si powder (0.4 g), deionized water (45 mL) and the alkaline pretreated kudzu powder (1.0 g) was examined. Illumination treatment was carried out stirring at 600 rpm.

The enzymatic saccharification was carried out with Acremozyme (*Acremonium cellulolyticum*) as the cellulase. The Acremozyme (Meiji Seika Kaisha, Ltd.) used in this study was kindly supplied by Kyowa Chemical Products Co., Ltd. The reactivity of this Acremozyme for the saccharification of napiergrass was examined and compared with other cellulase preparations in a previous paper\(^{10}\). A notable feature found for this Acremozyme was its high reactivity not only as a cellulase but also as a hemicellulase. The enzymatic hydrolysis of the residuals of napiergrass was carried out in a medium bottle containing 80 mL of the mixture (the pretreated residuals of napiergrass (1.5 g), Si powder (0.4 g), 0.2 wt% sodium azide (1 mL) and deionized H2O (35 mL), 40 mL of acetate buffer (pH 5.0, 0.2 M, 1 M = 1 mol・dm\(^{-3}\)) and 0.1 g of Acremozyme cellulase preparation). In the case of kudzu stalks, 100 mL of the mixture (the pretreated residuals of kudzu (1.0 g), Si powder (0.4 g), 0.2 wt% sodium azide (1 mL) and deionized H2O (45 mL), 50 mL of acetate buffer (pH 5.0, 0.2 M)) was hydrolyzed with 0.1 g of the Acremozyme. The hydrolysis was carried out by incubating the mixture at 318 K with stirring at 500 rpm for 72 h as reported previously\(^{10}\). The reaction was stopped by boiling the mixture for 5 min to inactivate the enzyme, and then the residual material was removed by centrifugation at 5500 rpm for 2 min. 50 μL was sampled on each time by a sample splitter. The amount of reducing sugars released in the supernatant solution was determined by the Somogyi-Nelson method\(^{13}\). The amount of glucose was determined by the GOD-POD method (glucose CII-test, Wako Pure Chemical Industries, Ltd.).

3. Results and Discussion

The napiergrass leaves used in this study were analyzed for their components as shown in Table 1. The moisture content (on a wet weight basis) of the napiergrass leaves was 3.0%. Cellulose and hemicellulose
contents were 31.8% and 19.6% on a dry weight basis, respectively. The proportion of cellulose was larger than that of the hemicellulose, and the percentage of the holocellulose, i.e., cellulose + hemicellulose, was 51.4%.

The components of the residuals after alkaline treatment with 1.0 wt% NaOH solution for 1 h at 373 K also were shown in Table 1. The alkaline treatment was effective in the elution of lignin because the proportion of total lignin decreased from 16.1 to 6.1% after the alkaline treatment. Thus the proportion of holocellulose increased from 51.4 to 79.7% caused by the elution of lignin. Since the ratio of cellulose after and before the alkaline treatment was larger than that of hemicellulose, we assumed hemicellulose also eluted somewhat. The degradation of lignin and hemicellulose after the alkaline treatment has been reviewed.14)

First, we examined the coexistent effects of semiconductor materials on the enzymatic saccharification with commercial crystallized cellulose (Avicel) as shown in Fig. 1. Without any semiconductor materials, glucose was produced steadily from avicel. Some semiconductors such as WO₃, Fe₂O₃, and Si did not affect the reactivity of Acremozyme, but TiO₂ and SrTiO₃ strongly deteriorated the reactivity.

The time course variations of reducing sugars and glucose obtained from napiergrass leaves through the enzymatic saccharification for several different combinations of NaOH pretreatment and the illumination with a Xe lamp in the presence of Si are shown in Figs. 2 and 3. Since the amounts of reducing sugars and glucose for both samples increased rapidly in a few hours, we assumed the amount of our used cellulase (0.1 g) was too much for these samples. The variations show the differences in the influence of the alkaline treatment and/or the treatment under visible light irradiation on the degradation of the napiergrass in Fig. 2. The effectiveness of the treatment in 1.0 wt% NaOH solution is clearly shown on the variation of reducing sugars. The increase of the glucose amount after the alkaline treatment was evident as shown in Fig. 3. The main target of the alkaline treatment is the removal of lignin from the biomass, and the proportion of lignin decreased after the treatment in the NaOH solution at 373 K for 1 h as shown in Table 1. Since the increment of the reducing sugar was originated from glucose as shown in Fig. 3, the elution of lignin was assumed.

Table 1 Compositional Proportion of Napiergrass Leaves before and after the Alkaline Treatment

<table>
<thead>
<tr>
<th>Component</th>
<th>Dried raw materials of napiergrass</th>
<th>Residue of alkaline pretreated napiergrass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>31.8</td>
<td>54.4</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>19.6</td>
<td>25.3</td>
</tr>
<tr>
<td>Acid-insoluble lignin (Klason lignin)</td>
<td>9.8</td>
<td>4.5</td>
</tr>
<tr>
<td>Acid-soluble lignin</td>
<td>6.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>8.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Ash</td>
<td>13.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Others</td>
<td>10.9</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Reactant: Avicel (2.0 g), Acremozyme cellulase preparation (0.1 g) deionized H₂O (75 mL), 0.2 M acetate buffer (pH 5.0, 25 mL), 0.1% sodium azide (250 μL). Reaction condition: incubating temperature (318 K) and rotation (180 rpm).

Fig. 1 Coexistent Effects of Semiconductor Materials on the Enzymatic Saccharification of Avicel

Fig. 2 The Variations of Reducing Sugars Obtained from Napiergrass in the Time Course of Enzymatic Saccharification under Several Different Conditions

Reactant: raw napiergrass or alkaline pretreated residuals of napiergrass (1.5 g), Si powder (0.4 g), 0.2 wt% sodium azide (1 mL) and deionized H₂O (35 mL), 40 mL of acetate buffer (pH 5.0, 0.2 M) and 0.1 g of Acremozyme cellulase preparation. Reaction condition: incubating temperature (318 K) and rotation (500 rpm). NaOH 0% means the napiergrass without alkaline pretreatment.
to make the enzyme easy to contact mainly with cellulose in the napiergrass.

The treatment under visible light illumination for 3 h without the alkaline pretreatment of the napiergrass was not effective in improving the production of reducing sugars by the enzymatic saccharification as shown in Fig. 2. The stirring treatment in the presence of silicon without the illumination for 3 h was also ineffective as shown in Fig. 2. The amount of reducing sugars obtained from the subsequently treated napiergrass under visible light illumination in the presence of Si for 3 h was distinctly larger in comparison to that without the illumination. The improvement effects of the illumination appeared as an increase in the initial rate, and then stabilized shortly after.

The effectiveness of the illumination with Si was observed for the time course variation of glucose also through the enzymatic saccharification as shown in Fig. 3. The increment of glucose by the illumination for 3 h with Si was smaller than that of the reducing sugars in Fig. 2. The obtained reducing sugars after the enzymatic saccharification for 24 h varied with the illumination time as shown in Fig. 4. The amount of reducing sugars clearly increased after the illumination time for 3 h but a longer illumination time over 3 h was not effective. We assumed that the increased reducing sugars were degraded by a longer photocatalytic treatment. We measured each amount of obtained glucose also depending on the variation of the illumination time. The illumination effects were not prominent through every illumination time. Thus the increase of the reducing sugars after the illumination in Fig. 2 was originated from that of xylose through the saccharification of hemicellulose with the enzyme.

Since the types of the incremental sugars were different between the sample for the alkaline treatment only and that for the subsequent visible light illumination, we assumed that a different type of chemical bond in the napiergrass leaves was cleaved through the visible light illumination with silicon. While all types of aryl ether bonds in a biomass are typically cleaved in alkaline medium, aryl_alkyl or alkyl_alkyl carbon-carbon bonds and diaryl ether are stable to some extent under the same conditions. The covalent linkage between lignin and hemicellulose such as ether linkages are reported also to be stable in alkaline medium. We therefore assumed that some of those bonds were cleaved through the complementary visible light illumination with silicon. The cleavage of ether linkages especially between hemicellulose and lignin was expected to increase the chance of production of xylose.

The variations of yield of reducing sugars of the alkaline treated napiergrass together with and without the visible light illumination through the enzymatic saccharification were shown in Fig. 5. The yield was calculated by using the content of holocellulose in the residue after the alkaline treatment with 1.0 wt% NaOH solution at 373 K for 1 h. The content of holocellulose in the residue of napiergrass was 797 mg/g. The yield was calculated from the ratio between the calculated amount from the measured concentration of reducing sugars in the supernatant solution after the enzymatic saccharification and that content (797 mg/g).
The yield of reducing sugars for the sample of the alkaline treatment only was 60.3% after the enzymatic saccharification for 24 h. On the other hand, the yield of that for the sample of the alkaline treatment plus subsequent photocatalytically treatment was improved up to 86.7% after the enzymatic saccharification for 24 h.

Woody kudzu stalks used in this study were analyzed for their components\(^{15}\). The moisture content (on a wet weight basis) was 8.4%. On average, the cellulose, hemicelluloses, and lignin were 35.9, 21.2, and 22.9% on dry weight basis, respectively. The rest was methanol extractives and others. In comparison with the proportion of lignin for herbaceous napiergrass, that for woody kudzu stalks was larger. The proportion of holocellulose was 57.1% and slightly larger than that for napiergrass.

Figures 6 and 7 show the variations of obtained amount of glucose and reducing sugars from kudzu stalks\(^{15}\). They are the time course variations of enzymatic saccharification with and without the subsequent visible light illumination with a Xe lamp after the alkaline treatment. The amount of glucose after the subsequent visible light illumination with Si for 2 h was zero at the start point but increased more rapidly, and attained ca. 1.5 times larger amount after the enzymatical hydrolysis for 72 h as shown in Fig. 6. The obtained line of glucose stirring Si in the dark for 2 h was almost the same with that for the alkaline treatment only.

Figure 7 shows the time course variation of the amount of reducing sugars and its yield obtained from kudzu stalks during the enzymatic saccharification. The obtained amount was zero at the start point even for the sample together with the subsequent visible light treatment for 2 h. The variation is similar to that of the glucose, and the proportion of the observed amounts with and without the subsequent visible light illumination with Si was similar to that of the glucose. This means that the increment of the reducing sugars by the illumination is derived mainly from that of the glucose. Thus, the cellulose in the kudzu stalks was able to be saccharified further by the subsequent visible light illumination with Si. This result of kudzu stalks disagreed with that concluded from the increment of xylose for the case of napiergrass. We suggested that the types of cleaved bonding were different between the treatment in the alkaline medium and photocatalytic treatment with Si. This reason, nevertheless, is not
clear but we assumed this difference was attributed to the different bonding structures between herbaceous napiergrass and woody kudzu stalks.

The variations of yield of reducing sugars of the alkaline treated napiergrass together with and without the visible light illumination through the enzymatic saccharification were also shown in Fig. 7. The yield was calculated in a uniform manner with that for the napiergrass. The content of holocellulose in the residue of kudzu stalks was 760 mg/g. The yield of reducing sugars of the sample for the alkaline treatment only was 57.4% after the enzymatic saccharification for 24 h. On the other hand, the yield of that of the sample for the alkaline treatment plus subsequent photocatalytically treatment was 88.6% after the same treatment for 24 h by subjecting the visible light illumination with Si for 2 h. The incremental component of reducing sugars after the illumination was different such as xylose for napiergrass and glucose for kudzu stalks. We assumed the different bond structures between herbaceous napiergrass leaves and woody kudzu stalks made the different results.

**4. Conclusions**

The yield of reducing sugars from the alkaline pretreated herbaceous napiergrass leaves was improved from 60.3 to 86.7% after the enzymatic saccharification for 24 h by subjecting the visible light illumination with Si for 3 h. That from the alkaline pretreated woody kudzu stalks also was improved from 57.4 to 88.6% after the same treatment for 24 h by subjecting the visible light illumination with Si for 2 h. The incremental component of reducing sugars after the illumination was different such as xylose for napiergrass and glucose for kudzu stalks. We assumed the different bond structures between herbaceous napiergrass leaves and woody kudzu stalks made the different results.

**References**

要　旨
シリコン存在下で可視光照射によるアルカリ前処理した草本系および木質系リグノセルロースからの糖収率の改善

田畑 宏*1, 廣　健*1, 松下 洋一*1, 西脇 亜也*2, 林 幸男*1, 小川 喜八郎*1, 田畑 研二*1

*1 宮崎大学工学部物質環境化学科, 889-2192 宮崎市学園木花台西1丁目1番地
*2 宮崎大学農学部附属フィールド科学教育研究センター, 889-2192 宮崎市学園木花台西1丁目1番地

持続できる社会の構築を目指し、バイオマスの有効利用、すなわちリグノンを含むバイオマスからの有用化学物質あるいは燃料への変換に大きな関心が寄せられている。リグノセルロースの酵素醸化の前処理としてアルカリ液を利用し方方式についてはこれまでに多くの研究がなされてきたが、醸化率の向上を目指しさらなる改善が求められている。我々は、草本系バイオマスであるネピアグラスの葉部と木質系であるクズ茎部からの醸化率の向上を目指した研究を行った。アルカリ処理を事前に施したこれらのリグノセルロースにシリコン存在下で可視光照射、その効果について調べた。草本系ネピアグラス葉部の場合は、醸素挿入24時間後では還元醸収率はアルカリ処理の
みでは60.3%であったものが、可視光照射を加えることににより86.7%に改善された。同様に木質系クズ茎部では57.4%から88.6%に改善された。