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Improvement of sugar recovery from *Sida acuta* (Thailand Weed) by NaOH pretreatment and application to bioethanol production

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Abstract−*Sida acuta*, a common type of weed in Thailand, contains relatively high cellulose (42.7%) content. We pretreated NaOH to improve glucose recovery from *S. acuta*. The effect of pretreatment temperature and NaOH concentration was fundamentally investigated based on hydrolysis efficiency with recovery of solid fraction. The pretreatment condition was determined to be 3% NaOH at 60 °C for 9 h, which showed the highest glucose recovery. The hydrolysates obtained by enzymatic hydrolysis of *S. acuta* were applied to the fermentation of *Saccharomyces cerevisiae* K35, and a theoretical yield of 97.6% was achieved at 18 h. This indicated that the hydrolysates medium without detoxification had no negative effects on the fermentation. The production of biomass into bioethanol was evaluated based on the material balance of 1,000 g basis. Following this estimation, approximately 28 g and 110 g bioethanol could be produced by untreated and pretreated *S. acuta*, respectively, and this production was improved about 3.9-fold by NaOH pretreatment. These results again show the importance of pretreatment in biorefinery process.

Keywords: Bioethanol, Pretreatment, *Sida acuta*, Sodium Hydroxide, Weed Biomass

INTRODUCTION

Bioethanol has been recognized as an alternative transportation fuel because it is environmentally friendly and has low carbon dioxide emissions [1]. In addition, it is primarily used as a blending component in the production of motor gasoline in the USA. According to the U.S. Energy Information Administration, the average production of fuel ethanol in the United States was previously 0.86 million barrels per day and increased to 1.03 million barrels per day in 2017 (setting a new record for annual production) [2]. In recent years, ethanol production has increased as a result of higher renewable fuel standard (RFS) targets and higher amounts of gasoline consumption; almost all gasoline is now blended with 10% ethanol (E10). However, the demand for highly concentrated ethanol blends such as E15 and E85 remains limited. In particular, the major feedstock for bioethanol is currently corn; therefore, it is necessary to investigate various feedstock alternatives such as non-food resources [3,4].

Lignocellulosic (second-generation) biomass has attracted attention as a feedstock since it is widely abundant, low-cost, and a non-food material. Lignocellulosic biomass includes hardwood and softwood, municipal solid waste, agricultural residue, dedicated energy crops, and weedy plants [5]. Among these, weed biomass holds great potential as an economically feasible feedstock in terms of biorefinery, because most weeds grow on marginal areas and can produce large quantities of biomass with limited nutrients and water. Weed biomass is particularly resistant to natural disasters such as droughts and pests, and does not require farmland, cultivation, or additional fertilizer and water supplies [6,7]. Several application studies have previously been conducted using specific species on order to demonstrate the potential of weeds as feedstocks, such as *Achyranthes aspera* [8], *Eicchornia crassipes* [9,10], *Eupatorium adenophorum* [11], *Lantana camara* [12], *Prosopis juliflora* [12,13], *Saccharum spontaneum* [14-16], *Sida acuta* [17], *Thysanolaena maxima* [18], and *Typha latifolia* [19,20].

Lignocellulosic biomass is a complex polymeric substance composed of cellulose, hemicellulose, lignin, and other materials, and the corresponding proportions of these depend on the plant species, type, and source. These biopolymers combine with each other to form a complex structure in plant cell walls, forming a tough barrier to enzymatic hydrolysis by cellulase [21-23]. Therefore, in the conversion of lignocellulosic feedstock to bioethanol, the pretreatment process is a crucial step for modifying the microscopic and macroscopic structure as well as for reducing and/or altering the physical barriers in order to improve enzymatic hydrolysis. Various pretreatment techniques have been investigated on lignocellulosic biomass, which can be categorized into physical, chemical, and biological pretreatments. However, studies have shown that no single pretreatment process can be applied to all types of lignocellulose feedstocks. A suitable pretreatment process should consider the diversity of other plant biomass, which differs in term of structural arrangement, physico-chemical composition and other

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factors.

Alkaline pretreatment has been widely used to remove lignin in biomass. The relevant mechanism is known to be decomposition of the intermolecular ester bonds by saponification with an alkali, and thus the biodegradability and enzyme accessibility of lignocellulose is improved by breaking the ester bonds between lignin, hemicelluloses and cellulose. Sodium hydroxide is a simple chemical in which that soda hydrates can penetrate the amorphous area of cellulose. Pretreatment by sodium hydroxide is economical due to its associated relatively low temperatures, chemical cost, and pressures compared to other methods. Recent studies focused on sodium hydroxide pretreatment have resulted in significant improvements in enzymatic digestion such as barley straw [24], canola residues [25], cotton gin dust [26], crofton weed stem [12], *Panicum virgatum* [27], *Populus deltoids* [28], *Prosopis juliflora* [13], *Saccharum* spontaneum [29], *Sorghum bicolor* L [30] and *Sorghum sudanense hybrid* [31].

In this study, *Sida acuta*, one the most abundant weeds in Thailand, is selected as feedstock for the production of bioethanol. *S. acuta*, a common perennial weed belonging to the families Malvaceae, is believed to have originated in Mexico and Central America; however, today it is widespread in the tropics with higher rainfall areas [17]. Fig. 1 shows the photograph of *S. acuta* from Phitsanilok province, Thailand. It typically ranges from 30 to 150 cm in height and the stems are fibrous, almost woody with a tough stringy bark. The leaves have toothed margins and have prominent veins on the undersurface. The flowers are yellow, solitary, 1-2 cm in diameter and on a short stalk 0.3-0.8 cm long, five petals on the underside and a shallow notch at the apex. To improve sugar recovery from *S. acuta*, sodium hydroxide pretreatment was carried out, and the effect of the reaction factors on the efficiency of enzymatic hydrolysis was investigated. In addition, the biomass hydrolysate was applied to the cultural medium of yeast for bioethanol production. Finally, fermentation profiling of *Saccharomyces cerevisiae* K35 was examined in standard and biomass hydrolysates media to confirm the cell performance and inhibitors.

MATERIALS AND METHODS

1. Materials

Sida acuta, the selected weed biomass, was collected from Phitsanilok province, Thailand. The fresh weed was chopped and dried, then crushed using a wood milling machine (Rtsch, Rheinische StraBe 36-D-42781, Haan, Germany) and sieved (Fig. 1). The fractions obtained between 150 μm and 300 μm were collected in plastic bags and stored at room temperature. Sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4) were purchased from Dea-jung Chemical, Korea. All reagents used in the current study were above analytical grade.

2. Alkaline Pretreatment

NaOH pretreatment of weed biomass was carried out in a 50 mL screw-capped tube (Falcon), loaded with 2 g (dry basis) of untreated biomass. The pretreatment of weed biomass was divided into two parts: 1) To determine the moderate temperature, 3% (w/w)

Fig. 1. Photograph of *Sida acuta*.

NaOH solution was added into the tube containing biomass at a ratio of 1 g substrate: 10 mL NaOH. All tubes were incubated in a water bath at temperatures of 50 °C, 60 °C, and 70 °C for 9 h. 2) To examine the effects of different NaOH concentrations, 0 (D.W.), 1, 3, 5, 7, and 10% (w/w) were added to the tube containing biomass at the same ratio. All of the tubes were incubated in a water bath at temperature of 60 °C for 9 h. Pretreated biomass of a solid fraction was collected by filtration using a standard sieve (no. 170, a mesh size of 90 μm) and neutralization was performed with deionized water until a neutral pH was achieved.

3. Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted in a 50-mL Falcon conical tube containing 0.15 g (dry basis) of treated biomass, 0.05 M of sodium citrate buffer (pH 4.8), and 2% sodium azide (w/v) for a total volume of 10 mL. Amounts of 240 FPU/g-glucan of cellulase, Celluclast 1.5 L (Novozyme), and 120 CBU/g-glucan of cellobiase (Sigma-Aldrich) were added into the solution. The cellulase activity of cellulase, Celluclast 1.5 L (Novozyme), was measured in terms of filter paper units (FPU) by NREL [32] and Jung [33] procedures. The beta-glucosidase unit (BU) was determined by using para-nitrophenyl-β-glucoside (*p*NPG) as the substrate. One unit of enzyme liberated 1 μg of glucose per minute [34].

The tubes of each reaction were incubated at 50 °C on a rotary shaker at 250 rpm. The liquid fractions (biomass hydrolysates) were then collected at 24, 48, 72, 96 and 120 h of incubation for the determination of monomer sugar using HPLC. The hydrolysis efficiencies and overall glucose recoveries from the biomass were calculated by the following Eq. (1) and (2), respectively:

$$\text{Hydrolysis efficiency (\%)} = \frac{\text{glucose released (g)}}{1.11 \times \text{glucan in biomass (g)}} \times 100 \quad (1)$$

$$\text{Glucose recovery (\%)} = [\text{solid recovery} \times \text{glucan content} \times 1.11 \times \text{hydrolysis efficiency}] \times 100 \quad (2)$$

4. Preparation of Biomass Hydrolysate Medium for Ethanol Fermentation

The biomass hydrolysate from the enzymatic hydrolysis step was collected and transferred to a 1-liter Erlenmeyer flask. To stop the enzymatic reaction, the hydrolysate in the flask was heated in a water bath at 100 °C for 20 min. The hydrolysate was then centrifuged at 8,000 × g for 2 h and filtered through a glass microfiber filter (Whatman). To obtain the glucose concentration, approximately 20 g/L biomass hydrolysate was concentrated by rotary evaporator. The pH of the hydrolysate was adjusted to 6 with NaOH solution, then stored at 4 °C overnight. The hydrolysate was then centrifuged at 8,000 × g for 2 h and filtered through a glass microfiber filter again. The biomass hydrolysate was stored at 4 °C for further experimentation.

5. Bioethanol Production

Saccharomyces cerevisiae K35 was used for fermentation of the biomass hydrolysates to ethanol. The culture strain maintained in the liquid YM medium was inoculated on 10 ml of fresh YM medium in a 50-mL Erlenmeyer flask for the preparation of a seed culture. The cultures were incubated overnight in a shaker at 30 °C at 180 rpm. The yeast cell growth was monitored and measured by optical density (OD) at 600 nm with a UV-Vis spectrophotometer (UV mini-1240, Shimadzu, Japan) [35].

The main culturing for bioethanol production was performed using a 250 mL Erlenmeyer flask containing 50 mL fresh medium. A standard medium for ethanol fermentation consisted of 20 g/L glucose, 2 g/L $MgSO_4$, 2 g/L K_2HPO_4, 10 g/L yeast extract and 10 g/L peptone. The biomass hydrolysate containing ~20 g/L glucose was supplemented with 2 g/L $MgSO_4$, 2 g/L K_2HPO_4, 10 g/L yeast extract and 10 g/L peptone. The pH of both media was adjusted to 6. The media were aseptically filtered through 0.2 μm filter. These media were then inoculated with 2% fresh seed cultures and incubated at 30 °C on a rotary shaker at 150 rpm. After that, liquid fraction was collected at 3, 6, 9, 12, 15, 18, 21, and 24 h of incubation time for the determination of sugar consumption and ethanol production using HPLC. The ethanol yield (%) was calculated based on a theoretical yield of 0.511 g ethanol per g glucose [35,36].

$$\text{Ethanol yield (\%)} = \frac{\text{ethanol released (g)}}{0.511 \times \text{glucose consumed (g)}} \times 100 \quad (3)$$

All experiments were conducted in duplicate with the data presented as the mean±standard deviation (SD).

6. Analytical Methods

The cellulose, hemicellulose, acid soluble lignin (ASL), and acid insoluble lignin (AIL) contents of the treated and untreated weed was analyzed according to the National Renewable Energy Laboratory (NREL) method [37]. The ash and extractive content of the untreated weed was also determined based on NREL procedures [38,39].

Monomer sugars and ethanol were analyzed using a high performance liquid chromatography (HPLC) system (Shimadzu Co., Ltd., Kyoto, Japan) equipped with a refractive index detector (RID-10A). Sugar monomers and ethanol were separated by Shodex sugar SH1011 column (300 mm×8 mm) and 10 mM H_2SO_4 was used for the mobile phase at a flow rate of 0.8 mL/min. The column temperature was maintained at 50 °C and the injection volume was 20 μL.

RESULTS AND DISCUSSION

1. Biomass Composition

The chemical composition of *S. acuta* (in dry weight) was found to be 42.7±0.8% glucan, 19.1±0.7% xylan, 16.1±0.1% acid insoluble lignin (AIL), 2.0±0.1% acid soluble lignin (ASL), 8.4±0.2% ash and 7.5±0.4% extractive. These results indicate that *S. acuta* has higher glucan content than other weed species, such as *Eicchornia crassipes* (30.0%) [9], *Eupatorium adenophorum* (37.1%) [11], *Thysanolaena maxima* (34.3%) [18], and *Typha latifolia* (34.5%) [19]. In addition, the cellulose content of *S. acuta* was found to be higher than that of other residues utilized for bioethanol production including barley straw (33.4%) [40], corn stover (37.1%) [41], rice straw (39.3%) [42], and switchgrass (32.0%) [27].

Therefore, the selected *S. acuta* has a higher potential sugar content than other biomass, and it will be more efficient to utilize sugar platform-based biorefinery such as the bioethanol production process. Previously, biomass potential was calculated by measuring the initial glucan content in the biomass [25,36]. Following this result, the theoretical maximum glucose recovery form *S. acuta*

was estimated to be 47%. However, this value did not reflect solid losses and hydrolysis efficiency after pretreatment. Therefore, it is necessary to estimate the glucose recovery including the solid loss following pretreatment.

2. Effect of Pretreatment Temperature

Pretreatment plays an important role in sugars production from lignocellulosic biomass and the major objective is to improve the enzyme digestibility by changing the solids properties such as surface area, porosity and crystallinity. One of the categories of alkali pretreatment is the use of NaOH as a catalyst, and the main factors of the pretreatment process have been discussed in many previous studies. In general, the reaction temperature, NaOH concentration, and time were considered to be important variables for soaking in NaOH pretreatment, and the range of parameter was determined at 40-80 °C, 0.5-10% NaOH, and 6-24 h, respectively [24-28].

In addition, the biomass loading in the process is an important pretreatment option. Conventional pretreatments designed at 5-10% (w/w) biomass loading have been shown to facilitate higher conversion of biomass into fermentable sugars. However, several studies have investigated the effect of high-biomass loading (>15%, w/w) on different unit operations within the process as a means of improving the economics. Utilizing high-biomass loadings in the process is required to overcome certain challenges such as high concentrations of inhibitors and limitations of mass transfer (high viscosity). The type of biomass used is known to be affected by the amount of feedstock associated water that enters the process, as well as on the way the solid and liquid phases interact. The density of the prepared biomass (*S. acuta*) in current study was 0.279 g/mL; thus, it is possible to immerse the biomass in the reactor at less than 30% biomass loading. As a result of the fundamental experiment, the biomass was not well soaked in the liquid at more than 20% solid loading, probably due to the water-holding capacity (hygroscopicity) of hemicellulose; thus, a special reaction system or equipment is required for high-biomass loading (>15%, w/w) [43]. In this study, the most conventional biomass loading of 10% was finally selected because an additional equipment (for mixing) is not required for the reaction system and most of the comparable data after alkali pretreatment are processed under this condition.

To determine the pretreatment temperature, *S. acuta* was pretreated with 3% NaOH at 50, 60 and 70 °C for 9 h. Table 1 shows the chemical composition, hydrolysis efficiency and glucose recovery of *S. acuta* before and after pretreatment. According to the results, there are no significant differences in the content of glucan, xylan and lignin according to variations in temperature (50 °C, 60 °C, and 70 °C). Meanwhile, solid recovery and hydrolysis efficiency were affected differently depending on the temperature of pretreatment used. Pretreatment at the temperatures of 50 °C, 60 °C, and 70 °C significantly improved hydrolysis efficiency up to 55.8%, 67.4%, and 68.9%, respectively. This phenomenon might be a result of the partial degradation and decomposition of polysaccharides in the biomass due to the high temperature application, resulting in an increase in the accessibility of the enzyme and substrate. The lowest hydrolysis efficiency (12.2%) and glucose recovery (5.2%) were achieved from untreated *S. acuta*. The glucose recovery refers to the overall conversion of biomass (polymer) into glucose (monomer) based on the pretreatment and enzymatic hydrolysis. Thus, the solid recovery (%) after pretreatment, glucan content (%) in the solid fraction, and hydrolysis efficiency (%) after pretreatment are required, and the detailed method is shown in Eq. (2). At the pretreatment temperature of 50 °C, the overall glucose recovery from biomass was about 17.7%, because the solid recovery, glucan content, and hydrolysis efficiency were about 52.5%, 60.5%, and 55.8%, respectively. The solid recovery, glucan content, and hydrolysis efficiency were about 49.7%, 61.4%, and 67.4%, respectively, at 60 °C pretreatment, and about 47.2%, 62.2%, and 68.9%, respectively, at 70 °C pretreatment. Thus, the glucose recovery achieved by the pretreatment at 60 °C and 70 °C was about 20.6% and 20.2%, respectively.

The maximum hydrolysis efficiency was obtained from pretreatment at a temperature of 70 °C. However, the maximum glucose recovery of about 20.6% was achieved at the pretreatment temperature of 60 °C, and the glucose recovery at this pretreatment temperature was increased about 3.96-fold in comparison with untreated *S. acuta*. According to Yoo et al. [25] even though the hydrolysis efficiency is the highest at a specific condition (determined to be in the current study at a temperature of 70 °C), the overall glucose recovery can be lower because the solid loss is significantly increased under the severe conditions. Therefore, the pretreatment factors used should be determined by the overall glucose recovery. The results of this study confirmed that the temperature at 60 °C is a more suitable condition, and this determined temperature is used for further applications.

3. Effect of NaOH Concentration

To investigate the effect of NaOH concentration on hydrolysis efficiency, *S. acuta* was pretreated with 0 (D.W.), 1, 3, 5, and 10% NaOH at 60 °C for 9 h. The chemical composition, hydrolysis efficiency, and glucose recovery of *S. acuta* after pretreatment are shown in Table 2. According to these results, pretreatment by different concentrations of NaOH had a significant effect on the chemical composition of *S. acuta*. In particular, the hydrolysis efficiency was significantly improved with increases in the NaOH concentra-

Table 1. Chemical composition of *Sida acuta* pretreated with 3% NaOH at various temperatures

Temperature (°C)	Solid recovery (%)	Glucan (%)	Xylan (%)	ASL (%)	AIL (%)	Hydrolysis efficiency (%)	Glucose recovery (%)
Untreated	100	42.7±0.2	19.8±0.3	2.0±0.1	16.1±0.1	12.2±0.5	5.2
50	52.5±2.0	60.5±0.8	23.2±0.2	0.6±0.1	15.3±0.2	55.8±1.2	17.7
60	49.7±1.3	61.4±0.2	23.3±0.4	0.5±0.1	14.2±0.6	67.4±0.8	20.6
70	47.2±1.6	61.9±0.6	23.4±0.3	0.5±0.1	14.1±0.4	68.9±0.9	20.1

Table 2. Chemical composition of *Sida acuta* pretreated with different concentrations of NaOH at 60 °C

NaOH (%, w/w)	Solid recovery (%)	Glucan (%)	Xylan (%)	ASL (%)	AIL (%)	Hydrolysis efficiency (%)	Glucose recovery (%)
Untreated	100	42.7±0.2	19.8±0.3	2.0±0.1	16.1±0.1	12.2±0.5	5.2
0 (D.W.)	78.7±0.8	43.3±0.2	22.3±0.1	1.5±0.1	20.3±0.1	20.5±0.8	7.0
1	68.2±0.7	46.3±0.4	23.2±0.1	0.9±0.1	15.3±0.1	38.8±1.0	12.3
3	49.7±1.3	61.4±0.2	23.3±0.4	0.5±0.1	14.2±0.6	67.4±0.8	20.6
5	41.1±2.6	66.8±1.0	nd	0.3±0.1	9.6±0.4	72.5±1.1	19.9
10	34.7±2.4	69.6±1.1	nd	0.3±0.1	8.5±0.8	82.8±0.5	20.0

tion, and the enzymatic hydrolysis efficiency by D.W., 1%, 3%, 5%, and 10% NaOH was found to be 20.5%, 38.8% 67.4%, 72.5%, and 82.8%, respectively. The maximum hydrolysis efficiency was obtained by pretreatment with 10% NaOH, and this efficiency is higher than that of other NaOH-pretreated biomass, such as poplar (41.5%) [28], *Picea abies* (40.0%) [44], *Typha angustifolia* (55.3%) [45], *Betula pendula* (72.0%) [46], switchgrass (74.0%) [47], rice straw (78.7%) [48], sugarcane bagasse (55.1%) [49], oil palm mesocarp fiber (60.0%) [50], and *Imperata cylindrical* (70.0%) [51]. The reason for the increase in hydrolysis efficiency is presumed to be the removal of components (hemicellulose, lignin, and others) that inhibit the enzymatic reaction of biomass (cellulose) to glucose conversion. Previously Kim et al. [42] reported that the removal or change of specific components in the biomass pretreatment is affected by the type or concentration of the catalyst used. Similarly, in this study, specific components were more affected by NaOH concentration (chemical selectivity). Xylan content was not detected following pretreatment with 5% and 10% NaOH, which means that the hemicellulose portion in the biomass is 100% removed. In addition, the removal of acid insoluble lignin (AIL) following pretreatment was found to be 0.8%, 29.3%, 56.2%, 75%, and 81% at NaOH concentrations of 0% (D.W.), 1%, 3%, 5%, and 10%, respectively. This indicates that AIL removal is improved as the NaOH concentration increases in pretreatment. However, as the concentration of NaOH increased, the solid recovery after pretreatment decreased, as the obtained solid recovery was about 78.7%, 68.2%, 49.7%, 41.1%, and 34.7% at NaOH concentration of 0% (D.W.), 1%, 3%, 5% and 10%, respectively. In particular, the lowest solids recovery was obtained at 10% NaOH concentration. The glucose recovery was calculated based on the results with Eq. (2), and it was estimated to be about 7.0%, 12.3%, 20.6%, 19.9%, and 20.0% in pretreatment at NaOH concentrations of 0% (D.W.), 1%, 3%, 5%, and 10%. The glucose recovery of untreated *S. acuta* (control) was about 5.2%, whereas pretreated biomass improved the recovery. The highest recovery was achieved at 3% NaOH concentration, which was approximately 3.96-fold improved from that of the control group. The hydrolysis efficiency was the highest when pretreated with 10% NaOH, suggesting that its total glucose recovery would be the highest. However, the experimental results show that the highest glucose recovery was achieved by pretreatment with 3% NaOH concentration. This is due to the losses of the solid fraction during the pretreatment process. Thus, despite the high hydrolysis efficiency achieved through the pretreatment process, a reduction in solid recovery could decrease the total glucose recovery. This phenomenon is very similar to the previously reported by Yoo et al. [25]. In addition, the pretreatment of raw material with a low chemical concentration is more environmentally friendly and cost effective than with a high concentration of chemicals, especially when it needs to be applied in bioethanol production [35,36]. Therefore, the final pretreatment condition was determined to be 3% NaOH at 60 °C for 9 h, which showed the highest glucose recovery in current study.

4. Effect of Enzyme Loading

The amount of enzyme used in the saccharification process is highly related to economic efficiency. To determine the appropriate enzyme dosage, various loadings of cellulase (60-240 FPU/g-biomass of Celluclast 1.5 L and 30-120 CBU/g-biomass of Cellobiase) were conducted. Fig. 2 shows the enzymatic hydrolysis efficiency of *Sida acuta* by various enzyme loadings. Pretreated means pretreatment of *S. acuta* using 10% NaOH at 60 °C for 9 h. The hydrolysis efficiency of untreated *S. acuta* (control group) at 120 h was found to be 12.2% by enzyme loading of 240 FPU+120 CBU. As a result of using the pretreated biomass as a substrate, the hydrolysis efficiency was increased with increasing enzyme loading. At 120 h hydrolysis, the efficiency by enzyme loading of 60 FPU+30 CBU, 120 FPU+60 CBU, and 240 FPU+120 CBU was achieved about 68.3%, 80.2%, and 82.8%, respectively. The hydrolysis efficiency by enzyme loading of 240 FPU+120 CBU was not significantly improved compared to the enzyme loading of 120 FPU+60 CBU. The

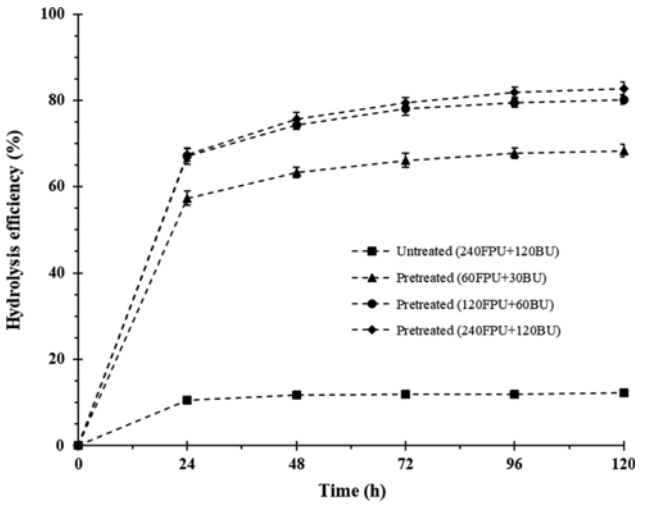

Fig. 2. Enzymatic hydrolysis efficiency of *Sida acuta* by various enzyme loadings.

initial reaction rates up to 24 h were particularly similar. Therefore, the effective amount of enzyme loading was finally determined to be 120 FPU+60 CBU. This hydrolysis efficiency (80.2%) was still higher than that of other biomass previously described in the "3. Effect of NaOH concentration of Results and Discussion section." The incomplete enzymatic hydrolysis of pretreated *S. acuta* biomass might have resulted from the retention of a complex structure of microcrystalline cellulose that inhibited cellulase activity [52].

5. Application to Bioethanol Production

The liquid fraction obtained by enzymatic hydrolysis of *S. acuta* was contained at about 20.4 g/L glucose. This biomass hydrolysate (BH), which was not detoxified by any process, was used for bioethanol production. As a standard (ST), a medium containing 20 g/L pure D-glucose of reagent grade (98%) was used as a control for bioethanol fermentation. The results revealed that the profiles of bioethanol production and growth rate of *S. cerevisiae* K35 in the ST medium were quite different from those in the BH medium. In the ST medium, glucose was rapidly utilized and depleted in 9 h. During this period, bioethanol production by *S. cerevisiae* K35 was sharply increased and reached a maximum concentration (10.1 g/L or 98.9% of the theoretical yield). After that point, the concentration of bioethanol was not improved. However, the glucose in BH medium was gradually utilized from 0-15 h and completely fermented in 18 h. During this period, bioethanol concentration was gradually increased and the maximum bioethanol concentration (10.2 g/L or 97.6% of the theoretical yield) was obtained at 18 h (Fig. 3). This amount of bioethanol was slightly lower than that of the ST medium. Moreover, *S. cerevisiae* K35 in the ST medium presented a relatively short lag-phase and a high growth rate. In the BH medium, *S. cerevisiae* K35 had a longer lag-phase and a slower growth rate than that observed in the ST medium (Fig. 4). Nevertheless, this performance had no effect on the bioethanol production of yeast. This indicates that BH medium without detoxification contained some inhibitory compounds which had

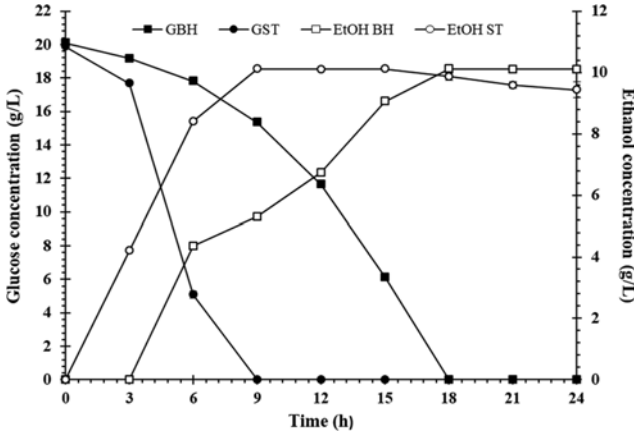

Fig. 3. Profiles of ethanol fermentation of *S. cerevisiae* K35 in standard (ST) and biomass hydrolysate (BH) media. GBH=Glucose consumption in biomass hydrolysate (BH) medium. GST=Glucose consumption in standard (ST) medium. EtOH BH=Ethanol production by biomass hydrolysate (BH) medium. EtOH ST=Ethanol production by standard (ST) medium.

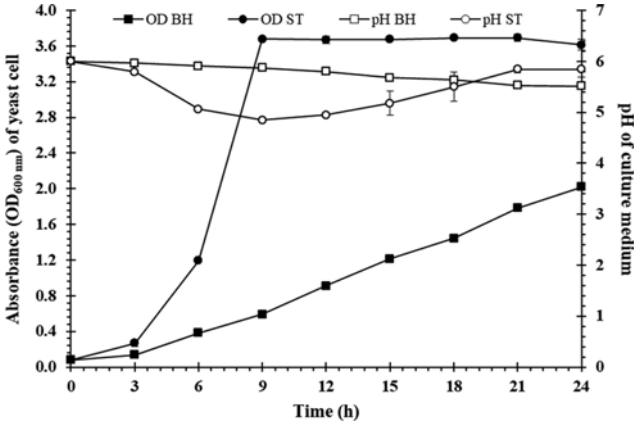

Fig. 4. Profiles of cell growth and pH change of *S. cerevisiae* K35 in standard (ST) and biomass hydrolysate (BH) media. OD BH= Optical density of yeast cell in biomass hydrolysate (BH) medium. OD ST=Optical density of yeast cell in standard (ST) medium. pH BH=pH change in biomass hydrolysate (BH) medium. pH ST=pH change in standard (ST) medium.

effects on yeast cell growth but had no effects on bioethanol production by *S. cerevisiae* K35. This phenomenon is comparable to the effect of inhibitors that may be formed during the pretreatment of lignocellulose. Almeida et al. [53] and Klinke et al. [54] reported that a low concentration of these inhibitory substances in lignocellulosic hydrolysate had no effect on bioethanol production by *S. cerevisiae*, whereas the cell growth of yeast was decreased. The use of high concentrations of hydrolysates significantly reduced the process cost such as ethanol distillation due to the increased concentration of ethanol. According to the reports, current technology has allowed the use of up to 30% solids content in the fermentation of starch; however, only 15-20% solids in lignocellulose conversion can be operated at the pilot plant-scale due to the inhibitors [42,43]. Pretreatment at high-biomass loading may be attractive to produce higher sugar concentrations; however, there is a risk of producing high concentrations of fermentation inhibitors. It is well known that the pretreatments lead to the production of degradation products such as acetic acid, furfural, hydroxymethylfurfural (HMF), and phenolic compounds. Therefore, optimization of pretreatment conditions to minimize inhibitor production, with consideration of the severity of the pretreatment and concentration of the biomass feedstock is necessary, as the combination of all of these variables is important when developing the efficient biorefinery process.

To evaluate the overall process, a material balance of biomass into bioethanol production was established; a schematic diagram of bioethanol production based on 1,000 g *Sida acuta* is presented in Fig. 5. Following this result, approximately 497 g solid can be recovered by NaOH pretreatment, and about 305 g glucan, 116 g xylan, 3 g acid soluble lignin, and 71 g acid insoluble lignin should be contained in the solid fraction. In enzymatic hydrolysis, a glucose conversion of 12.2% was obtained by untreated *S. acuta*, and about 57 g glucose was estimated based on a 1,000 g basis. However, a glucose conversion of 226 g was estimated by NaOH pretreatment and the theoretical maximum glucose recovery was

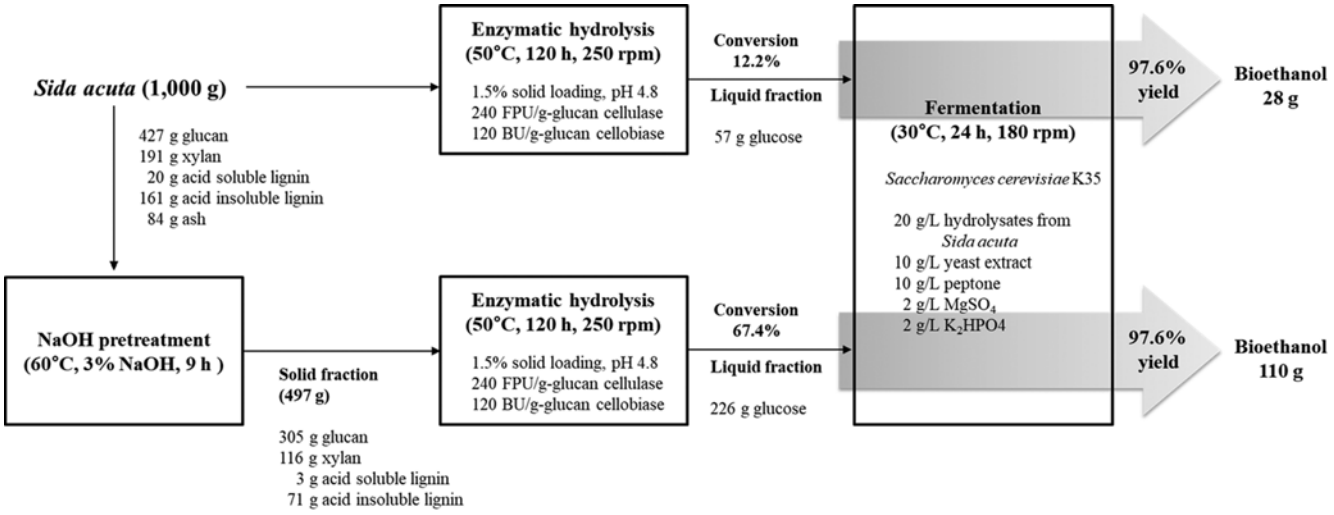

Fig. 5. Material balance of biomass to bioethanol production based on 1,000 g *Sida acuta*.

estimated to be 467 g based on initial glucan content in of *S. acuta*. Finally, the bioethanol yield of 97.6% was obtained by using biomass hydrolysates in the fermentation; thus, following the yield, bioethanol production by untreated and pretreated *S. acuta* was estimated as about 28 g and 110 g, respectively. Overall, the glucose conversion and bioethanol production of *S. acuta* was improved about 5.5-fold and 3.9-fold by NaOH pretreatment, respectively. This study provides the information necessary to improve bioethanol production through the pretreatment process. Statistical methods can be used to more effectively investigate the effect of major pretreatment factors such as temperature, catalyst concentration, and time on the solids recovery, hydrolysis efficiency and glucose recovery of *S. acuta*. In the near future, response surface methodology (RSM) will be used in our next study to investigate the correlation of factors. Thus, the results of this study will be beneficial as fundamental data for the experimental design of RSM.

CONCLUSION

Pretreatment of lignocellulosic biomass is significantly essential to improving the efficiency of the biorefinery process. A widely abundant weed in Thailand, *S. acuta*, was selected as a feed stock for bioethanol production. To improve glucose conversion, NaOH pretreatment was performed and the effects of major factors such as temperature and catalyst concentration were investigated. Following pretreatment, not only the hydrolysis efficiency but also the recovery of solid fraction was considered for the enhancement of overall glucose recovery from biomass. As a result, the highest glucose recovery was achieved at 60 °C with 3% NaOH for 9 h pretreatment. The theoretical maximum glucose recovery is estimated to be 467 g based on a 1,000 g *S. acuta* basis; however, glucose conversion by untreated and pretreated biomass was estimated as about 57 g and 226 g, respectively. Thus, the overall glucose recovery was about 5.5-fold improved by the pretreatment. In addition, the hydrolysates from *S. acuta* were applied to bioethanol production by *S. cerevisiae* K35 and the maximum bioethanol yield of 97.6% was achieved at 18 h. Finally, overall bioethanol production by untreated and pretreated 1,000 g of *S. acuta* can be estimated as about 28 g and 110 g, respectively.

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