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Research paper

Pretreatment of Sugarcane Bagasse by Hot Compressed Water In The Presences of Acid and Alkaline Promoters

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Abstract

Continual rise in utilization of the depleting fossil fuel leads to environmental concerns related to greenhouse gas release which makes finding sustainable alternative platform industry for the production of fuels, commodity chemicals, and materials from renewable plant biomass of great interest. Pretreatment of the starting biomass is a pre-requisite in sugar platform biorefinery in order to increase enzymatic digestibility of the recalcitrant lignocellulosic materials. In this work, pretreatment of sugarcane bagasse was studied under hot compressed water condition in the presence of several acid/alkali promoters (1.5% H_2SO_4, 5% NaOH) or alternatively in weak acid (acetic acid) or base (triethylamine (TEA) as the sole reaction medium under low reaction temperatures (80 to 120 °C) for 30 min with pressurized nitrogen gas at 20 bar with continuous stirring at 100 rpm. It was found that the use of base promoters strong base NaOH are efficient in pretreatment under the low temperature in experimental conditions enhancing biomass pretreatment efficiency with high delignification efficiency and high cellulose recovery.

***Keyword**: Acid/Alkali promoters; Biorefinery; Enzymatic hydrolysis; Lignocellulose*

1. Introduction

In Thailand, bagasse is one of most abundant crops, of which bagasse is the major waste and/or by-product of the sugar industry. Technically, bagasse is known as "lignocellulosic biomass", which refers to the plant compound that composes of cellulose, hemicelluloses, and lignin. Importantly, cellulose (as well as hemicelluloses) is nowadays considered as the important carbohydrate source for renewable energy and fuel production. Lignocellulosic ethanol is a biofuel produced from lignocellulose compounds i.e. wood, grasses, and the non-edible parts of plants. The native list of biomass residues in Thailand has been accumulated by the Ministry of Agriculture and Cooperatives (MoAC) and DEDE. The Table I are presented the net amount made available for energy purposes only. The compilation indicates a potential of 6040 MW for electricity generation in 2036. The government is also considering additional sources, such as dedicated energy cultivated land. Geographically, agricultural residues such as rice husk, cassava, and bagasse are mostly distributed in the central, northern and northeastern regions, while oil palm residues exist largely in the southern provinces, according to the Office of Agricultural Economics of Thailand.[1]

Table 1. biomass residue potential in thai land

Biomass type	Available residues for energy purposes 2014			Available residues in MoAC's development plan for energy		
	tonne/ year	ktoe	Power potential (MW)	tonne/ year	ktoe	Power potential (MW)
Rice husk	432	0.14	0.05	432	0.14	0.05
Rice straw	4,124,630	1,204	461	4,124,630	1,204	461
Sugar cane and leaf	2,928,140	1,073	411	5,265,619	1,929	738
Bagasse	-	-	-	21,280,000	3,712	1,421
Corn cob	80,889	18	7	80,889	18	7
Corn trunk	3,369,690	784	300	3,369,690	784	300
Cassava rhizome	2,838,125	369	141	3,372,560	439	168
Cassava trunk	1,052,636	388	149	2,084,755	769	294
Oil palm frond	14,606,671	2,265	867	33,586,191	5,208	1,993
Oil palm fiber	606,541	104	40	1,402,455	240	92
Oil palm shell	-	-	-	2,944,803	795	304
Oil palm empty fruit bunch	-	-	-	619,959	248	95
Para wood root	1,411,834	287	110	1,411,834	287	110
Coconut shell	79,678	31	12	79,678	31	12
Coconut fiber	71,875	27	10	71,875	27	10
Coconut bunch and frond	249,026	91	35	249,026	91	35
Total	31,420,166	6,642	2,542	79,944,394	15,783	6,040

*Based on data from MoAC and DEDE[1]

Theoretically, lignocellulose is a structural material that comprises much of the mass of plants. It is generally composed mainly of cellulose, hemicellulose, and lignin. Corn stover, switchgrass, miscanthus, woodchips and the byproducts of lawn and tree maintenance are some of the more popular cellulosic materials for ethanol production. Production of ethanol from lignocellulose has the advantage of abundant and diverse raw material compared to sources like corn and cane sugars, but it requires a greater amount of processing to make the sugar monomers available to the microorganisms that are typically used to produce ethanol by fermentation. Moreover, pretreatment of lignocellulosic biomass

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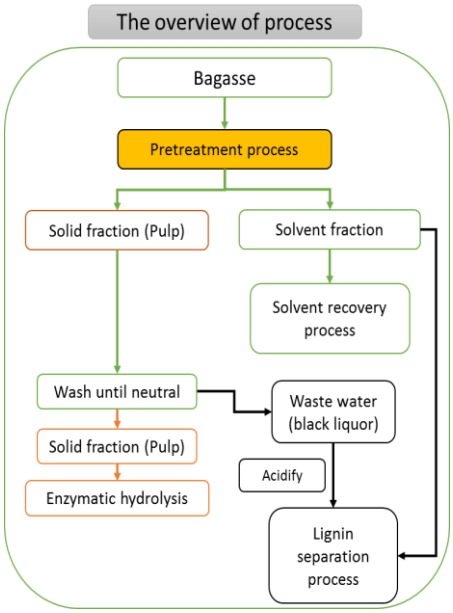
aims at rendering cellulose accessible to the action of hydrolytic enzymes by altering the lignocellulosic cell wall [2, 3]. Pretreatment effects include the increase of the accessible surface area, cellulose decrystallization, partial cellulose depolymerization, hemicellulose and/or lignin solubilization, and the modification of the lignin structure. Many pretreatment technologies have been proposed generally on the basis of combined physical and chemical actions. These include a steam explosion, liquid hot water (LHW), ammonia fiber expansion (AFEX), acid cooking, lime, Organosolv extraction. The main constraints are minimizing sugar degradation and the formation of inhibitors (furanic and phenolic compounds) and limiting the consumption of chemicals, energy and water, and the production of wastes.

To overcome the recalcitrance of lignocellulosic biomass is the goal of enzymatic hydrolysis. The process is applied to depolymerize the polysaccharides in the water-insoluble solid fraction that remains after pretreatment. After most pretreatments, the bulk of these remaining polysaccharides are cellulose. After the pretreatment, the glucose molecules are still imprisoned in long chains of cellulose and hemicellulose and therefore not readily available for fermentation. This is the reason that the hydrolysis is necessary for the fermentation process. [4]

In this work, focus on the comparative study of acid/alkaline promoters in LHW pretreatment to maximize the reducing sugar for the fermentation process.



2. Materials and Methodology

A. Raw material preparation

Sugarcane bagasse was obtained from PTT Global Chemical Public Company Limited (PTTGC). This biomass was proud by Retsch ZM200 cutting mill. (Retsch, Haan, Germany) and sieved through 18 mesh to < 2 mm^2. The chemical composition of the bagasse was analyzed by National Renewable Energy Laboratory (NREL) method [6]. All chemicals and reagents were analytical grade and purchased from major chemical companies.

B. Pretreatment

The pretreatment reaction was performed with the total volume of 100 mL in a 600-ml Parr reactor. The pretreatment process was studied using LHW in the presence of several acid/alkali promoters i.e. 1.5% sulfuric acid (H_2SO_4), 5% sodium hydroxide (NaOH), or alternatively in pure weak acid (acetic acid) or base (triethylamine (TEA)) The reaction contained 10% w/v sold per liquid ratio and heated at low reaction temperatures (80 to 120 °C) for 30 min with pressurized nitrogen gas at 20 bar. The reaction was continuously stirred at 100 rpm. The solid cellulose fraction was separated by filtration on filter paper (Whatman number 4), washed with distilled water and dried at 60°C before subjecting to enzymatic hydrolysis. The liquid fraction was collected for analysis of sugar and inhibitory by-products by high performance liquid chromatography (HPLC).

C. Enzymatic hydrolysis

The pretreatment efficiency was studied by saccharification with a commercial cellulase under standard reaction conditions. The hydrolysis reactions of 1 ml total volume was composed of 5% (w/v) pretreated substrate, with 10 FPU/g *Cellic CTEC2 (Novozymes)* in 50 mM sodium citrate buffer, pH 4.8 and 50 µl of 5% sodium azide. The reactions were incubated at 50°C for 72 h with vertical rotation at 30 rpm. The experiments were done in triplicate. The fermentable sugars profiles were analyzed by high performance liquid chromatography (HPLC).

D. Analysis

High Performance Liquid Chromatography (HPLC)

Composite sugar profiles were analyzed on a high performance liquid chromatography (LDC Model 4100) equipped with a refractive index detector using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 65˚C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.5 ml/min.

Chemical component analysis

The raw material and the residue were analyzed according to the standard Laboratory Analytical Procedures (LAP) for biomass analysis provided by the National Renewable Energy Laboratory (NREL).[6] The percentage of solid residue compositions can be calculated as below

$$Cellulose\ content\ (\%) = Glucose\ recovery\ after\ pretreated\ (\%) \times 0.9 \quad (1)$$

$$Hemicellulose\ content\ (\%) = Xylose\ recovery\ after\ pretreated\ (\%) \times 0.88 \quad (2)$$

$$Hemicellulose\ content\ (\%) = Arabinose\ recovery\ after\ pretreated\ (\%) \times 0.88 \quad (3)$$

The percentage of enzymatic hydrolysis efficiency can be calculated as below;

$$Enzymatic\ hydrolysis\ efficiency\ (\%) = \frac{Glucose\ after\ hydrolysis\ \left(\frac{mg\ glucose}{g\ pretreated}\right)}{Glucose\ in\ pretreated\ material\ \left(\frac{mg\ glucose}{g\ pretreated}\right)} \times 100 \quad (4)$$

The percentage of hemicellulose removal can be calculated as below;

$$Hemicellulose\ removal\ (\%) = \frac{(hemicellulose\ in\ raw\ - hemicellulose\ remaining\ in\ solid\ pulp\ )(m}{hemicellulose\ in\ raw\ (mg)} \times 100 \quad (5)$$

The percentage of lignin removal can be calculated as below;

$$Lignin\ removal\ (\%) = \frac{(lignin\ content\ in\ raw\ -\ lignin\ remaining\ in\ solid\ pulp\ )(mg}{lignin\ content\ in\ raw\ (mg)} \times 100 \quad (6)$$

The glucose hydrolysis can be calculated as below;

$$Glucose\ hydrolysis\ \left(\frac{mg}{g} pretreated\ biomass\right) = \frac{glucose\ after\ hydrolysis\ \left(\frac{mg\ glucose}{ml}\right)}{pretreated\ biomass\ loading\ \left(\frac{g\ pretreated\ biomass}{ml}\right)} \quad (7)$$

The percentage of glucose yield can be calculated as below;

$$Glucose\ yield\ (\%) = \frac{glucose\ hydrlysis\ \left(\frac{mg\ glucose}{g\ pretreated}\right) \times remaining\ pulp\ (\frac{g\ pret}{g\ native}}{glucose\ content\ in\ raw\ material\ (\frac{mg\ glucose}{g\ native\ biomass})} \times 100 \quad (8)$$

The solid residue after pretreatment was used in the enzymatic hydrolysis process, the concentration of monomeric sugar was determined by HPLC and calculate follow the step of the equation.

3. Results and Discussions

The native sugarcane bagasse used in experiment contained 42.39% cellulose, 15.16% hemicelluloses, 30.82% lignin and 8.22% ash. The composition of the biomass calculated from equation (1-3) is shown in table 2.

Table 2. the composition of native sugarcane bagasse in this study based on dry basic.

Native Bagasse (% w/w)			
Cellulose	42.39	glucose	47.1
Hemicellulose	15.1	xylose arabinose	16.26 0.9
Lignin	30.84		
Total Ash	8.22		

The effects of acid, alkaline and hot compressed water on bagasse pretreatment

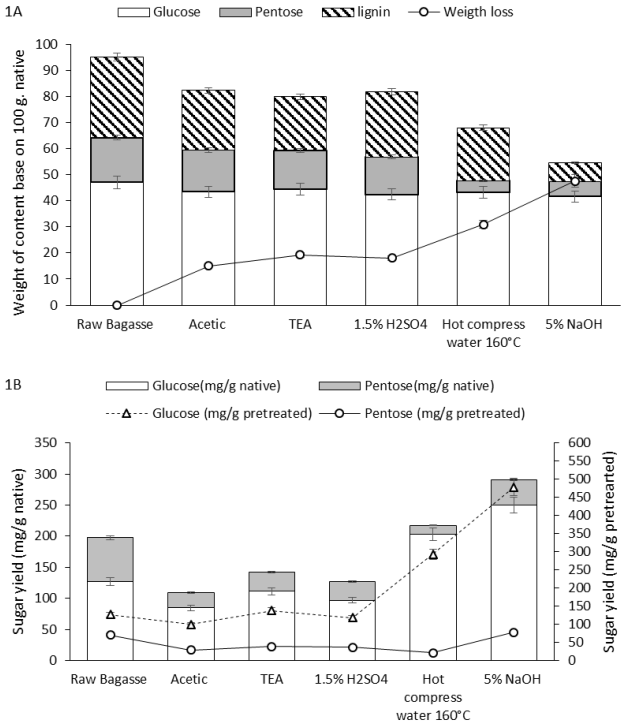


Figure1 Effects of acid and alkaline in the pretreatment of sugarcane bagasse. The reaction contained 10% solid loading in the presence of acid (1.5% H_2SO_4) and alkaline (5% NaOH) or solely in acetic acid The reactions were heated at 80°C for 30 min with 10% solid loading. The pretreatment reaction using liquid hot water at 160°C for 30 min was used for comparison.

This set of an experiment aimed to compare the effects of acid and alkaline pretreatment at low temperature to the conventional hot compressed water pretreatment with no promoter at high temperature. The result in figure 1 shows the pretreatment led to weight recovery of 52.44-84.93%. Acid pretreatment with H_2SO_4 led to the lowest lignin removal (18.74%) from the solid phase while alkaline pretreatment using NaOH resulted in the highest lignin removal (76.91%). Only slight hydrolysis of hemicellulose was observed for the acid pretreatment conditions using H_2SO_4 or acetic acid, it cannot activate and increase of enzymatic digestibility at the low temperature and short reaction time [7,8] while the high removal of hemicellulose was found with the NaOH pretreatment and liquid hot water. High recovery of cellulose (88.32-92.25%) was observed under all conditions, suggesting minimal degradation of the cellulose fraction under these pretreatment conditions.[10] The highest biomass digestibility (mg/g pretreated) was obtained from enzymatic hydrolysis of the solid fraction from NaOH pretreatment (glucose 477.60 mg/g pretreated) followed by liquid hot water (glucose 293.09 mg/g pretreated) while low biomass digestibility was found with the acid pretreated biomass. Because, the structure of the substrate (e.g. crystallinity, surface area, pore structure) and the mechanism and interactions of the cellulolytic enzymes are factors limiting the cellulose hydrolysis. In addition, lignin content and its distribution within the lignocellulose matrix have an impact on enzymatic hydrolysis of cellulose [8]. The highest glucose recovery yield (250.45mg/g native) was achieved with the NaOH pretreated biomass. In addition, the result suggested that the alkali pretreatment have a higher pretreatment efficiency than the acid pretreatment at the same temperature and hot compressed water process at the elevated temperature.

The effects of acid, alkaline and hot compressed water on the solid contents of pretreated bagasse compare with native bagasse

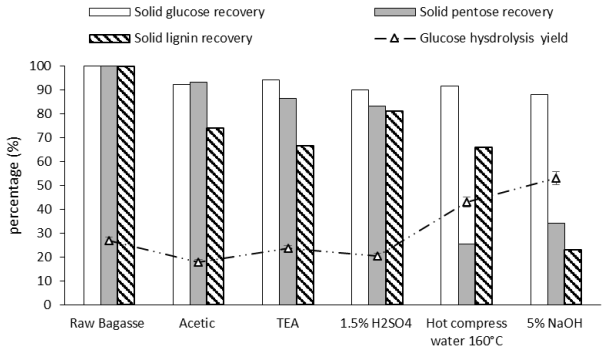


Figure 2 Effect of solid recovery and glucose hydrolysis yield after acid and alkaline in the pretreatment of sugarcane bagasse. The reaction contained 10% solid loading in the presence of acid (1.5% H_2SO_4) and alkaline (5% NaOH) or solely in acetic acid The reactions were heated at 80°C for 30 min with 10% solid loading. The pretreatment reaction using liquid hot water at 160°C for 30 min was used for comparison.

For a given reaction temperature and time (HCW at 160°C and 30 min), pentose removal has a high potential than other method and pentose removal equal to 74.5% of native biomass. In contrast, the lignin removal equal to 33.1% and refer to the inhibit enzyme digestibility [9]. The dilute acid generally increases surface area, removes hemicelluloses and alters lignin structure in the high reaction temperature. Wease, the low operating temperature is cannot remove lignin and hemicellulose as expected. [11]

The effects of temperature on TEA-based pretreatment

In this experiment, the use of weal organic base (triethylamine or TEA) as the sole solvent in biomass pretreatment was investigated. TEA has a low boiling point of 88.6°C and could be recycled after the pretreatment process using TEA as the sole solvent or as a promoter in other reaction media. The bagasse was pretreated with TEA at varying temperature. (60-120°C) for 30 min

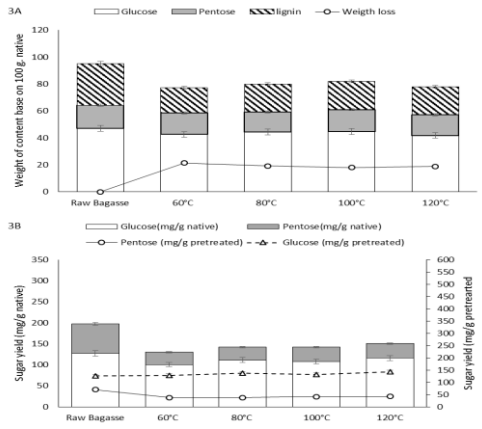


Figure 3 Pretreatment of bagasse using triethylamine (RX 1-4) at varying temperatures. The reactions contained 10% solid loading in TEA as the sole solvent and heated at varying temperatures for 30min.

Increasing temperature in the TEA-based reaction from 60-120℃ led to no difference in solid recovery (78.59-81.91%). No substantial difference in lignin removal (32.75-39.55%), hemicellulose removal (pentose 4.3-13.5%), and cellulose recovery (glucose 88.89-94.84%) were observed under all pretreatment conditions. No changes in biomass digestibility and glucose recovery after enzymatic hydrolysis were observed compared to the native biomass with no pretreatment suggesting no marked effects of pretreatment on the biomass. The results suggested the minimal effects of the TEA-based pretreatment to the biomass structure. In the future TEA-based pretreatment, it will be used on the sequential pretreatment or combine with another method (i.e. alkaline peroxide, supercritical fluid and microwave) to produce the microcrystalline cellulose because the reagent has a major effect on lignin removal only.[12-14]

4. Conclusion

The use of base promoters strong base NaOH is efficient in LHW pretreatment under the low temperature in experimental conditions enhancing biomass pretreatment efficiency with high delignification efficiency and high cellulose recovery. The results also suggested the minimal effects of the acid and TEA-based pretreatments to the biomass structure.

Acknowledgment

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