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Conversion of Sugarcane Shoots to Reducing Sugars

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***Abstract*—**Sugarcane Shoots is an abundantly available residual resources consisting of lignocelluloses which take it into the benefit. The present study was focused on utilizing of sugarcane shoot for reducing sugar production as a substrate in ethanol production. Physical and chemical pretreatments of sugarcane shoot were investigated. Results showed that the size of sugarcane shoot influenced the cellulose content. The maximum cellulose yield (60 %) can be obtained from alkaline pretreated sugarcane shoot with 1.0 M NaOH at 30 ^{o}C for 90 min. The cellulose yield reached up to 93.9% (w/w). Enzymatically hydrolyzed of cellulosic residual in 0.04 citrate buffer (pH 5) with celluclast 1.5L (0.7 FPU/ml) resulted in the highest amount of reducing sugar at a rate of 32.1 g/l after 4 h incubation at 50°C, and 100 ^{o}C for 5 min . Cellulose conversion was 55.5%.

***Keywords*—**Conversion, Sugarcane Shoots, Reducing Sugars.

I. Introduction

THE use of biomass as a renewable energy source has advantages for the development of application of new technologies, the creation of new jobs for biomass harvesting and manufacturing processes and the promotion of many environmental advantages [1]. Bioethanol, a renewable product from lignocellulosic biomass, has attacked much attention nowadays. Many lignocellulosic materials, particularly agricultural residues which are the big sources of the biomass (e.g., wheat straw, corn stover, sugarcane bagasse), can be converted to fermentable sugars, followed by fermentation to ethanol [9]. These materials generally contain cellulose, hemicelluloses and lignin, of which hemicelluloses and lignin makes the access of cellulase enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis to reducing sugar [3]. A great deal of research has been focused with rice straw and corn stover with many aspects of pretreatment processes. Ethanol can be made from the post-harvest sugarcane residue namely, leaf litter. The optimal pretreatment conditions for high efficiency ethanol production were accomplished using alkaline hydrogen peroxide pretreatment and sulfuric acid pretreatment, followed by three weeks of fermentation using the ATCC yeast, *Saccharomyces cerivisiae* strain765 [4].

After sugarcane was harvested and processed, there is a large amount of biomass waste. Sugarcane shoot is one of the most abundant agricultural residues in Thailand (about 6 million ton/year). Farmers generally burned them causing global warming [5]. The current study was initiated to determine a potential of sugarcane shoot for conversion to reducing sugar as a substrate for ethanol production. However, sugarcane shoot consists of three main polymers: cellulose, hemicelluloses and lignin that obstructs conversion to reducing sugar. Therefore, the research objectives were to 1) investigate the sugarcane shoot pretreatment methods: physical and chemical treatment and 2) study the reducing sugar from enzymatic hydrolysis of pretreated sugarcane shoot.

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II. Material and Method

A. Raw material and physical pretreatment

Three verities of sugarcane shoots : Sugarcane juice Suphanburi50, Sugarcane 95-2-156 and Chewing cane Suphanburi72 obtained from Office of Agricultural and Development Region 5 (OADR5) in suphanburi province, Thailand, were cleaned, chopped and dried in an oven at 50^{o}C for 3 days. The dried materials were milled with ultracentrifugal mill (ZM 200) and pass through a 40 mesh screen. A portion was then further passed through the 60 and 80 mesh screens to obtain a particle size of 0.250-0.425 mm, 0.180-0.250 mm and less than 0.180 mm. These materials were stored at room temperature for further analysis of the compositions (moisture and ash content by AOAC (2000), cellulose and hemicellulose content by TAPPI T 203 cm-99, lignin content by TAPPI T 222 om-06.

B. Alkaline pretreatment of Sugarcane shoots

The sugarcane shoot with particle size of 0.250-0.425 mm was pretreated with aqueous NaOH at concentrations of 0.5, 1.0, 2.0 and 4.0 M at a solid to liquid ratio of 1:10 and incubated in a shaker bath at 30, 50, 70 and 90°C for 90 min. The solid residual was separated from the mixture by filtration and washed thoroughly with tap water to pH 7, then dried at 50°C for 1 h. The washed solids were collected for composition analysis (cellulose, hemicelluloses and lignin) and enzymatic hydrolysis.

C. Enzymatic hydrolysis

In order to evaluate the influence of cellulose content on enzymatic hydrolysis, 50 mg (dry weight) of the solid residual from each pretreatment of sugarcane shoot with 0.5, 1.0, 2.0 and 4.0 M NaOH at 30 ^{o}C for 90 min, was suspended in 2 ml 0.04 M citrate buffer pH 5, and it was steam sterilized by autoclaving at 121 ^{o}C, 1 atm, for 15 mim. Enzymatic hydrolysis was performed with 0.2 ml celluclast 1.5L (Novozyme, Denmark) with activity of 0.7 FPU/ml at 50°C for 4 h, then was heated at 100 ^{o}C for 5 min, and finally centrifuged at 3,500 rpm/min. The

supernatant was determined for reducing sugar by DNS method [8].

III. Result and Discussion

A. The composition of sugarcane shoots

Three varieties of sugarcane shoots: sugarcane juice Suphanburi50, Sugarcane 95-2-156 and chewing cane Suphanburi72 have moisture content of 5.3±0.74 5.1±0.03 and 5.4±0.28 %, respectively which below the standard value of AOAC (not more than 10 %), therefore, there was no effect of moisture content to chemical component analysis. Analysis of chemical composition of three varieties of sugarcane shoots showed that cellulose was the major component accounts for up to 69.1-71.4%(dry weight), the rest were hemicellulose ranging from 12.7-15.7%(w/w), lignin ranging from14.4-15.9 %(w/w) and ash, respectively (Table 1). From the result, Chewing cane Suphanburi72 was used throughout the entire study.

B. Pretreatment of sugarcane shoot

Prior to enzymatic hydrolysis, sugarcane shoot was pretreated by physical method and chemical treatment with NaOH. The pretreatment results, expressed as chemical composition of pretreated sugarcane shoot are listed in Table 2 and 3. Table 2 showed that the smaller size of samples, the higher cellulose content was obtained. From the results, the particle size of sugarcane shoot between 0.180-0.250 mm was subsequently pretreated with NaOH at various concentrations and temperatures. Increasing the concentration of NaOH and temperature resulted in different weight loss and compositions. Treatment with 2.0 and 4.0 M NaOH gave higher percentage of cellulose significantly different ($p<0.05$) than at 0.5 and 1.0 M NaOH. However, pretreatment at 1.0 M NaOH gave the percentage of cellulose yield higher than 60%. While at higher concentrations of 1.0 M NaOH, the percentage of cellulose yields decreased. On the other hand, hemicellulose and lignin content substantially decreased in every pretreatment concentrations and temperatures (Table3). Based on the results of percentage of hemicellulose and lignin removal, NaOH - pretreated sugarcane shoot at concentration of 0.5, 1.0, 2.0 and 4.0 M at 30 ^{o}C for 30 min were selected for further experiments.

C. Enzymatic hydrolysis

The percentage of reducingEnzymatic hydrolysis of alkaline pretreated sugarcane shoot by 1.0 M NaOH, 30 ^{o}C for 4 h with celluclast 1.5L provided the maximum reducing sugar yield at a rate of 32.1 g/l. The percentage conversion of cellulose to reducing sugar was 55.5% (Table 4). Compared with enzymatic hydrolysis of recycled paper sludge using celluclast1.5L (14.7 FPU/ml, 50 ^{o}C, 144 h) by Margues *et al*. (2008), the highest reducing sugar yield was 12.4 g/l (glucose 9.9, cellobiose 1.6, xylose 1.9 g/l). Alkaline pretreated with 1.0 M NaOH gave higher content of reducing sugars in a shorter time from this study. However, a higher yield of reducing sugars might be obtained at the other pretreatment and hydrolysis conditions.

IV. Conclusion

The present study took a major step on pretreatment of sugarcane shoots by physical and chemical treatment. Varieties differences did not impact three main compositions: hemicelluloses, cellulose and lignin. Grinding of sugarcane shoot to a particle size less than 0.25 mm enhanced the cellulose yield. NaOH could be used to remove hemicelluloses and lignin and enhanced the accessibility and digestibility of the enzymes to the cellulose fibril. Conversion yield of cellulose to reducing sugar of 55.5% was achieved. The results suggested that sugarcane shoot is a potential biomass source of cellulose.

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TABLE IV ENZYMATIC HYDROLYSIS OF PRETREATED SUGARCANE SHOOT WITH NAOH AT DIFFERENT CONCENTRATIONS USING CELLUCLAST 1.5 L (0.7 FPU/ML)

NaOH (conc.)	Reducing sugar (g/l)	Conversion (%)
0.5	28.4±0.84^{a}	47.5±2.23^{a}
1.0	32.1±1.14^{b}	55.5±0.21^{b}
2.0	32.0±0.92^{b}	54.3±0.45^{b}
4.0	29.5±0.50^{b}	51.0±0.90^{b}

Mean within column followed by the same letter are not significant different at 5 % level using DMRT

TABLE I COMPOSITION OF 3 VARIETIES OF SUGARCANE SHOOTS

varieties	Composition (% w/w) [1]			Ash	Moisture
	Cellulose	Hemicellulose	Lignin	%	%
Sugarcane juice Suphanburi50	69.9±0.95[a]	15.7±1.73 [a]	14.4±0.86[b]	5.6±0.02 [a]	5.3±0.74 [a]
Sugarcane 95-2-156	71.4±0.36[a]	12.7±0.45[b]	15.9±0.35[a]	5.9±0.38 [a]	5.1±0.03 [a]
Chewing cane Suphanburi72	69.1±0.81[a]	15.3±0.98[a]	15.6±0.74[a]	5.7±0.16 [a]	5.4±0.28 [a]

[1]Calculated as % oven-dried raw material.
Means within column followed by the same letters are not significant different at 5 % level using DMRT

TABLE II COMPOSITION OF SUGARCANE SHOOT, CHEWING CANE SUPHANBURI 72 VARIETY, AT DIFFERENT SIZES

Size	Composition (%) [1]		
	cellulose	Hemicellulose	lignin
0.250-0.425 mm	69.1±0.81[a]	15.3±0.98[a]	15.6±0.74[a]
0.180-0.250 mm	86.0±3.93[b]	9.0±3.70[b]	7.0±0.52[b]
<0.180 mm	86.1± 1.37[b]	6.1±1.49[c]	7.9±0.69[b]

[1]Calculated as % oven-dried weight of raw material.
Means within column followed by the same letters are not significant different at 5 % level using DMRT

TABLE III COMPOSITION OF NAOH - PRETREATED SUGARCANE SHOOT AT VARIOUS CONCENTRATIONS AND TEMPERATURES

Conc. of NaOH (M)	Temp. (°C)	Composition (%) [1]			Weight loss (%)	Cellulose yield [2] (%)
		cellulose	Hemicellulose	lignin		
0.5	30	90.6[a]	6.8[a]	3.4[a]	48.3[a]	46.8[a]
	50	90.5[a]	5.1[a]	4.4[a]	45.1[a]	59.2[a]
	70	90.6[a]	6.3[a]	3.8[a]	43.9[a]	50.8[a]
	90	91.3[a]	6.5[a]	2.2[a]	45.0[a]	52.3[a]
1.0	30	93.9[b]	5.1[b]	1.0[b]	33.9[b]	60.7[b]
	50	94.2[b]	4.6[b]	1.2[b]	35.6[b]	60.7[b]
	70	93.1[b]	5.2[b]	1.7[b]	36.1[b]	59.5[b]
	90	94.8[b]	4.2[b]	1.2[b]	35.6[b]	60.3[b]
2.0	30	97.7[c]	1.2[c]	1.1[b]	55.2[c]	43.8[c]
	50	95.6[c]	2.4[c]	2.0[b]	52.4[c]	45.0[c]
	70	96.2[c]	2.0[c]	1.8[b]	54.4[c]	45.8[c]
	90	96.8[c]	2.0[c]	1.3[b]	53.8[c]	44.9[c]
4.0	30	98.4[c]	1.2[c]	0.4[b]	50.1[c]	47.6[c]
	50	97.4[c]	2.0[c]	0.6[b]	49.7[c]	48.9[c]
	70	96.4[c]	1.9[c]	1.7[b]	50.7[c]	47.5[c]
	90	97.2[c]	2.1[c]	0.7[b]	50.2[c]	48.0[c]

[1]Calculated as % oven-dried weight of sample after each pretreatment condition.
[2]Calculated as % oven-dried weight of raw material.
Means within column followed by the same letters are not significant different at 5 % level using DMRT