

Effect of Temperature and Time on Dilute Acid Pretreatment of Corn Cobs

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Abstract—Lignocellulosic materials are new targeted source to produce second generation biofuels like biobutanol. However, this process is significantly resisted by the native structure of biomass. Therefore, pretreatment process is always essential to remove hemicelluloses and lignin prior to the enzymatic hydrolysis. The goals of pretreatment are removing hemicelluloses and lignin, increasing biomass porosity, and increasing the enzyme accessibility. The main goal of this research is to study the important variables such as pretreatment temperature and time, which can give the highest total sugar yield in pretreatment step by using dilute phosphoric acid. After pretreatment, the highest total sugar yield of 13.61 g/L was obtained under an optimal condition at 140°C for 10 min of pretreatment time by using 1.75% (w/w) H₃PO₄ and at 15:1 liquid to solid ratio. The total sugar yield of two-stage process (pretreatment+enzymatic hydrolysis) of 27.38 g/L was obtained.

Keywords—Butanol production, Corn cobs, Phosphoric acid, Pretreatment

I. INTRODUCTION

THE unavoidable shortage of the world petroleum crisis and the increasing problem of the greenhouse gas are strongly effect the worldwide interest in an alternative fuels. A viable alternative for improving energy security and reducing greenhouse gas emission is conversion lignocellulosic biomass to biofuels. Biobutanol is one type of biofuels, which can be used for mixing with gasoline in order to decrease the amount of pollutants emitted from motor vehicles [1].

In order to produce biobutanol from lignocellulosic material involves 4 steps: pretreatment, hydrolysis, fermentation, and butanol separation/purification. Pretreatment is essential to remove lignin and separate hemicelluloses and improve enzymatic accessibility. After pretreatment process, cellulose will be less crystalline, allowing enzyme to hydrolyze it into fermentable sugars which mainly consist of six carbon sugar like glucose in hydrolysis step.

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After that, the hydrolysed cellulose and hemicelluloses were fermented to ABE (acetone, butanol, and ethanol) by using an anaerobic bacterium in fermentation step. Then the products were sent to butanol separation step.

Since many physicochemical structural and compositional factor in lignocellulosic biomass structure like a complex mixture of cellulose, hemicelluloses, and lignin [2]. Therefore, pretreatment process is a necessary process in order to achieve biobutanol yield. Various pretreatment techniques have been used to improve physical and chemical of lignocellulosic biomass such as physical, chemical, and biological pretreatment [3]. However, in this research focus on chemical pretreatment with dilute acid, which can modify the crystalline poly-saccharides form to a more reactive amorphous form [3]. In addition, dilute acid can solubilize hemicellulose and remain lignin and cellulose. The advantages of this step are enhancing the enzymatic digestibility of cellulose and significantly increasing value-added production yield [4]. Sulfuric and hydrochloric acids are widely used in dilute acid hydrolysis. In contrast to these acids, phosphoric acid is less toxic than other acids. Moreover, after neutralization of hydrolysate, the salt can be used as nutrient by microorganism. Therefore, the filtration process is not needed [5]. Dilute phosphoric acid, on hydrolysates from sugarcane bagasses, fermentable sugars with 21.4 g/L with less than 4 g/L of inhibitors was obtained at operating conditions of 6% acid concentration at 100°C for 300 min [6]. In this present work, dilute phosphoric acid was used to remove hemicellulose and lignin in order to increase reducing sugar.

II. EXPERIMENTAL

A. Material

Corn cobs was obtained from the Betagro Company. The collected corn cobs was stored in a sack bag at ambient room temperature. Prior to pretreatment process, it was dried in an oven at 65°C for 24 h and was ground to particle size of 1.6 mm homogenized in a single lot. The resulting ground biomass was stored in a sealed plastic bag at ambient temperature. Standard of glucose, xylose, arabinose, and furfural were purchased from Sigma Chemical Co., Ltd., Thailand.

B. Corn cobs Pretreatment

To study the important variables of pretreatment process such as pretreatment temperature and pretreatment time on the sugar yield in both the prehydrolysate and the hydrolysate as well as the generation of xylose degradation like furfural, which presented in prehydrolysate. Phosphoric acid 1.75%

(w/w) was examined at temperatures of 100, 120, 140 and 160°C and pretreatment time of 5, 10, 30 and 60 min.

Dilute phosphoric acid pretreatment was performed in a laboratory scale stirred Stainless Steel reactor. The reactor is an acid resistant alloy and has a total volume of 1 L, with an electric heater and mechanic agitation. A 5 g of corn cobs were placed in a 250 mL glass beaker and mixed well with 75 mL of 1.75% (w/w) phosphoric acid at desired pretreatment temperature and time. The pretreatment time was counted when the temperature reached set point.

After pretreatment process, the prehydrolysate was filtrated to separate liquid and solid phase by using a vacuum pump. A vacuum pump was used to pull a vacuum across a Whatman 40 filter paper in a standard vacuum flask and Buchner filter funnel setup. After that, the solid residues were thoroughly washed with tap water to neutralize pH. Then, the solid residues were dried at 65°C oven for 24 h and collected in a sealed plastic bag at ambient room temperature. While the liquid fraction was collected for monomeric sugar analysis by using HPLC (Perkin Elmer LC200) equipped with a refractive index detector and Aminex HPX-87H column under these following conditions: flow rate 0.30 mL/min, mobile phase 0.005 M of H₂SO₄, and column temperature of 65°C.

C. Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted with solid residues using a commercial enzyme, donated by Novozyme (Cellulase). Enzyme contains a mixture of cellulase, hemicellulase and higher level of betaglucosidase enzyme activities. They are a brown liquid. Enzymatic hydrolysis was performed by using the solid residues of pretreated corn cobs 0.5 g with 15 mL of 0.05 N citric acid–sodium citrate buffer (pH 4.8) at 50°C on an incubator shaker at 150 rpm for 48 h. After enzymatic hydrolysis, the hydrolysate was taken in the water bath at 50 °C to inhibit growing enzyme. Then, the hydrolysate was filtered to separate liquid and solid residue by using a vacuum pump. The liquid was determined the quantity of monomeric sugars yield by HPLC (Perkin Elmer LC200) using refractive index detector and Aminex HPX-87H column under these following conditions: flow rate 0.30 mL/min, mobile phase 0.005 M of H₂SO₄, and column temperature of 65°C.

III. RESULTS AND DISCUSSION

A. Chemical Composition of Corn cobs

Quantity of cellulose, hemicellulose and lignin in corn cobs was analyzed and the results are shown in Table I as a dry weight unit. Table I shows the composition of corn cobs which composed of 41.27% cellulose, 46.00 % hemicelluloses, and 7.4% lignin respectively. Cellulose is a main structural component in plant cell walls. The structure of cellulose is a homopolymer consists of β-D-glucose repeating units that are linear connected by β-1-4 glycosidic bonds. The cellulose in a lignocellulosic biomass comprises of parts with a crystalline structure and amorphous structure [2]. Crystalline cellulose is a major proportion of cellulose while a small

proportion is amorphous cellulose. Cellulose is a highly crystalline material, which is a major effect to resist enzymatic hydrolysis accessibility. Hemicellulose acts as a connection between cellulose and lignin which gave cellulose-hemicellulose-lignin network more rigidity. Hemicellulose is a heteropolymers of carbohydrate which consists of five-carbon sugars (e.g. xylose and arabinose) and six-carbon sugars (e.g. mannose, glucose, and galactose). Hemicellulose has a lower molecular weight than cellulose. The structure of hemicelluloses is random, amorphous, and branched that help hemicellulose is easily to hydrolyze [2]. Lignin is a very complex molecule made up of three types of phenolic acids (p-coumaryl, coniferyl, and sinapyl alcohol) [2], which linked in a three dimensional structure that make lignin particularly difficult to hydrolyze [7].

TABLE I
CHEMICAL COMPOSITION OF CORN COBS

Chemical components	Dry solid (%)
Cellulose	41.27
Hemicellulose	46.00
Lignin	7.40

B. Thermal Gravimetric Analysis

TG-DTG, Perkin Elmer/Pyris Diamond is an equipment to analyze thermal decomposition of biomass. The thermal decomposition of corn cobs is shown in Fig. 1.

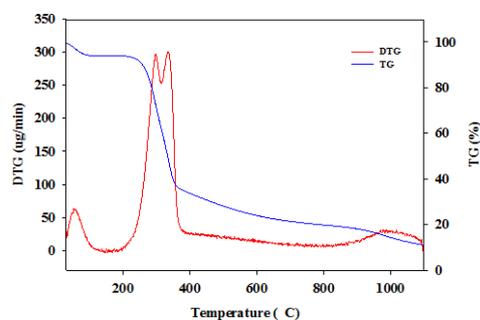


Fig. 1 Thermal gravimetric analysis curve of corn cobs

From thermal gravimetric analysis, it has four decomposition temperature range, implying corn cobs consist of four components. The different in decomposition temperature range depends on the physical and chemical structure of each component in corn cobs. The first decomposition presented around 100°C, implying that in this decomposition temperature was moisture contained in the corn cobs. The second decomposition generated in the range of 200-315°C, which was a hemicellulose. Decomposition of hemicellulose presented in the low temperature range because its structure is a random, amorphous, and branches. Cellulose was the third de-composition that presented in the range of 315-380°C. The structure of cellulose is mainly composes of both crystalline cellulose and the small percentage is

amorphous cellulose. Therefore, the cellulose decomposition temperature is higher than hemicelluloses. Among the four components in corn cobs, lignin was the most difficult component to decompose. The decomposition temperature range of lignin was higher than 900°C, owing to its structure that consists of aromatic rings with various branches.

C. Effect of Temperature and Time on Pretreatment Step

Corn cobs was pretreated with dilute H₃PO₄ under these following conditions: 1.75% (w/w) H₃PO₄, 15:1 of liquid to solid ratio, temperature in the range of 100-160°C and 5-60 min of pretreatment time. The highest yield of total sugar in prehydrolysate, 13.61 g/L, was measured after a pretreated at condition of 1.75% (w/w) H₃PO₄, 15:1 of liquid to solid ratio, 140°C, and 10 min of pretreatment time. Moreover, under the condition at 1.75% (w/w) H₃PO₄, 140°C for 5 min and 160°C for 5 min were found to have similar highest total sugar yield (as shown in Table II and Fig. 2).

TABLE II
THE TOTAL SUGAR YIELD OF CORN COBS IN PREHYDROLYSATE AFTER DILUTE PHOSPHORIC ACID PRETREATMENT BY USING 1.75% (W/W) H₃PO₄, 15:1 LSR, AND DIFFERENT PRETREATMENT TIMES AND TEMPERATURES

Temperature (°C)	Pretreatment time (min)	Total monomeric sugars (g/L)
100	5	2.01
	10	2.23
	30	3.27
	60	5.03
120	5	5.14
	10	5.29
	30	8.87
	60	12.86
140	5	13.24
	10	13.61
	30	12.77
	60	11.85
160	5	13.48
	10	11.37
	30	10.48
	60	9.38

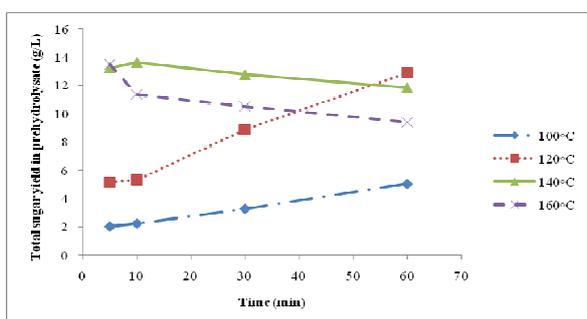


Fig. 2 The total sugar yield of corn cobs in prehydrolysate after dilute phosphoric acid pretreatment using 1.75% (w/w) H₃PO₄, 15:1 LSR, and different pretreatment times and temperatures

Table III shows monomeric sugar yield of corn cobs in prehydrolysate after dilute H₃PO₄ pretreatment using 1.75% (w/w) H₃PO₄, 15:1 LSR, and different pretreatment times and temperatures. The major component in prehydrolysate from pretreatment process was xylose. Since the structure of

hemicellulose which consists of short lateral chain as mentioned previously; therefore, xylose presented in hemicelluloses can be readily to hydrolyze than glucose. In term of xylose yield, a clear trend is observed, showing that it increased with pretreatment time from 5 min to 60 min and pretreatment temperature from 100°C to 120°C. While harsher pretreatment conditions (> 120°C), the xylose yield decreased owing to xylose degradation into furfural which can interfere the micro-organism growing in fermentation process [8]. The presence of furfural at 140 °C and 160 °C was first obtained at 10 min and the furfural yield was 0.17 g/L and 0.50 g/L, respectively (as shown in Table IV). The highest xylose yield of 11.40 g/L was obtained at 140°C for 10 min of pretreatment time. From table IV, the xylose lost reversed with the furfural generation. Moreover, the results showed that pretreatment temperature and time can drive xylose degradation into furfural and at higher temperatures and times there is faster furfural formation which is in a good agreement with previous results [9]. The mechanism and toxicity of furfural was reported in previous work [10]. The arabinose production was obtained as a function of temperature and time which is similar to the xylose trend under mild pretreatment conditions. After 140°C and 30 min of pretreatment time, the arabinose yield decreased since the mass of solid residue was burned under severity pretreatment conditions. For glucose yield, we can not detect the glucose yield because the retention of phosphoric acid is exactly match with glucose. However, same research groups reported that the glucose yield that formed in prehydrolysate has a small amount [5], [11].

TABLE III
THE MONOMERIC SUGAR YIELD OF CORN COBS IN PREHYDROLYSATE AFTER DILUTE PHOSPHORIC ACID PRETREATMENT USING 1.75% (W/W) H₃PO₄, 15:1 LSR, AND DIFFERENT PRETREATMENT TIMES AND TEMPERATURES

Temperature (°C)	Pretreatment time (min)	Xylose (g/L)	Arabinose (g/L)
100	5	1.24	0.77
	10	1.26	0.97
	30	1.82	1.45
	60	3.25	1.78
120	5	3.68	1.46
	10	3.72	1.57
	30	7.15	1.72
	60	10.9	1.96
140	5	11.26	1.98
	10	11.40	2.21
	30	10.51	2.26
	60	9.61	2.24
160	5	11.34	2.14
	10	9.25	2.12
	30	8.59	1.89
	60	7.80	1.58

TABLE IV
FURFURAL LEVELS IN PREHYDROLYSATE UNDER VARIOUS PRETREATMENT CONDITIONS

Temperature (°C)	Pretreatment time (min)	Average furfural (g/L)
140	10	0.17
	30	0.31
	60	0.52
160	10	0.50
	30	1.00
	60	1.40

D. Effect of Temperature and Time on Enzymatic Hydrolysis Step

After the enzymatic hydrolysis, the major component in hydrolysate was glucose and the others were xylose and arabinose. In pretreatment process, hemicellulose was removed in order to maximize glucose yield in hydrolysate after enzymatic hydrolysis step. Table V and Fig. 3 show the levels of total sugar yield measured in the hydrolysate after enzymatic hydrolysis of corn cobs under different pretreatment conditions. The maximum total sugar yield obtained after enzymatic hydrolysis was found to be 13.77 g/L at an optimum condition of 1.75% (w/w) H₃PO₄, 15:1 liquid to solid ratio, 140°C, and 10 min of pretreatment time. Table VI shows the monomeric sugar yield in the hydrolysate after enzymatic hydrolysis of corn cobs under different pretreatment conditions. The maximum glucose contained in the hydrolysate was 11.46 g/L at 1.75% (w/w) H₃PO₄, 15:1 liquid to solid ratio, 160 °C, and 60 min. The glucose yield increased with an increase of pretreatment temperature and time, due to hemicellulose removal during the pretreatment in the form of xylose and xylose degradation (furfural). Furfural formation is influenced by temperature and acid concentration so it can imply that the glucose in hydrolysate would be influenced by temperature and acid concentration as well. In addition, the pretreatment process can disorganize the crystalline cellulose to an amorphous form which made it was easily to hydrolyze by enzyme (as shown in Fig. 4). Fig. 4 shows the structure of corn cobs after pretreatment process under different pretreatment conditions by using scanning electron microscope. Fig. 4 (a) shows the corn cobs without pretreated with dilute phosphoric acid. Fig. 4 (b), (c) were the results of pretreated samples in different temperatures. Fig. 4 (c), (d) the samples pretreated in different times. It was found that high temperature and pretreatment time can disrupt crystalline structure in corn cobs. The minor products in hydrolysate were xylose and arabinose with small amount. Increasing pretreatment times and temperatures will decrease the yield of xylose and arabinose since the furfural formation and the pretreated xylose and arabinose during pretreatment step. It has been reported that 1 g/L furfural could inhibit yeast growing work [12]-[14].

TABLE V

THE TOTAL SUGAR YIELD OF CORN COBS IN HYDROLYSATE AFTER ENZYMATIC HYDROLYSIS UNDER THE CONDITION OF 1.75% (W/W) H₃PO₄, 15:1 LSR, AND DIFFERENT PRETREATMENT TIMES AND TEMPERATURES

Temperature (°c)	Pretreatment time (min)	Total monomeric sugars (g/L)
100	5	7.89
	10	7.96
	30	10.22
	60	11.00
120	5	10.38
	10	10.55
	30	12.55
	60	13.76
140	5	13.64
	10	13.77
	30	13.59

160	60	13.31
	5	13.71
	10	13.52
	30	13.34
	60	13.14

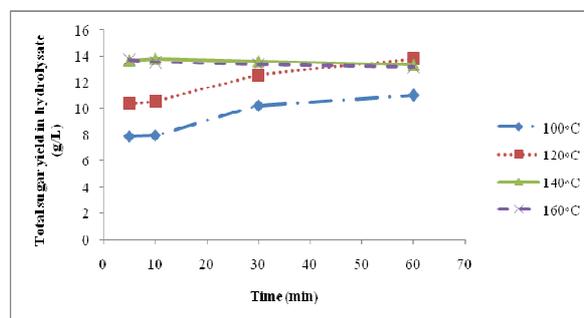


Fig. 3 The total sugar yield of corn cobs in hydrolysate after enzymatic hydrolysis under the condition of 1.75% (w/w) H₃PO₄, 15:1 LSR, and different pretreatment times and temperatures

TABLE VI

THE MONOMERIC SUGAR YIELD OF CORN COBS IN HYDROLYSATE AFTER ENZYMATIC HYDROLYSIS UNDER THE CONDITION OF 1.75% (W/W) H₃PO₄, 15:1 LSR, AND DIFFERENT PRETREATMENT TIMES AND TEMPERATURES

Temperature (°C)	Pretreatment time (min)	Glucose (g/L)	Xylose+Arabinose (g/L)
100	5	4.31	3.58
	10	4.52	3.44
	30	6.79	3.43
	60	7.84	3.16
120	5	7.29	3.09
	10	7.52	3.03
	30	9.80	2.75
	60	11.19	2.57
140	5	11.10	2.54
	10	11.30	2.47
	30	11.40	2.19
	60	11.43	1.88
160	5	11.34	2.37
	10	11.38	2.14
	30	11.41	1.93
	60	11.46	1.68

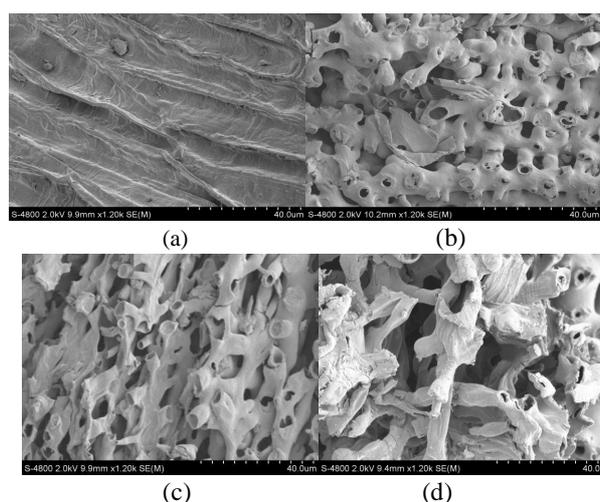


Fig. 4 Scanning electron microscope of samples after pretreated with dilute phosphoric acid under the condition of 1.75% (w/w) H₃PO₄, 15:1 LSR: (a) untreated, (b) 100°C 10 min, (c) 140°C 10 min, and (d) 140°C 30 min

IV. CONCLUSIONS

High temperature is an achievable way to disorder the complex structure of corn cobs for enzymatic hydrolysis step. However, an optimal condition in pretreatment step: pretreatment times, temperatures and the formation of furfural needs to be accounted. In this research, the overall highest total sugar yield in both pretreatment and enzymatic hydrolysis was 27.38 g/L under an condition at 140°C for 10 min of pretreatment time using 1.75% (w/w) H₃PO₄ and 15:1 liquid to solid ratio. In this optimal condition, furfural 0.17 g/L was obtained.

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