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A Comparison of Dilute Sulfuric and Phosphoric Acid Pretreatments in Biofuel Production from Corncobs

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***Abstract*—**Biofuels, like biobutanol, have been recognized for being renewable and sustainable fuels which can be produced from lignocellulosic biomass. To convert lignocellulosic biomass to biofuel, pretreatment process is an important step to remove hemicelluloses and lignin to improve enzymatic hydrolysis. Dilute acid pretreatment has been successful developed for pretreatment of corncobs and the optimum conditions of dilute sulfuric and phosphoric acid pretreatment were obtained at 120 °C for 5 min with 15:1 liquid to solid ratio and 140 °C for 10 min with 10:1 liquid to solid ratio, respectively. The result shows that both of acid pretreatments gave the content of total sugar approximately 34–35 g/l. In case of inhibitor content (furfural), phosphoric acid pretreatment gives higher than sulfuric acid pretreatment. Characterizations of corncobs after pretreatment indicate that both of acid pretreatments can improve enzymatic accessibility and the better results present in corncobs pretreated with sulfuric acid in term of surface area, crystallinity, and composition analysis.

***Keywords*—**Corncobs, Pretreatment, Sulfuric acid, Phosphoric acid.

I. Introduction

THE production of biobutanol from lignocellulosic biomass has four major steps: pretreatment, hydrolysis, fermentation, and separation. Pretreatment step is required to remove lignin and hemicelluloses, reduce cellulose crystallinity, and increase the porosity of the material [1]. Followed by enzymatic hydrolysis step which carried out by cellulase enzymes, the products of the hydrolysis were reducing sugars, especially glucose. After that, the hydrolysate from pretreatment and hydrolysis steps were fermented through acetone-butanol-ethanol (ABE) fermentation using solventogenic clostridia. Finally, the solvent products will be separated.

Dilute acid pretreatment was selected as the preferable method for pretreatment of lignocellulosic biomass which applied to a wide range of feedstocks, including softwood, hard wood etc [2]. The action mode of dilute acid was to solubilize hemicelluloses, enhance enzyme accessibility in hydrolysis process, and lower degree of polymerization and crystallinity of the cellulose component [2]. Dilute sulfuric acid pretreatment has been studied and widely used because it was inexpensive and effective process [1], [2]. From our previous work, it was found that the optimum condition of dilute sulfuric acid pretreatment was at 120 °C with a sulfuric acid concentration of 2% (w/w) and 5 min reaction time. This condition was released the total sugar (pretreatment + enzymatic hydrolysis) around 47.11 g/l [3].

One of the most widely used acids is phosphoric acid because phosphoric acid is less aggressive than other acids which give solution with lower concentration of growth inhibitors of microorganism such as furfural or acetic acid [5]. After neutralization of hydrolysates with NaOH, the salt formed is sodium phosphate. This salt can be remained in the hydrolysates because it is used as nutrient by microorganisms. Dilute phosphoric acid was used to remove hemicellulose and lignin in order to increase reducing sugar [4]. The overall highest total sugar yield in both pretreatment and enzymatic hydrolysis was 46.14 g/L under an condition at 140 °C for 10 min of pretreatment time using 2 % (w/w) H_3PO_4 and 10:1 liquid to solid ratio.

The aim of this work is to compare between the reducing sugar obtained from dilute sulfuric acid and phosphoric acid pretreatments.

II. Experimental

A. Material

Corncobs were a generous gift from Betagro Company and they were stored in a sack bag at ambient room temperature. Prior to pretreatment process, it was dried in an oven with 65 °C for 24 h and was minimized to particle size of 1.6 mm homogeneously in a single lot and kept in a sealed plastic bag at ambient temperature. Standards of glucose, xylose, arabinose, and furfural were purchased from Sigma Chemical Co., Ltd., Thailand.

B. Dilute Sulfuric and Phosphoric Acid Pretreatments of Corncobs

Dilute acid pretreatment was performed in a laboratory scale stirred Stainless Steel reactor, which is an 1 L of acid resistant alloy consisting of an electric heater and mechanic agitation. A 5 g of corncobs were suspended in 2% (w/w) sulfuric acid or phosphoric acid solution using liquid to solid

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ratios of 15:1 and 10:1 for sulfuric acid, and phosphoric acid (mL of solution:g of corncobs), respectively. The mixture was stirred until they are homogeneous before transferring to a reactor. The pretreatment of sulfuric acid was conducted under reaction condition of 120 °C for 5 min while phosphoric acid pretreatment was investigated with condition of 140 °C for 10 min. The pretreatment time was counted when the temperature reached the desired point.

After that, pretreatment process was completed and the residue was separated by filter paper subsequently. For liquid fraction, monomeric sugar will be analyzed by HPLC. While solid residues were thoroughly washed with tab water to neutralize pH followed by dried in an oven at 90 °C for 24 h.

C. Monomeric Sugar Analysis

HPLC system equipped with a refractive index detector (Model 6040 XR, Spectra-Physics, USA) was used to analyze glucose, xylose, and arabinose. An organic acid column (Aminex HPX- 87H column, Bio-Rad Lab, USA) was applied with 0.005 M sulfuric acid solution as a mobile phase at the total flow rate of 0.6 ml/min and the column temperature was maintained at 60 °C.

D. Component Analysis of the Biomass Samples

Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), and acid insoluble ash (AIA) of corncobs before and after pretreatment were determined by the Nakhonratchasima Animal Nutrition Research and Development Center (Nakhonratchasima province, Thailand). The detergent hemicellulose can be estimated by the difference between NDF and ADF. Detergent cellulose was calculated by subtracting the values for (ADL + AIA) from ADF.

E. Crystallinity Measurement

Cellulose crystallinity was determined by X-ray diffraction (Bruker AXS Model D8 Discover). All samples were scanned from 2θ = 5° to 40° with a step size of 0.02°. Determination time was 0.5 s/0.02°. Biomass crystallinity as expressed by crystallinity index (CrI) was determined according to:

$$CrI = 100 \text{ x } [(I_{002} - I_{amorphous})/I_{002}]$$

in which, I_{002} is the intensity for the crystalline portion of biomass (i.e., cellulose) at about 2θ = 22.5° and $I_{amorphous}$ is the peak for the amorphous portion (i.e., cellulose, hemicellulose, and lignin) at about 2θ = 18.7° [6].

F. BET Surface Area Analysis

BET surface area of corncobs before and after pretreatment was measured by N_2 adsorption/desorption measurements (Quantachrome instrument; model: BELSORP–max, BEL, Japan) done at 100 °C (373 K). Prior to measurement, all biomass materials were dried at 90 °C for 48 h and then 0.25-0.26 g of sample was put into tube of the Quantachrome instrument and degassed using a vacuum for 24 h. The BET surface area and pore volume were obtained from the N_2 adsorption/desorption curves using BELSORP–max software.

G. Surface Morphology

The surface morphology and porosity of untreated corn cobs pretreated sample after pretreatment process was observed by Scanning Electron Microscope (SEM) (HitachiS–4800 SEM instrument operated at 10–15 kV accelerated voltage).

III. Results and Discussion

A. Chemical Composition of Corncobs

Corncobs were measured the quantities of cellulose, hemicellulose, and lignin. The results are shown in Table I as the percentage of dry weight unit.

TABLE I
CHEMICAL COMPOSITION OF CORNCOBS

Chemical components	Dry solid (%)
Cellulose	39.31
Hemicellulose	34.46
Lignin	10.47

From Table I, the composition of corncobs consists of 39.31% cellulose, 34.46% hemicelluloses, and 10.47% lignin. Cellulose is a main structural component in plant cell walls, which its structure is a homopolymer consists of repeating β–D–glucose units. Cellulose is a highly crystalline material which mainly effects to resist enzymatic hydrolysis accessibility. 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). In addition, lignin made up of three types of phenolic acids (p–coumaryl, coniferyl, and sinapyl alcohol) and they linked in a three dimensional structures affected lignin particularly difficult to hydrolyzed [6].

B. Comparison of Total Sugar Concentration Obtained from Different Pretreatment Acids

Table II illustrates monomeric sugar and furfural yield of prehydrolysate after dilute sulfuric and phosphoric acid pretreatments. For sulfuric acid condition, the trend clearly presents that xylose yield increased when pretreatment liquid to solid ratio (LSR) was decreased from 15:1 to 10:1. The previous works [4] reported that the appropriate ratio which acid and corncobs are being well-mixed with highest monomeric sugar yield is 10:1. Furfural formation at a 10:1 LSR was higher than that at a 15:1 LSR because of high amount of xylose yield which presented in prehydrolysate can be converted to higher content of furfural. When comparing with phosphoric acid condition, monomeric sugar contents were lower than sulfuric condition at a 10:1 LSR according to an increase in pretreatment temperature and time could drive xylose degradation into furfural [7]. With too long residence time and too high reaction temperature, carbohydrates were degraded into furfural and HMF which in turn were degraded into levulinic acid and formic acid, respectively [8]. The highest total monomeric sugar (35.72 g/l) was obtained in

TABLE II
COMPARISON OF TOTAL SUGAR CONCENTRATION OBTAINED FROM DIFFERENT PRETREATMENT METHODS

Conditions	Glucose (g/l)	Xylose (g/l)	Arabinose (g/l)	Furfural (g/l)
2% (w/w) H_2SO_4 120 °C for 5 min and 15:1 LSR	1.74	17.89	3.47	0.11
2% (w/w) H_2SO_4 120 °C for 5 min and 10:1 LSR	2.79	27.60	5.33	0.17
2% (w/w) H_3PO_4 140 °C for 10 min and 10:1 LSR	2.08	26.86	5.15	0.37

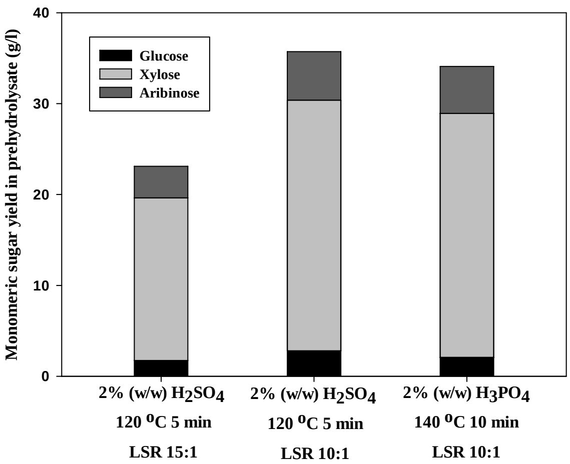

Fig. 1 Monomeric sugar yield of corncob in prehydrolysate after dilute sulfuric and phosphoric acid pretreatment by using 2% (w/w) with different pretreatment times, temperatures and LSR

pretreated corncobs with 2% (w/w) H_2SO_4 120 °C for 5 min and 10:1 LSR (35.72 g/l). However, the glucose, xylose, and arabinose concentrations were almost the same as those obtained in phosphoric acid pretreatment. While, the highest furfural content can be found in pretreated corncobs hydrolysate with 2% (w/w) H_3PO_4 at 140 °C for 10 min and 10:1 LSR (0.37 g/l).

C. Surface Area Analysis

Accessible cellulose surface area is one of the main factors which have influenced to the ease of enzymatic hydrolysis of pretreated lignocellulosic biomass. Table III shows the physical properties of dilute acid pretreated corncobs compared with untreated corncob. It was found that the surface area and total pore volume of pretreated corncobs were higher than those of the untreated corncobs. In case of sulfuric acid pretreatment conditions: 2% (w/w) H_2SO_4 120 °C for 5 min, surface area of the pretreated substrates with a 15:1 LSR, 4.41 m^2/g, was lower than that of a 10:1 LSR (4.58 m^2/g). The highest surface area was 4.58 m^2/g under the pretreatment

TABLE III
BET SURFACE AREA, TOTAL PORE VOLUME AND AVERAGE PORE DIAMETER OF SAMPLES

Sample	Surface area (m^2/g)	Total pore volume (cm^3/g)	Average pore diameter (nm)
Untreated corncobs	3.83	0.0062	6.47
Pretreated corncobs with 2% (w/w) H_2SO_4 at 120 °C for 5 min, 15:1 LSR	4.41	0.0136	12.34
Pretreated corncobs with 2% (w/w) H_2SO_4 at 120 °C for 5 min, 10:1 LSR	4.58	0.0120	10.48
Pretreated corncobs with 2% (w/w) H_3PO_4 at 140 °C for 10 min, 10:1 LSR	3.90	0.0132	11.61

condition: 2% (w/w) H_2SO_4 120 °C, 5 min with 10:1 LSR. On the other hand, surface area of pretreated corncobs with 2% (w/w) H_3PO_4 at 140 °C for 10 min with a 10:1 LSR (3.90 m^2/g) was close to untreated corncobs (3.83 m^2/g).

D. X–ray Diffraction Analysis

The degree of cellulose crystallinity is a major factor affecting enzymatic hydrolysis of the substrate [9]. Fig.1 presents XRD patterns of pretreated corncob and untreated corncobs. There are three peaks of cellulose at the 2θ of 15.61°, 22.09°, and 34.53°, respectively [10]. The higher intensity peak indicates that more crystalline part of pretreated corncobs. Crystallinity Index (CrI) refers to the fraction of crystalline material in the sample and CrI value depends on the compositions in lignocellulosic materials [6].

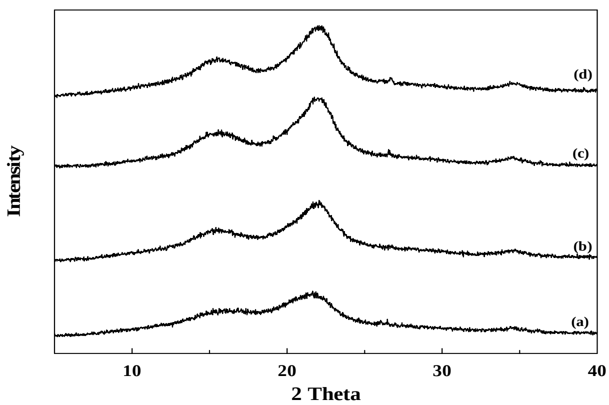

Fig. 2 X-ray diffraction patterns of the corncobs. Symbols; (a) untreated corncobs, (b) pretreated corncobs with 2% (w/w) H_2SO_4 120 °C, 5 min and 15:1 LSR, (c) pretreated corncobs with 2% (w/w) H_3PO_4 140 °C, 10 min and 10:1 LSR, (d) pretreated corncobs with 2% (w/w) H_2SO_4 120 °C, 5 min and 10:1 LSR

TABLE IV
CRYSTALLINITY INDEX (%) OF UNTREATED AND TREATED CORNCOBS

Sample	Crystallinity index
Untreated corncobs	26.94
Pretreated corncobs with 2% (w/w) H_2SO_4 120 °C for 5 min 15:1 LSR	34.63
Pretreated corncobs with 2% (w/w) H_2SO_4 120 °C for 5 min, 10:1 LSR	39.98
Pretreated corncobs with 2% (w/w) H_3PO_4 140 °C for 10 min, 10:1 LSR	39.25

The highest intensity peak is obtained with the pretreated corncobs with 2% (w/w) H_2SO_4 120 °C for 5 min and 10:1 LSR which corresponds to high Crystallinity Index (CrI) calculation of 39.98, as shown in Table IV. Pretreated corncobs with 2% H_3PO_4 140 °C for 10 min and LSR 10:1 does not give much different, 39.25. CrI of pretreated samples were increased due to the fact that amorphous portion of biomass was removed [10] such as lignin which is considered to be amorphous covering cellulose in lignocellulosic biomass [9].

E. Surface Morphology by SEM

The damaged structure of dilute acid pretreated corncobs has high surface area which can increase enzymatic accessibility, as shown in Fig. 3. The SEM image of fresh corncobs in Fig. 3 (a) shows non-porous, bulging, smooth, and uniform surfaces. In contrast, SEM images of the dilute acid pretreated corncobs, Fig. 3 (b), (c), (d), present significant collapse and destruction structures. For dilute sulfuric pretreated corncobs with 15:1 LSR, Fig. 3 (b), many of bulges have oval characteristic which become hole and the average shape size is 61 µm wide and 156 µm long. Meantime, SEM of sample pretreated at a 10:1 LSR in Fig. 3 (c) illustrates more porous and deeply cracks which can increase surface area than sample pretreated at a 15:1 LSR and has 150 µm of average diameter. Furthermore, pretreated corncobs with phosphoric acid cause circle shape crack with 132 µm of average diameter.

The dilute acid pretreated corncob had a rougher surface and more porous than fresh corncob. This kind of cracks was essential for enzymatic hydrolysis of cellulose because they can increase surface area and porosity of lignocellulosic biomass [11].

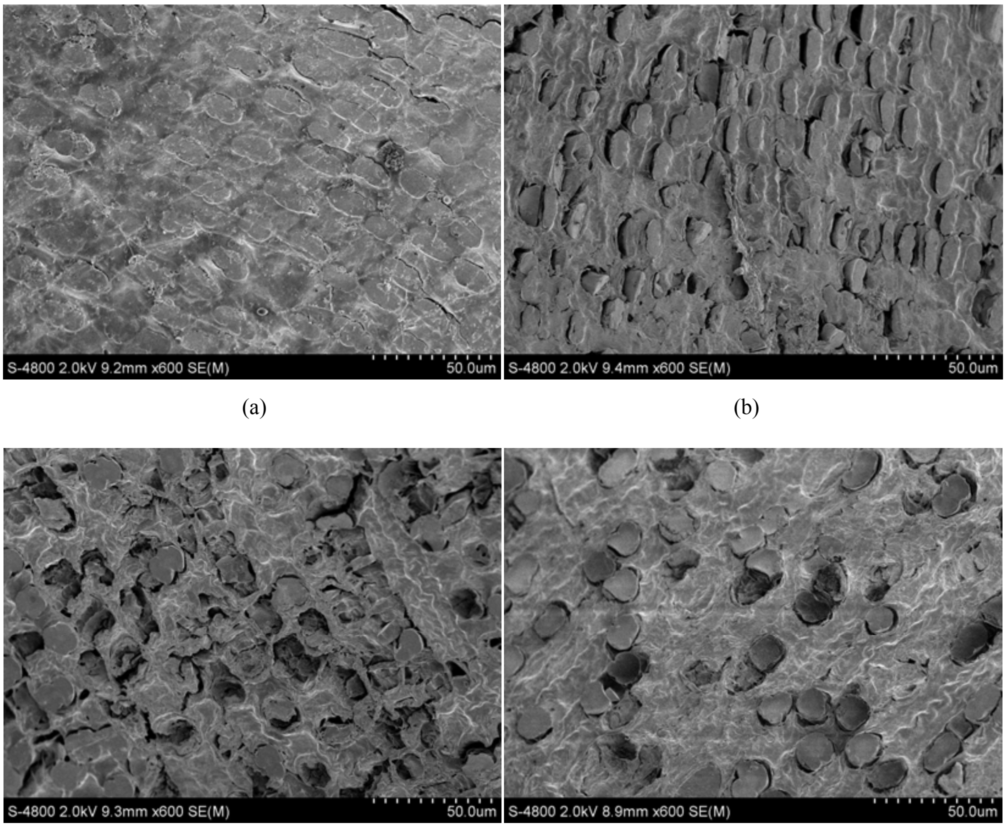

Fig. 3 Scanning electron microscope images of (a) untreated corncobs; (b) pretreated corncobs with 2% (w/w) H_2SO_4 15: 1 LSR at 120 °C for 5 min; (c) pretreated corncobs with 2% (w/w) H_2SO_4 10: 1 LSR at 120 °C for 5 min; (d) pretreated corncobs with 2% (w/w) H_3PO_4 with 15: 1 LSR at 140 °C for 10 min

IV. Conclusion

Dilute sulfuric acid and phosphoric acid pretreatments contributed to the successful method for improving enzymatic hydrolysis. The highest total monomeric sugar (35.72 g/l), surface area (4.58 m^2/g), and cystallinity index (39.98) were obtained in the corncobs pretreated with 2% (w/w) H_2SO_4 120 °C for 5 min and 10:1 LSR. While corncobs pretreated with 2% (w/w) H_3PO_4 140 °C 10 min and 10:1 LSR gave total monomeric sugar of 34.09 g/l and cystallinity index of 39.25 which are similar to the highest condition obtained from sulfuric acid pretreated sample. In term of furfural formation, sulfuric acid pretreatment gives lower content than phosphoric acid pretreatment.

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