CHAPTER III

EXPERIMENTAL

3.1 Materials and Chemicals

3.1.1 Corncob waste was obtained from the Betagro Company. The collected corncob waste was stored at the ambient room temperature in a large bag. The average particle size of corncob waste was around 1.6 mm homogenized in a single lot and stored until used.

3.1.2 Phosphoric Acid (H_3PO_4)

3.1.3 pH Meter

3.1.4 Citrate Buffer

3.1.5 Filter Paper

3.1.6 Enzyme (Novozyme)

3.1.7 Standard glucose, xylose, arabinose, cellobiose, mannose, rhamnose, galactose and furfural

3.2 Equipment

3.2.1 High Performance Liquid Chromatograph (HPLC)

3.2.2 Scanning Electron Microscope (SEM)

3.2.3 Thermogravimetric Analyzer (TGA)

3.2.4 X-Ray Diffraction Analyzer (XRD)

3.2.5 UV-VIS Spectrometer (UV)

3.2.6 Gas Chromatograph (GC)

3.2.7 Incubator Sharker

3.2.8 Oven

3.2.9 Stainless Steel Reactor

3.2.10 Waterbath

3.2.11 Glassware

3.3 Methodology

3.3.1 Dilute Phosphoric Acid Pretreatment

Dilute Phosphoric acid pretreatment was performed in a laboratory scale stirred stainless steel reactor. The reactor is an acid resistant stainless steel and has a total volume of 1 L, with an electric heater and mechanic agitation. Corncob waste was suspended in H_3PO_4 solution (1–10 % (w/w)) using different liquid-to-solid ratios (LSR, 10:1–20:1, mL of solution:g of corncob waste). The mixture was stirred until homogeneous before transferring to a stainless steel reactor. The pretreatment was conducted under different reaction temperatures (100–160 °C) and times (5–60 min). At the end of each run the reactor was removed from the heating jacket and the prehydrolysate agitated until the reactor was cooled to about 40 °C. Then the prehydrolysate was filtered to separate liquid and solid residue. 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 H_2SO_4 and column temperature was fixed at 65 °C. While, the solid was washed thoroughly with tab water until no colour and neutral pH in the resulting water was obtained. After that, the solid residue was dried for 24 h at 65 °C in an oven. The weight of dried samples was collected and stored in a ziplock bag prior to enzymatic hydrolysis step.

3.3.2 Enzymatic Hydrolysis

Enzymatic hydrolysis used a commercial enzyme, which given by Novozyme (Cellulase). Enzyme contains a mixture of cellulase, hemicellulase, and higher level of beta-glucosidase enzyme activities. They are a brown liquid. Enzymatic hydrolysis was performed by using the washed water-insoluble residue 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 baht at 50 °C to inhibit growing enzyme. Then, the hydrolysate was filtered to separate liquid and solid residue. The liquid was determined the quantity of monomeric sugars yield by 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 H_2SO_4 and column temperature was fixed at 65 °C.

3.3.3 Ethanol Production

After pretreatment and enzymatic hydrolysis step, the two fermentable sugars, which are readily to ferment were fermented by active yeast (*Saccharomyces Cerevisiae*) within ratio 10:1. Then samples were taken to waterbath at 37 °C for 1 day. After the process was completed, the fermented samples were kept in a refrigerator prior to measure the ethanol production by a gas chromatograph.

3.4 Analysis Method

3.4.1 High Performance Liquid Chromatography (HPLC)

The quantity of monomeric sugars yield was determined by High Performance Liquid Chromatography (HPLC) equipped with a refractive index detector (Series 200 LC/S/N291N5060508, Perkin Elmer) using an Aminex-HPX 87H column (300 mm x78 mm, Bio-Rad Lab, USA) and a guard column (30 mm x 4.6 mm, Bio-Rad Lab, USA) under these following conditions: flow rate 0.30 mL/min, mobile phase 0.005 M of H_2SO_4 and column temperature was fixed at 65 °C.

3.4.2 UV-VIS Spectrometer (UV)

After pretrement process, the product in liquid phase, prehydrolysate, was measured the total sugar yield by UV-VIS (1800). UV-Vis equipped with the photometric mode and UV wavelength of 540 nm.

3.4.3 Scanning Electron Microscope (SEM)

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).

3.4.4 X-Ray Diffraction (XRD)

The crystallinity index (CrI) of untreated corncob waste and pretreated corncob waste after pretreatment process was detected by X-Ray Diffraction (XRD) (Bruker D8 Advance). In addition, the chemical structure of lignocellulosic biomass changing between fresh and treated corncob waste were compared.

3.4.5 BET

BET surface area information of the pretreated residues 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 40°C for 48 h and then 1 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.

3.4.6 Gas Chromatography (GC)

The ethanol yield which was produced from fermentable sugars that got from two-stage (pretreatment and enzymatic hydrolysis) was measured concentration by gas chromatograph (Series SHIMAZU 7AT) equipped with flame ionization detector using PQ column length 1 m under these following conditions: flow rate N_2 45 mL/min, Detector temperature 220°C, Injection Temperature 220°C, Column temperature 170°C, and Volume injection 1 μL.

3.4.7 Fibertect M6

The methods to determine cellulose, hemicelluloses, lignin, and ash in solid residue after pretreatment and enzymatic hydrolysis were amylase neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose acid detergent lignin (ADL). Amylase neutral detergent fiber (NDF) method used to measure the quantity of cellulose, hemicelluloses, and lignin. After that, the hemicelluloses were detected by using acid detergent fiber (ADF) method. Then, cellulose was measured by using cellulose acid detergent lignin (ADL) method. The quantity of cellulose was measured when suspended with sulfuric acid. After this process was completed, the

sample was burned at 500°C for 2 h in order to determine lignin's quantity. The solid residue after this process is ash.