CHAPTER III
METHODOLOGY

3.1 Materials

3.1.1 Corn cobs obtained from Betagro company

3.1.2 Sulfuric acid (H_2SO_4)

3.1.3 pH meter

3.1.4 Citrate buffer

3.1.5 Filter paper

3.1.6 Enzyme (Novozyme, Genencer)

3.1.7 Standard glucose, xylose, arabinose, galactose and mannose

3.2 Equipment

3.2.1 Autoclave

3.2.2 Incubator shaker

3.2.3 Oven

3.2.4 Water bath

3.2.5 Glassware

3.2.6 Perkin Elmer Series 200 LC/S/N291N5060508: High Performance Liquid Chromatography (HPLC) with a refractive index detector using an Aminex–HPX 87H column (300 mm x78 mm, Bio–Rad Lab, USA)

3.2.7 Scanning Electron Microscope (SEM)

3.2.8 Thermogravimetric Analyzer (TGA)

3.2.9 X–ray Diffraction (XRD)

3.2.10 Gas Chromatography (GC)

3.3 Methodology

3.3.1 Pretreatment of Corn Cobs by Dilute Acid

The corn cobs hydrolyzate was obtained using sulfuric acid (H_2SO_4) in a batch reactor. Corn cobs were mixed with sulfuric acid solution in the ratio 1:15 (Acid concentration from 2–10 % (v/v)) and the sample was presoaked at room temperature in an appropriate concentration of acid solution at least 4 h. Temperature and time are in the range 100–160 °C and 5–60 min, respectively. The pretreatment procedure flow diagram is shown in Figure 3.1.

3.3.2 Enzymatic Hydrolysis

Enzymatic hydrolysis used a commercial enzyme, which were donated by Novozyme (Cellulase) and Genencor (ACCELLERASE@1500). Enzyme contains a mixture of cellulase, hemicellulase, and higher level of beta–glucosidase enzyme activities.

Enzymatic hydrolysis was carried out in experiment flasks consisted of solid substrate and citrate buffer in ratio 1:30. The pH of reaction system was kept at 4.8 with 0.05 N citric acid–sodium citrate buffers. The flasks were incubated at 48 °C in the incubator shaker agitated at 150 rpm. In all experiments, the citric acid buffer was supplemented with antibiotics tetracycline to prevent microbial contamination. Then the sample from the reaction will be stored for sugar analysis. The enzymatic hydrolysis procedure flow diagram is shown in Figure 3.1.

3.3.3 Activity of Enzyme

The substrate (Carboxymethyl cellulase sodium salt for cellulase) 320 µl was added in sample, blank, and standard tube. The sample tubes were heated in a heating box at 50 °C for 10 min and then added with enzyme samples at 50 °C. Glucose solution (0, 1, 2, 3, 4, 5 mM) was added in standard tubes. After 30 minutes, 680 µl DNS reagent was added. The enzyme samples were also added in the blank tube. All tubes were boiled in water 100 °C after 5 minutes. The tubes were

immediately transferred to cold water. UV–VIS Spectrometer at 550 nm was used to measure the color absorbed. The color formed in the blank tube is subtracted from that of the sample tube and translated the absorbance of the sample tube into glucose production during the reaction using standard curve. The sample tube was measured 2 times and the averages reported. The glucose solution calibration curve was used to calculate the activity of cellulase.

3.3.4 Ethanol Production

Active yeast was combined with sugar solution from enzymatic hydrolysis in ratio 1:10 and transferred to incubator shaker at 37 °C for 1 day to 3 days. After this process completed, the solution was stored in order to analyze the ethanol concentration by GC instrument.

3.3.5 Analysis Methods

Total reducing sugar was measured by using the HPLC equipped with an organic acid column (Aminex HPX–87H column, Bio–Rad Lab, USA) and a refractive index detector (Model 6040 XR, Spectra–Physics, USA). 0.005M sulfuric acid solution was used as a mobile phase at a flow rate of 0.6 mL min^{-1} and column temperature was fixed at 65 °C.

Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), and acid insoluble ash (AIA) of corn cobs were determined by Nakhonratchasima Animal Nutrition Research and Development Center (Nakhonratchasima province, Thailand). The difference between NDF and ADF estimates detergent hemicellulose. Detergent cellulose is calculated by subtracting the values for (ADL+AIA) from ADF. Carbohydrate content of untreated material was also determined by measuring the hemicellulose (xylan and araban) and cellulose (glucan) derived sugars in supernatants following concentrated acid hydrolysis as described by (NREL). Acid–insoluble lignin content of corn cobs and the solid fraction remaining after pretreatment was determined according to NREL methods. Likewise, water and ethanol soluble sugars were extracted from untreated sorghum straw and quantified according to NREL methods (McIntosh *et al.*, 2010).

The Scanning Electron Microscope (SEM) was used to investigate the structure of corn cobs both before pretreatment and after pretreatment.

The X–Ray Diffraction (XRD) was used to investigate the crystallographic structure, chemical composition, and physical properties of materials of corn cobs both before pretreatment and after pretreatment.

BET surface area information of the pretreated residues was measured by N_2 adsorption/desorption measurements (Quantachrome instrument; model: BELSORP–max,BEL Japan INC., Japan) done at 196 °C (77 K). Prior to measurement, all biomass materials were dried at 40 °C for 48 h and then a sample (0.5–1 g) was put into the sample tube of the Quantachrome instrument and degassed using a vacuum for 16 h. The BET surface area and pore volume were obtained from the N_2 adsorption/desorption curves using BELSORP–max software.

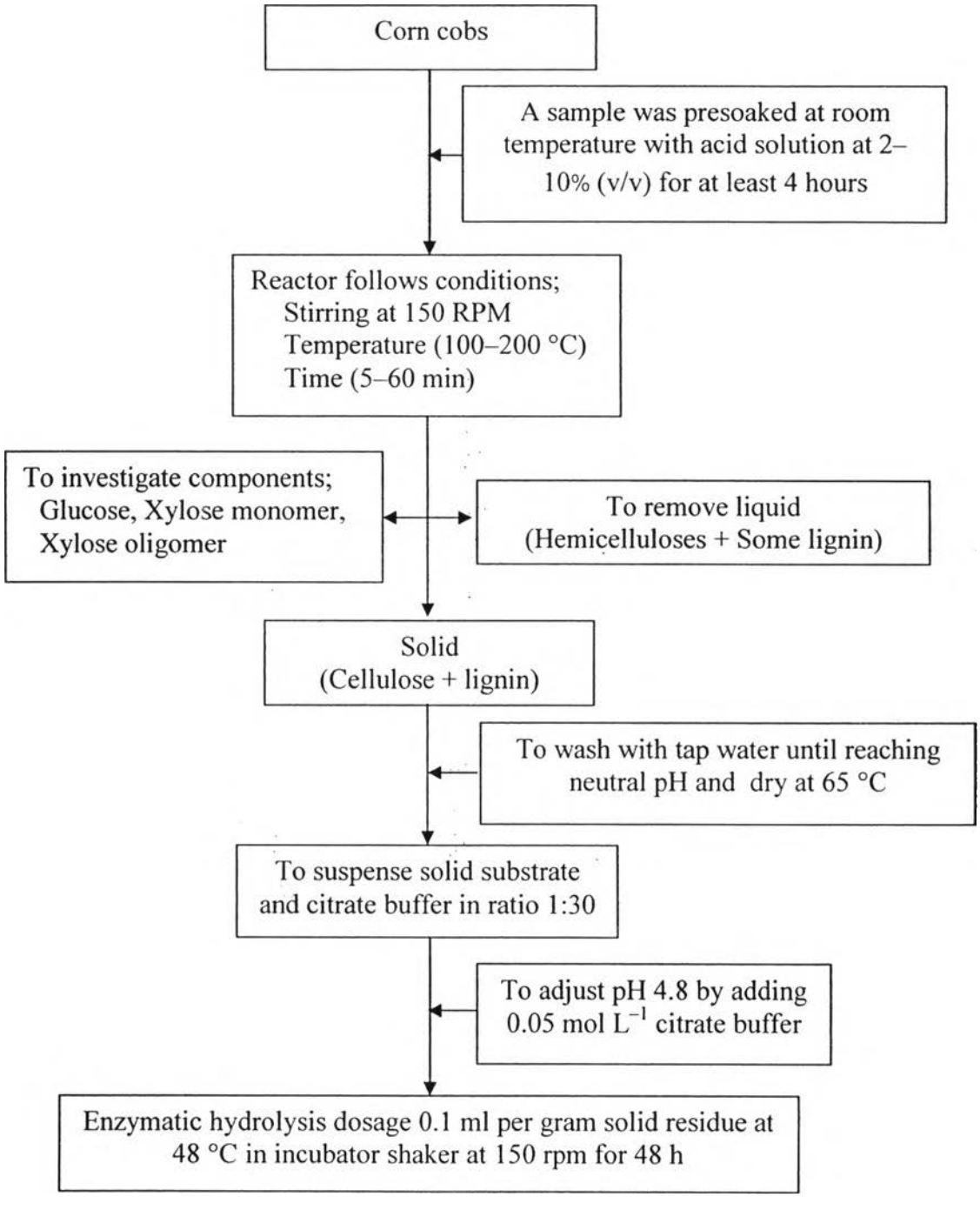

Figure 3.1 Pretreatment and hydrolysis procedure flow diagram.