CHAPTER III
EXPERIMENTAL

3.1 Materials

- Corn cobs from Betagro Corporation Limited, Thailand
- Potassium hydroxide
- Citrate (Citric acid/Citric sodium) buffer
- Standard glucose, xylose, arabinose
- Distilled water
- Cellulase enzyme

3.2 Equipment

- A CEM (Matthews, NC, USA) MAR-5 HP-500 microwave system
- 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)
- Varian GC-3800 simulated distillation gas chromatograph (Sim-Dist GC)
- Scanning electron microscope (SEM)
- Thermogravimetric analyzer (TGA)
- Brunauer-Emmett-Tellet (BET) Surface area analysis
- X-ray Diffraction (XRD)
- Glassware
- Oven
- pH meter
- Incubator shaker
- Water bath

3.3 Methodology

3.3.1 Pretreatment of Corn Cobs by Microwave Assisted Alkali

Microwave radiation system was used in this study for combination of microwave and alkali pretreatment. A 2 g of corn cobs was suspended in 30 mL of different potassium hydroxide concentrations (0.75 % to 3 %) and then transferred to a microwave oven to treat corn cobs at desired temperatures (60 °C to 120 °C) for 10 min to 30 min. After this process was completed, the residues were collected by filter paper and washed with tap water until neutral pH, dried at 65 °C.

Figure 3.1 A CEM (Matthews, NC, USA) MAR-5 HP-500 microwave system.

3.3.2 Enzymatic Hydrolysis

A hydrolysis mixture consisted of 0.5 g of pretreated corn cobs and 15 mL of 0.1 mol L^{-1} citrate buffer (pH 4.8). The mixture was added with 0.1 mL of the commercial cellulese enzymes that was incubated at 50 °C in an incubator shaker at 150 rpm for 48 h. Thereafter the hydrolysis solution was heated to 100 °C immediately for 3 min to denature the enzymes, cooled to room temperature, and

then centrifuged for 20 min at 8000 rpm (Zhu *et al.*, 2005). Then, the sample from the reaction was stored for sugar analysis.

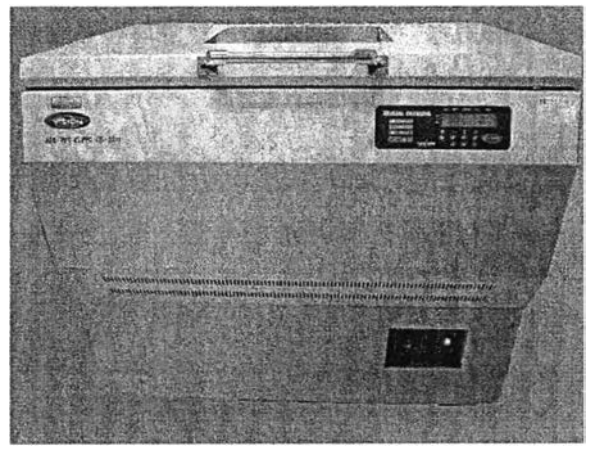

Figure 3.2 Incubator shaker.

3.3.3 Fermentation

A sugar solution from enzymatic hydrolysis step was adjusted with sodium hydroxide to natural pH before using as substrate in fermentation step. Than the 5 ml of sugar solution was mixed 0.5 mL of active yeast (Saccharomyces cerevisiae) and transferred to water bath at 37 °C for 1 day to 3 days. After that, the solution was then centrifuged for 5 min at 8000 rpm and collected to analyze the ethanol concentration by a GC instrument.

3.4 Component Analysis of the Biomass Samples

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

3.5 Monosaccharide Analysis

Glucose, xylose, and arabinose were determined using an HPLC system equipped with a refractive index detector (Model 6040 XR, Spectra-Physics, USA). An organic acid column (Aminex HPX- 87H column, Bio-Rad Lab, USA) was used with 0.005 M sulfuric acid solution as a mobile phase. The flow rate was controlled at 0.6 mL min^{-1} and the column temperature was 65 °C.

3.6 Thermal Gravimetric Analysis

For TG-DTA work, the untreated corn cobs were loaded with approximately 5 mg in high purity alumina pan in Perkin Elmer/Pyris Diamond. Nitrogen was used as a carrier gas for creating the inert environment. The heating rate was 10 °C/min from 50 °C to 1000 °C. In general, weight change of a sample was recorded as a function of time or temperature and characterized by a TG curve. DTA emphasized the zone of reaction where various reaction steps are taking place over the entire temperature range (Abdullah *et al.,* 2010).

3.7 Surface Characteristics

The physical structure changes of the untreated and pretreated of corn cobs were imaged by scanning electron microscope (SEM) using a Hitachi S-4800 microscope. The sample was located on a specimen holder by using carbon tape, which was sputter-coated with Au–Pd for reducing electrostatic charging. The surface structure images of the untreated and pretreated corn cobs were obtained with a 15 kV accelerating voltage.

3.8 Crystallinity Measurement

X-ray diffraction (XRD) was used for phase identification of a crystalline of the untreated and pretreated corn cobs. Samples were scanned and recorded by using

Rigaku X-Ray Diffractometer system (RINT-2200) with Ni filter and Cu K_α radiation (1.5406 Å) that generated at 30 mA and 40 kV. The scan speed of 5° (2θ)/min with scan step of 0.02 (2θ) was used for the continuous run in 5 to 90°C (2θ) range.

The crystalline index of cellulose samples were calculated from the X-ray diffraction patterns by the following equation (Xiao *et al.*, 2011):

$$CrI = \frac{I_{002} - I_{amorphous}}{I_{002}} \times 100\%$$

Where 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.

3.9 BET Surface Area Analysis

A BET surface area of corn cobs before and after pretreatment was measured by N_2 adsorption/desorption measurements (Quantachrome/Autosorb1). The dried sample (0.1–0.5 g) was put into the sample tube and outgassed to remove the humidity and volatile adsorbents adsorbed on surface under vacuum at 150 °C for 4 h prior to the analysis. Then, N_2 was purged to adsorb on surface, and the quantity of gas adsorbed onto or desorbed from their solid surface at some equilibrium vapor pressure by static volumetric method will be measured. The solid sample was maintained at a constant temperature of the sample cell until the equilibrium is established. The BET surface area and pore volume was obtained from the N_2 adsorption/desorption curves.

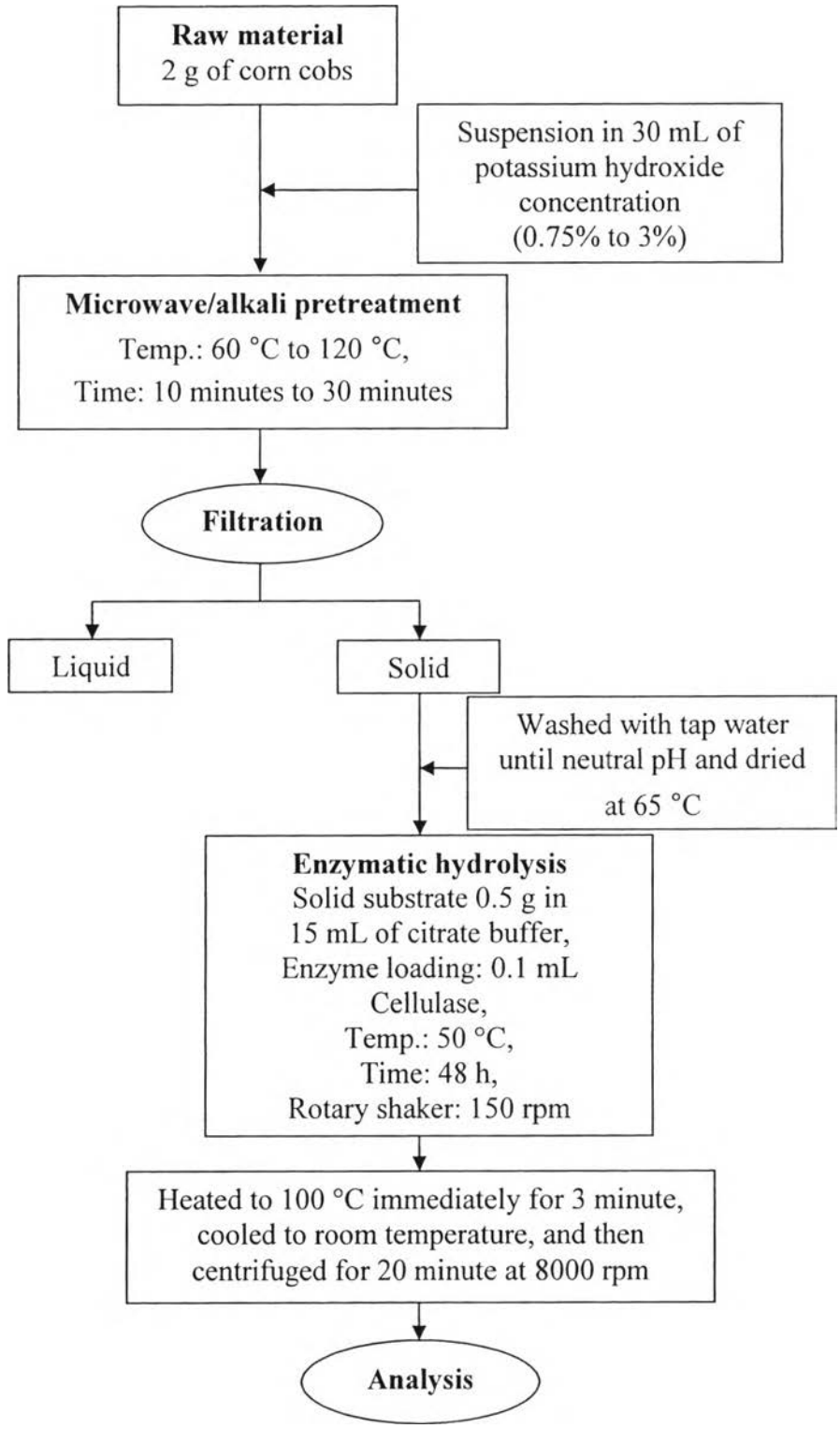

Figure 3.3 Schematic of pretreatment and hydrolysis procedure flow diagram.