CHAPTER III
EXPERIMENTAL

3.1 Materials and Chemicals

Miscanthus Sinensis was obtained from Cha-Chueng-Sao province, Thailand. Before any pretreatment, *Miscanthus Sinensis* (only leaves and stems) was washed with tap water and dried under sunlight. It was then milled to obtain small particles using herb grinder. The ground biomass (Moisture content 3.68 %) was then stored in sealed plastic bags prior to use. The main composition of the *miscanthus* was as follows: 8.6% extractives, 42.7% cellulose, 31.3% hemicellulose, and 17.4% lignin (Lin *et al.*, 2010).

Ammonium hydroxide (NH_4OH, Panreac Quimica Sau), hydrochloric acid (HCl, Labscan Asia Co.), nitric acid (HNO_3, Labscan Asia Co.), sulfuric acid (H_2SO_4, Merck Co., Germany), phosphoric acid (H_3PO_4, Merck Co., Germany), D-glucose anhydrous (Ajax Finechem, Australia), and DL-xylose (min. 99 %, Sigma Aldrich Chemicals Co. Inc., USA) were directly used without purification.

3.2 Equipments

3.2.1 Microwave Solvent Extraction Lab Station

Pretreatment of sugarcane bagasse was conducted using microwave solvent extraction lab station. Samples were heated in a Teflon-vessel sealed with a Teflon using with time-to-temperature program.

3.2.2 High Performance Liquid Chromatography (HPLC)

Monomeric sugars (glucose, xylose, and arabinose) were determined by HPLC with a refractive index detector (Series 200 LC/S/N291N5060508, Perkin Elmer) using an Aminex-HPX 87H column (300 mm x 7.8 mm, Bio-Rad Lab, USA) and a guard column (30 mm x 4.6 mm, Bio-Rad Lab, USA) under the following conditions: mobile phase 0.005 M of H_2SO_4 and a flow rate of 0.60 mL/min. Sample injection was 20 μL.

3.3 Methodology

Microwave heating was employed to digest *Miscanthus Sinensis* using different catalysts, as follows:

3.3.1 Dilute Ammonium Hydroxide Pretreatment

Prior to microwave pretreatment, *Miscanthus Sinensis* was suspended in NH_4OH solution (0.5–5 % (w/v)) using different liquid-to-solid ratios (LSR, 15:1–45:1, mL of solution:g of *Miscanthus Sinensis*). The mixture was stirred until homogeneous before transferring to a Teflon-vessel sealed with a Teflon cap. The microwave (300 W) pretreatment was conducted under various reaction temperatures (60–160 °C) and times (5–60 min). After the pretreatment, the mixture was filtered to separate solid residues from filtrate fraction. The liquid fraction was collected for monomeric sugar analysis. The solid residues were thoroughly washed with distilled water to neutral pH and dried in the oven. Then, the oven-dried samples were stored in valve bags for further dilute acid pretreatment in the two-stage pretreatment study. The liquid fraction was determined the main monomeric sugars, viz. glucose and xylose, using HPLC (Perkin Elmer LC200) using refractive index detector and Aminex HPX- 87H column under the following conditions: mobile phase 0.005 M of H_2SO_4 and a flow rate of 0.60 mL/min. Sample injection was 20 μL.

3.3.2 Dilute Acid Pretreatment

To optimize the pretreatment, *Miscanthus* was mixed with different concentrations of acid solution (0.5–5.0 % (w/v)) using 15:1–45:1 LSR. The pretreatment temperature and time were varied from 60–160 °C (300W microwave power) and 5–60 min, respectively. For different acid pretreatment comparison, H_2SO_4, HCl, HNO_3, and H_3PO_4 pretreatments were performed. After the pretreatment, the liquid fraction was collected for monomeric sugar analysis using HPLC.

3.3.3 Two-stage Pretreatment (Dilute Ammonium Hydroxide Followed by Dilute Phosphoric Acid Pretreatment)

The solid residues from the ammonium hydroxide pretreatment were treated with dilute phosphoric acid using the optimal conditions from the dilute acid

pretreatment. The obtaining solution mixture was filtered to collect the liquid part and solid residues were thoroughly washed with distilled water to neutral pH and dried in the oven for further characterization using FTIR (Nicolet nexus 670) recorded in the range of 4000–800 cm^{-1} with a resolution of 1 cm^{-1} and 64 scans per sample to compare with the untreated one.

3.3.4 Chemical Composition Analysis

To determine the amount of extractives in biomass, solvent extraction (60 ml acetone for 1 g of dried biomass sample) was used, and the temperature was held at 90 °C for 2 h. After that, the sample was dried at 105 °C until a constant weight was obtained. The weight difference before and after the extraction is the amount of the extractives.

To determine the amount of hemicelluloses, 10 ml 0.5 mol/L of sodium hydroxide solution was added to 1 g of extractive-free dried biomass, and the temperature was held at 80 °C for 3.5 h. After that, the sample was washed using DI water until pH value of the solution approach 7, then it was dried to a constant weight. The difference between the sample weight before and after this treatment is hemicellulose content.

To determine the amount of lignin, 30 mL of 98 wt.% sulfuric acid was added for each extractive-free dried biomass. After the sample was held at ambient temperature for 24 h, it was boiled at 100 °C for 1 h. The mixture was filtered, and then the residue was washed until the sulfate ion in the filtrate was undetectable (via titration of a 10% barium chloride solution); it was then dried to a constant weight. The weight of the residue was recorded as the lignin content.

The content of cellulose was calculated by the difference, assuming that extractives, hemicelluloses, lignin, and cellulose are the only components of the entire biomass. (Jiang *et al.*, 2010).