CHAPTER III
EXPERIMENTAL

3.1 Materials

Sugarcane bagasse was obtained from Ratchaburi, Thailand. Before any pretreatment, sugarcane bagasse was washed with tap water and dried under sunlight. It was then milled to obtain small particles using herb grinder before further pretreatment, as shown in Figure 3.1.

Figure 3.1 Powder of sugarcane bagasse.

Sodium hydroxide (NaOH), hydrochloric acid (HCl) and nitric acid (HNO_3) were purchased from Labscan Asia Co., sulfuric acid (H_2SO_4) and phosphoric acid (H_3PO_4) were supplied by Merck Co. (Germany), D-glucose anhydrous was obtained from Ajax Finechem (Aus.) and DL-xylose (minimum, 99 %) were purchased from Sigma Aldrich Chemicals Co. Inc. (USA). These chemicals were directly used without purification.

3.2 Equipments

3.2.1 Microwave Solvent Extraction Lab Station

Pretreatment of sugarcane bagasse was conducted in Microwave Solvent Extraction Lab Station (Ethos Sel, Milestone, Figure 3.2). Samples were heated in a Teflon-vessel sealed with a Teflon using with time-to-temperature program.

Figure 3.2 Microwave Solvent Extraction Lab Station (Ethos Sel, Milestone).

3.2.2 High Performance Liquid Chromatography (HPLC)

Monomeric sugars (glucose and xylose) 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

3.3.1 Pretreatment of sugarcane bagasse by microwave heating

The microwave/chemical pretreatment process of sugarcane bagasse in this project was investigated from NaOH/H_2SO_4 hydrolysis, using water as a controlled experiment.

3.3.1.1 Dilute Sodium Hydroxide Pretreatment

Prior to microwave pretreatment, sugarcane bagasse was suspended in NaOH solution (0.5–5 % (w/v)) with 15:1–40:1 liquid-to-solid ratio (LSR) (mL of solution/g of bagasse by weight). The mixture was stirred until homogeneous, and transferred to Teflon-vessel sealed with a Teflon cap. The microwave pretreatment was conducted under reaction temperature and time in the range of 40 °C to 80 °C (300 W microwave power) and 5–90 min, respectively. After pretreatment, the mixture was filtered to separate solid residues from filtrate fraction.

The liquid fraction was collected for reducing sugars analysis (Figure 3.3). 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. The liquid fraction was determined the main monomeric sugars, viz. glucose and xylose, by HPLC.

Figure 3.3 Sugarcane bagasse hydrolysates obtained from microwave/chemical pretreatment.

3.3.1.2 Dilute Sulfuric Acid Pretreatment

Sugarcane bagasse was mixed with different concentrations of H_2SO_4 solution (0.5–3.0 % (v/v)) using 15:1–40:1 liquid-to-solid ratio (LSR) (mL of solution/g of bagasse by weight). The mixture solution was treated as the dilute NaOH pretreatment. The reaction temperature and time were varied from 60 °C to 120 °C (300W and 500W microwave power for 60 °C to 100 °C and 120 °C, respectively) and 5–120 min, respectively. After pretreatment, the solution mixture was filtrated and solid residues were thoroughly washed with distilled water to neutral pH and dried in the oven. The liquid fraction was determined the main monomeric sugars, viz. glucose and xylose, by HPLC.

For comparison with dilute H_2SO_4 pretreatment, HCl, HNO_3, and H_3PO_4 pretreatment were performed at the optimal conditions of dilute H_2SO_4 pretreatment.

3.3.1.3 Water Pretreatment (Controlled Experiment)

Sugarcane bagasse was suspended in distilled water with a 30:1 liquid-to-solid ratio (LSR) (mL of water/g of bagasse by weight). The mixture solution was treated as the dilute NaOH pretreatment. The pretreatment was conducted under reaction temperature and time in the range of 40 °C to 80 °C

(microwave power 300 W) and 30–90 min, respectively. After pretreatment, the liquid faction and solid residues were collected as above (Adapted from Carrillo *et al.* (2005)). The liquid fraction was determined the main monomeric sugars, viz. glucose and xylose, by HPLC.

3.3.2 Pretreatment of sugarcane bagasse by conventional heating

Sugarcane bagasse were suspended in 0.5–2.0 % (v/v) H_2SO_4 solution with a 30:1 liquid-to-solid ratio (LSR) (mL of solution/g of bagasse by weight) in a 1000 mL flask and kept boiling at 120 °C for 90 min using oil bath (Adapted from Hu *et al.* (2008)). It is estimated that the solution mixture took about 5 min to be heated up from room temperature to the required temperature (120 °C), therefore the recording of time was started after the flask was placed in the oil bath for 5 min. During the heating period, the solution mixture was stirred to homogenize the solid and liquid fractions. After treatment, the reactor was removed from the oil bath and cooled to room temperature. The procedures for handling the solid and liquid fractions were the same as those used in microwave pretreatment.