CHAPTER III
EXPERIMENTAL

3.1 Materials and Equipment

3.1.1 Chemicals

- Carboxymethyl cellulose, (CMC), purchased from Fluka, Sigma-Aldrich Co., Inc., Singapore
- Glucose, ($C_6H_{12}O_6$), purchased from Merck KGaA, Germany
- Malt Extract, purchased from Lab Scan Analytical Sciences, Thailand
- Potassium tartrate, ($C_4H_4K_2O_6$. $4H_2O$), purchased from Merck KGaA, Germany
- Standard sugars (glucose, xylose, arabinose, mannose and galactose) for HPLC analysis
- Sodium hydroxide, (NaOH), purchased from Merck KGaA, Germany
- Yeast extract, purchased from Bio Springer, France

3.1.2 Equipment

- 4-Digit precision weighting balance: Model AG 204, Mettler Toledo, Switzerland
- Autoclave: Model Autoclave ES-315, Tomy Seiko Co., Ltd., Tokyo, Japan
- High speed refrigerated centrifuge: Beckman Coulter TM Avanti J-30I, Palo Alto, California, U.S.A.
- Hot air oven: Model UC 30, Memmert GmbH and Co. KG., Western Germany
- Incubator: Model 800, Memmert GmbH and Co. KG., Western Germany
- Incubator shaker: Model SK-737, Amerex Instruments, Inc., U.S.A.

- Kubota refrigerated microcentrifuge 6500: Kubota Corporation, Tokyo, Japan
- Laminar flow 'clean': Model V6, Lab Service Ltd., Thailand
- pH meter: Mettler-Toledo International Inc., New York, U.S.A.
- Spectrophotometer: Genesys 20 Model 4001/4, ThermoSpectronic, Rochester., New York, U.S.A.
- Surface area analyzer (SAA, Quantachrome/Autosorb 1)
- Water bath: Model WB14, Memmert GmbH and Co. KG., Western Germany
- X-ray diffractometer (XRD, Rigaku/Rint2200 HV)

3.2 Experimental Procedures

Firstly, the raw material (corncob) was milled and determined for its composition. The raw material was then mixed in the hydrolysis reactor in order to hydrolyze cellulose and hemicelluloses to sugar. Finally, produced sugar was separated from bacteria by centrifugation. Figure 3.1 illustrates the overall sugar production process. Details of each step as follows:

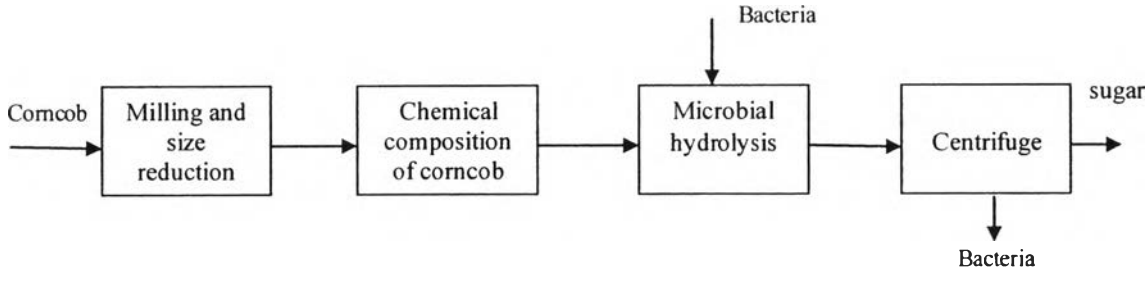

Figure 3.1 Schematic illustrating glucose production process.

3.2.1 Preparation of Corncob and Composition Analysis

Corncob (provided by River Kwai International Food Industry Co., Ltd.) was dried at 105 °C and stored in sealed plastic bags. Then, the dried corncob was milled to small size particles and sieved to sizes between 40 and 60 mesh. To identify the physical and compositional properties of the corncob, the particle size distributions and compositions were analyzed for two different batches.

To determine the amount of extractives in corncob, solvent extraction (60 ml acetone for 1 g of dried corncob sample) was used, and the extraction was performed 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 defined as the amount of extractives.

To determine the amount of hemicellulose, 10 ml of 0.5 M sodium hydroxide solution was added to 1 g of the extractive-free dried corncob, and the mixture was held at 80 °C for 3.5 h. After that, the sample was washed using distilled water until a neutral pH value of 7 was reached. Then, it was dried to obtain a constant weight. The weight difference before and after this alkaline treatment is defined as the hemicellulose content.

To determine the amount of lignin, 30 ml of 72 wt% sulfuric acid was added to the extractive-free dried corncob. The mixture was kept at 8–15 °C for 24 h. Then, it was transferred into a flask and diluted with 300 ml of distilled water. After that, the sample 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 not detected (via titration with a 10% barium chloride solution). It was dried to obtain a constant weight. The weight of the residue is defined as the lignin content.

The cellulose content was calculated by the difference of the biomass weight to that of extractives, hemicelluloses, and lignin (Lin *et al.*, 2010).

3.2.2 Preparation of Bacteria Cells for Microbial Hydrolysis

For the preparation of bacteria cells, an innoculum was prepared by transfering a loop of colonies into a 250 ml Erlenmayer flask containing 50 ml of 65 modified DSMZ broth medium 2 with pH of 7.2. The culture was incubated at 37 °C in a shaking incubator at 180 rpm for 12 h. Then, 50 ml of the prepared inoculum was transferred into a 500 ml bottle with a screw cap containing 450 ml of the production medium (65 modified DSMZ broth medium 2, pH 7.2) and incubated again at 37 °C in a shaking incubator at 180 rpm for 12 h. After that, the cells were harvested by centrifugation (8,000 rpm, 4 °C for 10 min).

3.2.3 Microbial Hydrolysis

For the hydrolysis, there are two sets of experiment. The first reactor was added with the production medium (65 modified DSMZ broth medium 2 without CMC, pH 7.2), whereas the second reactor was added with the mineral mixture (96 mg NH_4HCO_3, 30.59 mg K_2HPO_4, 100 mg $MgCl_2 6H_2O$, 15mg $MnSO_4 6H_2O$, 5 mg $CuSO_4 5H_2O$, 6720 mg $NaHCO_3$, 0.125 mg $CoCl_2 5H_2O$and 25 mg $FeSO_4 7H_2O$). The reactors were then added by the corncob powder, which was autoclaved under clean conditions. Each reactor contained 1.5-1.6 g corncob powders, 4-7 g bacteria cells, and 1 L of the production medium. The reactor temperature was controlled by the water jacket at 30 and 37 °C. Compressed air was applied to the reactor simultaneously, while all substrates were tranfered into the reactor in order to start-up the hydrolysis reaction.

3.2.4 Determination of Sugar and Bacteria Concentrations

Glucose, xylose, and arabinose were analyzed by a high performance liquid chromatography (HPLC) with an organic acid column (VertiSep^{TM} SUGAR LMP). Distilled water was used as the mobile phase at a flow rate of 0.3 ml/min. The column temperature was fixed at 80 °C. The concentration of bacteria was determined by the total nitrogen test kit.