CHAPTER III
EXPERIMENTAL

3.1 Materials and Equipment

Equipment:

1) Hot air oven: Model UC 30, Memmert GmbH and Co. KG., Western Germany
2) MFC grinder
3) Autoclave: Model Autoclave ES-315, Tomy Seiko Co., Ltd., Tokyo, Japan
4) Cold room: Model Compakt 880(B)H, Foster Refrigerator (U.K) Ltd., U.K.
5) 4-Digit precision weighting balance: Model AG 204, Mettler Toledo, Switzerland
6) High speed refrigerated centrifuge: Beckman Coulter TM Avanti J-30I, Palo Alto, California, U.S.A.
7) Hot plate stirrer: Model C-MAG HS 10, Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand
8) Incubator: Model 800, Memmert GmbH and Co. KG., Western Germany
9) Incubator shaker: Model SK-737, Amerex Instruments, Inc., U.S.A.
10) Kubota refrigerated microcentrifuge 6500: Kubota Corporation, Tokyo, Japan
11) Laminar flow 'clean': Model V6, Lab Service Ltd., Thailand
12) Microscope: Model CH 30RF200, Olympus Optical Co., Ltd., Japan
13) Orbital shaker: Innova Model, New Brunswick Co., Inc., U.S.A.
14) Pipetteman: Gilson, France
15) pH meter: Mettler-Toledo International Inc., New York, U.S.A.
16) Spectrophotometer: Genesys 20 Model 4001/4, ThermoSpectronic, Rochester., New York, U.S.A.

17) Vortex mixer: Model G-560E, Scientific Industries, Inc., Bohemia. N.Y., 11716, U.S.A.
18) Water bath: Model WB14, Memmert GmbH and Co. KG., Western Germany
19) High pressure liquid chromatography: Model 200LC, Perkin Elmer

Chemicals:

1) Corncob from Kanchanaburi
2) Carboxymethyl cellulose, (CMC), purchased from Fluka, Sigma-Aldrich Co., Inc., Singapore
3) Malt Extract, purchased from Lab Scan Analytical Sciences, Thailand
4) Yeast extract, purchased from Bio Springer, France
5) Sodium hydroxide, (NaOH), purchased from Merck KGaA, Germany
6) Ethanol absolute, (C_2H_5OH), Analytical grade, purchased from Scharlau Chemie S.A., Spain
7) Standard sugars (glucose, xylose, arabinose, mannose and galactose) for HPLC analysis

3.2 Experimental Procedures

3.2.1 Preparation of Corncob and Composition Analysis

Corncob 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 to 80 mesh.

3.2.2 Preparation of Bacteria Cells for Microbial Hydrolysis

For the preparation of bacteria cells, an inoculum was prepared by transferring a loop of colonies into a 250 ml Erlenmayer flask containing 50 ml of 65 modified DSMZ broth medium 2 (as shown in Appendix B) 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

Before the hydrolysis, reactors, production medium (65 modified DSMZ broth medium 2 without CMC, pH 7.2) and corncob powder were autoclaved under aseptic conditions. The reactor contained 1.0 corncob powder, 6-7 g bacteria cells, and 1 L of the production medium. The reactor temperature was controlled by the water jacket at 37 °C. Compressed air was applied to the reactor simultaneously, while all substrates were transferred into the reactor in order to start-up the hydrolysis reaction. All the samples were taken out every hour for 24 h.

3.2.4 Enzymatic Hydrolysis

The enzymatic hydrolysis was carried out by using commercial cellulase (Aspergillus niger), and incubation of corncob with varying 100U , 200U, 300U, 400U, and 500U cellulase per gram of corncob in 100 ml of 50 mM L^{-1} acetate buffer solution, pH 5.5, at 37 °C. A buffer solution of 0.01% sodium azide was added for the prevention of microorganism contamination. The hydrolytic mixture was incubated in a rotation incubator at 180 rpm for 72 h. During the hydrolysis, samples were collected and analyzed for glucose.

3.2.5 Determination of Sugar and Bacteria Concentrations

Glucose was analyzed by a high performance liquid chromatography (HPLC) (Shimadzu, Japan) with a refractive index detector (Model 6040 XR, Spectra-Physics, USA) and an organic acid column (BIORAD HPX-87H). 0.005M H_2SO_4 was used as the mobile phase at a flow rate of 0.6 ml/min. The column temperature was fixed at 65 °C. The concentration of bacteria was determined by the total nitrogen test kit (DR 2700, spectrophotometer).