CHAPTER III
EXPERIMENTAL

3.1 Materials and Equipment

3.1.1 Chemicals

- Rice Straw from Petchaburi province (Suphan–60 rice with three months plantation).
- Carboxymethyl cellulose, (CMC), purchased from Fluka, Sigma-Aldrich Co., Inc., Singapore.
- Malt Extract, purchased from Lab Scan Analytical Sciences, Thailand.
- Yeast extract, purchased from Bio Springer, France.
- Sodium hydroxide, (NaOH), purchased from Merck KGaA, Germany.
- Ethanol absolute, (C_2H_5OH), Analytical grade, purchased from Scharlau Chemie S.A., Spain.
- Standard sugars (glucose, xylose, arabinose, mannose and galactose) for HPLC analysis.

3.1.2 Equipments

- Hot air oven: Model UC 30, Memmert GmbH and Co. KG., Western Germany.
- MFC grinder.
- Autoclave: Model Autoclave ES-315, Tomy Seiko Co., Ltd., Tokyo, Japan.
- Cold room: Model Compakt 880(B)H, Foster Refrigerator (U.K) Ltd., U.K.
- 4-Digit precision weighting balance: Model AG 204, Mettler Toledo, Switzerland.
- High speed refrigerated centrifuge: Beckman Coulter TM Avanti J-30I, Palo Alto, California, U.S.A.

- Hot plate stirrer: Model C-MAG HS 10, Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand.
- 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.
- Microscope: Model CH 30RF200, Olympus Optical Co., Ltd., Japan.
- Orbital shaker: Innova Model, New Brunswick Co., Inc., U.S.A.
- Pipetteman: Gilson, France.
- pH meter: Mettler-Toledo International Inc., New York, U.S.A.
- Spectrophotometer: Genesys 20 Model 4001/4, ThermoSpectronic, Rochester., New York, U.S.A.
- Vortex mixer: Model G-560E, Scientific Industries, Inc., Bohemia. N.Y., 11716, U.S.A.
- Water bath: Model WB14, Memmert GmbH and Co. KG., Western Germany.
- High pressure liquid chromatography: Model 200LC, Perkin Elmer.

3.2 Experimental Procedures

3.2.1 Biomass Preparation

Rice Straw was dried in an oven at 105 °C for 12 hr. Then dried rice straw was milled to obtain small particles and extensively homogenized by using MFC grinder. After that, the rice straw power was sent to Nakhonratchasima Animal Nutrition Research and Development Center to determine cellulose, hemicellulose, lignin, and ash content of rice straw powder.

3.2.2 Bacteria Cells Preparation for Microbial Hydrolysis

For the preparation of bacteria cells, an inoculum was prepared by transferring a loop of colonies into a 250 mL Erlenmeyer flask containing 50 mL of 65 modified DSMZ broth medium 2, pH 7.2 (Taechapoempol, 2008). 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 at 37 °C in a shaking incubator at 180 rpm for 12 h. After that, the cells were harvested by centrifugation (8000 rpm, 4 °C for 10 min).

To determine the hydrolysis capacity value (HC value), the pure colonies of bacteria were single spotted on 65 modified DSMZ agar medium 2 and incubated at 37°C for 1 day. All colonies were measured for the diameter of colony, after that, they were flooded with 0.1 wt./vol.% congo red solution for 10 minutes and then washed by 0.1 M NaCl. Clear-zone appearance and diameter of each colonie were measured and calculated for HC value, which was calculated from the clear zone diameter divided by the colony diameter.

3.2.3 Microbial Hydrolysis

Before the hydrolysis experiments, the reactors, production medium (65 modified DSMZ broth medium 2 without CMC, pH 7.2), and rice straw powder were autoclaved under aseptic conditions. Each reactor contained 1 g of rice straw powder, 0.3 g of bacteria cells and 1 L of the production medium. The reactor temperature was controlled by the water jacket at 30 °C and 37 °C. Compressed air

will be applied to the reactor simultaneously with transferring of all substrates in order to start-up the hydrolysis reaction.

3.2.4 Determination of Sugar and Bacteria Concentrations

The enzymatic reaction was carried out with two repetitions and monitored by withdrawing samples from the supernatant periodically. Finally, the main monosaccharides including glucose were analyzed by a high performance liquid chromatography (HPLC) with a sugar column (LiChroCART-NH_2). Distilled water and acetronitrile were used as the mobile phase at a flow rate of 1.4 ml/min. The column temperature was fixed at 25 °C. The concentration of bacteria was determined by the total nitrogen test kit (HACH, Germany).