CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Samples

Sugarcane bagasse samples were collected from Mitr Phol Sugar Corp., Ltd., located at Dan-chang, Suphanburi province, Thailand.

3.1.2 Chemicals

- 3, 5-Dinitrosalicylic acid
- Acetone
- Carboxymethyl cellulose, CMC (Fluka, Sigma-Aldrich Co., Inc., Singapore)
- D-(+)galactose, standard grade (CHEMSERVICE, U.S.A)
- D-glucose, standard grade (CHEMSERVICE, U.S.A)
- D-mannose, standard grade (CHEMSERVICE, U.S.A)
- D-xylose, standard grade (CHEMSERVICE, U.S.A)
- Ethanol absolute, C_2H_5OH
- L-arabinose, standard grade (CHEMSERVICE, U.S.A)
- Malt Extract (Lab Scan Analytical Sciences, Thailand)
- Phenol
- Potassium sodium tartrate tetrahydrate, $C_4H_4KNaO_6.\ 4H_2O$
- Sodium chloride, NaCl
- Sodium hydroxide, NaOH
- Sodium sulfite
- Sulfuric acid, H_2SO_4
- Yeast extract (Bio Springer, France)

3.1.3 Identification Kits

- Glucose assay kit, GAGO-20 (SIGMA)
- Total nitrogen test kit (HACH, Germany)

3.2 Equipment

3.2.1 Instrument

- High performance liquid chromatography, HPLC: Model Prominence (Shimadzu Co., Japan) with VertiSep™ SUGAR LMP, 8um, HPLC Column, 7.8x300mm (VERTICAL®, Thailand) and a refractive index detector
- Surface area analyzer: Model Autosorb-1 (Quantachrome Instruments, Florida, U.S.A)
- Particle size analyzer: Model Mastersizer X (Malvern Instruments Ltd., UK)
- Scanning Electron Microscope, SEM: Model S4800 (Hitachi)
- CHNS Analyzer: Model TruSpec® Elemental Determinator (Leco corporation, Michigan, U.S.A)

3.2.2 Apparatus

- 0.25 μm filter
- 1.5 mL eppendorf tubes
- 4-digit precision weighting balance: Model AG 204 (Mettler Toledo, Switzerland)
- Alcohol Burner
- Aluminium tray
- Autoclave: Model Autoclave KT-40D (Alp Co., Ltd., Japan)
- Bottle with a screw cap
- Cell culture dish
- Desiccator
- Erlenmeyer flask
- Filtration unit
- Freezer: model FC27 (-20°C) (Sharp, Japan)
- Heating block: Digital PID control with thermal protection, M-lab, Bangkok, Thailand

- Hot plate stirrer: Model C-MAG HS 10 (Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand)
- Incubator shaker: Model SK-737 (Amerex Instruments, Inc., U.S.A)
- Incubator: Model 800 (Memmert GmbH and Co. KG., Western Germany)
- Kubota refrigerated centrifuge 6500 (Kubota Corporation, Tokyo, Japan)
- Laminar flow 'clean': Model V6 (Lab Service Ltd., Thailand)
- Magnetic bars
- Measuring cylinder
- MFC grinder
- Microcentrifuge
- Microliter pipette
- Oven: Model Universal Oven (Memmert GmbH and Co. KG., Western Germany)
- pH meter (Mettler-Toledo International Inc., New York, U.S.A)
- Sieve with 40-mesh, 60-mesh, and 100-mesh screens
- Spatula
- Syringe
- Thermometer
- Volumetric flask
- Weighting paper

3.2.3 Bioreactors

- Compressed air
- Glass reactor with water jacket and sampling value
- Water bath: Model WBC-15 (M-lab,Bangkok, Thailand)

3.3 Methodology

3.3.1 Biomass Preparation

Sugarcane bagasse samples were collected from Mitr Phol Sugar Corp., Ltd. Before any treatment, sugarcane bagasse was washed with tap water and dried in an oven at 105 °C for one day. Then dried bagasse was milled to obtain small particles and extensively homogenized by using an MFC grinder. It was sieved with a 40-mesh, 60-mesh, and 100-mesh screens. After that, cellulose, hemicellulose, lignin, and extractive content of the ground bagasse were determined using the following methods.

To determine for the extractive component, 1 g of the biomass was soaked in 60 ml acetone at ambient temperature for 24 h. After that, the residue was filtered and dried at 105 °C until a constant weight was obtained. Then, it was cooled to room temperature in a desiccator. The weight difference before and after the extraction is the extractives component.

To determine for the hemicellulose component, 10 ml 0.5 mol/l of sodium hydroxide solution was added into a glass containing 1 g of extractive-free biomass and held at 80 °C for 3.5 h. After that, the sample was washed to neutral pH and dried until the weight was constant. Then, it was cooled to room temperature in a desiccator. The difference between the weight before and after is the hemicellulose component.

To determine for the lignin component, 1 g of the extractive-free biomass was hydrolyzed with 30 ml of 72 wt% sulfuric acid at 8–15 °C for 24 h. Then, it was diluted with 300 ml of distilled water and boiled for 1 h. After cooling and filtration, the residue was washed until no sulfate ion was left in the filtrate which can be detected by titration with 10% barium chloride solution. After that, the residue was dried until a constant weight and cooled to room temperature in a desiccator. The weight of the residue is the lignin component.

To determine for the cellulose component, by assuming that components of biomass are only extractives, hemicellulose, lignin, and cellulose, cellulose component was calculated by the total difference.

3.3.2 Bacteria Preparation

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 growth media (65 modified DSMZ broth medium 2, pH 7.2). The culture was incubated at 37 °C in a shaking incubator at 180 rpm for 12-16 h. Then, 50 ml of the prepared inoculum was transferred into a 500 mL bottle with a screw cap containing 450 mL of the growth media and incubated at 37 °C in a shaking incubator at 180 rpm for 12-16 h. After that, the cells were harvested by centrifugation at 8,000 rpm and 4 °C for 10 min.

3.3.3 Hydrolysis Experiment

Before running the microbial hydrolysis experiment, reactors, fermentation media (65 modified DSMZ broth medium 2 without CMC, pH 7.2), and ground bagasse were autoclaved under aseptic conditions at 121 °C for 15 min. The experiment was setup as shown in Figure 3.1. Each reactor contained 1 g of ground bagasse, 7-9 g of bacteria cells (wet basis) and 1 L of the production media. The reactor temperature was controlled by the water bath and water jacket at 30° C and 37 °C. Compressed air was applied to the reactor simultaneously in order to maintain aerobic condition and ensure homogeneous of the system.

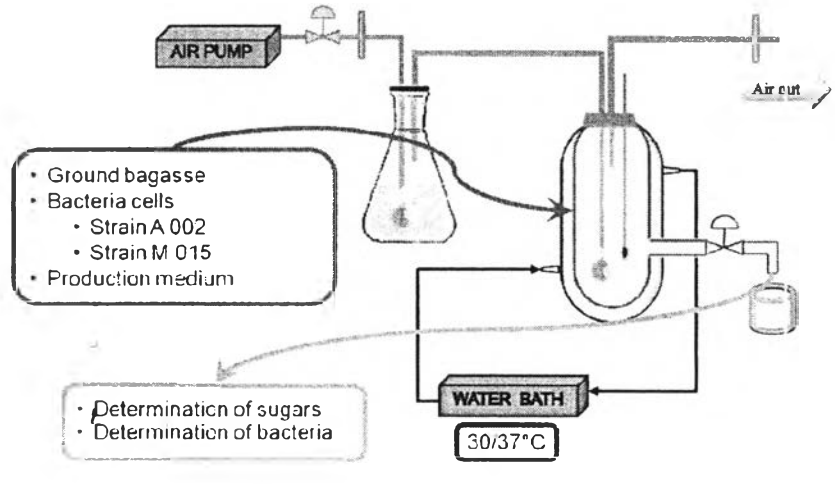

Figure 3.1 Bioreactor and experimental setup for using in microbial hydrolysis of sugarcane bagasse.

3.3.4 Sugar Analysis

The hydrolysis reaction was monitored by periodically withdrawing samples from the sampling value. Finally, the main monosaccharides including glucose, xylose, arabinose, mannose, and galactose in the liquid fraction product were analyzed by an HPLC using refractive index detector and VertiSep^{TM} SUGAR LMP 8μm 7.8x300mm column under the following conditions: mobile phase as DI-H_2O and a flow rate of 0.30 mL/min at 80 °C of column oven temperature. The growth of bacteria in the solid fraction product was determined by the total nitrogen test kit.