CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Chemicals

- 1-Butyl-3-methylimidazolium chloride, ($C_8H_{15}ClN_2$), Fluka, Sigma-Aldrich Co., Inc., Germany.
- Carboxymethyl cellulose, (CMC), Fluka, Sigma-Aldrich Co., Inc., Singapore.
- Ethanol absolute, (C_2H_5OH), Analytical grade, Scharlau Chemie S.A., Spain.
- Glucose, ($C_6H_{12}O_6$), Merck KGaA, Germany.
- Malt extract, Lab Scan Analytical Sciences, Thailand.
- Sodium chloride, (NaCl), Analytical grade, Merck KGaA, Germany.
- Sodium hydroxide, (NaOH), Merck KGaA, Germany.
- Whatman filter Paper No.1, 2, 4 and 5, Whatman, Germany.
- Yeast extract, Bio Springer, France.

3.1.2 Test Kits

- Glucose (HK) assay kit: GAHK-20, Sigma-aldrich, U.S.A.
- Total nitrogen test kit: HACH, Germany.

3.2 Equipment

- Autoclave KT-40D, Alp Co., Ltd., 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.
- Hot air oven: Model UC 30, Memmert GmbH and Co. KG., Western Germany.
- 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.
- Data Physics, Germany laminar flow hoods, Pennyful Thailand, Co., Ltd.,Germany
- Pipetteman: Gilson, France.
- pH meter: Mettler-Toledo International Inc., New York, U.S.A.
- Water bath: Model WBC-15, M-lab,Bangkok, Thailand.
- Heating block: Digital PID control with thermal protection, M-lab, Bangkok, Thailand.
- Microcentrifuge: Spectrafuge 24D, Labnet International, Inc. N.J., U.S.A.
- Autosorb-1MP, Quantachrome Instruments, Florida, U.S.A.
- TruSpec® Elemental Determinator, Leco corporation, Michigan, U.S.A.

3.3 Methodology

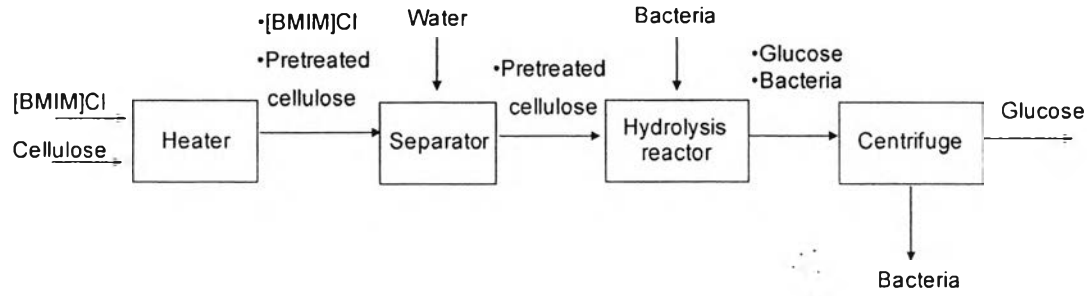


Figure 3.1 Schematic illustrating glucose production process.

Cellulose was first dissolved by [BMIM]Cl with an aid of a heater. Then, the pretreated cellulose was separated from [BMIM]Cl using water. After that, the pretreated cellulose and proper bacteria were mixed in a hydrolysis reactor in order to hydrolyze the cellulose to glucose. Finally, produced glucose was separated from the bacteria by centrifugation. Figure 3.1 illustrates the overall glucose production process. Details of each step are as follows:

3.3.1 Cellulose Pretreatment

Whatman filter paper No.1 and [BMIM]Cl mixtures were incubated in a 10-ml vial with various cellullose-to-[BMIM]Cl ratios of 2:100, 5:100, 7:100. Each vial was heated in a block heater at 80, 100, and 120°C for 0.5 to 24 h.

After that, deionized water was used as an anti-solvent for separating cellulose from [BMIM]Cl. The mixture was centrifuged, and the supernatant was removed. The precipitated sample was washed for three times with deionized water. The resulting cellulose was referred to as a the pretreated cellulose. The pretreated cellulose was characterized by XRD in order to obtain the change in the crytallinity to determine the optimal temperature and time for the pretreatment of cellulose. The change was calculated using Eq. (3.1) .

$$\text{Decrease in crystallinity (\%)} = \frac{[(CW - CP)\times 100]}{CW} \qquad (3.1)$$

CW = Crystallinity of Whatman cellulose

CP = Crystallinity of pretreated cellulose

The amount of [BMIM]Cl remained in the pretreated cellulose was also determined using TruSpec® Elemental Determinator by detecting the nitrogen content in [BMIM]Cl.

3.3.2. Design of a Hydrolysis Reactor

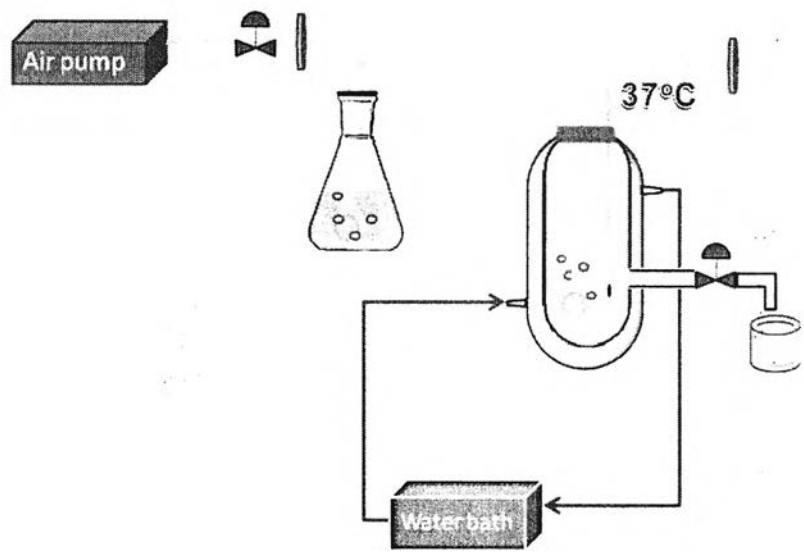
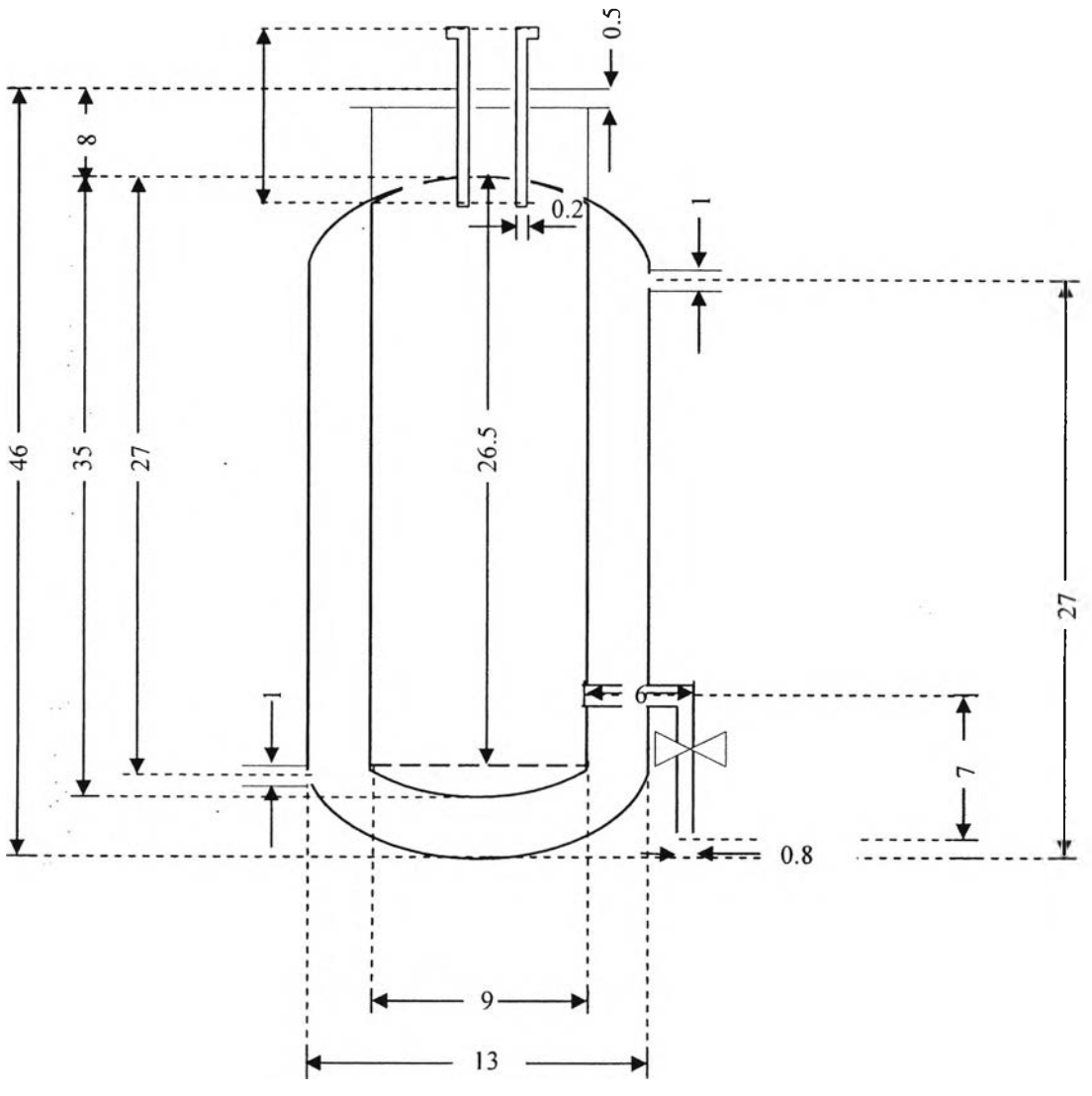


Figure 3.2 Schematic of a hydrolysis system.

The reactor was operated under aseptic conditions. The water jacket's temperature was controlled at 37°C by a water bath. Compressed air was filtered through a 0.2-μm pore size membrane to get rid of all contaminants. The air was also passed to distilled water to increase the humidity before going to the hydrolysis reactor, in order to maintain aerobic condition and complete mixing inside the bioreactor.

(a)



Unit : cm

Figure 3.3 Diagram of a hydrolysis reactor: (a) glass reactor, (b) hose, and (c) air spreader.

(b)

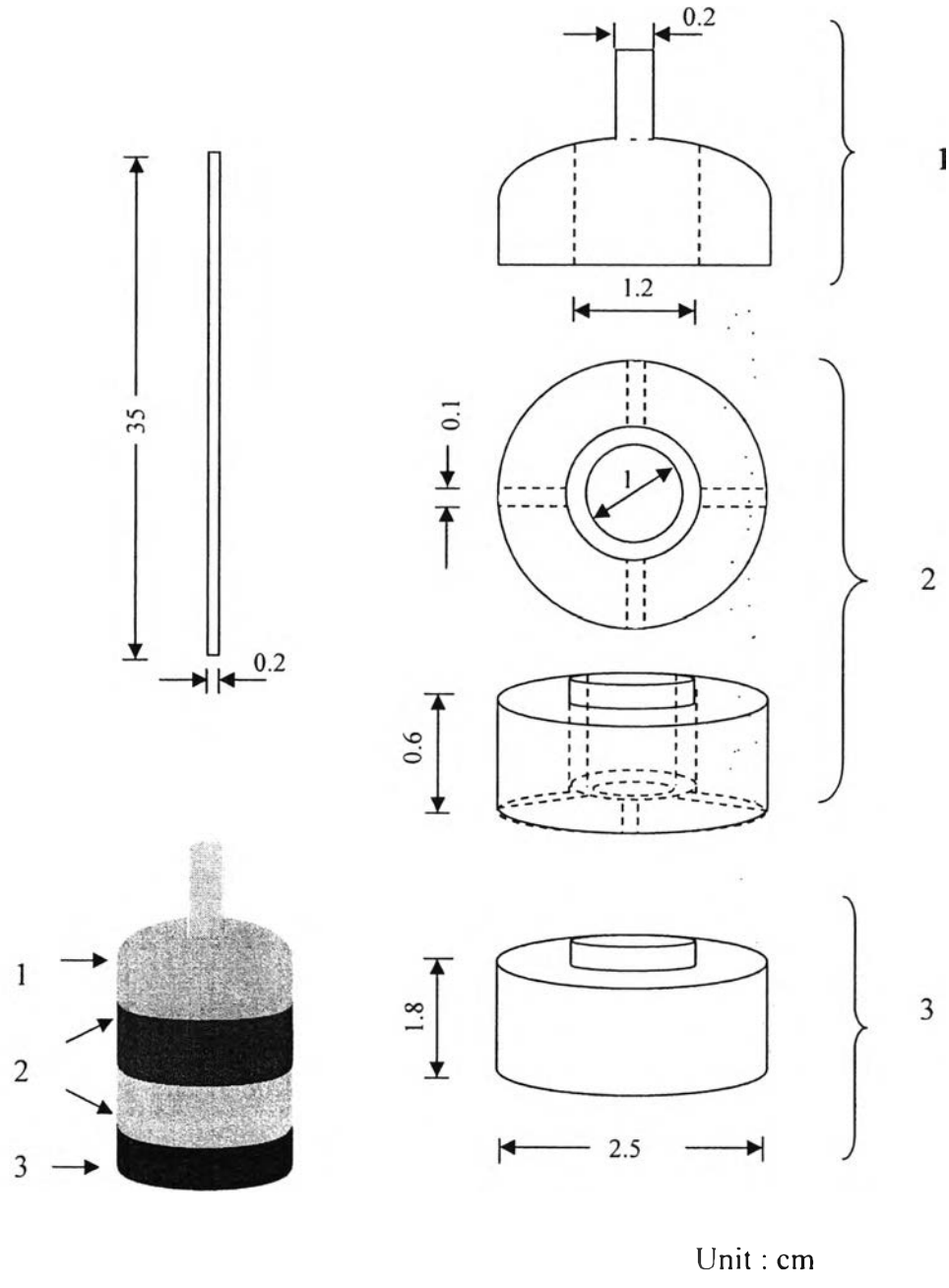


Figure 3.3 (continued) Diagram of a hydrolysis reactor a) glass reactor b) hose c) air spreader.

The hydrolysis reactor was constructed with a round-bottom glass vessel with a total volume of 2,000 mL and a working volume of 1,000 mL. A valve was made from teflon. Hoses were made from silicone and connected to an air spreader made from plastic, as shown in Figure 3.3. The hydrolysis reactor setup is shown in Figure 3.4.

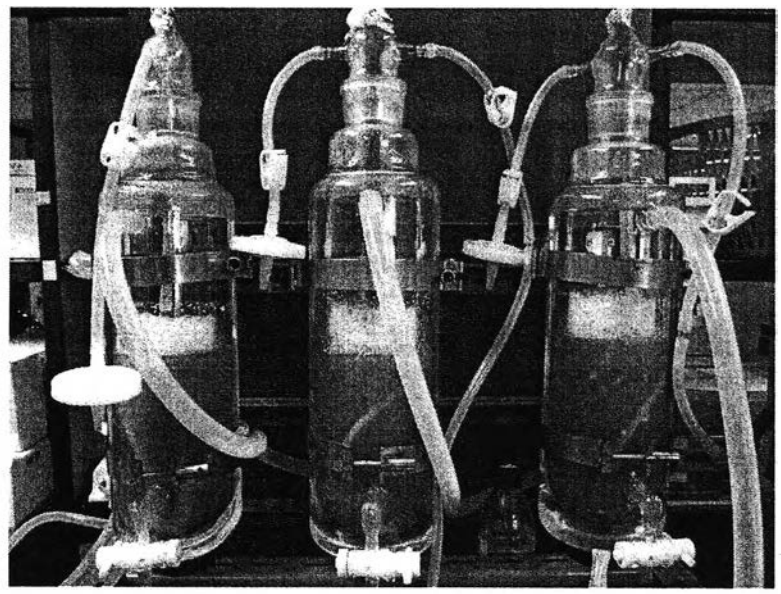


Figure 3.4 Hydrolysis reactor.

3.3.3 Preparation of Bacteria Cells for Enzymatic 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, pH 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 at 37°C in a shaking incubator at 180 rpm for 12 h. Afer that, the cells were harvested by centrifugation (8,000 rpm, 4°C for 10 min).

3.3.4 Reactor Start-up and Operating Condition

Before the start-up of an experiment, all reactors, production medium (65 modified DSMZ broth medium 2 without CMC, pH 7.2), and cellulose sources were autoclaved under aseptic conditions. Each reactor contained 1 g of untreated or pretreated cellulose. About 7-9 g of bacteria cells was gathered and transferred to the hydrolysis reactor, which contained 1 L of the production medium. The reactor temperature was controlled by the water jecket at 37°C. Compressed air was applied to the reactor simultaneously with transferring all substrates in order to start-up the hydrolysis reaction.

3.3.5 Determination of Glucose and Cellulose Conversion

Batch enzymatic hydrolysis of untreated and treated celluloses was carried out at 37°C in the hydrolysis reactor. Furthermore, the effect of cellulase enzymes, derived from bacteria isolated from higher termites, on the rate of hydrolysis of treated and untreated celluloses was investigated. The enzymatic reaction was monitored by withdrawing samples from the supernatant periodically. Untreated and IL-treated celluloses were hydrolyzed using the same cellulases, and all experiments were carried out with two repetitions. Finally, glucose concentration was determined by the glucose assay method, and a concentration of bacteria was determined by the total nitrogen test kit.