

CHAPTER III EXPERIMENTAL

3.1 Materials

Seed sludge and ethanol wastewater were collected from Saphip Lopburi Co., Ltd., Thailand. The anaerobic seed sludge was black and had total suspended solids (TSS) concentration of 15,270 mg/l. The ethanol wastewater had the chemical characteristics, as shown in Table 3.1. The ethanol wastewater was kept at 4 °C prior to use.

Table 3.1 Chemical characteristics of the ethanol wastewater

Chemical Characteristic	Concentration (mg/l)
Chemical oxygen demand (COD)	60,000 – 80,000
Total suspended solids (TSS)	2,600 – 5,200
Total nitrogen	600 – 4,600
Phosphorus	710 – 1430

3.2 Equipment

- Upflow anaerobic sludge blanket (UASB) reactors
- Gas chromatograph (GC), Perichrom, PR2100
- Gas chromatograph (GC), PerkinElmer, AutoSystem GC
- COD reactor, HACH
- Spectrophotometer, HACH D/R 2000
- pH electrode, Cole-palmer KH-27012-27

3.3 Chemicals

- Ammonium hydrogen carbonate (NH_4HCO_3), analytical reagent grade, AJAX Finechem Pty Ltd, Australia
- Di-potassium hydrogen orthophosphate (K_2HPO_4), analytical reagent grade, AJAX Finechem Pty Ltd, Australia
- Sulfuric acids (H_2SO_4) 98%, analytical reagent grade, Lab-scan, Thailand
- Sodium hydroxide (NaOH), analytical reagent grade, Lab-scan, Thailand
- Phenolphthalein ($\text{C}_{20}\text{H}_{14}\text{O}_4$), analytical reagent grade, Labchem, Australia

3.4 Methodology

3.4.1 Seed Sludge Preparation

Seed sludge was obtained from Saphthip Lopburi Co., Ltd., Thailand. It was boiled at 95 °C for 15 min to eliminate methane-producing bacteria that consume hydrogen before introducing into the UASB reactor (Sreethawong *et al.*, 2010).

3.4.2 Substrate Preparation

Ethanol wastewater was obtained from Saphthip Lopburi Co., Ltd., Thailand. It was diluted with water to obtain chemical oxygen demand (COD) of 60,000 mg/l. The wastewater was supplemented by nutrients to obtain COD:N:P ratio of 100:2:0.4. Ammonium hydrogen carbonate (NH_4HCO_3) and di-potassium hydrogen orthophosphate (K_2HPO_4) were added as the nitrogen and phosphorus source, respectively.

3.4.3 UASB Operation

Two identical upflow anaerobic sludge blanket (UASB) reactors were constructed from borosilicate glass with a 24-liter working volume for hydrogen production. The temperature and pH were controlled by a water jacket system with

heating bath and pH-controller, respectively. The schematic of the UASB process is shown in Figure 3.1. The ethanol wastewater was fed into the reactor from the feed tank. The feed was introduced at the bottom of the reactor (in upward direction) and passed through the microbial sludge. The organic compound in the feed was digested by the microorganisms, and then the gaseous products were generated. A three-phase separator was used for preventing outflow of flocculants and separating the gaseous products and the overflow liquid effluent. The gaseous products were analyzed for gas production rate and gas compositions by gas meter and gas chromatograph, respectively. The overflow liquid effluent was collected in a product tank, and then it was analyzed for the COD value, pH, and compositions of volatile fatty acids (VFA). The microbial concentration in the UASB reactor was also analyzed in terms of mixed liquor volatile suspended solids (MLVSS).

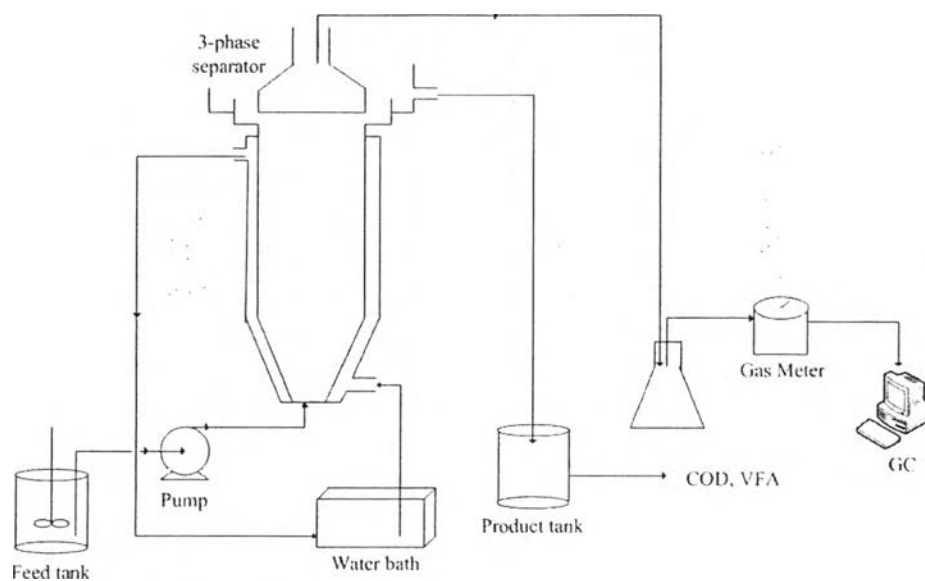


Figure 3.1 Flow diagram of UASB reactor.

Ethanol wastewater was fed into the UASB reactor with an initial feed COD value of 60,000 mg/l at a controlled temperature of 37 °C. The pH was controlled at 5.5 by a pH-controller and adjusted by a 5 wt./vol.% NaOH aqueous solution. In this research, the COD loading rate was varied from 10 to 35 kg COD/m³d, corresponding to the feed flow rate and hydraulic retention time (HRT)

shown in Table 3.2. The parameters, such as effluent COD, gas production rate, and gas composition, were analyzed until the system reached the steady state, at which these parameters were almost invariant (with less than 5 % standard deviation). Then, the COD removal, VFA composition, and MLVSS were determined.

Table 3.2 Conditions for investigating the effect of COD loading rate on hydrogen production

COD loading rate (kg COD/m ³ d)	Feed flow rate (l/d)	HRT (d)
10	4	6
15	6	4
20	8	3
25	10	2.4
30	12	2
35	14	1.7

3.5 Analytical Methods

3.5.1 COD Analysis

The liquid sample was heated by the COD reactor (HACH) for 2 h and left for 20 min. Then, the COD value was determined by the spectrophotometer (HACH DR 2700).

3.5.2 Amount of VFA

Amount of VFA in mg as acetic acid per liter was determined by a distillation-titration method. The effluent sample was distilled and titrated with 0.1 M NaOH aqueous solution using phenolphthalein as an indicator (Eaton *et al.*, 2005).

3.5.3 Composition of VFA

VFA composition was analyzed by a GC (PR2100, Perichrom) equipped with a flame ionization detector (FID) and a 50 m x 0.32 ID, 0.25 μ m film thickness DB-WAXetr (J & W Scientific) capillary column in the split mode (10 ml/min) with helium at a pressure of 82 kPa as a carrier gas, H₂ at 50 kPa as a combustion gas, and air zero at 50 kPa as a combustion-supporting gas. The column temperature program was started at 60 °C, heated to 125 °C at a ramping rate of

10 °C min⁻¹, held for 2 min, then heated to 180 °C at a ramping rate of 15 °C min⁻¹, and held for 15 min. The temperatures of injector and detector were 250 and 270 °C, respectively.

3.5.4 pH Analysis

pH value was determined by the pH electrode (Cole-palmer KH-27012-27).

3.5.5 Amount of Produced Gas

The volume of gas produced in the reactor was recorded daily using the water replacement method by a gas counter.

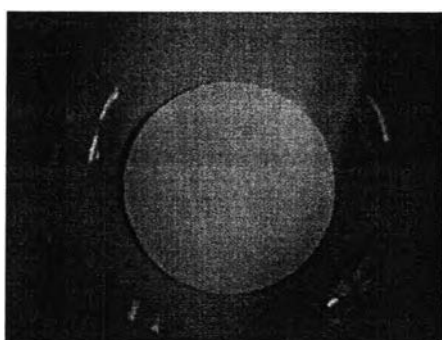
3.5.6 Gas Composition

The gas composition was determined by a GC (AutoSystem GC, PerkinElmer) equipped with a thermal conductivity detector (TCD) and a stainless-steel 10' x 1/8" x .085" HayeSep D 100/120 mesh (Alltech) packed column. Injector, column, and detector temperatures were kept at 60, 35, and 150 °C, respectively. Argon was used as the carrier gas at pressure of 345 kPa.

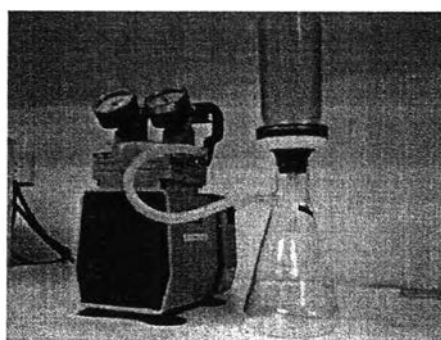
3.5.7 Total Suspended Solids (TSS) Analysis

3.5.7.1 *Procedure*

(1) Preparation of glass-fiber filter disk (Pall-61631 A/E, 47 mm, 1 µm):



(a)



(b)

Figure 3.2 (a) glass-fiber filter disk and (b) filtration apparatus.

- The glass-fiber filter disk with wrinkled side up was inserted in filtration apparatus, as shown in Figure 3.2(a) and (b), after that it was applied to vacuum and it was washed with three successive 20 cm³ of distilled water.

- The glass-fiber filter disk was dried in an oven at 105 °C for 1 h, left to be cooled in desiccator to balance temperature, and then weighed.

(2) Selection of filter and sample sizes:

- The sample volume was chosen to yield between 10 and 200 mg dried residue.

- If more than 10 min were required to complete filtration, either filter size was increased or sample volume was decreased.

(3) Sample analysis:

- The filtering apparatus and filter were prepared.

- The filter was wet with a small volume of distilled water to stick it to the apparatus.

- A sample was homogeneously mixed before test.

- A sample was pipetted onto the seated glass-fiber filter.

- The filter was washed with three successive 10 cm³ of distilled water, and suction was continued for about 3 min after complete filtration.

- The filter was carefully removed from filtration apparatus, dried at least 1 h at 103 to 105 °C in an oven, cooled in desiccator to balance temperature, and then weighed.

- The cycle was repeated until the weight of sample became nearly constant (less than 4% difference).

3.5.7.2 Calculation

$$\frac{\text{mg total suspend solids (TSS)}}{L} = \frac{(A - B) \times 10^6}{\text{Sample volume, (mL)}} \quad (3.2)$$

A = Weight of filter + dried residue [g]

B = Weight of filter [g]