CHAPTER III
EXPERIMENTAL

3.1 Materials

Seed sludge and alcohol wastewater was collected from Sapthip Lopburi Co., Ltd., Thailand.The alcohol wastewater had the chemical characteristics, as shown in Table 3.1. The alcohol wastewater was kept at 4°C prior to use.

Table 3.1 Chemical characteristics of the alcohol wastewater

Parameter	Concentration (mg/l)
Chemical oxygen demand (COD)	45,000
Total nitrogen	4,600
Phosphorus	600
Ammonia	46
Nitrate nitrogen	360
Nitrite nitrogen	1.6

3.2 Equipment

1. Upflow anaerobic sludge blanket (UASB) reactors
2. Gas chromatograph (GC), Perichrom, PR2100
3. Gas chromatograph (GC), Perkin-Elmer, AutoSystem GC
4. COD reactor, HACH
5. Spectrophotometer, HACH D/R 2000
6. Cathetometer (model TC–II) with digimatic height gauge (model 192–631)
7. 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
- Hydrochloric acid (HCl) 37%, analytical reagent grade, Lab-scan, Thailand
- Sodium hydroxide (NaOH), analytical reagent grade, Lab-scan, Thailand
- Phenolphthalein ($C_{20}H_{14}O_4$), analytical reagent grade, Labchem, Australia

3.4 Methodology

3.4.1 Seed Sludge Preparation

Seed sludge was obtained from the UASB reactor treating an alcohol wastewater of Sapthip Lopburi Co., Ltd., Thailand. In the hydrogen production step, it was boiled at 95 °C for 15 min to eliminate methane-producing bacteria before being introduced as a seed sludge into the UASB reactors (Sreethawong *et al.*, 2010). In the methane production step, it was introduced into the UASB reactor without such thermal pretreatment.

3.4.2 Substrate Preparation

3.4.2.1 Hydrogen Production Step

The ethanol wastewater was also obtained from Sapthip Lopburi Co., Ltd., Thailand. It had a chemical oxygen demand (COD) value about 45,000 mg/l with a ratio of COD: nitrogen: phosphorous of 100:10.2:1.3, indicating that the wastewater has sufficient nitrogen and phosphorous for anaerobic degradation (the theoretical ratio of COD: nitrogen: phosphorous is 100:2:0.4)

3.4.2.2 Methane Production Step

The effluent was obtained from hydrogen production step which operated under the optimum COD loading rate. It had a chemical oxygen demand (COD) value about 31,000 mg/l with a ratio of COD: nitrogen: phosphorous of 100:7.4:1.3, indicating that the wastewater has sufficient nitrogen and phosphorous for anaerobic degradation

3.4.3 UASB Operation

The upflow anaerobic sludge blanket reactors (UASB) were constructed from borosilicate glass with a 4 L working volume. The temperature and pH were controlled by a water jacket system with a circulating heating bath and a pH-controller, respectively. The schematic of the UASB unit is shown in Figure 3.1. The alcohol wastewater was fed into the reactor from a feed tank using a peristaltic pump. The feed was pumped into the bottom of the reactor in upward direction and passed through the microorganism sludge. A three-phase separator was used for preventing outflow of flocculants and separating the gaseous product and the overflown liquid effluent. The effluent was adjusted to pH 5.5 using a 5 wt. % NaOH solution and was recycled to the UASB at a recycle 1:1. The temperature of the UASB reactor was maintained at 37 °C by using the water jacket with the circulating water bath.

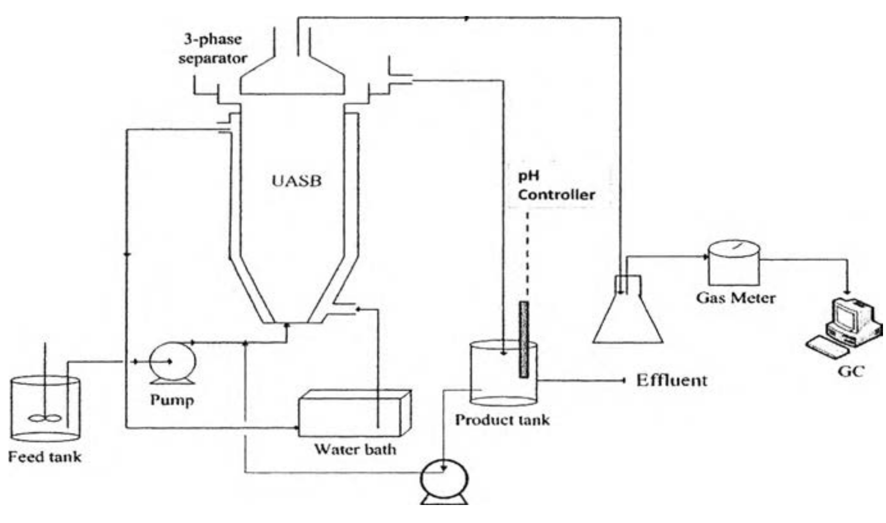

Figure 3.1 Flow diagram of UASB reactor.

3.4.3.1 Hydrogen Production Step

Alcohol wastewater was fed into the UASB reactor with an initial feed COD value of 45,000 mg/l at a controlled temperature of 37 °C. . The pH is controlled at 5.5 by a pH-controller and adjusted by NaOH 3 wt./vol.%. In this research, the COD loading rate is varied from 23 to 62 kg COD/m^3d, corresponding to the feed flow rate and hydraulic retention time (HRT) as shown in Table 3.2. The parameters, such as gas composition, gas production rate, hydrogen production rate, COD removal, specific hydrogen production rate and hydrogen yield, were determined from the averaged experimental data taken when the system reached steady state conditions, at which these parameters are almost invariant(with less than 5% standard deviation). After that, the COD removal, VFA composition, and MLVSS was determined.

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

COD loading rate (kg COD/m^3d)	Feed flow rate (l/d)	HRT (d)
23	2.07	1.93
31	2.76	1.45
46	4.14	0.96
62	5.52	0.72

3.4.3.2 Methane Production Step

In this step, the COD loading rate was varied from 4 to 12 kg/m^3d corresponding to the feed flow rate and hydraulic retention time (HRT) as shown in Table 3.3 without pH control at a temperature of 37 °C. The effluent of the hydrogen production step operated under the optimum COD loading rate was used as a feed solution. The reactor was also be operated until reaching steady state conditions, at which the produced gas composition and effluent COD become almost invariant before taking samples for analyses.

Table 3.3 Conditions for investigating the effect of COD loading rate on methane production step

COD loading rate (kg/m^3 d)	Feed Flow Rate (l/d)	HRT (d)
4.5	0.58	6.90
6.2	0.80	5.00
8.8	1.13	3.54
11.6	1.50	2.67

3.5 Analytical Methods

3.5.1 COD Analysis

The analytical of COD was followed the standards method. The sample is heated by the COD reactor (HACH) for 2 hours and left for 20 min. Then, the sample is determined for COD value by the spectrophotometer (HACH DR 2700).

3.5.2 Amount of VFA

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

3.5.3 Composition of VFA

VFA composition will be analyzed by a GC (PR2100, Perichrom) equipped with a flame ionization detector (FID) and a 50 m x 0.32 ID, 0.25 μm 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, H_2 at 50 kPa as a combustion gas, and air zero at 50 kPa as a combustion-supporting gas. The column temperature program is 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 are 250°C and 270 °C, respectively.

3.5.4 pH Analysis

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

3.5.5 Amount of Produced Gas

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

3.5.6 Gas Composition

The amount of gas composition was determined by a gas chromatograph (Auto System GC, Perkin-Elmer) 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 are kept at 60 °C, 35 °C, and 150 °C, respectively. Argon is used as the carrier gas at pressure of 345 kPa.

3.5.7 The Organic Content

The organic contents in the feed and the effluent samples of both UASB units were quantified by using the chemical oxygen demand method (COD).

3.5.8 The Microbial Concentration

The microbial concentration in the UASB bioreactor was measured by taking the whole sludge in the bioreactor at the end of operation for each COD loading rate. The sludge sample was filtered, and the filtered solids were dried at 110°C to obtain MLSS (mixed liquor suspended solids) and further burnt at 550°C to obtain MLVSS (mixed liquor volatile suspended solids) to represent the microbial concentration in the system. The analytical methods of SS and VSS were followed the standard methods