

## CHAPTER III EXPERIMENTAL

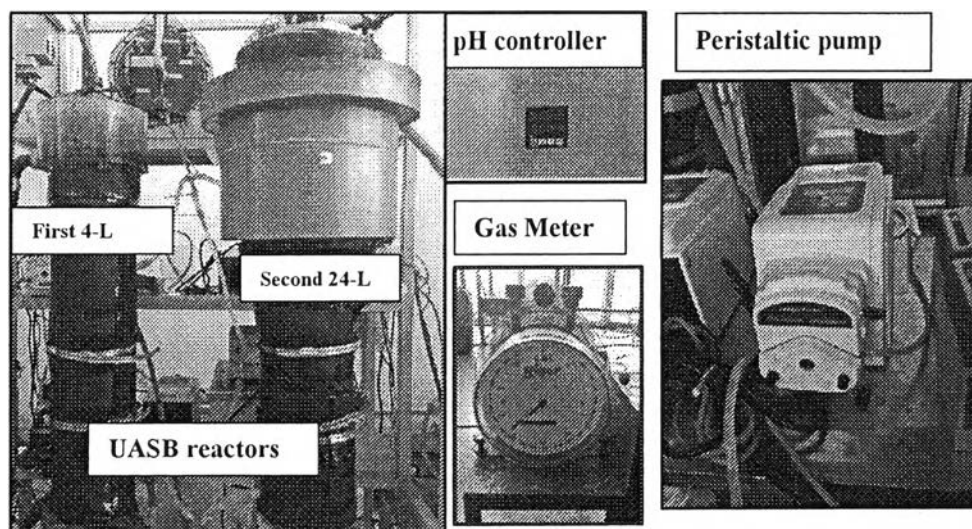
### 3.1 Materials

Seed sludge and alcohol wastewater were collected from Saphip Lopburi Co., Ltd., Thailand.

### 3.2 Equipment

#### 3.2.1 Equipment for setting two-stage Upflow anaerobic sludge blanket reactors

Upflow anaerobic sludge blanket reactors were constructed from borosilicate glass with a 4 and 24 L working volume. The alcohol wastewater was fed into reactors by Peristaltic pump (BT 100-2J Longer peristaltic pump). The temperature was kept constant at 37 °C by a water jacket system with a circulating heating bath. pH controller (Cole-palmer KH-27012-27) was used to control pH of first 4 L UASB. The produced gas was measured the gas production rate and gas composition by gas meter and gas chromatograph (GC) (Perkin-Elmer, AutoSystem GC), respectively. The UASB reactors used in this study are shown in Figure 3.1.



**Figure 3.1** Apparatus of UASB setup.

### 3.3 Chemicals

- Ammonium hydrogen carbonate ( $\text{NH}_4\text{HCO}_3$ ), analytical reagent grade, AJAX Finechem Pty Ltd, Australia
- Di-potassium hydrogen orthophosphate ( $\text{K}_2\text{HPO}_4$ ), analytical reagent grade, AJAX Finechem Pty Ltd, Australia
- Sulfuric acids ( $\text{H}_2\text{SO}_4$ ) 98%, analytical reagent grade, Lab-scan, Thailand
- Hydrochloric acid ( $\text{HCl}$ ) 37%, analytical reagent grade, Lab-scan, Thailand
- Sodium hydroxide ( $\text{NaOH}$ ), 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

A seed sludge sample collected from the anaerobic treatment plant of Saphip Co., Ltd located at Lopburi, Thailand were added into both UASB reactors without thermal pretreatment.

#### 3.4.2 Substrate Preparation

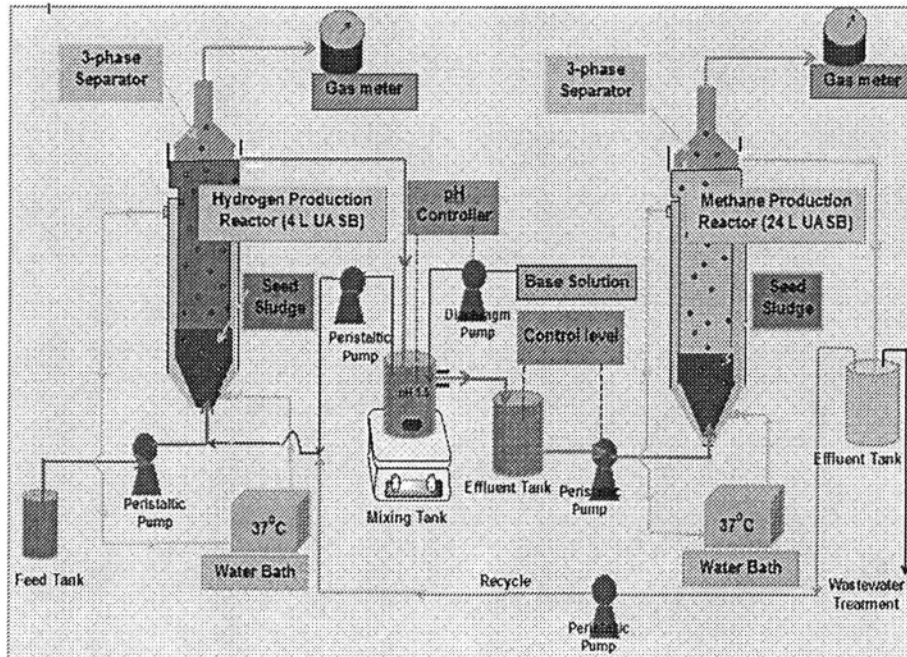
The ethanol wastewater was analyzed the chemical characteristic as shown in Table 3.1. The ratio of COD -total nitrogen-phosphorus achieved 100:3.1:2. This actual ratio was higher than the theoretical ratio which was 100:1:0.4. This ratio showed that the ethanol wastewater has sufficient nitrogen and phosphorous for anaerobic degradation. The alcohol wastewater was stored in a refrigeration prior to use

**Table 3.1** Chemical characteristics of the ethanol wastewater

Parameter	Concentration (mg/l)
Chemical oxygen demand (COD)	51,556
Total nitrogen	1,600
Phosphorus	1,040
Ammonia	88
Nitrate nitrogen	360
Nitrite nitrogen	2.1
COD:N:P	100:3.1:2

### 3.4.3 UASB Operation

The ethanol wastewater was fed into the bottom of first 4-L UASB for hydrogen production step which controlled pH at 5.5 by a pH-controller and adjusted by NaOH 3 wt./vol.%. The ethanol wastewater was flown up through microorganism sludge. The organic compound in feed was degraded by microorganism sludge and 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 liquid effluent from first UASB was pumped into the second 24-L UASB for methane production step in upward direction and passed through microorganism sludge to produce the gaseous products. The liquid effluent from this UASB was sent back to first UASB as a recycle 1:1 (Feed: Effluent from methane-producing stage). Both UASB was operated in mesophilic condition (37 °C). The schematic of the UASB unit is shown in Figure 3.2.



**Figure 3.2** Flow diagram of two-stage UASB reactor.

#### 3.4.3.1 Hydrogen Production Step

For hydrogen production step, an alcohol wastewater is fed into the first UASB reactor with an initial feed COD of 51,556 mg/l operated at different COD loading rates, pH 5.5 by a pH-controller and adjusted by NaOH 5 wt./vol.% and a recycle ratio of feed-to-effluent from methane-producing stage of 1:1. In this study, the COD loading rate is varied from 5 to 270 kg COD/m<sup>3</sup>d, corresponding to the feed flow rate and hydraulic retention time (HRT) as shown in Table 3.2.

#### 3.4.3.2 Methane Production Step

For methane production step, the liquid effluent from hydrogen producing stage was used as a feed for this step.

For each condition, Both UASBs were operated until reaching steady state conditions, at which the produced gas composition and effluent COD become almost invariant (with less than 5% standard deviation) before the samples was taken to analysis other parameters such as such as gas composition, gas production rate, hydrogen production rate, COD removal, specific hydrogen production rate and hydrogen yield.

**Table 3.2** COD loading rate, flow rate for determining the effect of COD loading rate on two-stage UASB system

COD loading rate (kg COD/m <sup>3</sup> d)	Feed flow rate (l/d)
5	0.47
10	0.93
20	1.86
48	3.72
60	4.66
120	7.60
180	11.40
270	17.11

### 3.5 Analytical Methods

#### 3.5.1 COD Analysis

COD value of sample was determined by the spectrophotometer (HACH DR 2700). Before the sample was placed into the spectrophotometer for reading a value, it was heated by the COD reactor (HACH) for 2 h and left for 20 min followed the standard method.

#### 3.5.2 Amount of volatile fatty acid (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).

#### 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  $\mu$ 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, H<sub>2</sub> 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<sup>-1</sup>, held for 2 min, then heated to 180 °C at a ramping rate of 15 °C min<sup>-1</sup>, and held for 15 min. The temperatures of injector and detector were 250°C and 270 °C, respectively.

### **3.5.4 Amount of Produced Gas**

The volume of gas produced in the reactor was recorded by gas meter.

### **3.5.5 Gas Composition**

The gas composition was determined by a GC (AutoSystem 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 were kept at 60 °C, 35 °C, and 150 °C, respectively. Argon was used as the carrier gas at pressure of 345 kPa.

### **3.5.6 The Microbial Concentration**

The microbial concentration, which was the parameter indicating the degradation of organic compounds in reactor presented in them of mixed liquor suspended solids or MLSS and MLVSS (mixed liquor volatile suspended solids) (Apha, 1995). The sludge sample was collected from the reactors, filtrated through a glass fiber filter, and dried in an oven at 110°C for 30 minute. The dried weight obtained MLSS. For the value of MLVSS, the sludge sample was dried at 110 °C was further bunt at 550 °C.