

## CHAPTER III METHODOLOGY

### 3.1 Materials

The seed sludge and ethanol wastewater were obtained from at Saphip, Lopburi Co., Ltd., Thailand. The seed sludge was dark, pH of 4.0 – 5.0, total solids (TS) concentration of 13,300 mg/l and the mixed liquor volatile suspended solid (MLVSS) of 9,100 mg/l. The substrate was brown, pH of 3.0 – 4.0 and COD of 59,000 mg/l. The characteristics of seed sludge and ethanol wastewater used in this study were shown in Table 3.1 and 3.2, respectively. The seed sludge and ethanol wastewater were kept at 4 °C before use.

### 3.2 Chemicals

#### 3.2.1 pH Control System

- Sodium hydroxide (NaOH), AR grade, Ajax, Thailand

#### 3.2.2 COD Analysis

- Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) 98 %, AR grade, RCI Labscan, Thailand
- Silver sulphate (Ag<sub>2</sub>SO<sub>4</sub>), AR grade, Sigma-Aldrich, Thailand
- Mercuric sulphate (HgSO<sub>4</sub>), AR grade, Sigma-Aldrich, Thailand
- Sodium thiosulfate pentahydrate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O), AR grade, RCI Labscan, Thailand

#### 3.2.3 Total VFA Analysis

- Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) 98 %, AR grade, RCI Labscan, Thailand

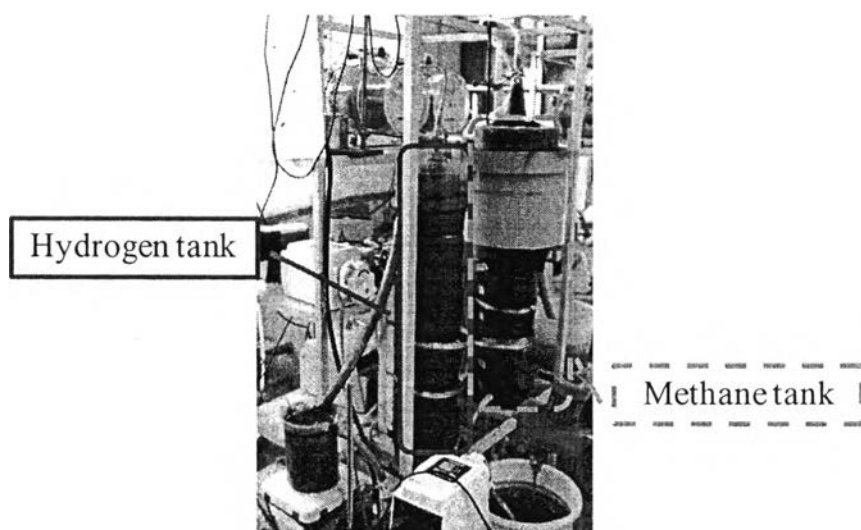
#### 3.2.4 Gas Measuring Method

- Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) 98 %, AR grade, RCI Labscan, Thailand

### 3.3 Equipment

#### 3.3.1 A Two-stage UASB System

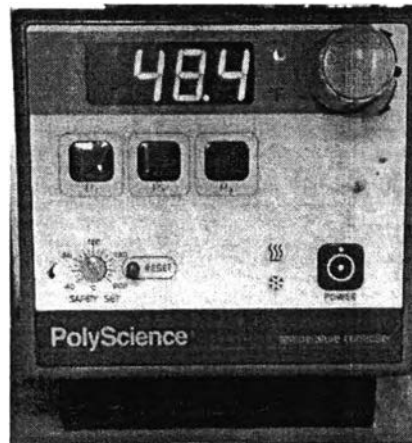
The two-stage UASB system consisted of 4 and 24 l working volume of hydrogen and methane production tank, respectively. Both tanks were made from borosilicate glass and covered by a black sheet to prevent the light. The two-stage UASB unit in this stage was shown in Figure 3.1.



**Figure 3.1** A two-stage UASB system used in this study.

#### 3.3.2 Temperature-controlling System

From Figure 3.2, the temperature-controlling system including a heating bath, thermocouple and temperature controller was used to control the system temperature by circulating hot water through heat jacket of outside UASB tanks. The system temperature was adjusted to be 37 °C.



**Figure 3.2** Temperature controller used in this study.

### 3.3.3 pH-controlling Systems

From Figure 3.3, the pH-controlling system consists of a pH controller (Extech model 48PH2), a pH electrode (Cole-Parmer Double-Junction Electrode), a diaphragm pump and a magnetic stirrer for mixing. The pH of the system was adjusted automatically by feeding a 1 M NaOH solution via the diaphragm pump to the effluent of methane tank which some of effluent was recycled to hydrogen UASB unit with equal flow rate to feed flow rate.



**Figure 3.3** pH sensor at the effluent of methane tank (a) methane effluent tank (b) pH phobe used in this study.

**Table 3.1** Characteristics of the seed sludge in this study

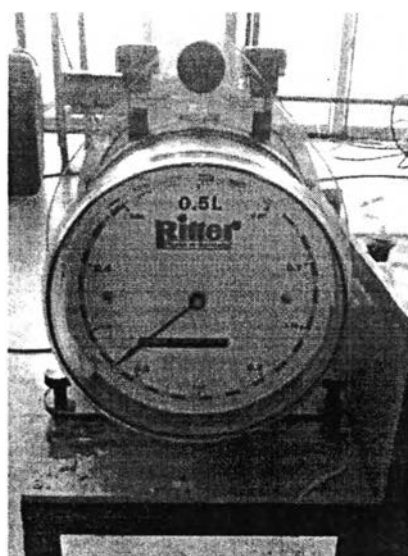
Parameters	Unit	Value
Color	-	Dark
TSS (Total suspended solids)	mg/l	13,000
TVS (Total volatile solids)	mg/l	9,100

**Table 3.2** Characteristics of the ethanol wastewater in this study

Parameters	Unit	Value
Total COD (Total chemical oxygen demand)	mg/l	60,500
Settled COD (Settled chemical oxygen demand)	mg/l	59,000
Total nitrogen	mg/l	590
Total phosphorous	mg/l	354
COD:N:P	-	100:1.5:1.6
Ammonium	mg/l	25.00
Nitrate	mg/l	108.67
Nitrite	mg/l	15.07
pH	-	4.50
Total VFA (Total volatile fatty acid)	mg/l	870
VS (Volatile solids)	mg/l	1,500
Ethanol concentration	mg/l	2,720

### 3.3.4 Gas-measuring System

This system composed of 2 flasks of water trap and a wet gas meter (Ritter, TGO5/5). The flask was filled with 1 M  $H_2SO_4$  in order to prevent dissolution of the produced  $CO_2$  gas (Ueno et al., 1996). The gas-measuring system was shown in Figure 3.4.



**Figure 3.4** Wet gas meter used in this study.

## 3.4 Methodology

### 3.4.1 Seed Sludge Preparation

The seed sludge was settled to concentrate the sludge and screened to remove inorganic particles, such as sand and grit before adding to the UASB units.

### 3.4.2 Substrate Preparation

The feed substrate contained high concentration of soluble organic compounds categorized as carbohydrate-rich wastewater (Gerardi, 2006). The chemical oxygen demand: nitrogen: phosphorus ratio (COD:N:P) of fresh ethanol wastewater was 100:1.5:1.6 which is higher than a minimum COD:N:P ratio of

100:0.5:0.1 for microbes under mesophilic condition to confirm the sufficient nutrient amount (Argun et al., 2008).

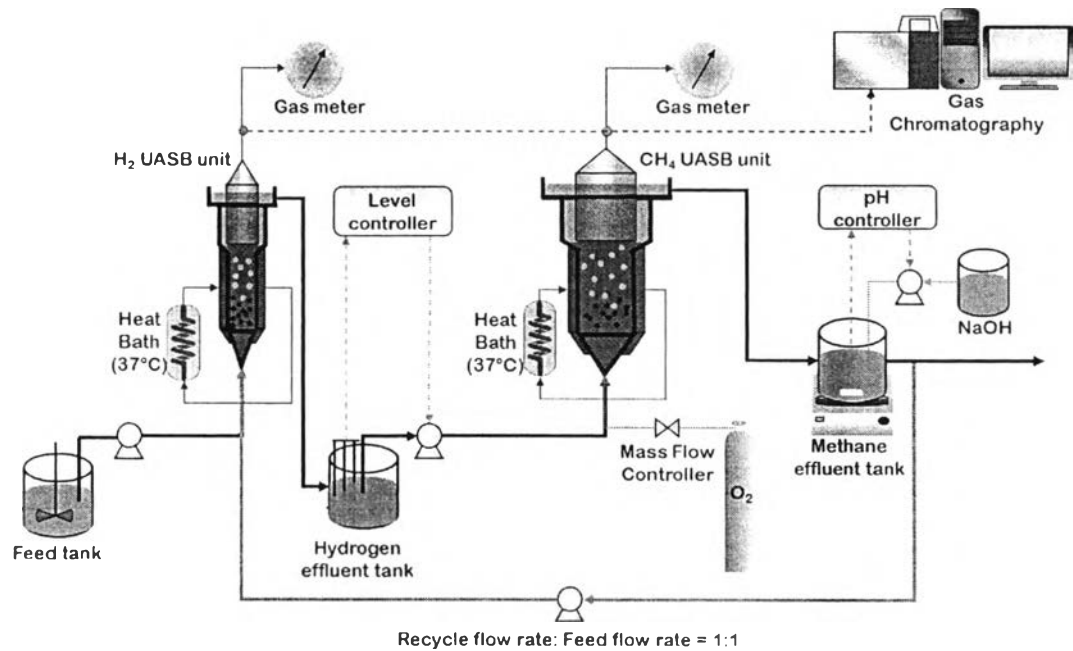
### 3.4.3 The Two-Stage UASB System

The Dark fermentation in this research was separately produced in a two-stage UASB system consisted of 4 and 24 l working volume of hydrogen and methane UASB unit, respectively. The working load determined in term of COD loading rate shown in Equation 3.1.

$$\text{COD loading rate (kg/m}^3 \text{ d)} = \frac{(\text{Feed COD}) \times (\text{Feed flow rate})}{(\text{Working volume})} \quad (3.1)$$

The feed substrate was transported to bottom of hydrogen UASB unit by peristaltic pump, then the effluent of hydrogen UASB unit was directly pumped to methane UASB unit. The schematic of a two-stage of UASB system was shown in Figure. 3.5.

The pH of hydrogen UASB unit was adjusted to 5.5, when the pH of methane UASB unit was not controlled. The temperature system was maintained at 37 °C.



**Figure 3.5** The schematics of a two-stage UASB system used in this study.

There are two scopes in this research. Firstly, to determine the optimal COD loading rate of a separate hydrogen and methane production from ethanol wastewater, the different COD loading rate was defined as 2, 4, 6, 8 and 10  $\text{kg}/\text{m}^3 \cdot \text{d}$  based on methane UASB volume and 12, 24, 36, 48 and 60  $\text{kg}/\text{m}^3 \cdot \text{d}$  based on hydrogen UASB volume, shown in Table 3.3. The second scope was to find the optimum microaeration condition which defined by an oxygen supply load. The system was operated at fixed COD loading rate of 6  $\text{kg}/\text{m}^3 \cdot \text{d}$  based on methane UASB volume and added a different oxygen supply load of 3, 4, 4.5 and 6  $\text{ml O}_2/\text{LR} \cdot \text{d}$ , shown in Table 3.4.

**Table 3.3** The relationship between COD loading rate and feed flow rate for a two-stage UASB system

COD loading rate (kg/m <sup>3</sup> d) Based On CH <sub>4</sub> UASB Unit	COD Loading Rate (kg/m <sup>3</sup> d) Based On H <sub>2</sub> UASB Unit	Feed Flow Rate (ml/min)
2.0	12.0	0.56
4.0	24.0	1.13
6.0	36.0	1.69
8.0	48.0	2.26
10.0	60.0	2.82

**Table 3.4** The oxygen flow rate according to the oxygen supply load

Oxygen Supply Load (ml O <sub>2</sub> /L <sub>R</sub> d)	Oxygen Flow Rate (ml O <sub>2</sub> /min)
3.0	0.0500
4.0	0.0667
4.5	0.0750
6.0	0.1000



### 3.5 Measurement and Analytical Method

The system was operated until reaching a steady state condition indicated by the constant gas composition, gas production rate and COD removal. The effluent and inoculum sample was taken to analyze by the standard methods listed below.

#### 3.5.1 COD Analysis

COD value was determined by the closed reflux, colorimetric method. Sample was added to a digestion vial (HACH, 16×100 mm). The digestion reagent was added to the vial. Afterwards, the sulfuric acid reagent was slowly dropped into the vial. The vial was inverted several times to homogeneously mix the contents and the vial was placed in the COD reactor (HACH) that sample was heated for 2 hrs and left for 20 min to be cooled. Then, the sample was determined for COD value by spectrophotometer (HACH DR 2700).

#### 3.5.2 Nitrogen Analysis

Nitrogen value was determined in term of organic nitrogen by the diazotization and cadmium reduction method and in term of inorganic nitrogen by the salicylate method. Sample was carried out with the TNT persulfate digestion. The sample cell was placed into the spectrophotometer (HACH DR 2700) for determining nitrogen value.

#### 3.5.3 Phosphorous Analysis

Phosphorous value was determined by the molybdovanadate method with acid persulfate digestion. The sample cell was placed into the spectrophotometer (HACH DR 2700) for determining phosphorous value.

#### 3.5.4 Total VFA and VFA Composition Analysis

The liquid effluent was analyzed by a high performance liquid chromatography (HPLC) (Shimadzu Prominence UFLC), equipped with a reflective index detector and a 300 x 7.8 mm, carbohydrate analysis column (APX-87H,

Aminex), using 0.5 ml/min of 4 mM H<sub>2</sub>SO<sub>4</sub> as a mobile phase. The column temperature program was preserved at 45 °C with retention time of 60 minute.

### 3.5.5 pH Analysis

The pH value was determined by a pH electrode (ECFG7350401B).

### 3.5.6 Amount of Produced Gas

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

### 3.5.7 Gas Composition Analysis

The gas composition of the produced gas was determined by the 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. The 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.

### 3.5.8 TSS Analysis

#### 3.5.8.1 *Procedure*

##### 3.5.8.1.1 *Preparation of Glass-Fiber Filter Disk (Pall-61631 A/E, 47 mm, 1 μm)*

- The glass-fiber filter disk with wrinkled side up was inserted in the filtration apparatus. After that, it was to vacuumed and washed with three successive 20 cm<sup>3</sup> of distilled water.

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

##### 3.5.8.1.2 *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 was required to complete the filtration, either the filter size was increased or the sample volume was decreased.

#### 3.5.8.1.3 Sample Analysis

- The filtering apparatus and filter were prepared.  
- The filter was wet with a small volume of distilled water.

- A sample was homogeneously mixed before testing.

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

- The filter was washed with three successive 10 cm<sup>3</sup> of distilled water and suction was continued for about 3 min after complete filtration.

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

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

- The TSS is calculated by Equation (3.2)

#### 3.5.8.1.4 Calculation

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

Where A = Weight of filter + dried residue (g)

B = Weight of filter (g)

### 3.5.9 VSS Analysis

#### 3.5.9.1 Procedure

- The residue produced by TSS method was ignited in a furnace at a temperature of 500 ± 50 °C.

- A furnace was heated up to 500 °C for 1 h after inserting sample.

- The filter disk was left to partially cool in air until most of the heat was dissipated.

- The disk was transferred to desiccator and weighed as soon as it was cooled to balance temperature.

- the TSS is calculated by Equation (3.3)

### 3.5.9.2 Calculation

$$\frac{\text{mg volatile suspended solids (VSS)}}{L} = \frac{(A - B) \times 10^6}{\text{Sample volume, mL}} \quad (3.3)$$

Where A = Weight of residue + disk before ignition (g)

B = Weight of residue + disk after ignition (g)

## 3.6 Parameters

The biogas production performance of hydrogen and methane is listed below.

### 3.6.1 COD removal

The COD removal relation is shown in Equation (3.4)

$$\text{COD removal (\%)} = \frac{\text{Effluent COD} - \text{Feed COD}}{\text{Feed COD}} \times 100 \quad (3.4)$$

### 3.6.2 Hydrogen Yield (ml H<sub>2</sub>/g COD removed) or Methane Yield (ml CH<sub>4</sub>/g COD removed)

The hydrogen yield in term of ml H<sub>2</sub>/g COD removed and methane yield in term of ml CH<sub>4</sub>/g COD removed are shown in Equations (3.5-3.6)

$$\text{Hydrogen yield } \left( \frac{\text{ml H}_2}{\text{g COD removed}} \right) = \frac{\text{Hydrogen production rate}}{\text{Feed flow rate} \times \text{COD removed}} \quad (3.5)$$

$$\text{Methane yield } \left( \frac{\text{ml CH}_4}{\text{g COD removed}} \right) = \frac{\text{Methane production rate}}{\text{Feed flow rate} \times \text{COD removed}} \quad (3.6)$$

### 3.6.3 Hydrogen Yield (ml H<sub>2</sub>/g COD applied) or Methane Yield (ml CH<sub>4</sub>/g COD applied)

The hydrogen yield in term of ml H<sub>2</sub>/g COD applied and methane yield in term of ml CH<sub>4</sub>/g COD applied are shown in Equations (3.7-3.8)

$$\text{Hydrogen yield } \left( \frac{\text{ml H}_2}{\text{g COD applied}} \right) = \frac{\text{Hydrogen production rate}}{\text{Feed flow rate} \times \text{COD applied}} \quad (3.7)$$

$$\text{Methane yield } \left( \frac{\text{ml CH}_4}{\text{g COD applied}} \right) = \frac{\text{Methane production rate}}{\text{Feed flow rate} \times \text{COD applied}} \quad (3.8)$$

### 3.6.4 Specific Hydrogen Production Rate (SHPR, ml H<sub>2</sub>/L<sub>R</sub> d) or Specific Methane Production Rate (SMPR, ml CH<sub>4</sub>/L<sub>R</sub> d)

The SHPR in term of ml H<sub>2</sub>/L<sub>R</sub> d and SMPR in term of ml CH<sub>4</sub>/L<sub>R</sub> d are shown in Equations (3.9-3.10)

$$\text{SHPR } \left( \frac{\text{ml H}_2}{\text{L}_R \text{ d}} \right) = \frac{\text{Hydrogen production rate}}{\text{Reactor Volume}} \quad (3.9)$$

$$\text{SMPR } \left( \frac{\text{ml CH}_4}{\text{L}_R \text{ d}} \right) = \frac{\text{Methane production rate}}{\text{Reactor Volume}} \quad (3.10)$$

3.6.5 Specific Hydrogen Production Rate (SHPR, ml H<sub>2</sub>/g MLVSS d) or Specific Methane Production Rate (SMPR, ml CH<sub>4</sub>/g MLVSS d)

The SHPR in term of ml H<sub>2</sub>/g MLVSS d and SMPR in term of ml CH<sub>4</sub>/g MLVSS d are shown in Equations (3.11-3.12)

$$\text{SHPR} \left( \frac{\text{ml H}_2}{\text{g MLVSS d}} \right) = \frac{\text{Hydrogen production rate}}{\text{g MLVSS} \times \text{Reactor Volume}} \quad (3.11)$$

$$\text{SMPR} \left( \frac{\text{ml CH}_4}{\text{g MLVSS d}} \right) = \frac{\text{Methane production rate}}{\text{g MLVSS} \times \text{Reactor Volume}} \quad (3.12)$$