CHAPTER III
EXPERIMENTAL

Materials and Equipment

3.1 Materials

The two sample seed sludge taken from the anaerobic pond of a palm oil plant of Suksomboon palm oil at Chonburi and the activated sludge of biodiesel plant of Bangchak Biofuel at Ayudtaya. These seed sludge were mixed and screened in order to remove large sand or other impurities. The mixed seed sludge had dark-brown color and total suspended solid (TSS) concentration of 11,000 mg/l. The biodiesel wastewater was obtained from Bangchak Biofuel public Co., Ltd., Ayudtaya and a chemical oxygen demand was 2,300 mg/l as show in Table 3.1. Therefore, biodiesel wastewater was added glycerine 3.5% w/v in order to obtain the chemical oxygen demand (COD) 45,000 mg/l. However, a ratio of COD: N: P was 100:0.44: 0.044 which was not sufficient for running in anaerobic fermentation. Hence, additional nutrients were required in this biodiesel wastewater and it was kept 4 °C prior to use as show in table 3.2.

Table 3.1 Chemical characteristics of the biodiesel wastewater

Parameter	Concentration (mg/l)
Chemical oxygen demand (COD)	2,300
Total suspended solids (TSS)	11,000
Total nitrogen	400
Total phosphorus	146.6
Glycerine	4,580

Table 3.2 Chemical characteristics of the biodiesel wastewater with added 3.5% w/v glycerine

Parameter	Concentration (mg/l)
Chemical oxygen demand (COD)	45,000
Total suspended solids (TSS)	11,000
Total nitrogen	1,200
Total phosphorus	300
Glycerine	27,700

3.2 Equipment

- Anaerobic Sequencing Batch Reactor (ASBR) in working volume of hydrogen 4 L and methane 24 L
- Time-controlling System: Timer(OMRON model H5CX-A)
- Temperature-controlling System

  This system comprise of a heater rot, thermocouple, and control box
- pH-controlling and mixing Systems

They consist of a pH controller (Extech model 48PH2), a pH electrode (Cole-Parmer Double-Junction Electrode), a diaphragm pump, and a magnetic stirrer (40×20 mm, egg shape) for mixing.

- Gas-measuring system

They composed of 2 flasks with a 1 M HCl solution, in order to prevent dissolution of the produced CO_2 gas (Ueno *et al.*, 1996), and a wet gas meter (Ritter, TGO5/5) was used to measure the volume of produced gas at room temperature.

- Gas chromatograph (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.
- High performance liquid chromatography (HPLC, Aminex HPX-87H column), with ultraviolet (UV,210 nm) and refractive index detectors.

- COD rector (HACH)
- Spectrophotometer (HACH DR 2700)
- A glass-fiber filter disk (Pall-61631 A/E, 47 mm, 1 μm)
- Filtration apparatus

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
- Digestion solution

Distilled water mixed with 10.216 g $K_2Cr_2O_7$ (primary standard grade) previously dried at 103 °C for 2 h, 167 ml 98% H_2SO_4, and 33.3 g $HgSO_4$. The mixture was left for complete dissolution, cooled to room temperature, and finally diluted to 1 L.

- Sodium hydroxide (NaOH), analytical reagent grade, Lab-scan, Thailand
- Phenolphthalein ($C_{20}H_{14}O_4$), analytical reagent grade, Lab-scan, Australia
- 80% of crude glycerol. Bangchak Biofuel Co., Ltd., Ayudtaya, Thailand

3.4 Experimental Procedures

3.4.1 Mixed Seed Sludge Preparation

Mixed seed sludge was prepared by sedimentation and filtered through the sieve in the size of 1 mm.

3.4.2 Biodiesel Wastewater Preparations

Biodiesel wastewater was filtered through sieve size 0.2 μm to remove debris and was added 3.5% w/v of glycerine.

3.4.3 ASBR Operation and Bioreactor Design

There are two identical ASBR reactor for hydrogen production at working volume 4 L and methane production at working volume 24 L. They operated under mesophilic temperature of 37 °C and have four sequencing steps: feed, react, settle, and decant. The mixing was achieved by using magnetic stirrer at 400 rpm (Chen and Chen, 2009). pH-controller and heater were used to maintain the pH and temperature in both bioreactors. Moreover, the timer controlled time in each step as show on Figure 3.1. The short hydraulic retention time (HRT) was operated in order to prevent hydrogen consumption by methanogenesis process (Hawkes *et al.*, 2002).

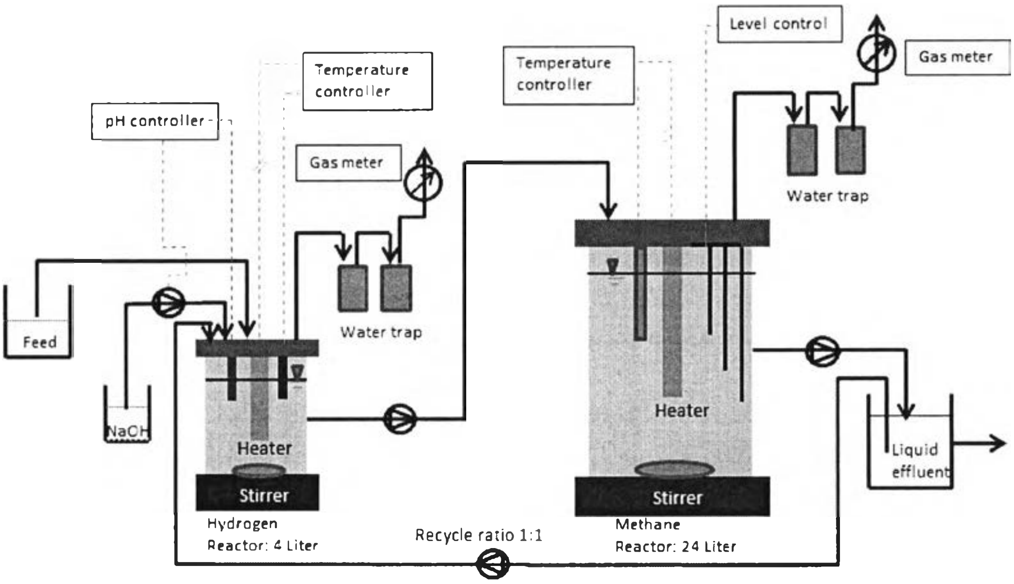


Figure 3.1 Schematic of two-stage anaerobic sequencing batch reactor (ASBR)

The experiment was carried out in two ASBR unit for the hydrogen production (4 L) and methane production (24 L) at a fixed HRT of 24 h and based on cycle duration of 4 h corresponding to 6 cycles per day. The operation times of ASBR operation are shown in Table 3.3.

Table 3.3 Operation conditions for the ASBR system

Operating Parameter		**6 cycles/day**
HRT (h)		24
Cycle time (min)	Feed	15
	React	90
	Settle	120
	Decant	15
	Total	240

Biodiesel wastewater was fed at an initial organic loading rate of 33.75 kg COD/m^{3}d based on hydrogen volume or initial organic loading rate of 5.63 kg COD/m^{3}d based on methane volume. The reactor was run around 3-4 weeks to reach the steady state and then the organic loading rate was changed in order to study the effect of organic loading rate on the hydrogen and methane production. Steady state conditions were reached when the variation in the production of produced gas was constant (less than 15 % variation).

Hydrogen Production Step

Biodiesel wastewater with an initial feed COD of 45,000 mg/l and liquid effluent from methane ASBR unit were fed into the hydrogen ASBR unit at a ratio of 1:1 and controlled temperature of 37 °C. The pH is controlled at 5.5 by a pH-controller and adjusted by NaOH 1 M. In this research, the COD loading rate was varied at 33.75, 50.63, 67.50 and 84.38 kg COD/m^{3}d based on hydrogen volume. The feed and decant flow rates were varied at a constant feed COD of 45,000 mg/L with 6 cycles per day. Therefore, the COD loading rate was varied according to the Equation (3.1)

$$\text{COD loading rate, kg/m}^3\text{ d} = \frac{\text{Feed COD, kg/m}^3 \times \text{Feed Flow Rate, m}^3\text{/d}}{\text{Working Volume, m}^3} \qquad (3.1)$$

Methane Production Step

The effluent wastewater from hydrogen ASBR unit will be used for methane production. In this step, the COD loading rate will be varied at 5.63, 8.44, 11.25 and 14.06 kg COD/m^3d based on methane volume. The methane ASBR unit operated without control pH at a temperature of 37 °C.

Both reactors were also operated until reaching the steady state condition, at which the produced gas composition and effluent COD become almost invariant. The parameters, such as effluent COD, pH, gas production rate, and gas composition are analyzed until the system reaches the steady state, these parameters are almost invariant (with less than 5 % standard deviation). After that, the COD removal, VFA composition, and MLVSS were determined.

3.4.4 Inorganic and Organic Analytical Methods

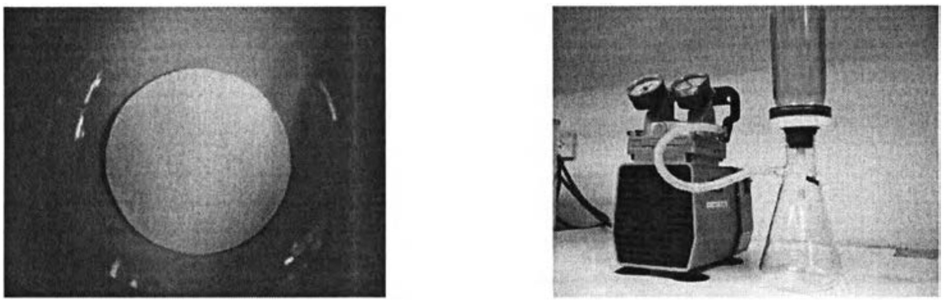
The steady-state condition of each experimental run was achieved when the properties of liquid product and produced gas, such as percentage of hydrogen, COD, and VFA concentration, were nearly constant (less than 15 % variations).

3.4.4.1 Total Suspended Solids (TSS) Analysis

TSS analyzed by using a glass-fiber filter disk (Pall-61631 A/E, 47 mm, 1 μm).The calculation of TSS is shown in the following equation:

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

A = Weight of filter + dried residue [g]
B = Weight of filter [g]



(a) (b)

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

3.4.4.2 Volatile Suspended Solids (VSS) Analysis

The residue product from TSS method was burned in the furnace at temperature of 500 ± 50 °C for 1 hour. The calculation of VSS is shown in the following equation.

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

A = Weight of residue + disk before ignition [g]

B = Weight of residue + disk after ignition [g]

3.4.4.3 COD Analysis (Closed Reflux, Colorimetric Method)

The sample was heated in COD reactor (HACH) and read the COD value by spectrophotometer (HACH DR 2700). (Chatsiriwatana, 2009) reported that the system operated at 6 cycles per day showed higher process performance in terms of hydrogen production rate; thus; 6 cycle per day was selected in this study.

3.4.4.4 Total VFA Analysis

The amount of VFA is determined by distillation-titration method. This technique recovers acids containing up to six carbon atoms and reports the results in terms of acetic acid (Eaton *et al.*, 1992).

3.4.4.5 VFA Composition Analysis

VFA determined by high performance liquid chromatography (HPLC, Aminex HPX-87H column), a 4 mM H_2SO_4 mobile phase (0.5 ml/min, 45 °C) with ultraviolet (UV, 210 nm) and refractive index detectors.

3.4.4.6 Phosphorous Analysis

The sample was determined by the molybdovanadate method with acid persulfate digestion (Hach Company) and placed into the spectrophotometer (HACH DR 2700).

3.4.4.7 Nitrogen Analysis

Both influent and effluent samples were determined by TNT persulfate digestion and placed into the spectrophotometer (HACH DR 2700).

3.4.4.8 Gas Composition Analysis

Produced gas is determined by a gas chromatograph (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) The temperatures of column, injector, and detector were kept at 35, 60, and 150°C and argon gas was used as the carrier gas.

3.4.4.9 Glycerine Analysis

Glycerine was measured by high performance liquid chromatography (HPLC, Aminex HPX-87H column), a 4 mM H_2SO_4 mobile phase with ultraviolet (UV,210 nm) and refractive index detectors.

3.4.4.10 Microbial Concentration (MLVSS)

This parameter was measured at steady state. Collecting the whole liquid and solid are drained out from reactor under steady state, washing out with distilled water and dried in an oven at 105 °C for 1 h. The dried residue sample is determined for MLSS. The MLVSS is the difference between the dried residue sample at 105°C and the dried residue sample at 550 °C for 1 h.

3.4.4.11 Microbial Washout (Effluent VSS)

This parameter was measured at steady state. The effluent of liquid and solid after steady state were collected and then dried in oven at 105 °C for 1 h. The effluent VSS is the difference between the dried residue sample at 105°C and the dried residue sample at 550 °C (for 1 h.).