CHAPTER III
METHODOLOGY

3.1 Materials and Equipment

Equipment:

1. Anaerobic sequencing batch reactor (ASBR)
2. Gas chromatograph, Perkin-Elmer, AutoSystem GC
3. Gas chromatograph, Perichrom, PR2100
4. Shimadzu UV-VIS spectrometer 2550, Barawindsor Co., Ltd.
5. Total organic carbon analyzer (TOC), Shimadzu TOC-5000A
6. COD reactor, HACH
7. Spectrophotometer, HACH D/R 2000

Materials:

1. Seed sludge and alcohol distillery wastewater

Seed sludge and alcohol distillery wastewater was collected from the wastewater treatment plant of Red Bull Distillery (1988) Co., Ltd. Part., Samuthsakorn, Thailand. The pH, total suspended solids (TSS), and chemical oxygen demand (COD) of the wastewater was measured.

2. Supplementary nutrient for bacterial growth
   - 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

Apparatus of ASBR:

1. Time-controlling system

The timers were used to control the time of each operation steps: (1) feed, (2) react, (3) settle, and (4) decant.

2. Temperature-controlling system

This system was used to control the system temperature. The system temperature was adjusted to be around 55°C in the thermophilic condition. This system was used during the reaction period.

3. pH-controlling and mixing system

This system was used to investigate the effect of pH on biohydrogen production. The pH of the mixed solution was controlled automatically by feeding NaOH (1 M) and H_2SO_4 (1 M) solutions via diaphragm pumps. The liquid was homogeneously mixed using magnetic stirrer at 400 rpm.

4. Gas-measuring system

This system consisted of two flasks filled with 1 M of HCl solution in order to prevent dissolution of the gas (Ueno *et al.*, 1996) and a wet gas meter that was used to measure the volume of produced gas at room temperature.

3.2 Experimental Procedures

3.2.1 Feed Preparation

Alcohol distillery wastewater was diluted with water to reduce chemical oxygen demand (COD) to 40,000 mg/l to decrease the effect of toxicity on hydrogen-producing bacteria.

3.2.2 Bioreactor Design and Operation

Two ASBR reactors were used in order to perform the biohydrogen production experiments. To inhibit the activity of photosynthetic bacteria, the system was operated without light illumination in 5 L PVC reactors. Each of them has an

inner diameter of 13 cm and a height of 30 cm. The reactors were operated with working volume of 4 L. The schematic of the ASBR process is shown in Figure 3.1.

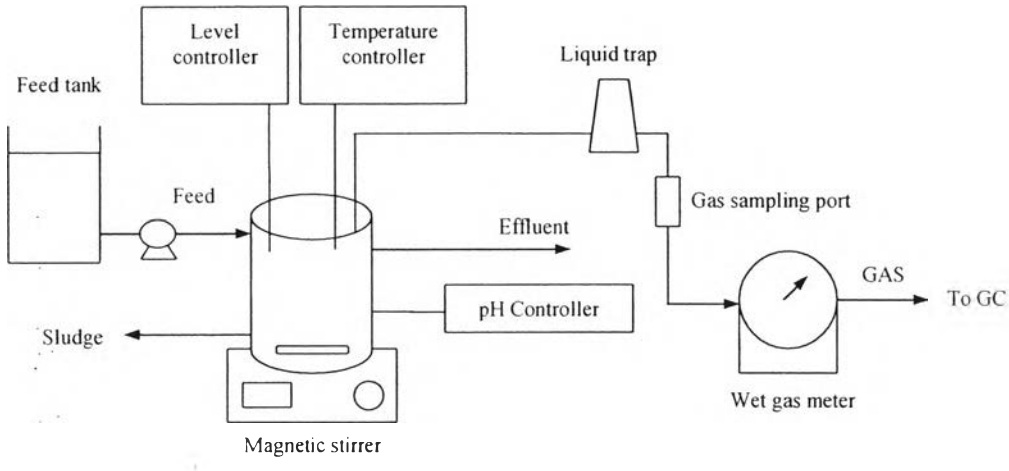


Figure 3.1 Schematic of the studied ASBR process.

ASBR operation composes of four steps: feed, react, settle, and decant. The operating conditions for the ASBR and time for each step are shown in Table 3.1. In the operation, time for each step was controlled by timers, which allowed the feed pump to pump the wastewater during the feed period. The short hydraulic retention time (HRT) was operated in order to prevent hydrogen consumption by methanogenesis process (Hawkes *et al.*, 2002). Furthermore, the system temperature and pH were controlled by using a heater and pH-controlling system, respectively. Recirculation pumps were used for mixing purpose during the reaction period.

Four liters of wastewater were fed at an initial organic loading rate of 20 kg COD/m^3d. The reactor was run around 3-4 weeks to reach the steady state then the organic loading rate was changed in order to study the effect of organic loading rate on the hydrogen production. Steady state conditions were reached when the variation in the production of produced gas was constant (less than 15 % variation).

3.2.2.1 Effects of Organic Loading Rate

The experiment was carried out in two anaerobic sequencing batch reactors for the biohydrogen production 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 3.2.

Table 3.1 Operating conditions for the ASBR

Operating parameter	**Value**
HRT (h)	24
Influent volume (L/cycle)	1
Cycle time (min)	
Feed	20
React	180
Settle	140
Decant	20
Total	360

Table 3.2 Operation conditions for the ASBR system at two different cycles per day

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

The feed and decant flow rates were varied at a constant feed COD of 40,000 mg/L, depending on the number of cycles per day. Therefore, the COD loading rate was varied according to the Equation (3.1)

$$\text{COD loading rate} = \frac{(\text{Feed COD}) \times (\text{Feed Flow Rate})}{(\text{Working Volume})} \quad (3.1)$$

The system was performed at different COD loading rates of 37.5, 56.25, 75, and 93.75 kg/m^{3}d with 18.75 kg/m^{3}d increment. According to the literature, Bhaskar *et al.* (2008) found that the pH range of 5.5-6 was considered to be the optimum pH range effective for hydrogen production. In addition, Lee *et al.* (2008) indicated that an excellent hydrogen production was obtained under the mesophilic condition at 37°C and pH 5.5 with better hydrogen yield (9.91 mmol H_2/g starch) compared to pH 6. So the mesophilic temperature at 37°C and pH 5.5 were selected for this experiment. Volume and compositions of produced gas, COD of the effluent liquid, and composition and amount of VFA were analyzed. Only those obtained under the steady state conditions were reported. For any fixed experimental conditions, the steady state data was averaged to assess the process performance.

Table 3.3 Conditions for investigating the effect of number of cycles per day

Number of cycle/d	**Feed and Decant volume (L/cycle)**	**Feed and Decant flowrate (L/d)**	**COD loading rate (kg/ m^{3}d)**
6	0.5	3	37.5
	0.75	4.5	56.25
	1	6	75
	1.25	7.5	93.75

3.2.3 Analytical Techniques

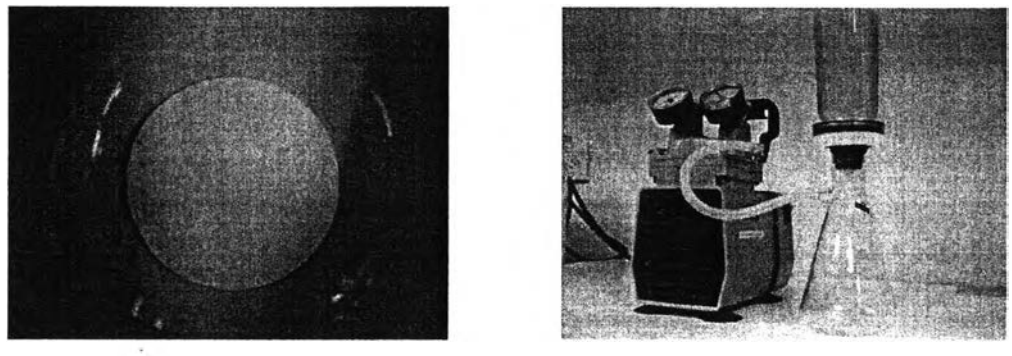
3.2.3.1 Steady-State Analysis

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).

3.2.3.2 Total Suspended Solids (TSS) Analysis

- *Procedure*

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



(a) (b)

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

- The glass-fiber filter disk with wrinkled side up is inserted in filtration apparatus, as shown in Figure 3.2(a) and (b). After that, it was apply to vacuum and wash with three successive 20 cm^3 of distilled water

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

(2) Selection of filter and sample sizes:

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

- If more than 10 min was required to complete filtration, the 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 the test.

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

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

- The filter was carefully removed from filtration apparatus and 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 weight of the sample was nearly constant (less than 4 % difference).

- *Calculation*

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

A = Weight of filter + dried residue [g]

B = Weight of filter [g]

3.2.3.3 Volatile Suspended Solids (VSS) Analysis

- *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 the sample.

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

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

- *Calculation*

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

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

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

3.2.3.4 COD Analysis (Closed Reflux, Colorimetric Method)

- *Reagents*

- *Digestion solution.* The following reagents were added into 500 ml distilled water: 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.

- *Sulfuric acid reagent.* Ag_2SO_4 (reagent grade, crystals or powder) was added to 98 % H_2SO_4 at the ratio of 5.5 g Ag_2SO_4/kg H_2SO_4. The mixture was left to stand for 1 to 2 days to completely dissolve Ag_2SO_4.

- *Procedure*

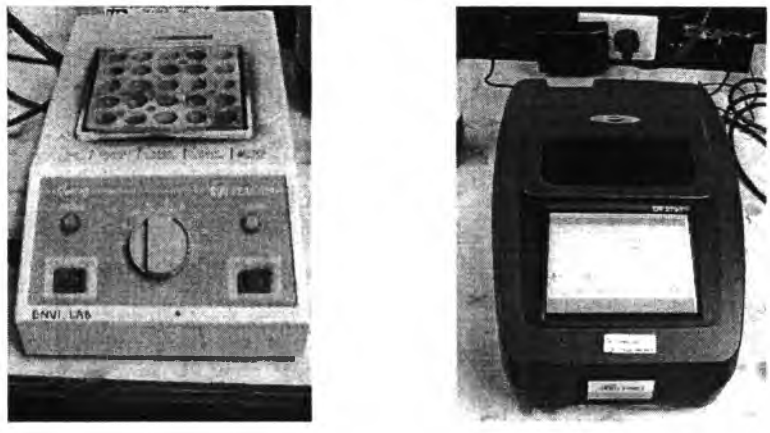
- Sample (dilute 100 times) of 2.5 ml was added to a digestion vial (HACH, 16× 100 mm).

- Digestion reagent of 1.5 ml was added to the vial. Afterwards, 3.5 ml 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 preheated COD reactor (HACH) (Figure 3.3(a)).

- The vial was heated for 2 h, and then left for about 20 min to be cooled.

- The vial was placed into a spectrophotometer (HACH DR 2700) for reading COD value, as shown in Figure 3.3(b).



(a) (b)

Figure 3.3 (a) COD reactor and (b) spectrophotometer.

3.2.3.5 Total VFA Analysis

The amount of VFA was 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).

3.2.3.6 VFA Composition Analysis

The liquid composition was determined by a gas chromatograph (PR2100, Perichrom) equipped with a flame ionization detector 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 was started at 60°C, heated to 125°C at a ramping rate of 10°C/min, held for 2 min, then heated to 180°C at a ramping rate of 15°C/min, and held for 15 min. The temperatures of injector and detector were 250 and 270°C, respectively.

3.2.3.7 Gas Composition Analysis

The gas composition was determined by a gas chromatograph (AutoSystem GC, Perkin-Elmer) equipped with 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.

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