CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Anaerobic Seed Sludge and Alcohol Distillery Wastewater

Seed sludge and alcohol distillery wastewater were collected from the wastewater treatment plant of Red Bull Distillery (1988) Co., Ltd. Part., Samuthsakorn, Thailand. The anaerobic seed sludge is black color, and has pH of 3.0–4.0, and total suspended solids (TSS) concentration of 60,760 mg/l. The alcohol distillery wastewater has the chemical characteristics as shown in Table 3.1. The anaerobic seed sludge and the alcohol distillery wastewater were kept at 4 °C prior to use.

Table 3.1 Chemical characteristics of the alcohol distillery wastewater

Parameter	Concentration (mg/L)
Chemical oxygen demand (COD)	100,000–120,000
Total suspended solids (TSS)	4,100
Total nitrogen	3,500
Total phosphorous	900
Potassium	9,000
Sulphate	4,400

3.1.2 Chemicals

Ammonium hydrogen carbonate (NH_4HCO_3, analytical reagent grade, AJAX Finechem), hydrochloric acid (HCl) 37 %, sodium hydroxide (NaOH), and phenolphthalein ($C_{20}H_{14}O_4$, analytical reagent grade, Lab-scan) were used in this work.

3.2 Equipment

3.2.1. Time-controlling System

Timers (OMRON model H5CX-A), as shown in Figure 3.1, were used to control the time of each operation steps: (1) feeding, (2) reacting, (3) settling, and (4) decanting.

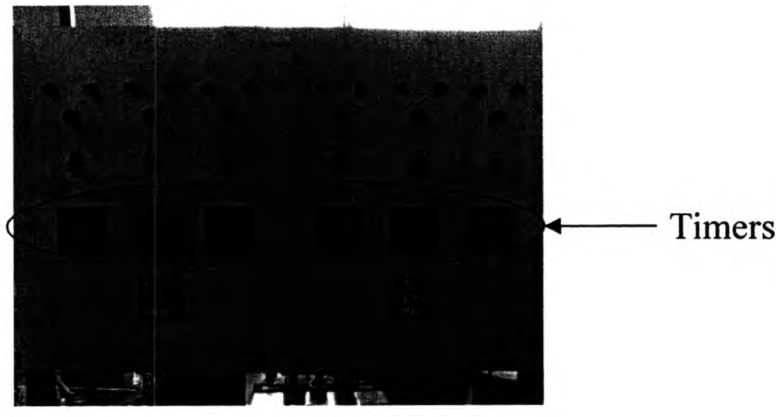


Figure 3.1 Time-controlling system.

3.2.2 Temperature-controlling System

This system comprising a heater rod, thermocouple, and control box (Figure 3.2) was used to control the system temperature. The system temperature was adjusted to be around 37 °C, and it was only used during the reacting period.

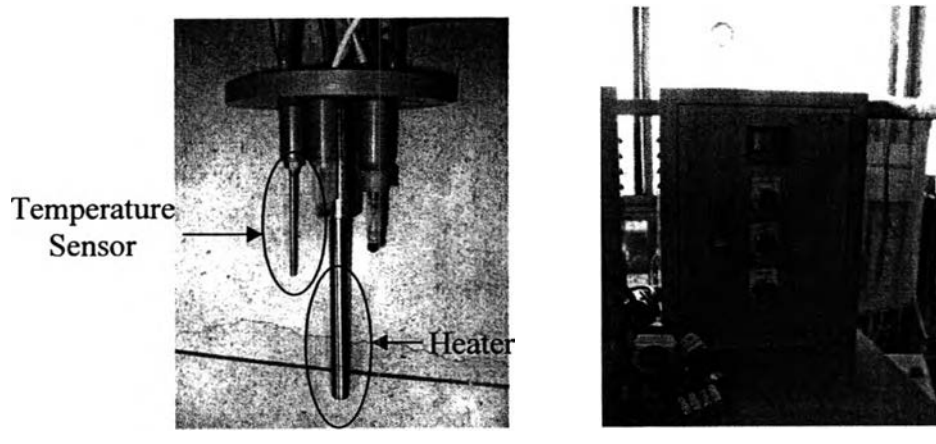


Figure 3.2 Temperature-controlling system installed at a cover of reactor.

3.2.3 pH-controlling and mixing Systems

This system consisted of a pH controller (Extech model 48PH2), a pH electrode (Cole-Parmer Double-Junction Electrode) (Figure 3.3), a diaphragm pump, and a magnetic stirrer (40×20 mm, egg shape) for mixing. The pH of the mixed solution was controlled automatically by feeding 1 M NaOH solution via the diaphragm pump. The liquid in the bioreactor was homogeneously mixed using the magnetic stirrer at 400 rpm.

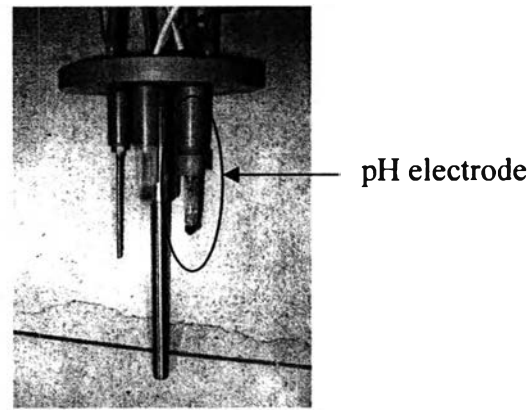


Figure 3.3 pH sensor installed at a cover of reactor.

3.2.4 Gas-measuring System

This system was composed of 2 flasks filled with 1 M HCl solution, in order to prevent dissolution of the produced gas (Ueno *et al.*, 1996), and a wet gas meter (Figure 3.4) that was used to measure the volume of produced gas at room temperature.

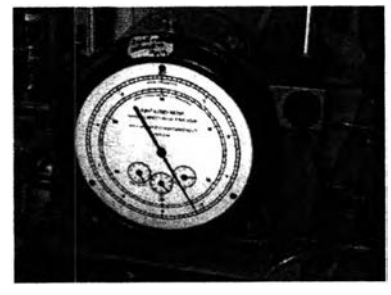


Figure 3.4 Wet gas meter.

3.3 Methodology

3.3.1 Seed sludge preparation

The seed sludge for hydrogen production experiments was concentrated by sedimentation, and the concentrated sludge was ground and filtered through the sieve in the size of 1 mm in order to remove debris and large particles. Then, it was pretreated before seeding the bioreactor by boiling at 95 °C for 15 min in order to select spore-forming of hydrogen-producing acidogenic bacteria and to eliminate hydrogen-consuming methanogens (Argun *et al.*, 2008).

3.3.2 Substrate preparation

Alcohol distillery wastewater was filtered through sieve size of 0.2 μm to remove debris and then was diluted with water to obtain a chemical oxygen demand (COD) of 20,000, 40,000, and 60,000 mg/l for reducing the effect of toxicity on hydrogen-producing bacteria.

3.3.3 Bioreactor design and operation

Two identical 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-liter opaque 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 liters under a mesophilic temperature of 37 °C. The schematic of the ASBR process is shown in Figure 3.5.

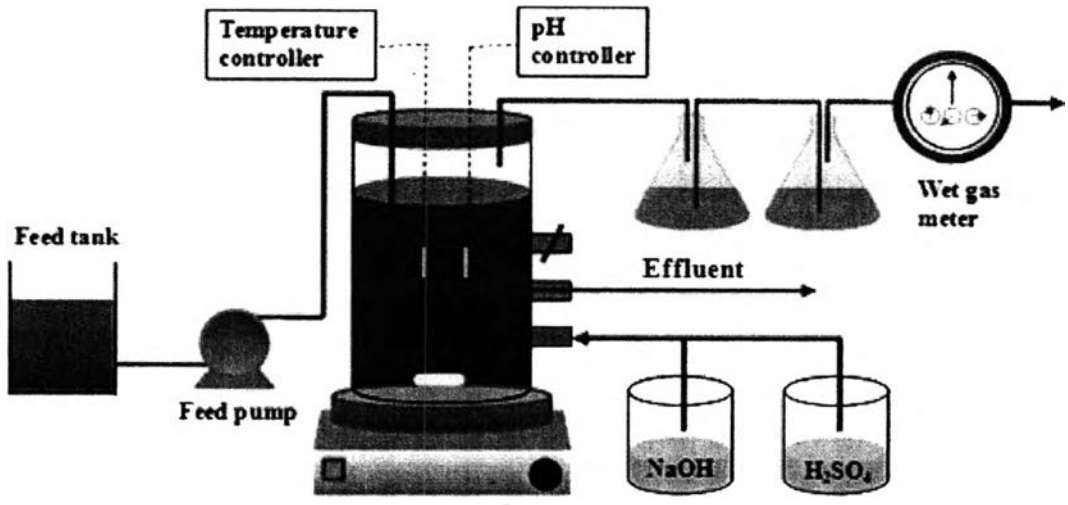


Figure 3.5 Schematic of the studied ASBR process.

ASBR operation composes four steps: feed, react, settle, and decant. During the operation, time for each step was controlled by timers, which allow the feed pump to pump wastewater during the feeding period. Mixing was achieved by using a magnetic stirrer at 400 rpm during the reacting phase (Chen and Chen 2009). The pH-controller and heater were used to maintain a constant pH and temperature of the system, respectively.

During the start-up, 500 ml of pretreated seed sludge was completely mixed with wastewater at initial feed COD of 20,000 mg/l and COD loading rate of 15 kg/m^3d, which corresponded to the hydraulic retention time (HRT) of 32 h. The COD loading rate was then increased stepwise by reducing HRT. Under any studied conditions, the reactor was operated until the system reached the steady state around two weeks. Then, the effect of COD loading rate on hydrogen production was studied. Steady state conditions were justified when the variation in the production of produced gas was nearly constant (standard deviation less than 5%).

The effects of initial feed COD value and COD loading rate on the biohydrogen production at a fixed cycle time of 4 h or 6 cycles per day were investigated since Chatsiriwatana (2009) reported that the system operated at 6 cycles per day showed higher process performance in terms of hydrogen production rate and yield than that operated at 4 cycles per day. So, this operation time was

selected based on practical range of possible operation cycles in a single day. The operation times of four steps, i.e. feed, react, settle, and decant, in the ASBR operation are shown in Table 3.2.

Table 3.2 Operation conditions for the ASBR system at 6 cycles per day

Operating step	Cyclic time (min)
Feed	15
React	90
Settle	120
Decant	15
Total	240

The feed and decant flow rates were varied at different feed COD values of 20,000, 40,000, and 60,000 mg/l. Therefore, the COD loading rate was varied as expressed in Equation (3.1):

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

The experiments were conducted at different initial feed COD values and COD loading rates, as shown in Table 3.3. In the study of Bhaskar *et al.* (2008) the pH range of 5.5–6 was considered as the optimum pH range, which is effective for hydrogen production. In addition, Lee *et al.*, (2008) reported that an excellent hydrogen production was obtained when a bioreactor was operated under mesophilic temperature of 37 °C and pH 5.5. Moreover, Lin *et al.,* (2006) reported that the toxicity of sulphate can be depressed by lowering the pH to 5.5, which is the desired value for suppressing the activity of sulphate-reducing bacteria (SRB). To avoid the effect of sulphate toxicity and reach the highest hydrogen production, the mesophilic temperature of 37 °C and pH 5.5 were selected for this research. Volume and compositions of produced gas, COD of the effluent liquid, and compositions and amount of VFA were then analyzed. Only those obtained under steady state

conditions were reported. For any fixed experimental conditions, the steady state data was averaged to assess the process performance.

Table 3.3 Operation conditions for the ASBR system at 6 cycles per day

Feed and decant volume (l/cycle)	Feed and decant flow rate (l/d)	HRT (h)	COD loading rate (kg/m^3d)		
			Initial feed COD of 20,000 mg/l	Initial feed COD of 40,000 mg/l	Initial feed COD of 60,000 mg/l
0.5	3	32	15	30	45
0.75	4.5	21	22.5	45	67.5
1	6	16	30	60	90
1.25	7.5	13	37.5	75	112.5

3.4 Analytical Methods

3.4.1 Total Suspended Solids (TSS) Analysis

3.4.1.1 Procedure

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

(a) (b)

Figure 3.6 (a) glass-fiber filter disk and (b) filtration apparatus.

- The glass-fiber filter disk with wrinkled side up was inserted in filtration apparatus, as shown in Figure 3.6(a) and (b), after that it was applied to vacuum and it was washed with three successive 20 cm^3 of distilled water.

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

(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 were required to complete filtration, 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 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 was 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 desiccator to balance temperature, and then weighed.

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

3.4.1.2 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]

3.4.2 Volatile Suspended Solids (VSS) Analysis

3.4.2.1 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.

3.4.2.2 Calculation

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

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

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

3.4.3 COD Analysis (Closed Reflux, Colorimetric Method)

3.4.3.1 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 liter.

- *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 d to completely dissolve Ag_2SO_4.

3.4.3.2 Procedure

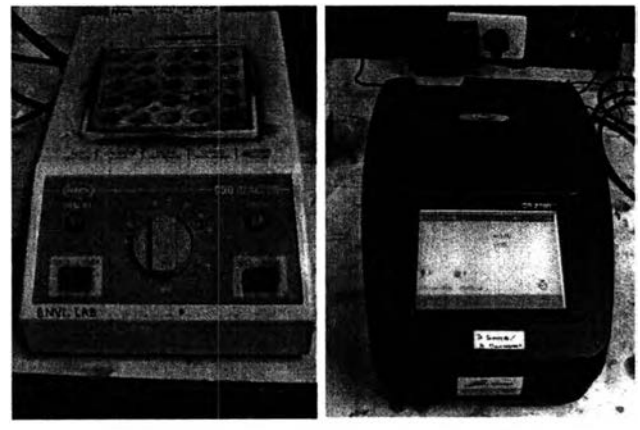
- Sample (dilute 100 times) of 2.5 ml was added to digestion vial (HACH, 16 x 100 mm).

- Digestion reagent of 1.5 ml was added to the vial. Afterwards, sulfuric acid reagent was slowly dropped for 3.5 ml into the vial.

- The vial was inverted several times to homogeneously mix the contents, and the vial was then placed in the preheated COD reactor (HACH) (Figure 3.7(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 3.7(b).



(a) (b)

Figure 3.7 (a) COD reactor and (b) spectrophotometer.

3.4.4 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 (Greenberge *et al.*, 1992).

3.4.5 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^{-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 were 250 and 270 °C, respectively.

3.4.6 Sulphate Analysis

- A sample (feed or effluent) of 10 ml was added to a square sample cell (HATC, 1 × 1 inch). A SulfaVer 4 Reagent Powder Pillow was added to the sample cell. The mixture was swirled continuously to dissolve powder (The mixture will become turbid due to the formation of white powder if there is sulphate present in the sample.) The sample cell was left to complete the reaction for 5 min. The sample cell was placed into a spectrophotometer (HACH DR 2700) for determining sulphate content.

3.4.7 Potassium Analysis

The potassium concentration contained in the feed and effluent samples was analyzed by using an atomic absorption spectrophotometer (AAS) at the wavelength of 766.5 nm.

3.4.8 Gas Composition Analysis

The gas composition was 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) 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.