



## CHAPTER III EXPERIMENTAL

### 3.1 Materials

#### 3.1.1 Anaerobic Seed Sludge and Cassava Wastewater

Anaerobic seed sludge and cassava wastewater were collected from the cassava wastewater treatment plant of Sahamitr Tapioca Chonburi Ltd., Part., Chonburi, Thailand. The anaerobic seed sludge has dark yellow color, pH 4.0-4.6, a total suspended solids (TSS) concentration of 44,000 mg/L, and a volatile suspended solids (VSS) concentration of 38,000 mg/L. The cassava wastewater has the chemical characteristics, as shown in Table 3.1. The anaerobic seed sludge and the cassava wastewater were kept at 4°C prior to use.

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

Parameter	Concentration (mg/L)
Chemical oxygen demand (COD)	20,000
Total suspended solids (TSS)	6,000
Total nitrogen	140-380
Total phosphorous	460-780

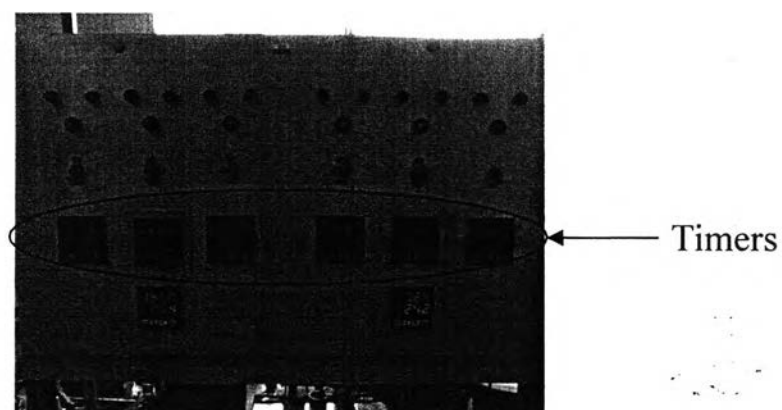
#### 3.1.2 Chemicals

Ammonium hydrogen carbonate ( $\text{NH}_4\text{HCO}_3$ ), analytical reagent grade, AJAX Finechem Pty Ltd (Australia). Hydrochloric acid (HCl) 37%, Sodium hydroxide (NaOH) and Phenolphthalein ( $\text{C}_{20}\text{H}_{14}\text{O}_4$ ), analytical reagent grade, Lab-scan (Thailand).

## 3.2 Equipment

### 3.2.1. Time-controlling System

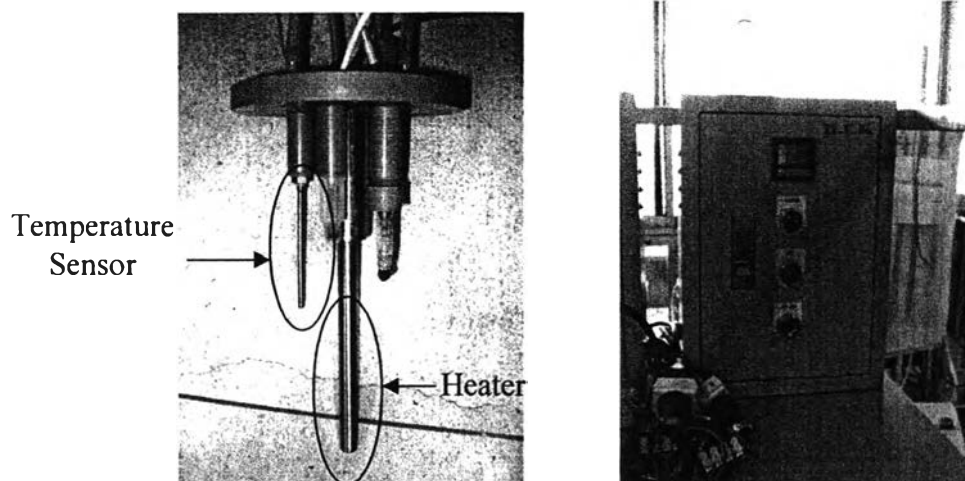
Timers (OMRON model H5CX-A), as shown in Figure 3.1, were used to control the time of each operation steps: (1) feeding, (2) reacting, (3) settling, and (4) decanting.



**Figure 3.1** Time-controlling system.

### 3.2.2 Temperature-controlling System

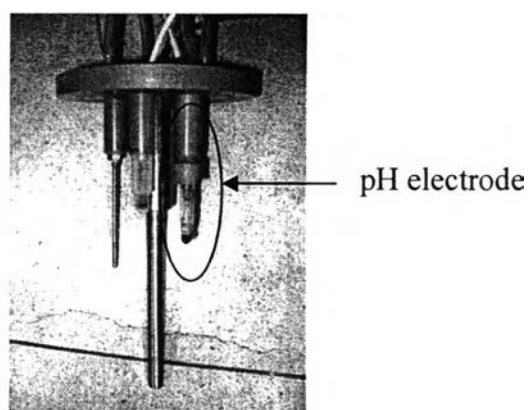
This system comprising a heater rod, thermocouple, and control box (Figure 3.2) was used to control the system temperature. The system temperature was adjusted to be around 37°C and it was used only during the reacting period.



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

### 3.2.3 pH-controlling and Mixing Systems

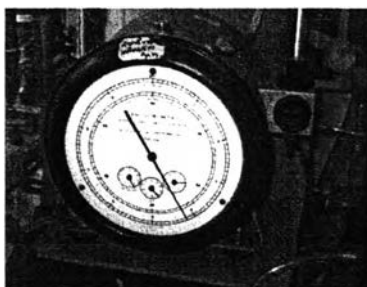
This system consisted of a pH controller (Extech model 48PH2), a pH electrode (Cole-Parmer Double-Junction Electrode) (Figure 3.3), a diaphragm pump, and a magnetic stirrer (40×20 mm, egg shape) for mixing. The pH of the mixed solution was controlled automatically by feeding 1 M NaOH solution via the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

An anaerobic sludge sample obtained from the first anaerobic pond treating a cassava wastewater was first concentrated by sedimentation and the concentrated sludge was ground and filtered through the sieve in the size of 1 mm to remove debris and large particles. After that, it was pretreated to enrich hydrogen-producing bacteria and inhibit methane-producing bacteria activity (or hydrogen consumers). Wang *et al.* (2008) reported that the heat-shock pretreatment method could obtain the maximum hydrogen production rate, specific hydrogen production rate, COD removal efficiency, and biomass concentration. Therefore, this method was conducted in this study by boiling the sludge at 95°C for 15 min. The heat-treated sludge was used as the seed sludge in both studied bioreactors.

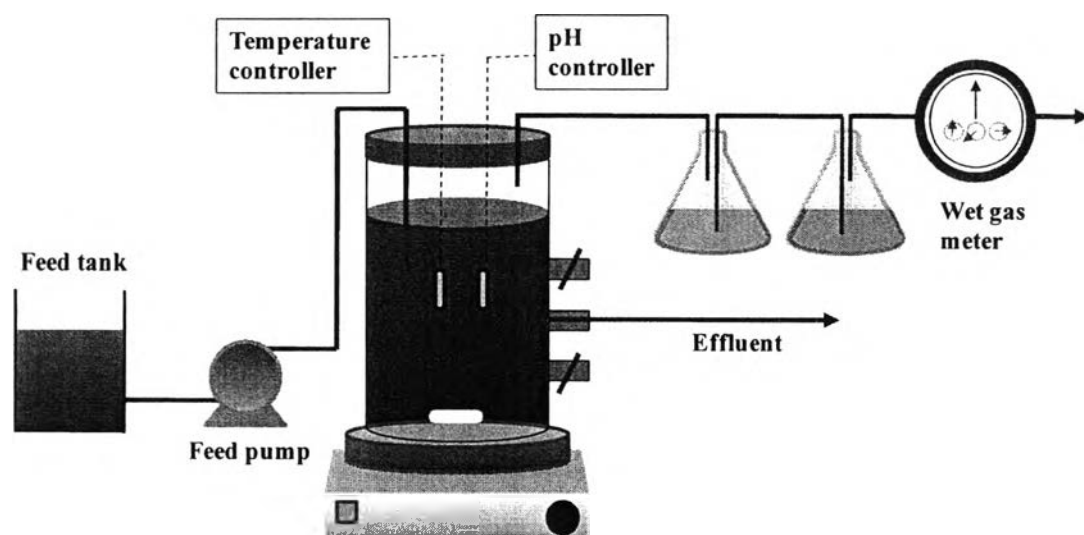
### 3.3.2 Feed Preparation

The cassava wastewater obtained from a cassava starch production plant was filtered through the sieve with the size of 0.2  $\mu\text{m}$  to remove debris. The

cassava wastewater was also added with  $\text{NH}_4\text{HCO}_3$  as the nitrogen source to adjust the COD:N ratio of 100:2.2 (Thailand Institute of Scientific and Technological Research, 1988), 100:3.3 (50% excess of nitrogen), and 100:4.4 (100% excess of nitrogen) in the second part of experiments in order to study the effect of nutrient supplementation.

### 3.3.3 ASBR Operation

The biohydrogen production experiments were carried out in two ASBR reactors. To inhibit the activity of photosynthetic bacteria, the system was operated without light illumination in 5-L PVC reactors. Each of them had an inner diameter of 13 cm and a height of 30 cm. The reactors were operated with a liquid working volume of 4 L. The schematic of the ASBR system is shown in Figure 3.5.



**Figure 3.5** Schematic of the studied ASBR process.

ASBR operation was composed of four sequential steps: feed, react, settle, and decant. In the operation, time for each step was controlled by timers, which allowed the feed pump to feed the wastewater into the bioreactor during the feed period. A short hydraulic retention time (HRT) of 24 h was used to operate the system in order to prevent the hydrogen consumption by methanogens (Hawkes *et al.*, 2002). Furthermore, the system temperature and pH were controlled by using

temperature-controlling and pH-controlling systems, respectively. A magnetic stirrer was used for mixing purpose during the reaction period.

Two series of experiments were consecutively conducted in the 4 L PVC reactors. Series 1 was conducted to examine the effect of number of cycles per day and COD loading rate. The series 2 was conducted to investigate the effect of nutrient supplementation on the biohydrogen production.

During the system start-up, the reactors were seeded with the heat-treated anaerobic seed sludge, which was allowed to acclimate to the cassava wastewater for 3-4 weeks at a mesophilic temperature of 37°C and a fixed HRT of 24 h. The COD loading rate into each reactor was 20 kg/m<sup>3</sup>d during this acclimation phase since . Acclimatization was considered to be completed when the reactors reached steady state at this COD loading rate. In this study, steady state was defined by an invariant composition of produced gas (within ±15% deviation). After that, the COD loading rates in each reactor were changed to investigate the effect of COD loading rate.

The effect of number of cycles per day (or cycle-frequency) on the biohydrogen production at a fixed HRT of 24 h and different COD loading rate was investigated since Chen *et al.* (2008) reported that the operational cycle-frequency greatly affects the biohydrogen production. Cycle durations of 4 and 6 h corresponding to 6 and 4 cycles per day operation modes, respectively, were selected based on practical range of possible operation cycles in a single day. The operation times of four steps: feed, react, settle, and decant in ASBR operation are shown in Table 3.2.

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

Operating Parameter		4 cycles/day	6 cycles/day
HRT (h)		24	24
Cycle time (min)	Feed	15	15
	React	210	90
	Settle	120	120
	Decant	15	15
	Total	360	240

The feed and decant flow rates were varied at a constant feed COD of 20,000 mg/L, depending on the number of cycles per day. Therefore, the COD loading rate was varied according to the Equation (3.1)

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

Table 3.3 summarizes the conditions for investigating the effect of number of cycles per day. The first set of experiments was performed at a 4 cycles per day. The COD loading rates were adjusted at 10, 15, 20, and 25 kg/m<sup>3</sup>d with a 5 kg/m<sup>3</sup>d increment. For a 6 cycles per day, the system was performed at different COD loading rates of 15, 22.5, 30, and 37.5 kg/m<sup>3</sup>d with a 7.5 kg/m<sup>3</sup>d increment. The COD loading rates of 25 and 37.5 kg/m<sup>3</sup>d for 4 and 6 cycles per day were the processible maximum values due to the limited available time of 24 h each day. Fang *et al.* (2002) found that the optimum pH for production of biohydrogen was observed at a pH of 5.5. So, the system pH was controlled at 5.5 by a 1 M NaOH solution. The operational temperature was under mesophilic condition of 37°C, and each run lasted over 10-14 days to ensure that the system reached steady state (composition of produced gas became nearly constant). Volume and compositions of produced gas, COD of the effluent liquid, and composition 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 were averaged to assess the process performance.

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

Number of Cycles/day	Feed and decant volume (L/cycle)	Feed and decant flow rate (L/d)	COD loading rate (kg/m <sup>3</sup> d)
4	0.50	2	10
	0.75	3	15
	1.00	4	20
	1.25	5	25
6	0.50	3	15
	0.75	4.5	22.5
	1.00	6	30
	1.25	7.5	37.5

Nutrient supplementation has been used for improving the treatment of wastewater containing is sufficient amount of the nutrient. From the chemical analysis results characteristics of the cassava wastewater, it shows an excess amount of phosphorous, but it is deficient in nitrogen based on the optimum ratio for anaerobic (or dark) fermentation which is a COD:N:P ratio of 100:2.2:0.4 (Thailand Institute of Scientific and Technological Research, 1988). Since nitrogen is a very important component for proteins, nucleic acids, and enzymes in hydrogen-producing bacteria cells, it is one of the most essential nutrients needed for their growth. Thus, an appropriate level of nitrogen addition is beneficial to the growth of hydrogen-producing bacteria and to fermentative hydrogen production accordingly (Wang *et al.*, 2008 and Wang *et al.*, 2009). Therefore, the effect of COD:N ratio was investigated in order to optimize the anaerobic hydrogen production from the cassava wastewater. The optimum conditions obtained in the previous part was used to study the effect of nutrient supplementation in this part. The raw cassava wastewater was nutrient-supplemented by adding NH<sub>4</sub>HCO<sub>3</sub> as nitrogen source at different COD:N



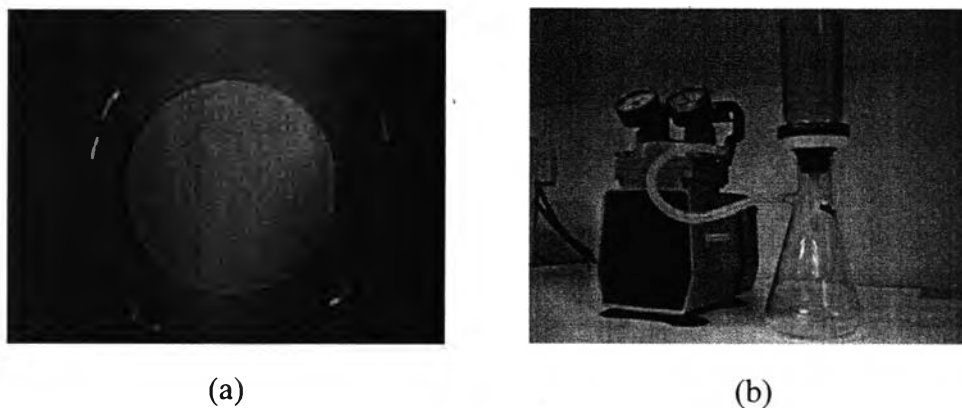
ratios of 100:2.2 (optimum nitrogen ratio), 100:3.3 (50% excess of nitrogen ratio), and 100:4.4 (100% excess of nitrogen ratio). The system pH was controlled at 5.5 by adding 1 M NaOH solution. The operational temperature was also under mesophilic condition of 37°C. The steady-state condition of each 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% deviation). When the system reached steady state for each operating condition, COD of the effluent, volume and compositions of produced gas, as well as VFA, and VFA composition were analyzed.

### 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  $\mu\text{m}$ ):



**Figure 3.6** (a) glass-fiber filter disk (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 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 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<sup>3</sup> 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*

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

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

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

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

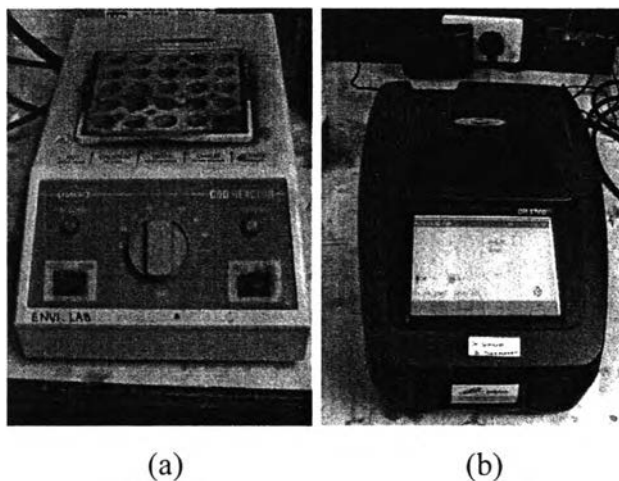
- *Digestion solution.* The following reagents were added into 500 ml distilled water: 10.216 g  $\text{K}_2\text{Cr}_2\text{O}_7$  (primary standard grade) previously dried at 103°C for 2 h, 167 ml 98%  $\text{H}_2\text{SO}_4$ , and 33.3 g  $\text{HgSO}_4$ . The mixture was left for complete dissolution, cooled to room temperature, and finally diluted to 1 L.

- *Sulfuric acid reagent.*  $\text{Ag}_2\text{SO}_4$  (reagent grade, crystals or powder) was added to 98%  $\text{H}_2\text{SO}_4$  at the ratio of 5.5 g  $\text{Ag}_2\text{SO}_4/\text{kg H}_2\text{SO}_4$ . The mixture was left to stand for 1 to 2 days to completely dissolve  $\text{Ag}_2\text{SO}_4$ .

#### 3.4.3.2 Procedure

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



**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 (Greenberg 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  $\mu\text{m}$  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,  $\text{H}_2$  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  $\text{min}^{-1}$ , held for 2 min, then heated to 180°C at a ramping rate of 15°C  $\text{min}^{-1}$ , and held for 15 min. The temperature of injector and detector were 250 and 270°C, respectively.

#### 3.4.6 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.