CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Chemicals

The supplementation nutrients for bacterial growth in this study were laboratory reagent grade ammonium hydrogen carbonate (NH_4HCO_3) as a nitrogen source and analytical reagent grade di-potassium hydrogen orthophosphate (K_2HPO_4) as a phosphorus source. The sodium hydroxide (NaOH) aqueous solution was used to control pH in the system.

3.1.2 Substrates

Seed sludge was collected from a full-scale anaerobic plant treating cassava wastewater at Sahamitr Tapioca Chonburi Ltd., Part, Thailand. Cassava wastewater was collected after being discharged from cassava manufacturing processes of Sahamitr Tapioca Chonburi Ltd., Part, Thailand.

3.2 Equipments

3.2.1 Apparatus for Setting up an Upflow Anaerobic Sludge Blanket (UASB) Reactor

Two identical UASB reactors were constructed from borosilicate glass with a 24-L working volume. Water jacket system was used to provide heat to the reactor with hot water from heating bath (Memmert). Peristaltic pump (Master Flex Model Easy-load II) was used to carry hot water to a water jacket system and also to carry the influent to the reactor. Moreover, the effluent pH was measured by a pH electrode (Cole-palmer KH-27012-27). The UASB reactors used in this study are shown in Figure 3.1. The effluent pH was re-adjusted by mixing with base solution using a magnetic stirrer in the mixing tank, and this partially pH-controlled effluent was pumped back to the reactor with the ratio of fresh influent-to-recycled effluent of 1 to 1.

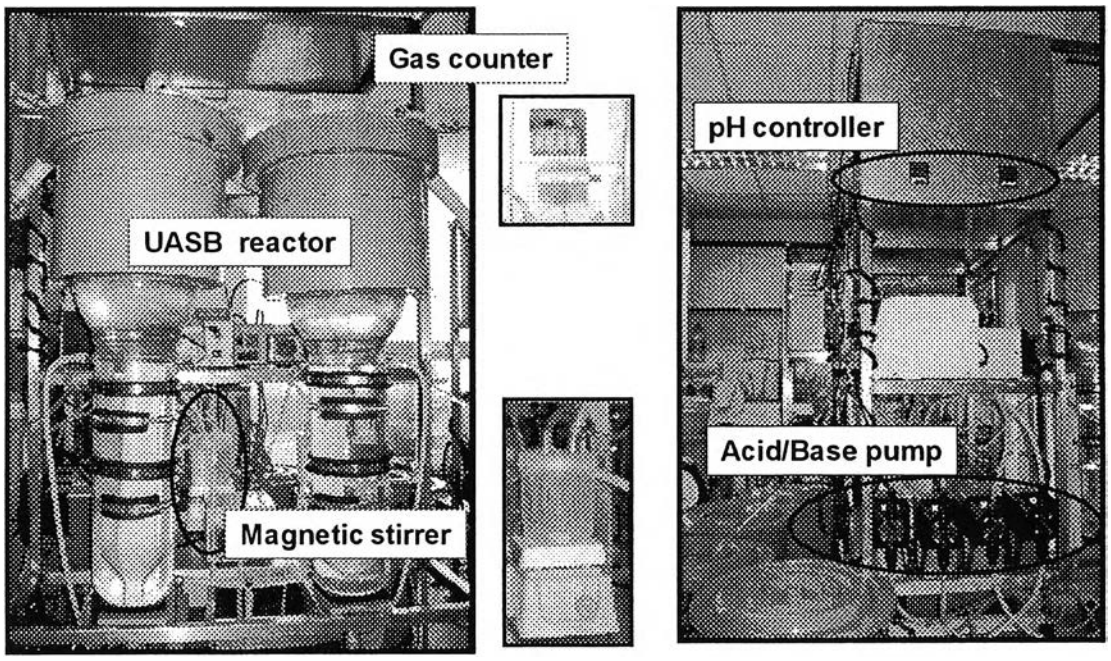

Figure 3.1 Apparatus of UASB setup.

3.3 Methodology

3.3.1 Bacteria and Cultivation

Bacterial seed sludge collected from the wastewater treatment plant was stored at a temperature below 4°C. The pH and total suspended solids (TSS) of the mixed seed sludge were 4.5 and 54 g/L, respectively. Before being introduced into the reactor, the sludge was screened by sieving to eliminate large particulates and inorganic materials. The sludge with TSS of 20 g/L was supplied to the reactor.

3.3.2 Feed Preparation

Cassava wastewater used for experiments had COD of 19-22 g/L. The influent was maintained at 4°C. The wastewater was screened by sieving before being fed into the reactor.

3.3.3 UASB Operation

The UASB reactors were constructed from borosilicate glass with a 24- L working volume for hydrogen and methane production. Temperature inside the reactors was controlled at 37°C by water jacket system with heating bath. The flow diagram of UASB operation is shown in Figure 3.2.

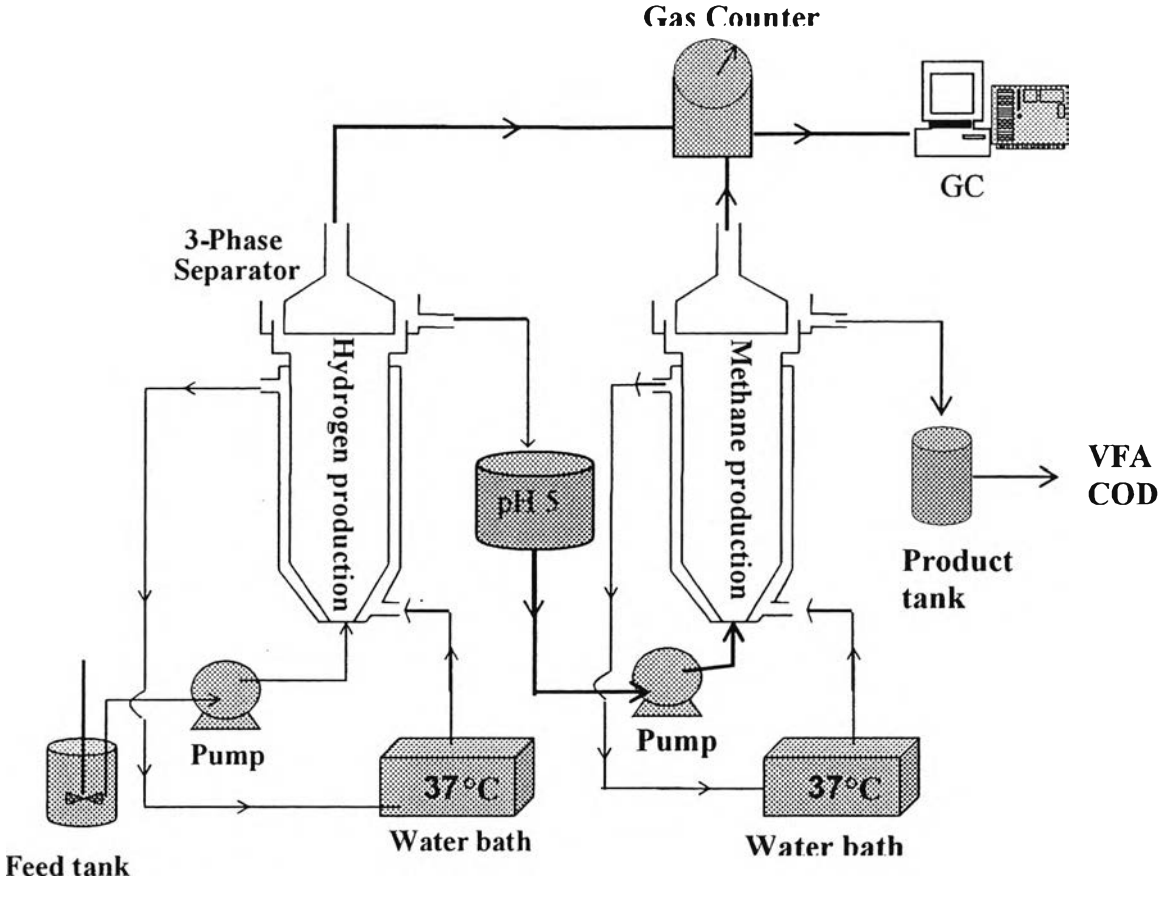

Figure 3.2 Flow diagram of UASB operation.

Cassava wastewater was pumped into the bottom of the hydrogen production reactor and was flown up through the flocculants of microorganisms. The organic compounds in the feed, especially carbohydrates, were digested by the microorganisms, and gaseous products were generated. A three-phase separator was used to prevent an outflow of flocculants, to collect the gaseous products, and to collect the overflown liquid effluent to the mixing tank. The pH of the effluent from the hydrogen production reactor was controlled at 5.5, and this pH-controlled effluent was fed as a feed substrate into the methane production reactor without further pH control.

3.3.3.1 Hydrogen Production Step

For the hydrogen production step, the sludge was boiled for 15 min to eliminate methane-producing bacteria or hydrogen consumers before being added to the reactor. The pH of liquid effluent was controlled at 5.5 by adding 1 M

NaOH solution. The reactor was operated at various COD loading rates of 10, 20, 25, and 30 kg/m^3d in order to obtain an optimum condition, at which the maximum hydrogen yield was achieved.

3.3.3.2 Methane Production Step

The effluent from hydrogen production reactor at an optimum COD loading rate was used as a feed for methane production. The methane production reactor was also operated without pH control.

For each run, the reactor was operated unit reaching the steady-state condition, at which produced gas composition and effluent COD became almost invariant.

3.3.4 Effect of COD Loading Rate on Biohydrogen Production

To determine the effect of COD loading rate on biohydrogen production, the organic concentration of the studied cassava wastewater, in terms of COD, was in the range of 19,000 to 22,000 mg/L. The amount of organic compounds in the reactor is the one of the parameters affected the production of hydrogen production (Islam *et al.*, 2006). The cassava wastewater was fed into the reactor by varying COD loading rate. The COD loading rate was calculated by the following relationship:

$$\text{COD loading rate} = \frac{(\text{Feed COD}) \times (\text{Feed flow rate})}{(\text{Working volume})}$$

The hydraulic retention time corresponding to the cassava wastewater loading rate is calculated by following equation:

$$\text{Hydraulic retention time (d)} = \frac{\text{Working volume of reactor (L)}}{\text{Feed flow rate (L/d)}}$$

The feed flow rate and hydraulic retention time corresponding to the cassava wastewater loading rate are shown in Table 3.1.

Table 3.1 COD loading rate, flow rate, and hydraulic retention time (HRT) for determining the effect of COD loading rate on hydrogen production at pH 5.5 and 37°C (COD = 20 g/L)

COD loading rate (kg COD/ m^3d)	Flow rate (L/d)	HRT (d)
10	6	4
20	12	2
25	15	1.6
30	18	1.33

3.4 Analytical Methods

3.4.1 Analysis of Gas Production

3.4.1.1 The Composition of Gas Produced

The composition of produced gas was analyzed by a gas chromatograph (AutoSystem GC, Perkin-Elmer) equipped with a thermal conductivity detector (TCD), or GC-TCD, and a packed column (stainless-steel 10'x 1/8' x .085" HayeSep D 100/120 mesh (Altech) 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.

3.4.1.2 The Amount of Produced Gas

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

3.4.2 Analysis of Volatile Fatty Acids (VFA)

3.4.2.1 The Amount of Volatile Fatty Acids

The VFA concentration in the effluent was determined by the distillation method. This technique recovers acids containing up to six C atoms and reports the result in terms of acetic acid by titrating with 0.1 M NaOH using phenolphthalein as an indicator (Greenberg *et al.*, 1992).

3.4.2.1.1 Apparatus

- Distillation flask
- Heater
- Condenser

3.4.2.1.2 Reagents

- Sulfuric acid
- Standard sodium hydroxide solution, 0.5 N
- Phenolphthalein indicator solution
- Acetic acid stock solution, 2000 mg/l

3.4.2.1.3 Procedure

A. Recovery factor

The recovery factor (f) was determined for a given apparatus by taking 150 ml of acetic acid stock solution to distillation apparatus. Then, the sample was distilled. Finally, the recovery factor was calculated by the flowing equation.

$$f = \frac{a}{b}$$

where

a = volatile acid concentration recovered in distillate [mgL^{-1}]

b = volatile acid concentration in standard solution used [mgL^{-1}]

B. Sample analysis

- 3 ml of H_2SO_4 were added to 150 ml of effluent solution in a beaker.
- The solution was mixed homogeneously.
- The mixed solution was placed into the distillation apparatus.
- The solution was continuously distilled.
- The first 5 mL of distillate were discarded.
- The 80 mL of distillate were collected.
- The 20 mL of distillate were titrated with 0.1 M NaOH using phenolphthalein as an indicator.

3.4.2.1.4 Calculation

$$\frac{\text{mg volatile acids as acetic acid}}{\text{L}} = \frac{\text{ml NaOH} \times \text{N} \times 60{,}000}{\text{ml sample} \times \text{f}}$$

where

N = Normality of NaOH solution

F = recovery factor

3.4.2.2 The Composition of Volatile Fatty Acids

The volatile fatty acids and alcohol composition of the effluent was analyzed by a gas chromatograph (PR2100, Perichrom) equipped with a flame ionization detector (FID), or GC-FID, and a 50 m x 0.32 ID, 0.25 μm film thickness DB-WAXetr (J &W scientific) capillary column in the splitless mode with helium at a pressure of 22 kPa as carrier gas, hydrogen at 50 kPa as 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 both injector and conductor were 250°C.

3.4.3 COD Analysis (Closed Reflux, Colorimetric method)

3.4.3.1 Apparatus

- Digestion vessel 16×100 mm
- COD reactor
- Spectrophotometer for reading COD value at 600 nm

3.4.3.2 Reagents

- Digestion solution: the following agents were added to 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 $HgSO_4$. The mixture was left for complete dissolution, cooled to room temperature, and finally diluted to 1000 ml.

- 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 for 1 to 2 d to completely dissolve Ag_2SO_4.

3.4.3.3 Procedure

- 2.5 ml of sample (diluted 100 times) were added to digestion vessel.

- 1.5 ml of digestion reagent were added to the vessel. Then, sulfuric acid reagent was slowly dropped for 3.5 ml into the vessel.

- The vessel was gently inverted several times to homogeneously mix the content, and the vessel was then placed in the preheated COD reactor.

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

- The vessel was placed into the spectrophotometer for reading COD value at 600 nm.

3.4.4 Mixed Liquor Suspended Solid (MLSS) Analysis

The microbial growth, which was a parameter for determining the degradation of organic compounds present in the reactor, can be measured in terms of mixed liquor suspended solids or MLSS (Apha, 1995). At steady state, the whole liquid and solid components were drained out from the reactor and then stirred with a stirrer until homogeneous mixing. 5 ml of welled-mixed sample were collected, filtrated through a glass fiber filter, washed with distilled water, and dried in an oven at 103-105°C for 1 h. The dried residue sample was determined for MLSS.

3.4.4.1 Apparatus

- Filtration apparatus
- Suction flask
- Glass-fiber filter disk (Pall-61631 A/E, 47 mm, 1μm)
- Drying oven
- Desiccators

3.4.4.2 Procedure

A. Preparation of glass-fiber filter disk

- The disk with wrinkle side up was inserted in the filtration apparatus.

- The disk was applied to vacuum and washed with three successive 20 ml of distilled water.

- The glass-fiber filter disk was dried in an oven at 105°C for 1 h.

- The disk was cooled in a desiccator to balance temperature and weighed.

B. 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 for complete filtration, filter size was increased or sample volume was decreased.

C. Sample analysis

- The filtering apparatus and filter were assembled.

- The filter was wet with a small volume of distilled water to stick it to the apparatus.

- A measured volume was pipetted onto the seated glass-fiber filter.

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

- The filter was carefully removed from filtration apparatus.

- The filter was dried at least 1 h at 103-105°C in an oven, cooled in desiccator to balance temperature, and weighed.

- The cycle was repeated.

3.4.4.3 Calculation

$$\frac{\text{mg mixed liquid suspended solid}}{\text{L}} = \frac{(\text{A-B}) \times 1000}{\text{Working volume of reactor}}$$

A= Weight of filter + dried residue [mg]

B= Weight of filter [mg]