# CHAPTER III
# EXPERIMENTAL

## 3.1 Materials

### 3.1.1 Cassava Wastewater

The cassava wastewater was screened to remove any large solid particles and used to feed the bioreactor without dilution and addition of any nutrient.

### 3.1.2 Seed Sludge and Substrates

Cassava wastewater was collected from the biogas pant at Ubon Biogas Co., Ltd., UbonRatchathani, Thailand.Seed sludge, a full-scale anaerobic plant treating cassava wastewater, was collected from SapthipCo., Ltd.,Lopburi, Thailand. Both seed sludge and cassava wastewater were kept at 4 °C before using. The anaerobic seed sludge is black color. A chemical oxygen demand (COD) value of the cassava wastewater used in this work was around 14,000mg·$L^{-1}$, as shown in Table 3.1. The ratio of COD:nitrogen:phosphorous was 100:1.43:0.85 which is higher than the theoretical ratio (COD:N:P = 100:1:0.4 for anaerobic decomposition for biogas production (Intanoo*et al.*, 2012) suggesting that the nitrogen and phosphorous contents in the wastewater were sufficient for bacteria growth.

**Table 3.1** Characteristics of the studied cassava wastewater

| **Parameters** | **Unit** | **Value** |
|---|---|---|
| pH | - | 4.51 |
| Total COD (Total chemical oxygen demand) | mg·$L^{-1}$ | 14,000 |
| Total nitrogen | mg·$L^{-1}$ | 200.01 |
| Total phosphorous | mg·$L^{-1}$ | 120 |
| Ammonium | mg·$L^{-1}$ | 2.00 |
| Nitrate | mg·$L^{-1}$ | 46.48 |
| Nitrite | mg·$L^{-1}$ | 1.04 |
| COD : N : P | - | 100 : 1.43 : 0.85 |

3.1.3 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
- Sodium hydroxide (NaOH), analytical reagent grade, Lab-scan, Thailand
- Phenolphthalein ($C_{20}H_{14}O_4$), analytical reagent grade, Labchem, Australia
- Sulfuricacid ($H_2SO_4$) 98 %, analytical reagent grade, Lab-scan, Thailand
- Methyl orange($C_{14}H_{14}N_3NaO_3S$), analytical reagent grade, Lab-scan, Thailand
- Sodium Thiosulfate Pentahydrate($Na_2S_2O_3 \cdot 5H_2O$), analytical reagent grade, Lab-scan, Thailand

## 3.2 Equipments

- Upflow anaerobic sludge blanket reactors (UASB)
- Wet gas meter, Ritter, TGO5/5
- Gas chromatograph(GC), AutoSystem, Perkin-Elmer
- High-performance liquid chromatography(HPLC), RI detector, UFLC, Shimadzu
- COD reactor, HACH DR3800
- Spectrophotometer, HACH DRB200
- pH electrode ,Cole-palmer, KH-27012-27
- Peristaltic pump

### 3.3 UASB Setup and Operation

Each of the upflow anaerobic sludge blanket (UASB) reactors was constructed from borosilicate glass with a 4 and 24 L working volume for hydrogen and methane UASB bioreactors, respectively. The operating temperature of both reactors was controlled at 37 °C.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. A schematic of the studied two-stage UASB unit used in this work is shown in Figure 3.2.

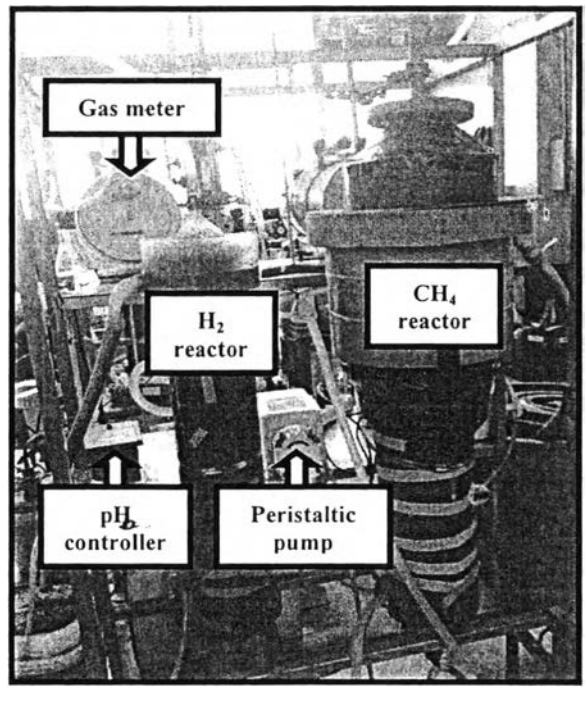


**Figure 3.1** Apparatus of UASB setup.

The 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 weregenerated.Athree-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.In the first part, cassava wastewater was digested in the anaerobic digestion without oxygen supply. The COD loading rate was varied from 12 to 60 $kg \cdot m^{-3} \cdot d^{-1}$corresponding to the feed flow rate and hydraulic retention time (HRT) shown in Table 3.2 to determine the optimum COD loading rate.

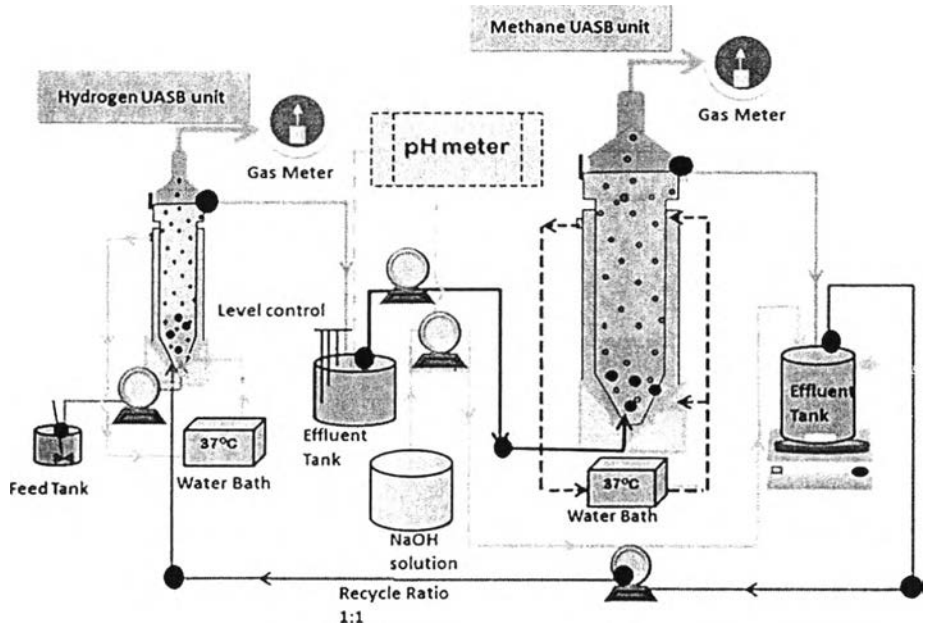


**Figure 3.2** Schematic of two stage upflow anaerobic sludge blanket (UASB) unit.

**Table 3.2** COD loading rate, flow rate, and hydraulic retention time (HRT) for determining the effect of COD loading rate on hydrogen UASB volume at pH 5.5 and on methane UASB volume without pH controlled under mesophilic temperature (COD = 14 $g \cdot L^{-1}$)

| Hydrogen UASB volume | | | Methane UASB volume | | |
|---|---|---|---|---|---|
| COD loading rate ($kg \cdot m^{-3} \cdot d^{-1}$) | Feed flow rate ($L \cdot d^{-1}$) | HRT (d) | COD loading rate ($kg \cdot m^{-3} \cdot d^{-1}$) | Feed flow rate ($L \cdot d^{-1}$) | HRT (d) |
| 12 | 1.715 | 4.640 | 2 | 3.430 | 14.000 |

| | | | | | |
|---|---|---|---|---|---|
| 24 | 3.430 | 2.320 | 4 | 6.860 | 7.000 |
| 36 | 6.860 | 1.160 | 6 | 13.720 | 3.500 |
| 48 | 13.720 | 0.580 | 8 | 27.440 | 1.750 |
| 60 | 27.440 | 0.290 | 10 | 54.880 | 0.875 |

In the second part, the optimum obtained in the first part were used to operate the two-stage UASB unit to investigate the effect of microaeration on methane UASB volume by vary oxygen supply rate from 0.0 to 6.1 $mLO_2 \cdot L^{-1}$wastewater$\cdot d^{-1}$(or 5.04 to 7.55 $mgO_2 \cdot g^{-1}$CODapplied$\cdot d^{-1}$)shown in Table 3.3. to determine the optimum value for the anaerobic hydrolysis of the cellulosic fraction.

**Table 3.3** Oxygen supply rate for determining the effect of oxygen added on methane UASB volume without pH controlled under mesophilic temperature(COD = 14 $g \cdot L^{-1}$)

| Oxygen supply rate ($mLO_2 \cdot L^{-1}$wastewater$\cdot d^{-1}$) | Oxygen supply rate ($mgO_2 \cdot g^{-1}$CODapplied$\cdot d^{-1}$) |
|---|---|
| 5.20 | 5.04 |
| 6.10 | 5.88 |
| 7.00 | 6.72 |
| 7.90 | 7.55 |

## 3.4 Measurements and Analytical Methods

### 3.4.1 COD Analysis

#### *3.4.1.1 Reagents*

- *Digestion solution.* 10.216 g of dried $K_2Cr_2O_7$ (primary standard grade), 167 ml of 98% $H_2SO_4$, and 33.3 g of $HgSO_4$ were added into 500 ml distilled water. Then 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% $H_2SO_4$. The mixture was left to stand for 1 to 2 d to completely dissolve the $Ag_2SO_4$.

*3.4.1.2* *Procedure*

Adilute sample of 2.5 ml was added to a digestion vial (HACH, 16×100 mm).Thedigestion 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 heated for 2 h in the preheated COD reactor (HACH, DR3800) shown in Figure 3.3a.After that,the vial was cooled to room temperature. Lastly, it was placed into the spectrophotometer (HACH, DRB200)for reading COD value, as shown in Figure 3.3b.

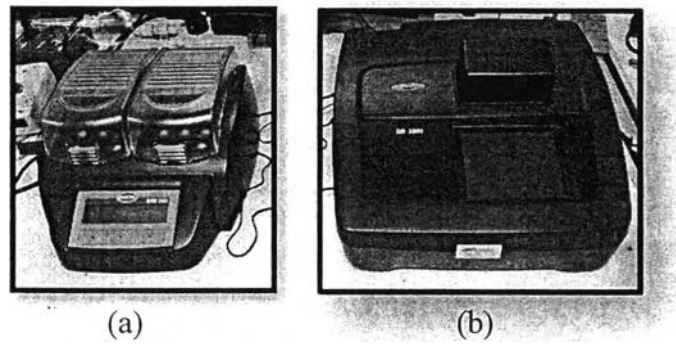


**Figure 3.3** (a)COD reactor and (b) spectrophotometer.

3.4.2 Total VFA and VFA Composition Analysis

The amount of VFAs in mg as acetic per liter and VFAs composition were analyzed by a high-performance liquid chromatography (HPLC), UFLC shimadzu equipped with a refractive index detector(RID detector). An HPLC instrument consists of 0.004 M of sulfuric acid which used to be mobile phase, deionized water(DI) filtrated by paper filter 0.45 micron used to bea solvent pump, and arefractive index detector used, all connected by tubing. Samples are injected into a stream of solvent being pumped through the column at flow rate 0.5 $mL \cdot min^{-1}$ under temperature 45 °C by using columnof C18. While passing through the column,

the analysts separate from one another. When they exit the column, a RID detector produces a signal proportional to the amount of each analyst.

3.4.3 *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 and detector temperatures were kept at 60, 35, and 150 °C, respectively. Argon was used as the carrier gas at pressure of 345 kPa.

3.4.4 Phosphorous Analysis

The total phosphorous in feed and effluent samples was determined by the molybdovanadate method with acid persulfate digestion (Hach Company). The sample cell was placed into the spectrophotometer (HACH DRB200)for determining phosphorous content.

3.4.5 Nitrogen Analysis

The nitrogen concentrations (in terms of organic-nitrogen by the diazotization, and cadmium reduction method and inorganic nitrogen by the salicylate method) in feed and effluent samples were carried out with the TNT persulfate digestion. The sample cell was placed into the spectrophotometer (HACH DRB200) for determining nitrogen content.

3.4.6 Microbial Concentration (MLVSS)

The microbial concentration in the system, which is a parameter for determining the degradation of organic compounds present in the reactor, can be measured in terms of microbial concentration or MLVSS. At steady state, the whole liquid and solid components are drained out from the reactor and then stirred with a stirrer until homogeneous mixing. The collected sample was filtrated through a glass fiber filter, washed 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.).

3.4.7 Microbial Washout (Effluent VSS)

The microbial washout from the system can be measured in terms of Effluent VSS. At steady state, the effluent liquid and solid components are drained out from the reactor. The collected sample was filtrated through a glass fiber filter,

washed with distilled water, and dried in an oven at 105 °C for 1 h. The dried residue sample was determined for TSS. 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.).