CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Substrates

Seed sludge, cassava wastewater, and cassava residue were collected from the biogas pant (Ubon Biogas Co., Ltd., Ubon Ratchathani, Thailand). Seed sludge and cassava wastewater were kept at 4 °C before use. The anaerobic seed sludge is black color, and has total suspended solids (TSS) concentration of 9,000 mg/l. A chemical oxygen demand (COD) value of the cassava wastewater used in this work was around 10,557 mg/l, as shown in Table 3.1. The ratio of COD:nitrogen:phosphorous was 100:2.5:0.8 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. The elemental and chemical compositions of cassava residue are shown in Tables 3.2.

Table 3.1 Characteristics of the studied cassava wastewater

Parameters	**Unit**	**Value**
pH	-	4.34
TS (Total solids)	mg/L	1,330
Total COD (Total chemical oxygen demand)	mg/L	10,557
Total nitrogen	mg/L	266.67
Total phosphorous	mg/L	80
Ammonium	mg/L	2.00
Nitrate	mg/L	46.67
Nitrite	mg/L	1.07
COD : N : P	-	100 : 2.5 : 0.8

Table 3.2 Elemental and chemical compositions of the studied cassava residue

Elemental composition	**wt%, dry basis**
Carbon	37.07
Hydrogen	5.89
Nitrogen	0.20
Oxygen	56.77
Sulfur	0.072
Chemical composition	**wt%, dry basis**
Starch	41.05
Hemicellulose	23.41
Cellulose	18.95
Lignin	5.63
Extractives	9.34
Ash	1.62

3.1.2 Chemicals

Sodium hydroxide (NaOH), and phenolphthalein ($C_{20}H_{14}O_4$, analytical reagent grade, Lab-scan) are used in this work.

3.2 Equipments

- Upflow anaerobic sludge blanket)UASB(reactors
- Wet gas meter, Ritter, TGO5/5
- Gas chromatograph)GC(, Perichrom, PR2100
- Gas chromatograph) GC(, Perkin-Elmer, AutoSystem GC

- COD reactor, HACH
- Spectrophotometer, HACH D/R 2700
- pH electrode, Cole-palmer KH-27012-27
- Elemental analyzer (TruSpec-CHN)
- Peristaltic pump

3.3 Methodology

3.3.1 Cassava Wastewater and Cassava Residue Preparation

The cassava wastewater was screened to remove any large solid particles and used to feed the bioreactor without dilution and addition of any nutrient. The cassava residue sample was dried at 105 °C. After that, it was crushed and milled to reduced particle size and finally sieved through a 60 mesh screen. (average diameter of the fermentation residue was about 213 μm).

3.3.2 Bioreactor Design and Dperation

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 55 °C. A schematic of the studied two-stage UASB unit used in this work is shown in Figure 3.1.

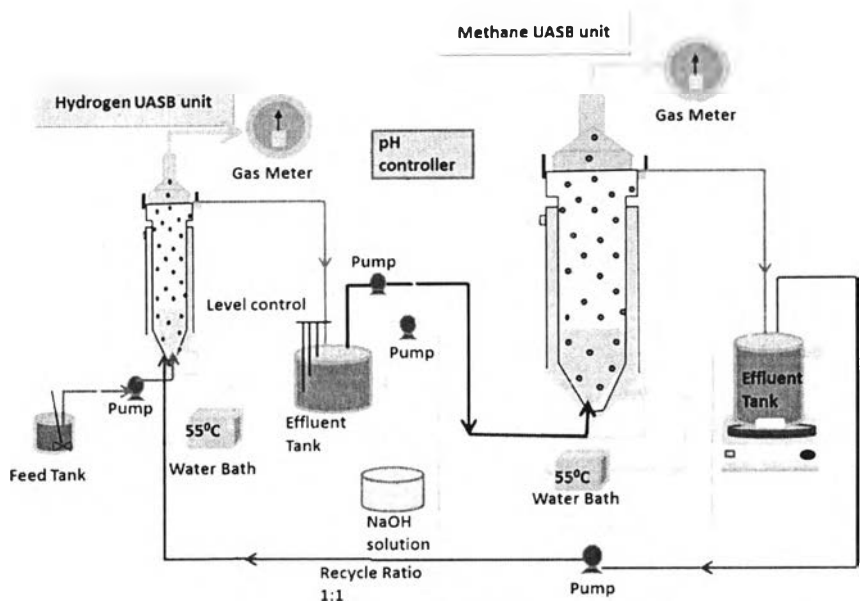


Figure 3.1 Schematic of two stage upflow anaerobic sludge blanket (UASB) unit.

The cassava wastewater with added different concentrations (300-1,500 mg/l) of the cassava residue was fed continuously to the bottom of the hydrogen UASB bioreactor under a COD loading rate of 12 kg/m^{3}d based on the methane bioreactor or 72 kg/m^{3}d based on the hydrogen bioreactor without added cassava residue.Yu et al. (2002) found that the pH value of 5.5 was the optimum pH for hydrogen production. So, the pH in this work was controlled at this value for the hydrogen bioreactor by using a pH controller with a 1 M NaOH solution. The effluent from the hydrogen UASB unit was directly pumped into the methane UASB bioreactor with a level control probe. The pH in the methane bioreactor was not controlled. The effluent of the methane bioreactor was pump to the hydrogen bioreactor with the recycle ratio of 1:1. At any cassava residue concentration, the system was operated for approximately 4 weeks to reach a steady state before taking the samples for analysis.

3.4 Analytical Methods

3.4.1 COD Analysis

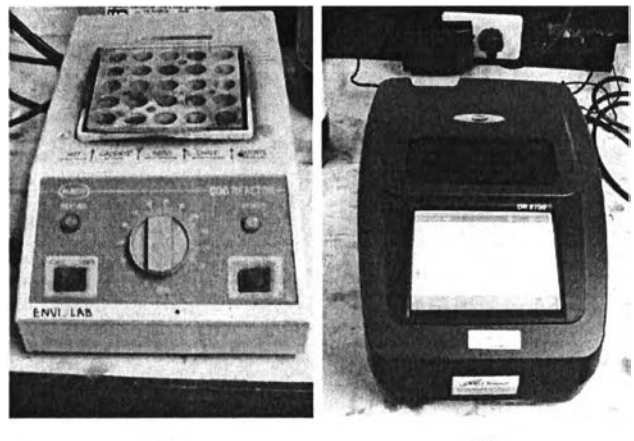
3.4.1.1 Reagents

- *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

A dilute sample of 2.5 ml was added to a digestion vial (HACH, 16×100 mm). The 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 heated for 2 h in the preheated COD reactor (HACH) (Figure 3.2a). Then, the vial was cooled to room temperature. Finally, it was placed into the spectrophotometer (HACH DR 2700) for reading COD value, as shown in Figure 3.2b.



(a) (b)

Figure 3.2 (a) COD reactor and (b) spectrophotometer.

3.4.2 Total VFA Analysis

The amount of VFAs in mg as acetic per liter was determined by a distillation-titration method. The effluent sample was distillated and titrated with 0.1 M NaOH using phenolphthalein as an indicator (Eaton *et al.*, 2005).

3.4.3 VFA Composition Analysis

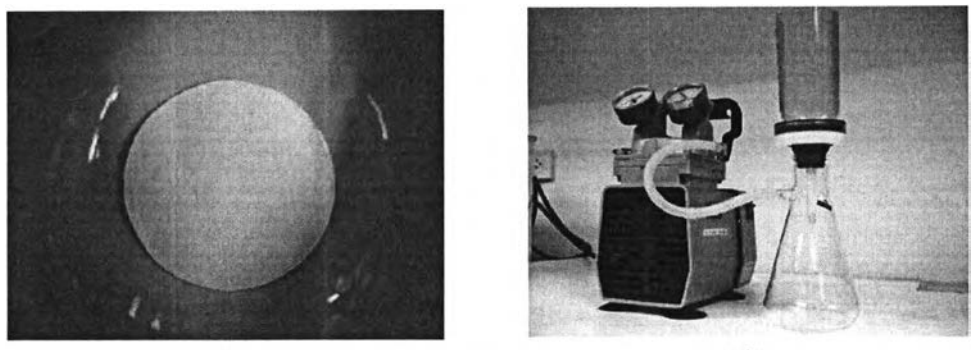
VFAs composition was analyzed by a gas chromatograph (PR2100, Perichrom) equipped with a flame ionization detector and a 50 m x 0.32 ID, 0.25 μm film thickness DB-WAXetr (J & W Scientific) capillary column in the split mode (10 mL/min) with helium at a pressure of 82 kPa as a carrier gas, H_2 at 50 kPa 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 are 250 and 270 °C, respectively.

3.4.4 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.

3.4.5 Total Suspended Solids (TSS) Analysis

A glass-fiber filter disk (Pall-61631 A/E, 47 mm, 1 μm) (Figure 3.3) was used for analysis of TSS according to the standard method:



(a) (b)

Figure 3.3 (a) glass-fiber filter disk and (b) filtration apparatus.

The calculation of ss is shown in the following equation:

$$\frac{\text{mg total suspend solids (TSS)}}{\text{L}} = \frac{(\text{A}-\text{B})\times 10^6}{\text{Sample volume, (mL)}} \qquad (3.1)$$

A = Weight of filter + dried residue [g]

B = Weight of filter [g]

3.4.6 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 DR 2700) for determining phosphorous content.

3.4.7 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 DR 2700) for determining nitrogen content.

3.4.8 Cassava Residue Composition Analysis

The dried sample of cassava residue was analyzed for elemental and chemical compositions. An elemental analyzer (TruSpec-CHN) was used to determine C, H, O, N and S contents in the sample. Combustion and burner temperatures were kept at 950 °C for C, H, 0, N and 850 °C for S with oxygen, helium, and air used as carrier gases. For the chemical composition analysis, a dried sample was extracted by acetone (60 ml acetone for 1 g of dried residue sample) at 90 °C for 2 h. After that, the sample was dried at 105 °C until a constant weight was obtained. The weight difference before and after the extraction was represented the extractive (oils and phenolic compounds) fraction in the sample. Then, 10 ml of a 0.5 M sodium hydroxide solution was added to the cassava residue from the previous step. The mixture was held at 80 °C for 3.5 h. After that, the sample was washed using distilled water until a neutral pH value of 7 is reached and dried to obtain a constant weight. The difference constant weight of the residue indicated the hemicellulose and starch fractions. The starch fraction was determined by the amylase/amyloglocosidase method using a starch assay kit (Sigma-Aldrich, Inc). To determine the amount of lignin, 30 ml of a 72 wt% sulfuric acid is added to the extractive-free dried residue. The mixture was maintained at 8–15 °C for 24 h. Then, it was transferred into a flask and diluted with 300 ml of distilled water. After that, the sample was boiled at 100 °C for 1 h. The mixture was filtered. The weight loss from this step showed the lignin fraction. So, the fraction of cellulose and ash was calculated by the difference of the cassava residue weight to that of extractives, hemicellulose, starch, and lignin. Finally, the undissolved solids were calcined at 550 °C for 1 h. The weight loss in this step indicated the cellulose fraction and the remained weight represented ash.

3.4.9 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.).

3.4.10 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.).