CHAPTER III

EXPERIMENTAL

3.1 Materials

- Anaerobic seed sludge was collected from the biogas plant at Sapthip Lopburi Co.,Ltd., Thailand. Its chemical characteristics was measured as follows: colour, pH, TSS, TVS and VSS.
- Cassava wastewater was collected from Ubon Biogas Co.,Ltd., Thailand. Its chemical characteristics was measured as follows: COD, total nitrogen, ammonia, nitrite, nitrate, and total phosphorous.
- Anaerobic seed sludge and cassava wastewater will be kept at 4 °C before experiments.

3.2 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
- Sulfuric acids (H_2SO_4) 98 %, analytical reagent grade, Lab-scan, Thailand
- Hydrochloric acid (HCl) 37 %, analytical reagent grade, Lab-scan, Thailand
- Sodium hydroxide (NaOH), analytical reagent grade, Lab-scan. Thailand
- Phenolphthalein ($C_{20}H_{14}O_4$), analytical reagent grade, Labchem, Australia
- N,N-bis (carboxymethyl) glutamic acid (GLDA), Akzo Nobel, America
- Ethylenediaminetetraacetic acid (EDTA), Carlo Erba, Rodano Ni, America

- N,N-bis (carboxymethyl) glutamic acid (GLDA), Akzo Nobel, America

Table 3.1 Characteristics of the seed sludge

Parameters	Unit	Value
Color	-	Dark
TSS (Total suspended solids)	mg/l	8,940
TVS (Total volatile solids)	mg/l	8,880
Nitrogen content in 1 g of dried seed sludge	g/L	0.4

Table 3.2 Characteristics of the cassava wastewater

Parameters	Unit	Value
Total COD (Total chemical oxygen demand)	mg/l	14,000
Total nitrogen	mg/l	200
Total phosphorous	mg/l	120
COD:N:P	-	100:1.43:0.86
Ammonium	mg/l	2.00
Nitrate	mg/l	167
Nitrite	mg/l	1.31
pH	-	4.34

3.3 Equipment

- Continuous stirred tank reactor (CSTR)
- Gas chromatograph (GC), Perichrom, PR2100
- Gas chromatograph (GC), Perkin-Elmer, AutoSystem GC
- COD reactor, HACH
- Spectrophotometer, HACH DR 2700
- pH electrode, ECFG7350401B
- Spectro photo meter, AAS VArience
- Feed pump
- Wet gas meter, Ritter, TGO5/5

3.4 Methodology

3.4.1 Seed Sludge Preparation

Anaerobic seed sludge will be filtered through the sieve with size of 1 mm in order to remove the large particles and debris. Then, it was added to CSTR reactor with volume of 500 mL for methane tank.

3.4.2 Substrate Preparation

Cassava wastewater contained high concentration of soluble organic compounds categorized as carbohydrate-containing wastewater (Gerardi, 2006). Its COD:N:P (Chemical Oxygen Demand: Nitrogen: Phosphorus) ratio were investigated to show value of 100:0.43:0.86, corresponding. It will be filtered through the sieve with size of 0.2 μm in order to remove the large particles and debris.

3.4.3 The CSTR Operation

A continuous stirred tank reactor (CSTR) will be used as the anaerobic digester in order to perform the experiments for anaerobic digestion of cassava wastewater. The schematic of the CSTR process is shown in Figure 3.1. The system will be operated without light illumination in a PVC reactor to inhibit the activity of photosynthetic bacteria. The CSTR will be operated with liquid working volume of 4 litres and has the magnetic stirrer for mixing during the period of anaerobic digestion. The cassava wastewater will be fed to the top of the CSTR by carrying it from the feed tank through the feed pump. The temperature of experiments is at ambient temperature. pH was not be controlled. The biogas will be analyzed for the production rate and compositions by a gas meter and GC, respectively. Besides, the overflown liquid effluent will be collected and analyzed for chemical characteristics.

The cassava wastewater with micronutrients will be fed to the top of the CSTR by carrying it from the feed tank through the feed pump and analyzed the overflown liquid effluent for chemical characteristics.

The cassava wastewater with different concentrations of EDTA, GLDA, $FeCl_3$ and $CoCl_2$ will be fed to the top of the CSTR by carrying it from the feed tank through the feed pump and analyzed the overflown liquid effluent for chemical characteristics.

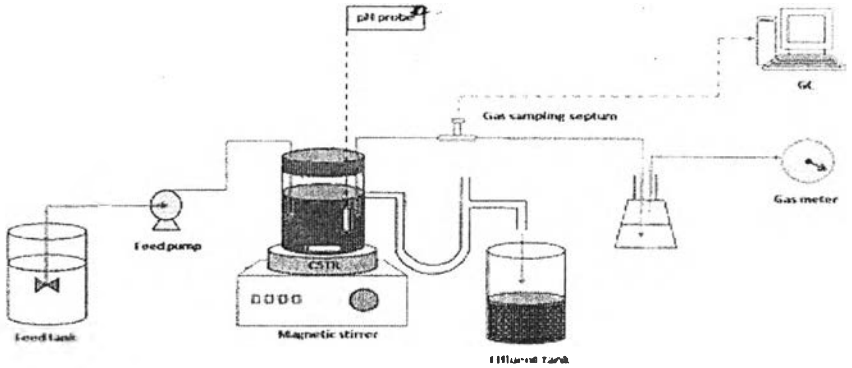

Figure 3.1 Schematic of the studied CSTR process.

3.5 Measurement and Analytical Method

At constant COD loading rate, the system was operated until reaching steady state condition which indicated by the gas composition and gas production rate. The effluent and inoculums sample was taken foe analysis and measurement. The standard method used in this research was listed below.

3.5.1 COD Analysis

COD value was determined by the closed reflux, colorimetric method. Sample was added to a digestion vial (HACH, 16 × 100 mm). The digestion reagent was added to the vial. Afterwards, the sulfuric acid reagent was slowly dropped into the vial. The vial was inverted several times to homogeneously mix the contents, and the vial was placed in the COD reactor (HACH) that sample was heated for 2 hr and left for 20 min to be cooled. Then, the sample was determined for COD value by spectrophotometer (HACH DR 2700).

3.5.2 Nitrogen Analysis

Nitrogen value was determined in term of organic nitrogen by the diazotization and cadmium reduction method and in term of inorganic nitrogen by the salicylate method. Sample was carried out with the TNT persulfate digestion. The sample cell was placed into the spectrophotometer (HACH DR 2700) for determining nitrogen value.

3.5.3 Phosphorous Analysis

Phosphorous value was determined by the molybdovanadate method with acid persulfate digestion. The sample cell was placed into the spectrophotometer (HACH DR 2700) for determining phosphorous value.

3.5.4 Total VFA and VFA Composition Analysis

The liquid composition was determined by a High performance liquid chromatography (HPLC) (Shimadzu Prominence UFLC), equipped with a reflective index detector and a 300 x 7.8 mm, carbohydrate analysis column, hydrogen form, 9 μm particle size, 8 % cross linkage, pH range 1–3, 0.5 ml/min (APX-87H, Aminex) in the split mode (0.5 ml/min) with 4 mM H_2SO_4 as a mobile phase. The column temperature program was preserved at 45 °C with retention time of 60 minute

3.5.5 pH Analysis

The pH value was determined by a pH electrode (ECFG7350401B).

3.5.6 The Amount of Produced Gas

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

3.5.7 Gas Composition Analysis

The gas composition will be determined by the GC (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 will be kept at 60, 35, and 150 °C, respectively. Argon is used as the carrier gas at pressure of 345 kPa.

3.5.8 TSS Analysis

3.5.8.1 Procedure

3.5.8.1.1 Preparation of Glass-fiber Filter Disk (Pall-61631 A/E, 47 mm, 1 μm):

- The glass-fiber filter disk with wrinkled side up was inserted in the filtration apparatus. After that, it was to vacuumed and 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 a desiccator to balance the temperature, and then weighed.

3.5.8.1.2 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 was required to complete the filtration, either the filter size was increased or the sample volume was decreased.

3.5.8.1.3 Sample Analysis:

- The filtering apparatus and filter were prepared.
- The filter was wet with a small volume of distilled water.
- A sample was homogeneously mixed before testing.
- 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 the filtration apparatus, dried at least 1 h at 103 to 105 °C in an oven, cooled in a desiccator, and then weighed.

- The cycle was repeated until the sample weight nearly constant (less than 4 % difference).
- The TSS is calculated by Equation (3.1)

3.5.8.1.4 Calculation

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

where A = Weight of filter + dried residue (g)

B = Weight of filter (g)

3.5.9 VSS Analysis

3.5.9.1 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.
- The VSS is calculated by Equation (3.2)

3.5.9.2 Calculation

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

A = Weight of residue + disk before ignition (g)

B = Weight of residue + disk after ignition (g)

3.5.10 Micronutrients Analysis

All micronutrients were determined by Spectrophotometer (AAS Varience). The collected effluent after the system reached to steady state condition were added acid for digestion depend on type of micronutrient. After that boil at 110°C for 15 minutes and filter through filter paper Measurement by AAS Varience used three different concentration of standard per element

3.6 Parameters

There are many parameters shown the performance of methane production. The reactions were listed below.

3.6.1 COD removal

The COD removal relation is shown in Equation (3.3)

$$\text{COD removal } (\%) = \frac{\text{Effluent COD - Feed COD}}{\text{Feed COD}} \times 100 \qquad (3.3)$$

3.6.2 Methane Yield (ml CH_4/g COD removed)

The methane yield in term of ml CH_4/g COD removed is shown in Equations (3.4)

$$\text{Methane yield } \left(\frac{\text{ml } CH_4}{\text{g COD removed}}\right) = \frac{\text{Methane production rate}}{\text{Feed flow rate} \times \text{COD removed}} \qquad (3.4)$$

3.6.3 Methane Yield (ml CH_4/g COD applied)

The methane yield in term of ml CH_4/g COD applied is shown in Equations (3.5)

$$\text{Methane yield}\left(\frac{\text{ml } CH_4}{\text{g COD applied}}\right) = \frac{\text{Methane production rate}}{\text{Feed flow rate} \times \text{COD applied}} \qquad (3.5)$$

3.6.4 Specific Methane Production Rate (SMPR) (ml CH_4/L_R d)

The SMPR in term of ml CH_4/L_R d is shown in Equations (3.6)

$$\text{SMPR}\left(\frac{\text{ml } CH_4}{L_R \text{ d}}\right) = \frac{\text{Methane production rate}}{\text{Reactor volume}} \qquad (3.6)$$

3.6.5 Specific Methane Production Rate (SMPR) (ml CH_4/g MLVSS d)

The SMPR in term of ml CH_4/g MLVSS d is shown in Equations (3.7)

$$\text{SMPR}\left(\frac{\text{ml } CH_4}{\text{g MLVSS d}}\right) = \frac{\text{Methane production rate}}{\text{g MLVSS} \times \text{Reactor volume}} \qquad (3.7)$$