

CHAPTER III EXPERIMENTAL

3.1 Materials

Seed sludge, cassava wastewater, and cassava residue were collected from the biogas plant at Ubon Biogas Co., Ltd., Ubon Ratchathani, Thailand. Their characteristics were investigated and are shown in Tables 3.1 - 3.7.

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
- Sodium hydroxide (NaOH), analytical reagent grade, Lab-scan, Thailand
- Phenolphthalein ($\text{C}_{20}\text{H}_{14}\text{O}_4$), analytical reagent grade, Labchem, Australia
- Sulfuric acid (H_2SO_4) 98 %, analytical reagent grade, Lab-scan, Thailand
- Methyl orange ($\text{C}_{14}\text{H}_{14}\text{N}_3\text{NaO}_3\text{S}$), analytical reagent grade, Lab-scan, Thailand
- Sodium Thiosulfate Pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$), analytical reagent grade, Lab-scan, Thailand

Table 3.1 Characteristics of the seed sludge

Parameters	Unit	Value
Colour	-	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	0.3998

Table 3.2 Characteristics of the cassava wastewater

Parameters	Unit	Value
Total COD (Total chemical oxygen demand)	mg/L	10,557
Settled COD (Settled chemical oxygen demand)	mg/L	10,417
Soluble COD (Soluble chemical oxygen demand)	mg/L	9,408
Total nitrogen	mg/L	266.67
Total phosphorous	mg/L	80
COD : N : P	-	100 : 2.5 : 0.8
Ammonium	mg/L	2.00
Nitrate	mg/L	46.67
Nitrite	mg/L	1.07
pH	-	4.34
Total VFA (Total volatile fatty acid)	mg/L	576.78
Total acidity	mg/L	838.33
TS (Total solids)	mg/L	1,330
VS (Volatile solids)	mg/L	1,324

Table 3.3 Characteristics of the cassava residue

Parameters	Unit	Value
TS (Total solids)	(g/g of dried TS)	0.994
TVS (Total volatile solids)	(g/g of dried TS)	0.976
Ash	(g/g of dried TS)	0.018
COD (Chemical oxygen demand)	(g/g of dried TS)	0.806
Total nitrogen	(g/g of dried TS)	0.031
Total phosphorous	(g/g of dried TS)	0.004
COD : N : P	-	100 : 3.8 : 0.5
Moisture	%	11.63
Carbon	%	37.07
Hydrogen	%	5.89
Nitrogen	%	0.20
Sulphur	%	0.07
Oxygen	%	56.77
Extractives	%	9.34
Hemicellulose	%	23.41
Starch	%	41.05
Lignin	%	5.63
Cellulose	%	18.95
Ash	%	1.62
Particle size (Use air as medium) -	µm	153.32
Particle size (Use water as medium)	µm	212.91

Table 3.4 Characteristics of the cassava wastewater with added cassava residue of 250 ppm

Parameters	Unit	Value
Total COD (Total chemical oxygen demand)	mg/L	10,861
Settled COD (Settled chemical oxygen demand)	mg/L	10,671
Soluble COD (Soluble chemical oxygen demand)	mg/L	9,612
Total nitrogen	mg/L	304.43
Total phosphorous	mg/L	112
COD : N : P	-	100 : 2.8 : 1.0
Ammonium	mg/L	2.17
Nitrate	mg/L	48.33
Nitrite	mg/L	1.07
pH	-	4.33
TS (Total solids)	mg/L	1,576
VS (Volatile solids)	mg/L	1,569

Table 3.5 Characteristics of the cassava wastewater with added cassava residue of 500 ppm

Parameters	Unit	Value
Total COD (Total chemical oxygen demand)	mg/L	11,114
Settled COD (Settled chemical oxygen demand)	mg/L	10,873
Soluble COD (Soluble chemical oxygen demand)	mg/L	9,765
Total nitrogen	mg/L	342.18
Total phosphorous	mg/L	134
COD : N : P	-	100 : 3.1 : 1.2
Ammonium	mg/L	2.17
Nitrate	mg/L	48.33
Nitrite	mg/L	1.10
pH	-	4.33
TS (Total solids)	mg/L	1,820
VS (Volatile solids)	mg/L	1,813

Table 3.6 Characteristics of the cassava wastewater with added cassava residue of 1,000 ppm

Parameters	Unit	Value
Total COD (Total chemical oxygen demand)	mg/L	11,632
Settled COD (Settled chemical oxygen demand)	mg/L	11,291
Soluble COD (Soluble chemical oxygen demand)	mg/L	10,083
Total nitrogen	mg/L	417.69
Total phosphorous	mg/L	198
COD : N : P	-	100 : 3.6 : 1.7
Ammonium	mg/L	2.33
Nitrate	mg/L	50.00
Nitrite	mg/L	1.13
pH	-	4.32
TS (Total solids)	mg/L	2,310
VS (Volatile solids)	mg/L	2,302

Table 3.7 Characteristics of the cassava wastewater with added cassava residue of 1,500 ppm

Parameters	Unit	Value
Total COD (Total chemical oxygen demand)	mg/L	12,180
Settled COD (Settled chemical oxygen demand)	mg/L	11,740
Soluble COD (Soluble chemical oxygen demand)	mg/L	10,431
Total nitrogen	mg/L	493.21
Total phosphorous	mg/L	242
COD : N : P	-	100 : 4.0 : 2.0
Ammonium	mg/L	2.33
Nitrate	mg/L	53.33
Nitrite	mg/L	1.15
pH	-	4.32
TS (Total solids)	mg/L	2,800
VS (Volatile solids)	mg/L	2,791

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
- DO meter, DØ-5512SD
- Feed pump

3.4 Methodology

- Seed sludge was filtered through the sieve with the size of 1 mm in order to remove large particles and debris.

- Cassava wastewater was filtered through the sieve with the size of 0.2 μm in order to remove large particles and debris.

- A continuous stirred tank reactor (CSTR) was used as the anaerobic digester for cassava wastewater. The schematic of the CSTR process is shown in Figure 3.1. The system was operated without light illumination in a PVC reactor to inhibit the activity of photosynthetic bacteria. The CSTR was operated with liquid working volume of 4 litres and had a magnetic stirrer for mixing during the anaerobic digestion. The cassava wastewater was fed to the top of the CSTR by carrying it from the feed tank through the feed pump. Oxygen was supplied to the top of the CSTR. Oxygen concentration of the system was measured by a DO meter. The temperature was at ambient temperature. The biogas was analyzed for the production rate and composition by a gas meter and GC, respectively. Besides, the overflown liquid effluent was collected and analyzed for chemical characteristics.

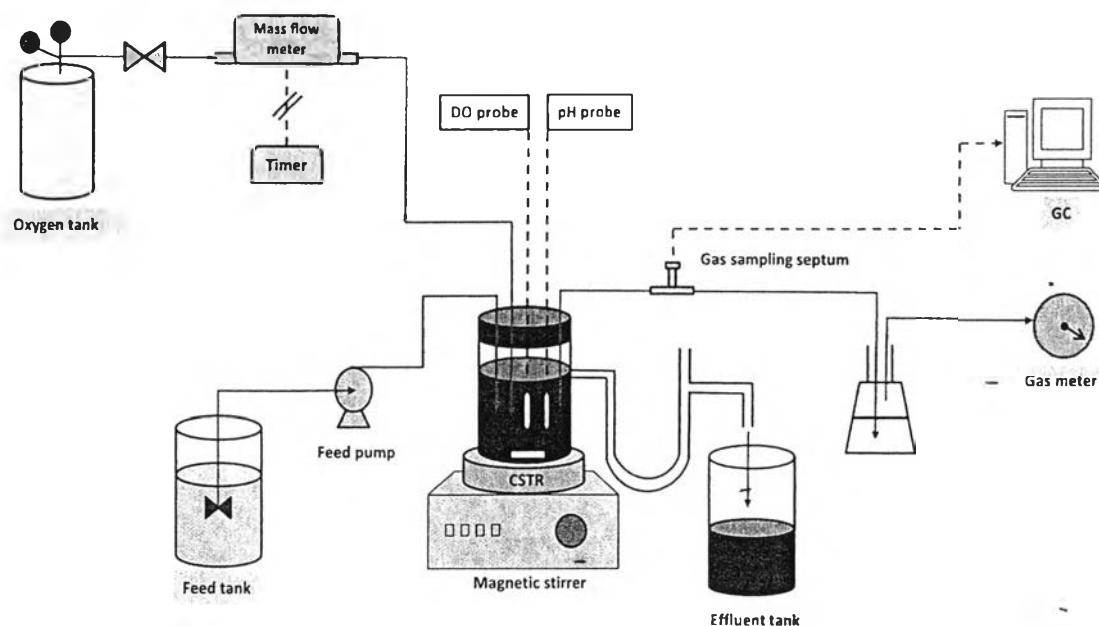


Figure 3.1 Schematic of the studied CSTR process.

- During the system start-up, the CSTR was seeded with the anaerobic seed sludge, which was allowed to acclimatise with the cassava wastewater at ambient temperature. Supplementary nutrient for bacterial growth was supplied depending on ratio of COD : N : P. Experiments were carried out as follows:

- The cassava wastewater was digested in the anaerobic digestion without oxygen supply. The COD loading rate was varied from 0.604 to 2.500 kg/m³ d corresponding to the feed flow rate and hydraulic retention time (HRT) shown in Table 3.8 to determine the optimum COD loading rate.

Table 3.8 Operating conditions for the CSTR system to determine the optimum COD loading rate

COD loading rate (kg/m ³ d)	Feed flow rate (x10 ⁻⁴ m ³ /d)	HRT (d)
0.604	2.29	17.47
1.212	4.59	8.71
1.710	6.48	6.17
2.500	9.48	4.22

- Contents of cassava residue were varied and mixed with the cassava wastewater at the feed tank. The cassava wastewater with added cassava residue was fed to the CSTR and digested without microaeration under the optimum COD loading rate. Contents of cassava residue were varied from 250 to 1,500 ppm shown in Table 3.9 to determine the optimum content of added cassava residue for methane production.

Table 3.9 Operating conditions for the CSTR system to determine the optimum content of added cassava residue

Cassava residue concentration (ppm)	Total COD loading rate (kg/m ³ d)
250	1.760
500	1.800
1,000	1.884
1,500	1.973

- Oxygen was supplied to the CSTR for 5 min every 2 hr, while the cassava wastewater with the optimum content of added cassava residue was digested under the total optimum COD loading rate. The oxygen supply rate was varied from 1.5 to 6.0 mL O₂/L_R d shown in Table 3.10 to determine the optimum value for the anaerobic hydrolysis of the cellulosic fraction.

Table 3.10 Operating conditions for the CSTR system to determine the optimum oxygen supply

Oxygen supply rate (mL O ₂ /L _R d)	Oxygen flow rate (mL/d)
1.5	6.0
3.0	12.0
4.5	18.0
6.0	24.0

Finally, hydrolysis efficiency of cassava wastewater was calculated according to;

$$\text{Hydrolysis efficiency} = (\text{SCOD}_{\text{in}} - \text{SCOD}_{\text{out}}) / \text{SCOD}_{\text{in}} \quad (3.1)$$

where

SCOD_{in}	=	Inlet soluble COD	(mg/L)
SCOD_{out}	=	Outlet soluble COD	(mg/L)

3.5 Analytical Techniques

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 Analysis

The amount of VFA was determined by distillation-titration method. This technique recovered acids containing up to six carbon atoms and reported the results in term of acetic acid.

3.5.5 VFA Composition Analysis

The liquid composition was determined by a gas chromatograph (PR2100, Perichrom) equipped with a flame ionization detector and a 50m 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 $^\circ\text{C}$, heated to 125 $^\circ\text{C}$ at a ramping rate of 10 $^\circ\text{C}/\text{min}$, held for 2 min, then heated to 180 $^\circ\text{C}$ at a ramping rate of 15 $^\circ\text{C}/\text{min}$, and held for 15 min. The temperatures of injector and detector were 250 and 270 $^\circ\text{C}$, respectively.

3.5.6 pH Analysis

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

3.5.7 Dissolved Oxygen (DO) Analysis

DO value was determined by a DO meter (DO-5512SD).

3.5.8 Amount of Produced Gas

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

3.5.9 Gas Composition Analysis

The gas composition of the produced gas was 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. The injector, column, and detector temperatures were kept at 60, 35, and 150 $^\circ\text{C}$, respectively. Argon was used as the carrier gas at pressure of 345 kPa.

3.5.10 TSS Analysis

3.5.10.1 *Procedure*

- 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 vacuumed and washed with three successive 20 cm^3 of distilled water.

- The glass-fiber filter disk was dried in an oven at 105 $^{\circ}\text{C}$ for 1 h, left to be cooled in a desiccator to balance the temperature, and then weighed.

- 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.

- 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 $^{\circ}\text{C}$ in an oven, cooled in a desiccator, and then weighed.

- The cycle was repeated until the sample weight nearly constant (less than 4% difference).

3.5.10.2 Calculation

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

where

A = Weight of filter + dried residue (g)

B = Weight of filter (g)

3.5.11 VSS Analysis

3.5.11.1 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.

3.5.11.2 Calculation

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

where

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

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

3.5.12 Preparation of Cassava Residue and Composition Analysis

The cassava residue sample was dried at 105 °C and stored in sealed plastic bags. Then, the dried cassava residue sample was milled to reduce the particle size and sieved to size below 60 mesh. Afterwards, the sieved cassava residue sample was investigated for the physical and compositional properties as follows:

- The 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 and 850 °C, respectively, with oxygen, helium, and air used as carrier gases.

- To determine the amount of extractives in the sample, solvent extraction (60 mL acetone for 1 g of dried cassava residue sample) was used, and the extraction was performed at 90 °C for 2 hr. After that, the sample was dried at 105 °C until a constant weight was obtained. The weight difference before and after the extraction was defined as the amount of extractives. -

- To determine the amount of hemicellulose, 10 mL of 0.5 M sodium hydroxide solution was added to 1 g of the extractive-free dried cassava residue, and the mixture was held at 80 °C for 3.5 hr. After that, the sample was washed using distilled water until a neutral pH value of 7 was reached. Then, it was dried to obtain a constant weight. The weight difference before and after this alkaline treatment was defined as the hemicellulose content. The starch fraction in the NaOH dissolution solution was then determined by the amylase/amyloglucosidase method using a starch assay kit (Sigma-Aldrich, Inc).

- To determine the amount of lignin, 30 mL of 72 wt% sulfuric acid was added to the extractive-free dried cassava residue. The mixture was kept at 8 - 15 °C for 24 hr. 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 hr. The mixture was filtered, and then the sample was washed until the sulfate ion in the filtrate was not detected (via titration with a 10% barium chloride solution). The remaining solid was finally dried to obtain a constant weight. The weight of the residue was defined as the lignin content.

- The cellulose content was calculated by the difference of the biomass weight to that of extractives, hemicelluloses, starch, and lignin.

3.5.13 Total Acidity Analysis

Total acidity value was determined by indicator method that was the sample titration with standard NaOH solution.

$$\text{Total acidity, mg CaCO}_3/\text{L} = \frac{A \times 1,000}{\text{Sample volume, mL}} \quad (3.4)$$

where $A = \text{Volume of standard NaOH solution used (mL)}$

3.5.14 Total Alkalinity Analysis

Total alkalinity value was determined by indicator method that was the sample titration with standard H₂SO₄ solution.

$$\text{Total alkalinity, mg CaCO}_3/\text{L} = \frac{A \times 1,000}{\text{Sample volume, mL}} \quad (3.5)$$

where $A = \text{Volume of standard H}_2\text{SO}_4 \text{ solution used (mL)}$