CHAPTER III

METHODOLOGY

3.1 Materials

3.1. 1 Configuration of the completely-mixed anaerobic reactors

The two simulated completely-mixed anaerobic reactors were constructed using clear acrylic tubing. The reactors had a diameter of 14 cm and a height of 18 cm. The reactors were assembled with two 0.8 cm outer diameter acrylic flanges at both ends to provide support for top lid. A coating of silicon was applied to the interior and exterior of the flanged joints to ensure that the junctions would be water and gas tight. The design and operation features of the simulated completely-mixed anaerobic reactors are shown in Figure 3.1.

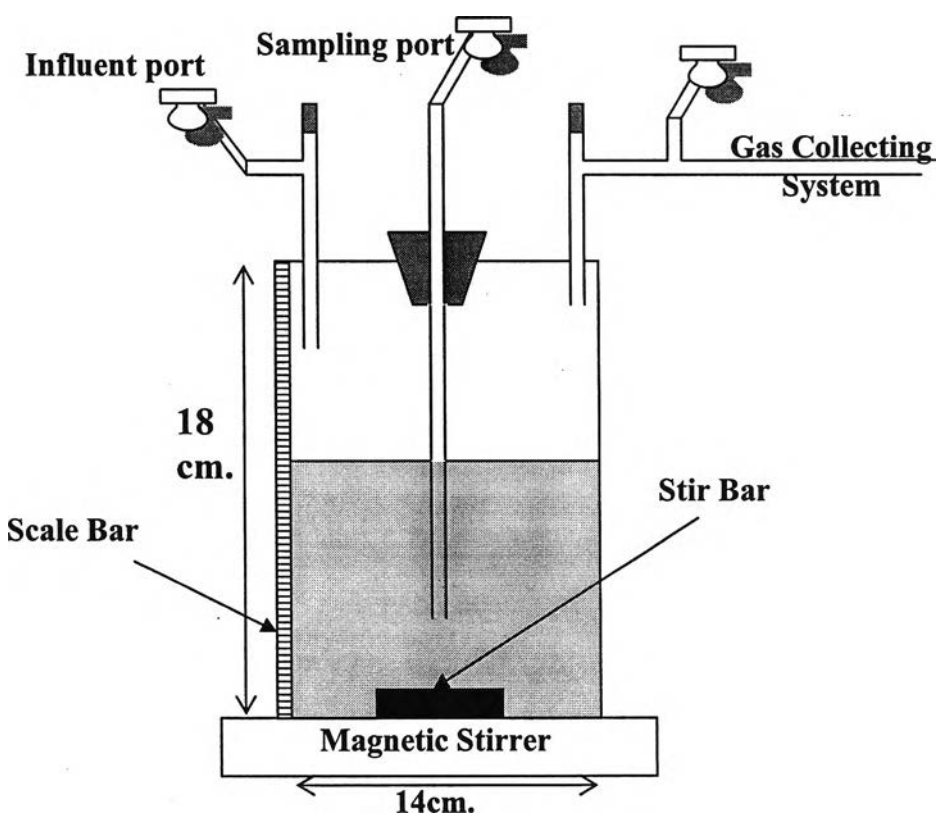

Figure 3.1 Configuration of the completely-mixed anaerobic reactors.

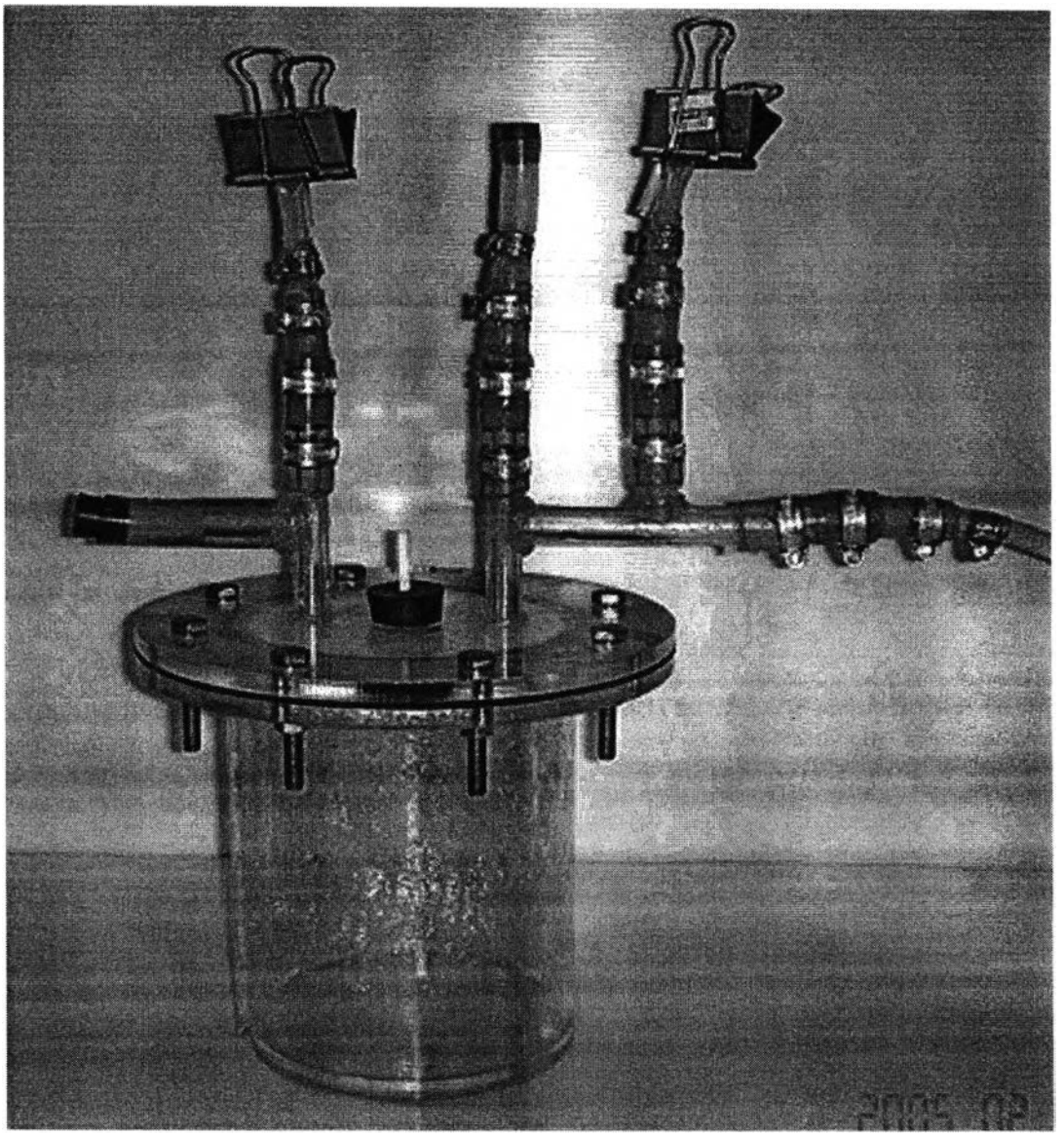

Figure 3.2 Completely-mixed anaerobic reactors

3.1.2 Sludge seeding characteristics

To facilitate initiation of anaerobic condition, sludge from a digested sludge sample obtained from an anaerobic digester at Nongkam wastewater treatment plant was prepared for completely-mixed anaerobic reactors. The seeding characteristics were presented in tables 3.1.

Table 3.1 Analysis of sludge from Nongkam wastewater treatment plant.

Parameter	Analysis
pH	6.97
Oxidation Reaction Potential (ORP)	-190.2 mV.
Biochemical Oxygen Demand (BOD)	400 mg/l
Chemical Oxygen Demand (COD)	2000 mg/l
Total Solid	42 g/l
Volatile Fatty Acid	40 mg/l
Alkalinity (mg/l as $CaCO_3$)	95 mg/l

3.1.3 Carbon source

The saw dust was slow releases carbon source because it was difficult to be digested by bacteria. So, the generation of the volatile fatty acids was quite low. It was easy to control VFA/Alk ratio in the reactors. The saw dust from saw factory was used as carbon source for this experiment. The properties of saw dust were reported in table 3.2

Table 3.2 Analysis of the saw dust added to reactor mixture.

Parameter	Analysis
Particle size	Less than 0.425 mm
Biochemical Oxygen Demand (BOD)	500 mg/g saw dust
Chemical Oxygen Demand (COD)	212,000 mg/g saw dust

3.1.4 Nutrients

The nutrients were necessary for microorganism. So the nutrient was prepared for experiment with composition in Table 3.3.

Table 3.3 Composition of Nutrient added to the reactor mixture (Tuppurainen et al., 2002).

Compositions	Amount
NaCl	3.20 g
NH_4Cl	4.00 g
KCl	0.40 g
KH_2PO_4	0.40 g
$MgSO_47H_2O$	0.80 g
$CaCl_2.2H_2O$	0.16 g

3.1.5 Reactor mixture

The reactor mixture was prepared by mixed seed sludge (for enriching bacteria), water (for diluting the reactor mixture), saw dust (for carbon source), nutrient, and $MgSO_4.7H_2O$ (For sulfur source) according to the ratio was presented in Table 3.4 (Tuppurainen et al., 2002).

Table3.4 The composition of reactor mixture (adapted from Tuppurainen et al., 2002).

Compositions	Amount
Seed Sludge	500 ml/l
Water	490 ml/l
Saw Dust	18 g/l
Nutrient	10 ml/l
$MgSO_4.7H_2O$	0.08g/l

3.1.6 Synthesized wastewater

The industrial wastewater contaminated with zinc was prepared for experiments. The synthesized wastewater was made using $ZnSO_4$.

The industrial wastewater was synthesized by diluting 4.4g. $ZnSO_4 7H_2O$ in 100 ml. water (1 ml. of synthesized water contains 10 mg of Zn).

3.2 Methods

3.2.1 Material processing and preparation

The materials (sludge, saw dust) were analyzed for basic properties as showed in table 3.1 and table 3.2. The basic properties analysis method were showed in table 3.5

Table 3.5 Methods of basic properties analyses

Parameter	Method
BOD*	Standard Methods for water and wastewater Examination# 4500-O. (Titration Method)
COD*	Standard Methods for water and wastewater Examination# 4500-COD. (Titration Method)
TS	Standard Methods for water and wastewater Examination# 2540 B. (Total Solids Dried at 103-105 °C)
VFA	Standard Methods for water and wastewater Examination# 4500. (Titration Method)
Alk	Standard Methods for water and wastewater Examination# 4500-P. (Titration Method)

* For saw dust, put 0.01g into 10 ml of water and analyze it like wastewater.

3.2.1.1 Preparation of sludge seeding analysis

The sludge seeding was prepared before mixing into reactor mixture by keeping in the 25 liter dark gallon. Saw dust was added into the dark gallon to start up sludge. The sludge was measured for temperature, oxidation reduction potential (ORP) and pH every day. ORP and pH in figure 3.3 and 3.4 showed the anaerobic condition that was established in 39 days during storage.

ORP

From figure 3.3 the ORP was dropped from -103 mV at the beginning to -190 mV at the 39th day. It showed the anaerobic condition was established in the sludge storage.

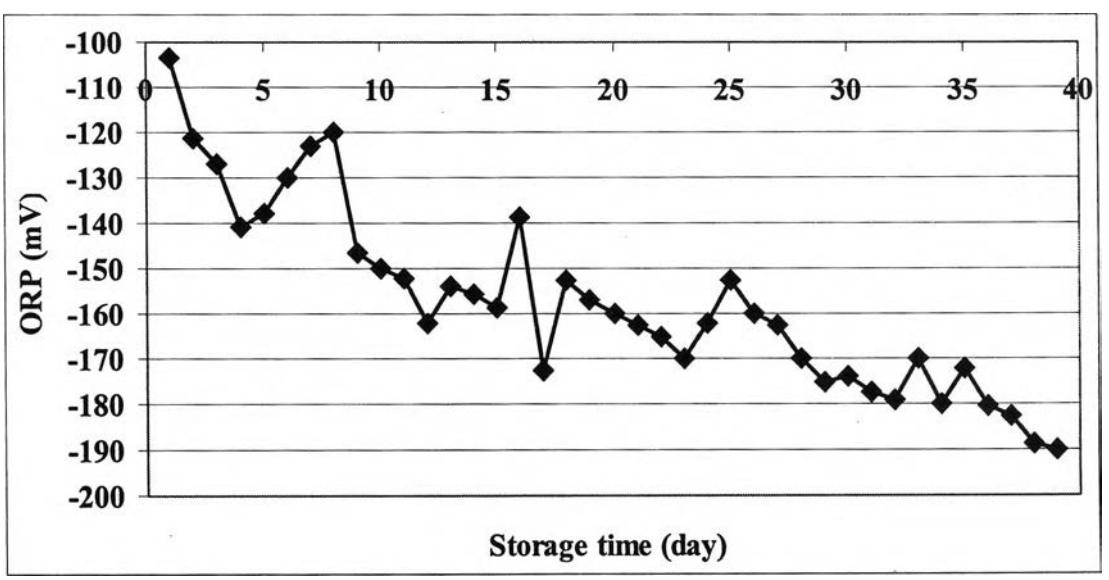

Figure 3.3 Daily ORP in storage (dark gallon)

pH

In figure 3.4, the pH was at the level of 6.75 for the first time and would increase a little bit within seven days. The next three days (from the 8th to the 10th day) pH would decrease (from 6.9 to 6.4). After the 10th day, the pH would increase until the 16th day. During this early period the anaerobic bacteria would have an adjustment and become still later on. There was a change from 6.3-7.1 between the 10th and the 16th day. The pH has become stable afterwards. The stable of pH showed the balance between organic acid productions (by acidogenic bacteria) and organic acid digestions (by sulfate reducing bacteria).

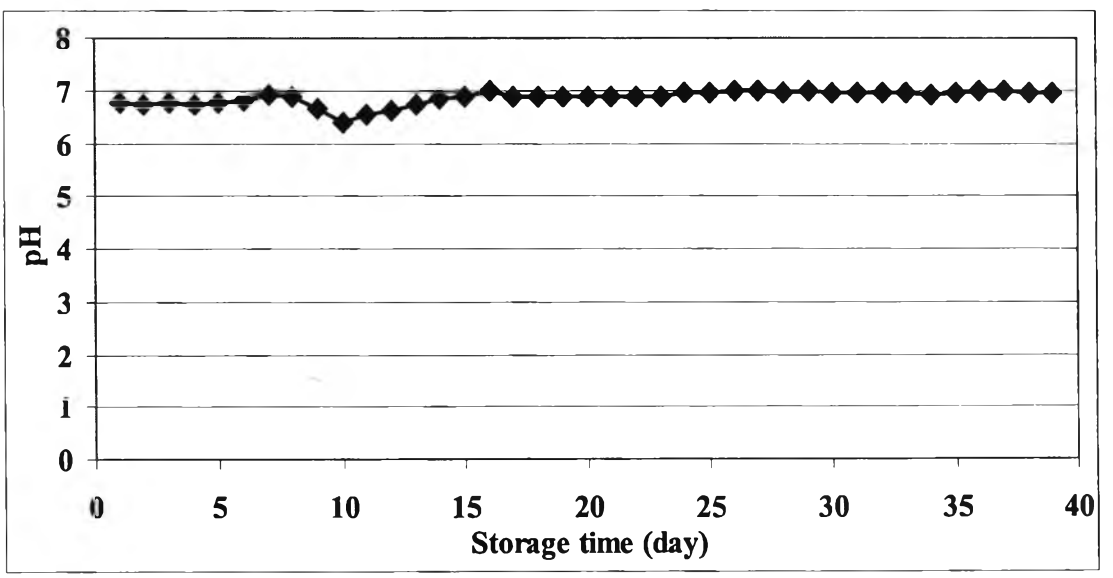

Figure 3.4 Daily pH in storage (dark gallon)

Temperature

In figure 3.5 it showed the temperatures in the dark gallon which indicated the changes in the range between 28 and 31 degrees Celsius that would suitable for the bacteria (between 25-35 °C).

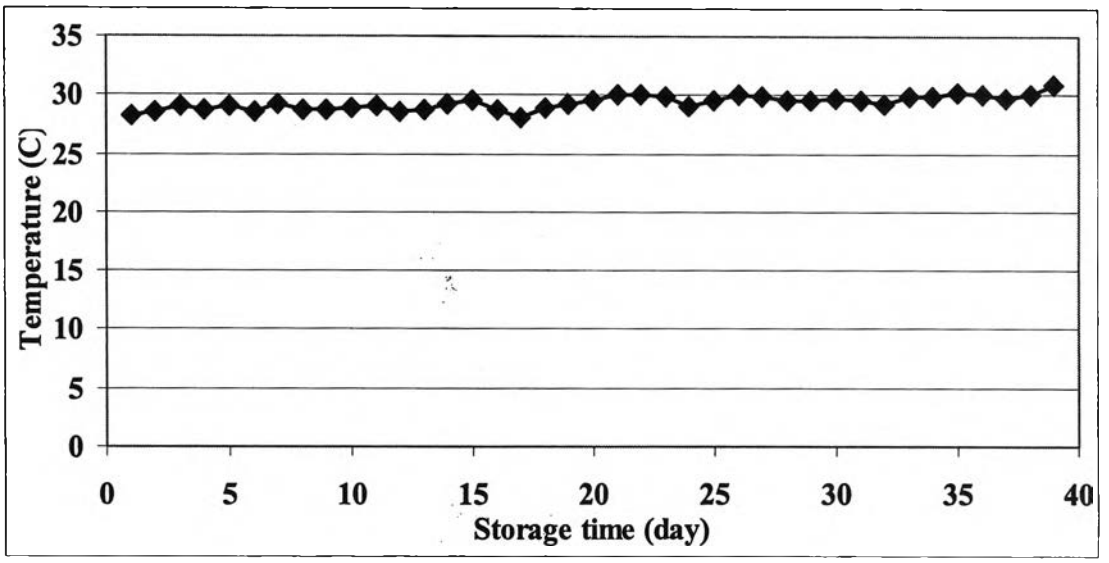

Figure 3.5 Daily temperatures in storage (dark gallon)

3.2.2 Reactor startup

The reactor mixture was prepared with sludge seeding, water, saw dust, nutrient and $MgSO_4 7H_2O$ as shown in table 3.4. The sludge seeding and saw dust characteristic was analyzed and shown in tables 3.1 and 3.2. 2 liters of reactor mixture were filled into two 2 liters completely mixed anaerobic reactors.

The two completely-mixed anaerobic reactors were operated under ambient temperature and completely mixed by magnetic stirrer at 500 rpm. They were necessary to measure pH, ORP, COD, sulfate, and sulfide every day during 8 days. After 8 days, the steady state was assumed when COD, sulfate, and sulfide become stable. The second reactor was intended to use as a refill for the first and was kept order the same condition.

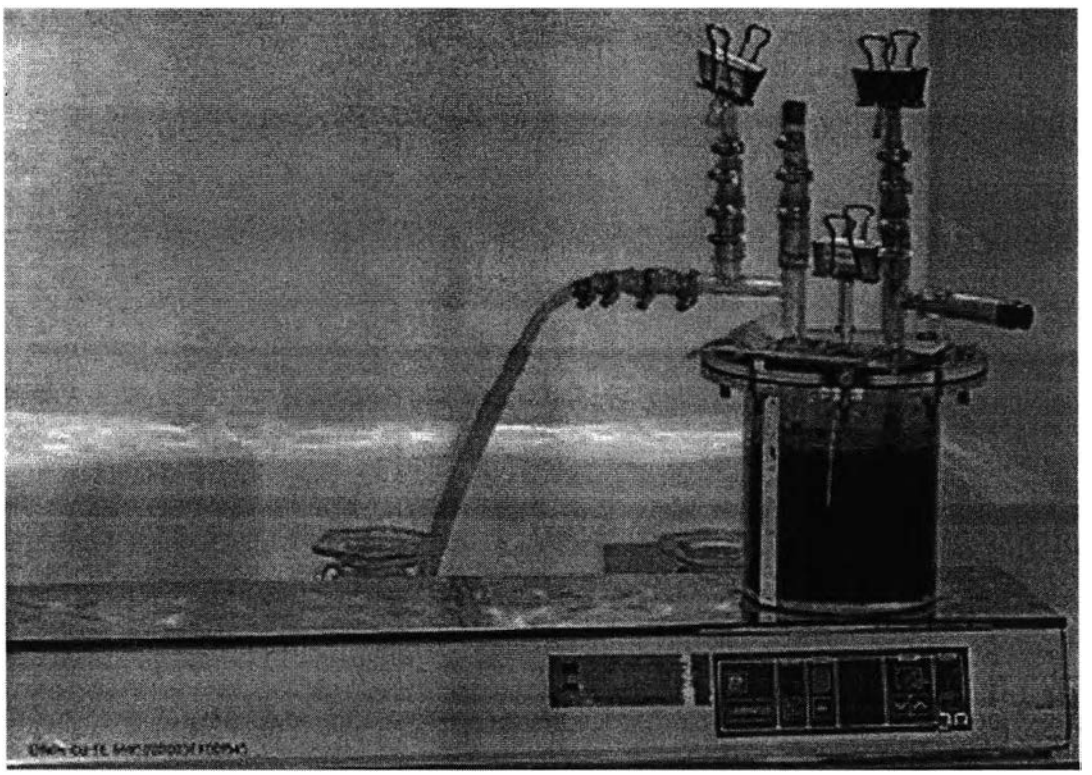

Figure 3.6 Reactor with magnetic stirrer

3.2.3 Reactor operation

The two reactors were injected with the synthesized wastewater nine times as shown in table 3.6. For the first reactor, temperature, ORP, pH, sulfate, sulfide, Zn, total COD, dissolved COD, and gas production were measured every day during the experiment. TKN, Alk and VFA were also measured every 2 days during experiment. For every injection of the synthesized wastewater into 2 liter of reactor mixture, Zn and sulfide was additionally measured with the frequency as shown in table 3.7. For the second reactor, temperature, ORP, pH, and gas production were measure every day during the experiment.

Table 3.6 Injection of synthesized wastewater.

Injection	Day	Conc. of Zn (mg/l)	Vol. Zn added (ml)	Total Conc. of Zn (mg/l)	Mass of Zn (mg)
1	9	10,000	2	10	20
2	23	10,000	4	20	40
3	25	10,000	4	20	40
4	28	10,000	4	20	40
5	29	10,000	4	20	40
6	31	10,000	10	50	100
7	32	10,000	10	50	100
8	33	10,000	20	100	200
9	34	10,000	20	100	200

After every sampling, the sample for the first reactor, the same amount of reactor mixture from the second reactor was filled into the first reactor to balance the volume of the first reactor.

Table 3.7 Frequency of sampling for measurement of sulfide and zinc after every injection

Injection	Frequency
1	Every 30 minutes for the first 2 hrs. after the injection and every 3 hours for 15 hrs.
2	Every 10 minutes for 1 hr. after the injection
3	Every 10 minutes for 1 hr. after the injection
4	Every 10 minutes for 1 hr. after the injection
5	Every 10 minutes for 1 hr. after the injection
6	Every 10 minutes for 1 hr. after the injection
7	Every 10 minutes for 1 hr. after the injection
8	Every 10 minutes for 1 hr. after the injection
9	Every 10 minutes for 1 hr. after the injection

3.2.4 Analysis of results

Collection and analysis of data from the reactor were carried out. As a result the optimum condition of sulfide, sulfate, zinc, pH, TKN, COD, gas production, as well as the analysis the amount of zinc in reactor and maximum of zinc content were maintained.

3.3 Sampling and analytical protocols

Wastewater samples were collected from the completely-mixed reactors, 40ml of sample were analyzed for chemical oxygen demand (COD), pH, oxidation-reduction potential (ORP), sulfide, sulfate, and zinc, and 40 ml of sample used for measure total kjedahl nitrogen, alkalinity, and volatile fatty acid. The daily temperature, daily gas production rate were also observed. Gas production measured by U-type collection was determined for volume. Detail about frequency and method of analyses are listed in Table 3.8.

3.3.1 Gas analysis

The gas produced by the completely-mixed reactors was collected and analyzed for quantity and composition. The amount of gas was measured using U-type collection method. Figure 3.7 showed U type collection.

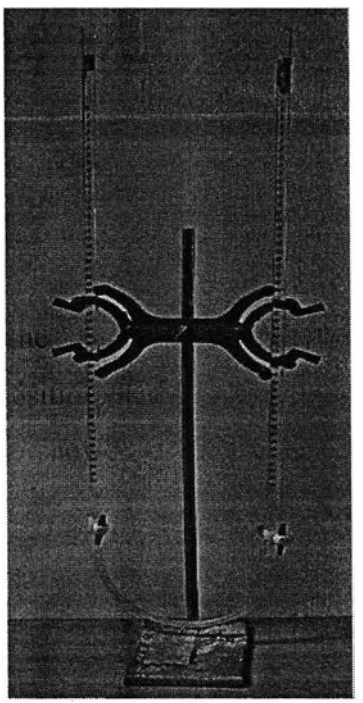

Figure 3.7 U-Type gas collection

3.3.2 Wastewater analysis

Wastewater samples from each of the completely-mixed anaerobic reactors were collected and characterized for gross parameters and heavy metals.

Wastewater parameters

The wastewater parameters included chemical oxygen demand (COD), pH, oxidation-reduction potential (ORP), alkalinity, sulfide, sulfate, ammonia-nitogen. Sulfides were measured periodically to confirm their presence under reducing conditions. pH and ORP were measured with pH meter and ORP meter.

Remaining analyses of wastewater parameters were performed according to The Standard Methods for the Examination of Water and Wastewater (1980) as shown in table 3.8.

3.3.3 Heavy metals analysis

Wastewater samples were also analyzed for Zn. The concentration of Zn was determined using a flame atomic absorption Spectroscopy (FAAS). Everyday 10 ml. samples of wastewater were acidified to pH of less than 2 with concentrated HNO_3 and stored in polyethylene bottles at $4^{o}C$. These results were described in the Appendix D

Table 3.8 Methods and frequency of wastewater parameters analyses

Measurement	Procedure	Frequency
Sulfate**	Standard Methods for water and wastewater Examination# 4500-SO_4^{2-} E. (Turbidimetric)	Every day
pH	pH Meter	Every day
ORP	ORP Meter	Every day
MLSS	Standard Methods for water and wastewater Examination# 2540-B. (Dried at 103-105C.)	Every day
COD	Standard Methods for water and wastewater Examination# 4500-COD. (Titration Method)	Every day
Gas Production	U-type Collection	Every day
Alkalinity	Standard Methods for water and wastewater Examination# 4500-P. (Titration Method)	Every 2days
Volatile fatty acids	Standard Methods for water and wastewater Examination# 4500. (Titration Method)	Every 2 days
Total Kjedahl Nitrogen	Standard Methods for water and wastewater Examination# 4500. (Kjedahl Method)	Every 2days

Measurement	Procedure	Frequency
Zn	Flame atomic absorption spectroscopy (FAAS)	Everyday*
Sulfide**	Standard Methods for water and wastewater Examination# 4500-S^{2-} (Iodometric Method)	Everyday*

* For every 30 minutes for the first 2 hours for the First injection of synthesized wastewater, thereafter every 3 hours for 24 hours. Every 10 minutes for 1 hour for the second, third, forth and so on injection of synthesized wastewater.

** The procedures were applied from the standard methods for water and wastewater. The measurement process were showed in appendix P