CHAPTER III

METHODOLOGY

3.1 Methodology

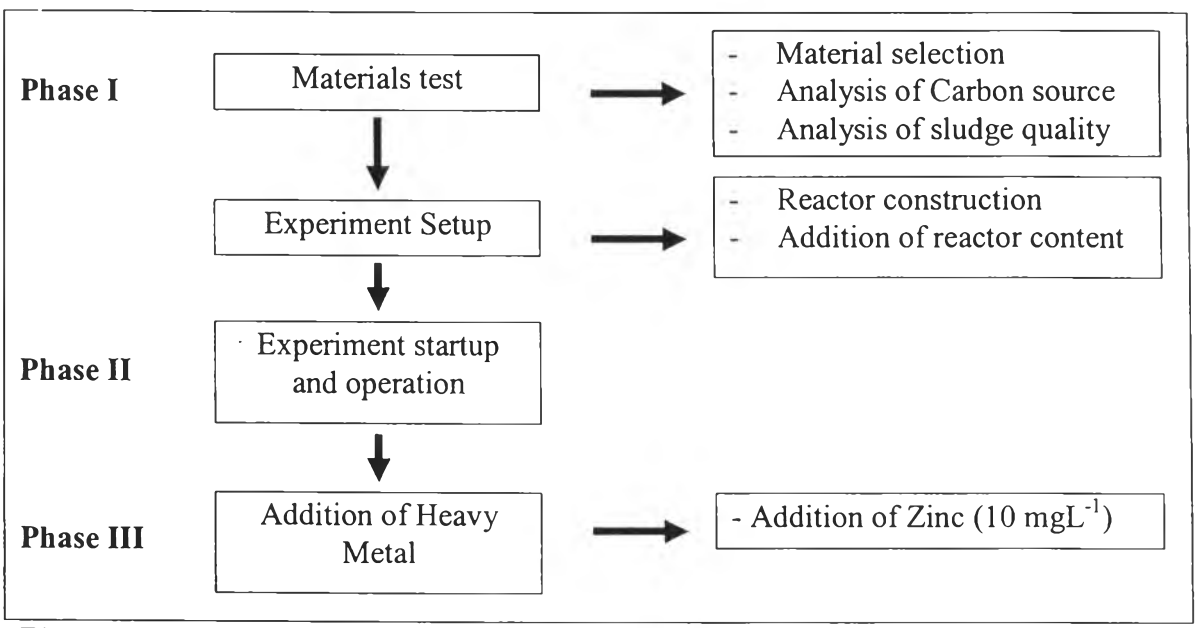


Figure 3.1: Experiment methodology

3.1.1 Materials test:

3.1.1.1 Materials selection of the reactive mixture in the column

1) Test the leaching property of Lime stone and Spinel mineral under neutral and acidic condition.

Lime stone and Spinel mineral as showed in figure 3.2 and 3.3 were tested under two conditions which were soaking in the DI water solution of pH 7 and acidic solution of pH 5 over the period of 24 hour as showed in table 3.1. The purposed of this test was to inform on the characteristic of stone reactions after the stones were conducted. By examine for the changes in the

alkalinity, pH, turbid production from the stone and weight differences after soaking in the solution.

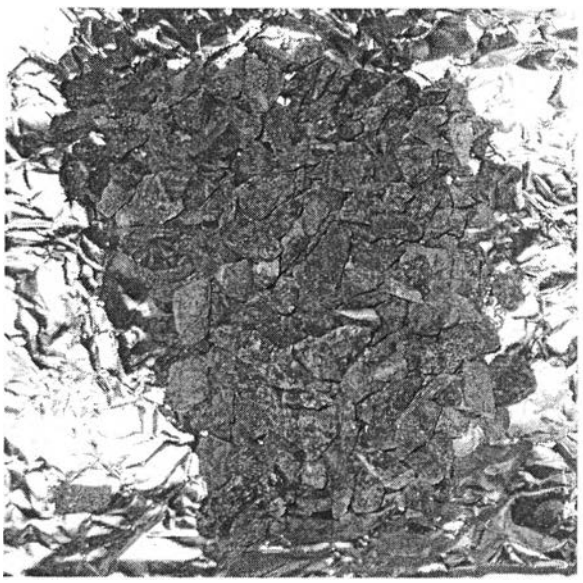


Figure 3.2: Lime stone

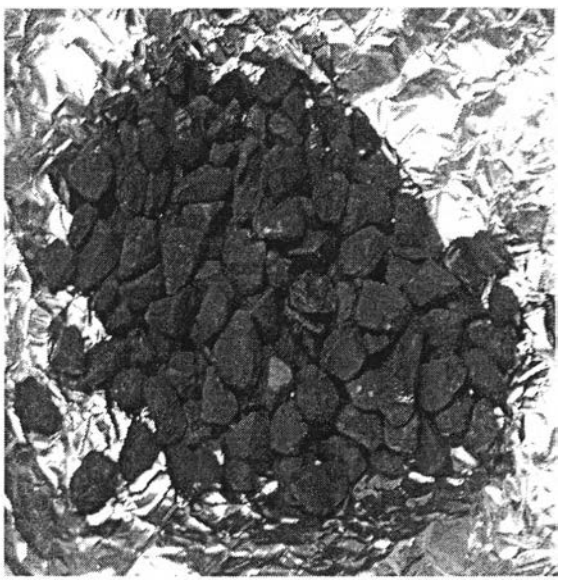


Figure 3.3: Spinel mineral

Table 3.1: The leaching tests on Lime stone and Spinel mineral were:

Test 1: No washed in tap water and place in DI water.	1) Test for changes in pH 2) Test for changes in alkalinity
Test 2: Washed in tap water before placed in DI water.	1) Test for changes in pH 2) Test for changes in alkalinity
Test 3: No washed in tap water and place in pH 5 solution.	1) Test for changes in pH 2) Test for changes in alkalinity 3)Test for changes in weight
Test 4: Washed in tap water before placed in pH 5 solution.	1) Test for changes in pH 2) Test for changes in alkalinity 3) Test for changes in weight

The results after testing as showed in table 3.2 can be concluded that the spinel mineral would provide better performances for the reactor. As the result of the stone test, it showed no changes in the spinel mineral characteristic under an acidic condition. In contrast, the lime stone had shown an increase in the alkalinity and lost of stone weight due to the leaching of

carbonate. Therefore, the spinel mineral was selected to be one of the mixtures.

Stone was selected to be parts be the mixture due characteristic of durability, cost effectiveness and ease to be found. It will provide housing for microorganisms.

The spinel mineral had properties of specific gravity of 3.6-4.0 with a diameter of 5 to 15 mm. It can be purchase in local warehouse store. The void volume was 0.3 when filled with spinel mineral to the full capacity of the reactor. The void volume was tested with water replacement method (void volume = volume of air / volume of spinel mineral).

Table 3.2: The summary of the leaching tests were:

Test 1: No washed in tap water and place in DI water.

Lime stone			**Spinel mineral**		
Sample no.	pH	Alk (mg/L)	Sample no.	pH	Alk (mg/L)
1	7.30	40	1	7.25	30
2	7.24	40	2	7.23	30

Test 2: Washed in tap water before placed in DI water.

Lime stone			**Spinel mineral**		
Sample no.	pH	Alk (mg/L)	Sample no.	pH	Alk (mg/L)
1	7.51	60	1	7.34	30
2	7.46	60	2	7.28	30

Test 3: No washed in tap water and place in pH 5 solution.

Lime stone				**Spinel mineral**			
Sample no.	pH	Alk (mg/L)	Wt Diff (g)	Sample no.	pH	Alk (mg/L)	Wt Diff (g)
1	5.49	11,800	2.05	1	5.07	4,740	0.3
2	5.44	12,000	1.71	2	5.06	4,680	0.35

Test 4: Washed in tap water before placed in pH 5 solution.

Lime stone				**Spinel mineral**			
Sample no.	pH	Alk (mg/L)	Wt Diff (g)	Sample no.	pH	Alk (mg/L)	Wt Diff (g)
1	5.46	11,000	1.91	1	5.07	4,490	0.43
2	5.48	11,500	1.61	2	5.07	4,430	0.45

2. Carbon source

The amount of organic from the Red wood chips as showed in figure 3.4, was tested by COD (Open Reflux Method) and BOD according to the Standard Method for examinations of water and wastewater 20th edition. The wood chip that was selected to test was sizes between 10 to 20 mm.

The purpose of this test was to inform on the amount of organic from the wood chips can provided for the microorganism in the system when conducted.

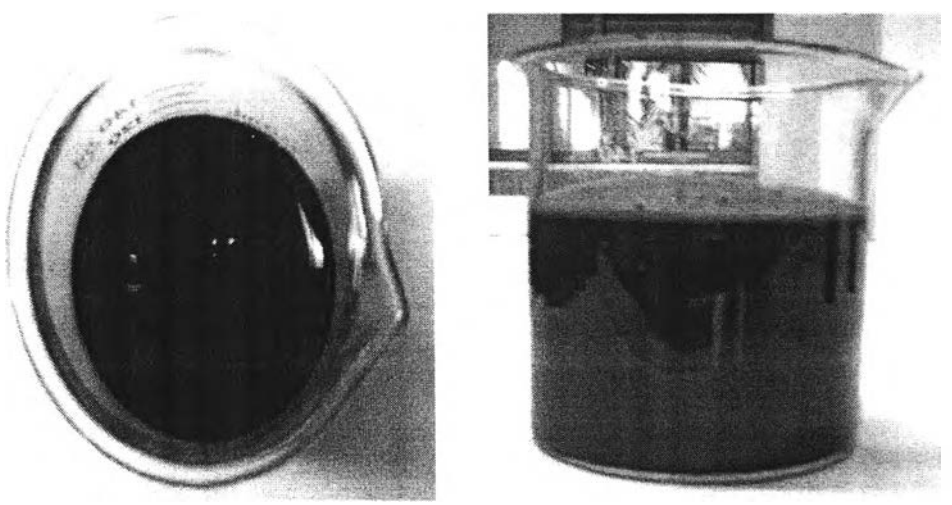
Figure 3.4: Red wood chips from local sawmill

The result of the wood chips tested from the local saw mill provided the amount of BOD of 240 mg/L and total COD of 65,800 mg/L per gram of wood chips. Such high amount of BOD and COD value showed that, the SRB would be able to consume and uptake the organic source and can survived in length of time without other organic source. The wood chips would also act as a slow releasing carbon source, structure and attaching surface.

3. Characteristic of the dewatered anaerobic digested sludge

The dewatered anaerobic digested sludge as showed in figure 3.5 was analyzed for pH by pH meter, total solids (TS) by Examination # 2540-B, total volatile solids (TVS) by Examination # 2540-E, alkalinity by Examination # 2320-B Titration Method, BOD by Examination # 5210-B and COD by Examination # 5220-C Titration Method, all was tested according to the Standard Method for examinations of water and wastewater 20th edition.

The purpose of this test as to inform on the amount of microorganism by TVS and amount of organics in form of BOD and COD in the dewatered anaerobic digested sludge in the system when conducted.



(a) (b)

Figure 3.5: Digested sludge (a) front view and (b) top view.

The summarized on characteristic of the dewatered anaerobic digested sludge was showed in table 3.3.

Table 3.3: Characteristic of the dewatered anaerobic digested sludge

BOD	8.7 mg/L
COD	760,000 mg/L
pH	6.5
Alkalinity	2,060 mg/L as $CaCO_3$
Moisture Content	90% of sludge
Total Solids (TS)	10% of sludge
Total Volatile Solids (TVS)	20% of TS

The conclusions on the characteristic of the dewatered anaerobic digested sludge were as following.

pH and Alkalinity

The pH of the dewatered anaerobic digested sludge obtained from treated wastewater from Nongkam wastewater treatment plant was pH of 6.5 which was in a slightly acidic condition with an alkalinity of 2,060 mg/L as $CaCO_3$.

Moisture Content, Total solid (TS) and Total volatile solid (TVS) of sludge

The dewatered anaerobic digested sludge obtained from treated wastewater from Nongkam wastewater treatment plant was dewatered, until the sludge was thickened and low in water content as shown in Figure 3.4. The properties of the dewatered sludge shown that the moisture contents in the sludge was 90% by weight and the total solids (TS) was 10% by weight due to the dewatering of the sludge. The amount of total volatile solids in the sludge was 20% of the total solids, which are the microorganisms that were available in the sludge.

BOD and COD

The value BOD of 17.4 mg/L and COD of 7600 mg/L from the dewatered anaerobic digested sludge provided good information about the sufficient amount of the total organic source for the microorganism to consume. The result of BOD showed that the actual food source for the SRB in sludge was not that much when compared with the amount of COD.

3.2 Experimental set-up and procedure

3.2.1 Configuration of the anaerobic reactors

The experimental set-up is illustrated in figure 3.6. Two simulated reactors were constructed using an acrylic pipe. Each reactor had a diameter of 8 cm and a height of 30 cm. The columns were assembled with two 10 cm

outer diameter acrylic flanges at both ends to provide support for top and bottom lids. A coating with silicone was applied to the interior and exterior of the flanged joints to ensure that the junctions would be water and gas tight.

The reactors were equipped with six ports, four ports were used for leachate drainage and sampling while the other two inlet/outlet ports were placed at the top lid to collect gas samples and to add leachate/wastewater.

Both 2 cm diameter holes on top of the lid functioned as liquid addition and gas sampling ports, respectively. A 2 cm diameter acrylic pipe connected with a PE tube was used for liquid addition. While the other port was connected to a 2-cm silicon tube and connected to the gas collection line, whereas, the other end of the tee was capped by rubber septum and functioned as a gas sampling port.

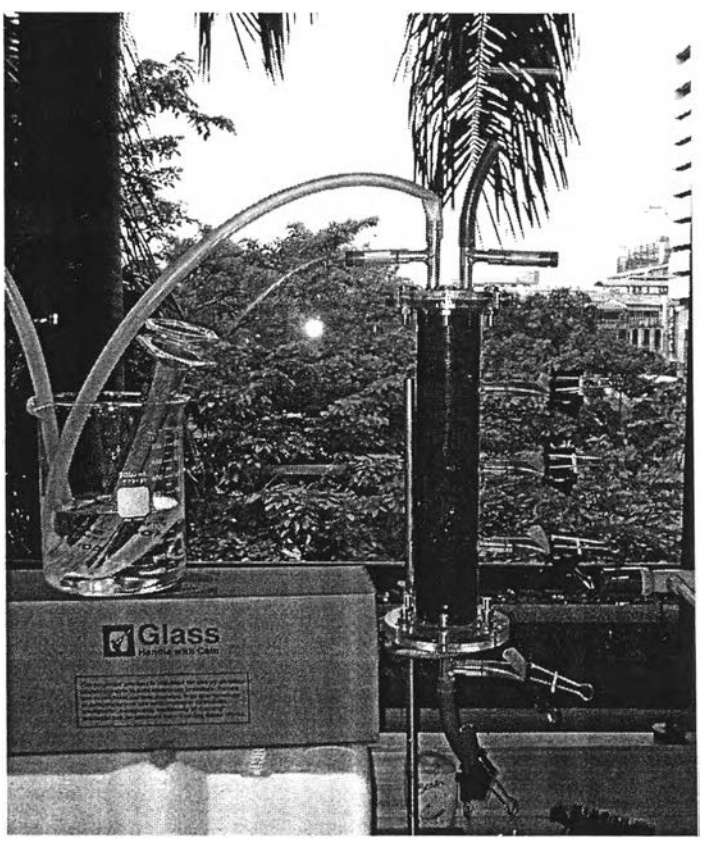


Figure 3.6: Experiment Set-up. (Adapted from Erses and Onay, (2003)).

The gas produced in the reactors was collected and measured by an inverted glass cylinder method. This technique utilized one 1-L glass cylinder placed inversely in 2-L glass cylinder which was filled with confining solution (20% Na_2SO_4 in 5 % H_2SO_4) (Sawyer and McCarty, 1989). The inner cylinder was lifted until the level of the confining solution in both cylinders equilibrated, and the amount of gas produced in a certain period was indicated by the volume occupied by gas in the inner cylinder.

3.2.2 Characteristics of mixture matrix

The volume of both reactors was 1500 cubic centimeter. The mixture matrixes were consist of spinel mineral, wood chip and dewatered anaerobic digested sludge. Reactor A and B were loaded with spinel mineral with a diameter of 5 to 15 mm with a specific gravity of 3.6-4.0, and a mixture of wood chip sizes between 10 to 20 mm obtained from the local sawmill plant in the ratio of 1:1 (by volume of spinel mineral and mixture) (Benner et al., 2002) and 200 ml of dewatered anaerobic digested sludge obtained from treated wastewater from Nongkam wastewater treatment. The reactor would be able to hold water in a capacity of 1 liter of water when the mixture was added. . The void volume was 0.5 when filled with all mixture matrixes in the reactor. The void volume was tested with water replacement method (void volume = volume of air / volume of spinel mineral). After all mixture matrixes were loaded into reactor A and B, they provided the HRT of 2 liters per hour to both reactors.

The experiment would be conducted in two conditions:

Reactor A was filled with deionized water, and Reactor B was filled with the filtered water of digested sludge of treated wastewater to the full capacity of the reactor after loaded with the entire mixture matrixes.

3.2.3 Experiment startup and operation

The experiment startup of this study was showed in figure 3.7. The leachates from both reactors were recirculated at 60 ml to stimulate the

suitable condition for sulfate reducing bacteria (SRB). Recirculation of leachate was done daily at the interval of 24 hour. Addition of nutrient was performed base on the needed of SRB. Formula for nutrient mixture was in table 3.4. The startup operation was conducted over a period of 31 days. An example of leachate from the effluent was showed in the figure 3.8.

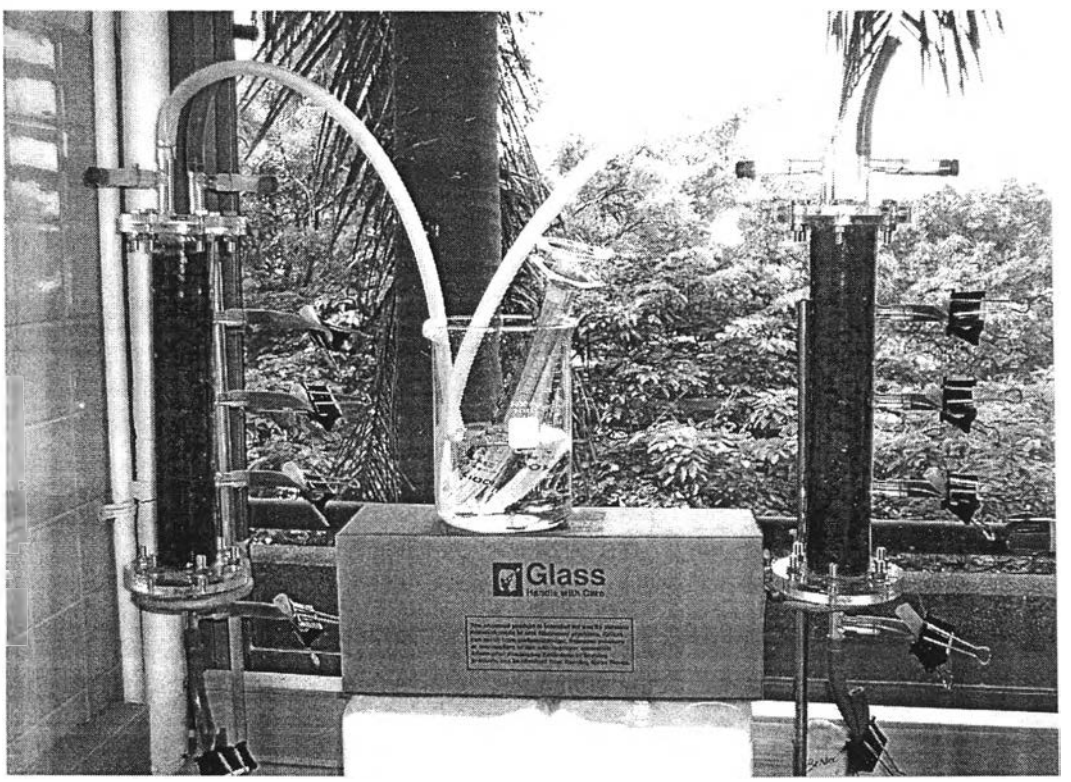
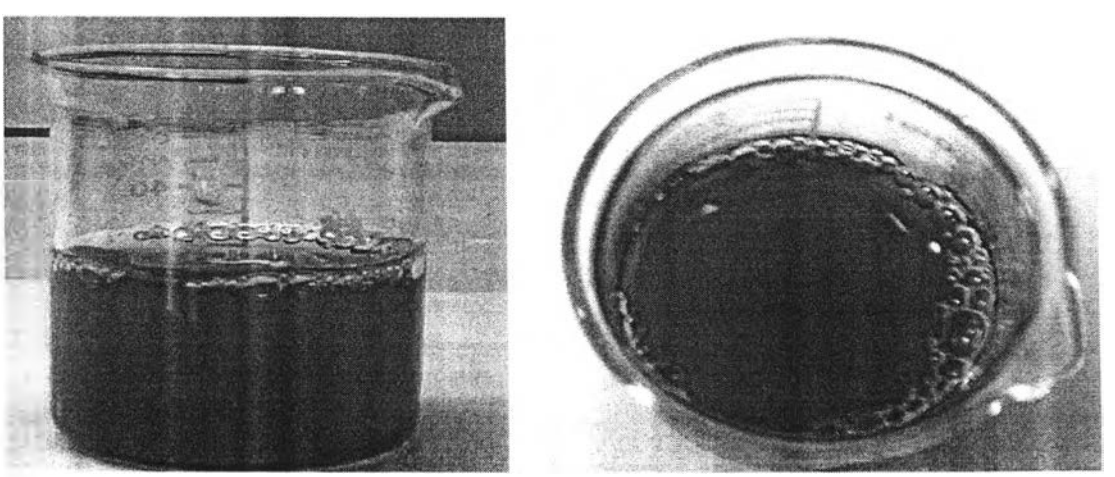


Figure 3.7: Experiment startup



(a) (b)

Figure 3.8: Wastewater Leachate, (a) front view and (b) top view.

Table 3.4: Nutrient composition.

Composition	Concentration (mgL^{-1})
NaCl	32
NH_4Cl	40
KCl	4
KH_2PO_4	4
$MgSO_47H_2O$	8
$CaCl_2.2H_2O$	1.6
Glucose (SCOD)	1700-2000
Sulfate in form of $NaSO_4$	2200-2300

Source: Tuppurainen, Vaisanen, and Rintala, (2002).

3.2.4 Addition of zinc (Zn)

The zinc addition was performed three times; first, second and third additions were added on day 32, 39 and 43, respectively.

First addition was added with 200 ml of synthetic wastewater at the concentration of 10 mg/L of zinc (Zn) (Gong et al., 1997).

Second addition was added with 200 ml of synthetic wastewater at the concentration of 50 mg/L of zinc (Zn).

Third addition was added with 200 ml of synthetic wastewater at the concentration of 50 mg/L of zinc (Zn).

Effluent leachates were recirculated at the interval of 24 hour. The samples were taken out from the effluent sampling ports of both reactors.

3.3 Sampling and Analytical Protocols

The collected leachate and gas samples were monitored on a regular basis to track fate of zinc (Zn). The quality and quantity of gas and leachate varied as the anaerobic phases of the system occurred. Therefore, monitoring for changes in

parameters indicative of anaerobic condition were used to identify the suitable condition for sulfate-reducing bacteria to active.

The leachate samples were collected from the bottom of the recycle reactors, and were analyzed for chemical oxygen demand (COD), pH, oxidation-reduction potential (ORP), sulfide, sulfate, alkalinity and heavy metals. The daily temperature, daily gas production rate, and gas composition were also observed. Gas composition, was measured as percent of methane by volume. Detail about frequency and method of analyses are listed in Table 3.5.

3.4 Gas Analysis

The gas produced by the simulated reactors were collected and analyzed for quantity and composition. The amount of gas was measured using an inverted glass cylinder collection method. The gas composition was analyzed using Gas Chromatograph (GC) (19091P-MS4) equipped with a Thermal Conductivity Detector (TCD). The glass packed column used to separate neon, argon, oxygen, nitrogen, methane, carbon monoxide was 30m× 0.32 mm ID. The typical operating conditions for the GC were:

Detector	Thermal Conductivity Detector (TCD) (Agilent Technology 6890 N Network GC system)
Oven program	40° Isothermal
Sample	250μL, split (75:1)
Carrier gas	Helium, 2mL/min
Column	HP-Molsiv Agilent Technologies, I.D.0.32 mm (widebore) Length 30 m, Film 12 μm

3.5 Wastewater Analysis

The wastewater parameters including chemical oxygen demand, pH, oxidation-reduction potential (ORP), alkalinity, sulfide, and sulfate of the influents and effluents will be analyzed routinely. Sulfides were measured periodically to confirm the amount of sulfate-reducing bacteria (SRB) presence under reducing conditions. pH and ORP were measured with pH meter and ORP meter.

Remaining analyses of wastewater parameters were performed according to Standard Methods for the Examination of Water and Wastewater, 20th edition 1998 and also include:

BOD by Examination # 5210-B
COD – Chemical oxygen Demand- Examination # 5220-C (Titration Method)
Total Alkalinity- Examination # 2320-B (Titration Method)
Sulfate- Examination # 4500-E (Turbidimetric Method)
Sulfide- Examination # 4500-G (Ion Selective Electrode Method)

3.6 Zinc Analysis

Wastewater samples were analyzed for zinc (Zn). The concentrations of zinc metal were determined routinely by using an atomic absorption spectrophotometer (A.A.S). 20 ml of samples of wastewater were collected at the effluent sampling ports to examine for the concentration of zinc.

Table 3.5: Frequency and Analysis Methods for the test of wastewater and gas parameters.

Measurement	Procedure	Frequency
pH	pH meter	Everyday
ORP	ORP meter	Everyday
COD	Standard Method for examinations of water and wastewater 20th edition. Examination # 5220-C (Titration Method)	* Every 3 days at the start of system ** Every week after system stable
Sulfides	Standard Method for examinations of water and wastewater 20th edition. Examination # 4500-G (Ion Selective Electrode Method)	Everyday
Sulfate	Standard Method for examinations of water and wastewater 20th edition. Examination # 4500-E (Turbidimetric Method)	Everyday
Alkalinity	Standard Method for examinations of water and wastewater 20th edition. Examination # 2320-B (Titration Method)	Every week
Zn	Atomic Absorption Spectrophotometer (A.A.S)	* At the start of the startup process ** Every hour after the addition of Zn
Gas production	Inverted glass cylinder method	Everyday
Percent Methane	Gas Chromatography (TCD)	Every 3 days