# APPENDICES

# APPENDIX A
# METHODOLOGY OF ANALYSIS

**Appendix A1** Chemical Oxygen Demand (COD) analysis (Closed reflux, Titration method)

**Apparatus**

- Digestion vessel 16 x 100 mm
- Hot air oven
- Heating block

**Reagents**

- Potassium standard solution. The following reagents were added into 500 ml distilled water. 4.913 g $K_2Cr_2O_7$ (Primary standard grade) previously dried at 103 ºC for 2 h, then added 167 ml conc. sulfuric and 33.3 g $HgSO_4$. The mixture was done until complete dissociation, thrown away to be cooled in room temperature, and finally diluted to 1000 ml.
- Sulfuric acid reagent. Added 1% W/V into conc. $H_2SO_4$. The mixture was let for 1-2 days for completely dissolved $Ag_2SO_4$.
- Ferroine Indicator
- 0.1 M Ferrous Ammonium Sulfate standard solution (FAS) 39.2 g $Fe(NH_4)_2(SO_4)_2$ dissolved into 500 ml distilled water, then added 20 ml conc. $H_2SO_4$, and finally diluted to 1000 ml.

Note: 0.1 M Ferrous Ammonium Sulfate standard solutions compare with Potassium Dichromate standard solution. Using 2.5 ml distilled water, 1.5 ml $K_2Cr_2O_7$, 3.5 ml $H_2SO_4$ and 1-2 drops Ferroine Indicator into flask titrate with 0.1 Fereous Ammonium Sulfate standard solution end point green blue to red brown.

$$\text{Concentration of FAS (M)} = \frac{\text{ml } K_2Cr_2O_7 \text{ x } 0.1}{\text{ml FAS}}$$

## Procedure

- 2.5 ml of Sample was added to digestion vessel
- Add 1.5 ml digestion reagent, and then 3.5 ml sulfuric acid was slowly into the vessel.
- Seal was tightly with cork tube. Afterward, the vessel was gently inverted several times. (As mixing should put on grove and mask), and the vessel was then placed in the hot air oven.
- The vessel was hearted at 150 ºC for 2 h, and then left to be cooled in room temperature.
- Afterward, Ferroine Indicator was dropped for 1-2 drop, and finally titrate with 0.1 M 0.1 M Ferrous Ammonium Sulfate standard solutions. (End point green blue change to red brown)

## Calculation

$$\text{COD (mg/l)} = \frac{(A-B) \times M \times 8000}{\text{ml of Sample}}$$

where A = ml of FAS titrate with blank
B = ml of FAS titrate with sample
M = Molarity of FAS

Note: Distilled water was used to blank

**Appendix A2** Total Suspended Solids (TSS) Analysis

## Apparatus

- Desiccators
- Drying oven
- Glass-fiber filter disk (47 mm ID)
- buchner funnel
- Suction flask

**Procedure**

- The glass-filter disk was dried in a hot air oven at 105 ºC for 1 h. The disk was cooled in desiccators to balance temperature and weighed.
- The Sample was chosen to yield between 10 and 200 mg dried residue.
- The glass-filter was placed in buchner funnel which connect vacuum machine.
- The filter was wet with a bit volume of distilled water for fix in buchner funnel
- The sample volume was sieved in the Buchner funnel, after complete filtration also suction and wash filter for 3 min.
- The filter was carefully removed from Buchner funnel.
- The filter was dried at least 1 h at 105 ºC in an oven, afterward was cooled in desiccators in room temperature, and weight.

**Calculation**

$$\text{mg Total Suspended Solids/l} = \frac{(A-B)\times 1000 (\text{mg})(\text{ml/l})}{\text{sample volume(ml)}}$$

A = Weight of filter + dried residue (mg)
B = Weight of filter (mg)

**Appendix A3** Volatile Suspended Solids (VSS) Analysis

**Procedure**

- The residue produced by TSS method was ignited in a furnace at 550 ± 50 ºC.
- A furnace was heated up to temperature after inserting sample.
- Usually, 15 to 20 min ignition is required for 200 mg residue.
- The filter disk was left to partially cool in air until most of the heat was dissipated.
- The dist was transferred to desiccators, and weight as soon as if was cooled in room temperature.

**Calculation**

$$\text{mg Volatile Suspended solid/L} = \frac{(A-B)\times 1000}{\text{sample volume, ml}}$$

A = Weight of residue + disk before ignition (mg)
B = Weight of residue + disk after ignition (mg)

**Appendix A4** Total Solid (TS) Analysis

**Apparatus**

- 50 ml crucible
- Water bath
- Analytical balance
- Muffle furnace
- Desicator
- Drying oven 103 to 105°C

**Procedure**

- Preparation of evaporation crucible : If volatile solids are to be measured ignite clean evaporaring crucible at 550°C for 1 h in a muffle furnace. Store and cool crucible in desiccator until needled. Weigh immediately before use.
- 25 ml sample , that homogeneous samples and then evaporate to dryness on water bath.
- Dry evaporate sample for 1 h in an oven at 103 to 105°C, and then cool crucible in desiccators for 30 min or until a constant weight.
- Determine weight in a weight balance.

**Calculation**

$$\text{mg Total solid/l} = \frac{(A-B)\times 1000}{\text{sample volume, ml}}$$

A = Weight of residue + crucible before ignition (mg)
B = Weight of residue + crucisle after ignition (mg)

**Appendix A5** Volatile Solids Analysis

**Apparatus**

- The residue from Total Solids
- Muffle furnace
- Weight balance

**Procedure**

- The residue from Total Solids is ignited in the muffle furnace at 550°C for 1 h.
- Cool in desiccators to a constant temperature and weigh.
- Weigh Analysis in weigh balance.

**Calculation**

$$\text{mg Volatile solid/l} = \frac{(A-B)x1000}{\text{sample volume, ml}}$$

A = Weight of residue from total solids + crucible before ignition

B = Weight of residue + crucisle after ignition (ml)

**Appendix A6** Total Nitrogen Analysis (Kjeldahl Methods)

**Apparatus**

- Weigh balance
- Hood
- Digestor
- Distilling unit
- 250 ml Digestor tube
- 250 ml Erlenmyer flask
- 100, 500 and 1000 ml of volumetric flasks
- 250 ml beaker
- 50 ml burette

- 5, 10, 15 and 20 ml of pipettes
- 25 and 100 ml of Cylinder
- Stir glass and rubber pump

**Reagents**

- conc.$H_2SO_4$
- 30% $H_2O_2$
- Sodium hydroxide Solution. The following reagents were added 1 kg sodium hydroxide (commercial grade NaOH) in to 1000 ml of distilled water
- Mixed catalyst, that consists of $K_2SO_4$ and $CuSO_4 \cdot 5H_2O$ in 100:10 ratio.
- Indicator (collected in a light brown bottle)

0.2 g of methyl red dissolve into 100 ml of 95% ethyl alcohol then collect in bottle

0.1 g of methyl blou dissolve into 100 ml of 95% ethyl alcohol and then collect in bottle

- 4% Boric indicator. Add 40 g of boric acid into 1 l of volumetric flak, that have a bit distilled water in it and add 10 ml of indicator (mixed 10 ml methyl red and 5 ml methyl blou) and then diluted to 1000 l.
- 0.1 N Sulfuric acid standard solution: pipette 1.5 ml conc.$H_2SO_4$ transfer to a 500 ml volumetric flask, fill flask to mark with distilled water. Titration certainly determine concentration of sulfuric acid standard solution with 40 ml of 0.05 N sodium carbonate. The following calculation express:

$$\text{Normality of sulfuric acid} = \frac{AxB}{53xC}$$

where: A = g $Na_2CO_3$ into 1 l flask

B = ml of $Na_2CO_3$ solution taken for titration,

C = ml acid used

**Procedure**

**Digestor**

- Add 100 ml saple in 250 ml digestor tube (blank use distilled water) and add 10.5 g mixed catalyst.
- Add 15 ml conc. Sulfuric acid and 2 ml hydrogen peroxide.
- Initial in digestor machine at temperature about 250°C, when temperature increase to 420°C count time for 2 hours.

**Distillation**

- Sample is distilled by auto Distillation unit (Kjeltec$^{tm}$2200). The program is fixed by Add 50 ml water and 30 ml sodium hydroxide distill for 4 min until get 150 ml and then was analyzed by titration.
- Add 25 ml boric indicator in 250 ml erlenmyer flask, which is supported from auto distillation.

**Titration**

- Titration with 0.1 sulfuric acid standard solution. The end-point change green to purple.

**Calculation**

$$\%N = \frac{(A-B) \times N \times 1400}{\text{ml sample}}$$

where: A = ml acid used titrate sample
B = ml acid used titrate Blank
N = Normality of standard acid

**Appendix A7** Grease and oil Analysis via Soxhlet (Ruibal-mendieta et al., 2001)

**Apparatus**

- 150 ml beaker
- Soxhlet & distill bottle
- Extraction thimbles
- Glass beads

- Electric heating mantle
- Vacuum pump
- buchner funnel
- Water bath
- Clean cotton
- Desiccator
- Muslin remnant of cloth

**Reagent**

- Conc. HCl
- n-hexane

**Procedure**

- Add muslin remnant of cloth and filter paper, respectively in the Buchner funnel, that connect with vacuum pump and then add 100 mL water into funnel.
- Add 25 ml sample pass filter in funnel until dry.
- Collect muslin cloth and filter paper in extraction thimble. The residue of oil is wiped by n-hexane. Dry in oven for 30 min at 103 to 105°C.
- Extration thimble is add in soxhlet, that connect with distill bottle and soxhlet extraction for 4 h. n-hexane is Solvent in distill bottle. Extraction ratio 20 rounds per h.
- Afterward, distill extraction bottle until dry (n-hexane is distilled). Dry constant weight in oven at 103 to 105°C and place in desiccators until weigh constant.
- Determine weight by weigh blance.

**Calculation**

$$\text{mg/l oil and grease} = \frac{\text{mg increase in weight of flask x 1,000}}{\text{ml sample}}$$

## **Appendix A8** Carbohydrate Analysis (Colorimetric method)

**Apparatus**

- Tube
- Water bath
- Spectro photometer
- Micro pipette

**Reagent**

- 80% (w/w) phenol: 20 g phenol dissolve in 80 ml deionize water at 25°C.
- conc. Sulfuric acid
- 100 ppm of D-glucose standard solution:

**Procedure**

Standard curve

- Series of sugar containing 2, 4, 6, 8 and 10 µl of sugar solution is pipetted into colorimetric tube, 50 µl of 80% phenol is added. Then 5 ml of concentrated sulfuric acid is added rapidly.
- The tubes are allowed to stand 10 min, and then they are shaken and placed for 20 min in a water bath at 30°C.
- Characteristic of solution is yellow-orange color can be measured at 480, 484 and 490 nm with spectro photometer. Blank are prepared by substituting deionize water for the sugar solution

Sample Analysis

- Add 2 ml sample, that it is diluted 1000 times by deionize water in tube
- Add 50 µl of 80% phenol and then add 5 mL conc. Sulfuric acid is rapidly.
- The tubes are allowed to stand 10 min, then they are shaken and placed for 20 min in a water bath at 30°C. The same previously method.
- The sample is analyzed by spectro photometer at 480, 484 and 490 nm. Plot graph in standard curve and calculate concentration.

# APPENDIX B
# GAS CHROMATOGRAPH’S STANDARD GAS

**Table B1** Gas chromatograph’s calibration on standard curve (60% Hydrogen and 10% Methane)

| Order | Hydrogen | | Methane | |
|---|---|---|---|---|
| | % $H_2$ | Area | % $CH_4$ | Area |
| 1 | 60 | 1810012 | 10 | 59149 |
| 2 | 60 | 1669967 | 10 | 61181 |
| 3 | 60 | 1568000 | 10 | 62757 |
| 4 | 60 | 1693790 | 10 | 61549 |
| 5 | 60 | 1894693 | 10 | 72672 |
| 6 | 60 | 1940139 | 10 | 73603 |
| 7 | 60 | 1912454 | 10 | 72642 |
| 8 | 60 | 1956328 | 10 | 72083 |
| 9 | 60 | 1874962 | 10 | 71123 |
| 10 | 60 | 1899974 | 10 | 70349 |
| Average | | 1822031.90 | | 67710.80 |

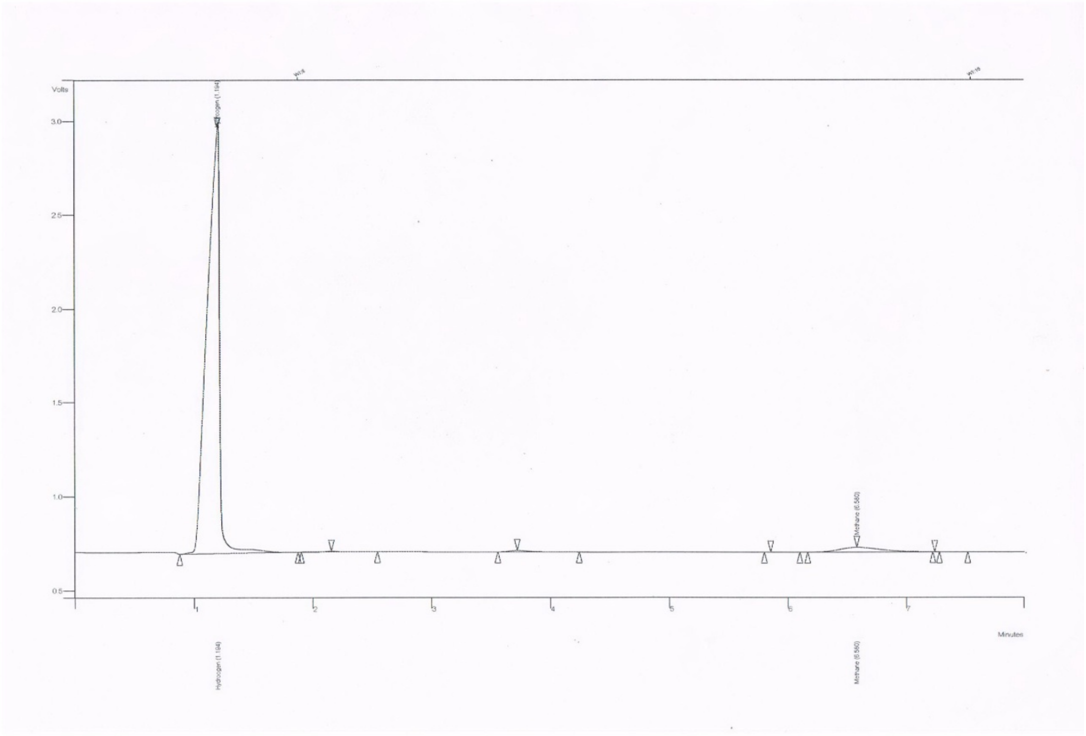

**Figure B1** Graph of standard gas (Hydrogen and Methane)

**Table B2** Gas chromatograph's calibration on standard curve (30% Carbon dioxide)

| Order | % $CO_2$ | Area |
|---|---|---|
| 1 | 30 | 28325 |
| 2 | 30 | 27869 |
| 3 | 30 | 28738 |
| 4 | 30 | 28279 |
| 5 | 30 | 29111 |
| 6 | 30 | 29176 |
| 7 | 30 | 27006 |
| 8 | 30 | 31940 |
| 9 | 30 | 29337 |
| 10 | 30 | 27830 |
| Average | | 28761.10 |

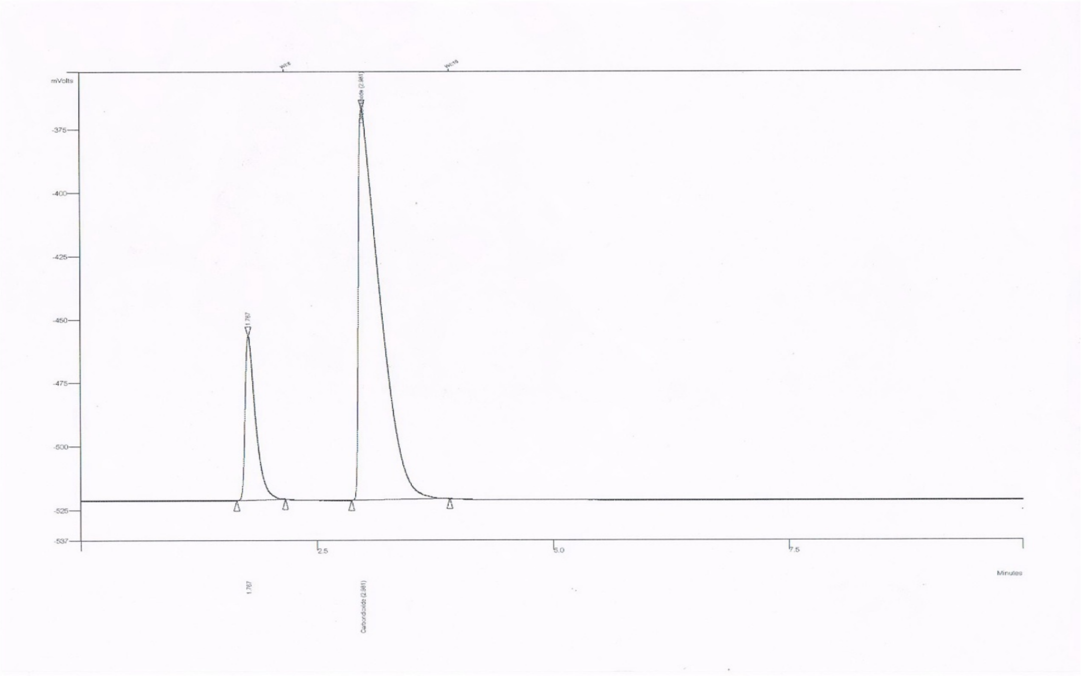

Figure **B2** Graph of standard gas (Carbon dioxide)

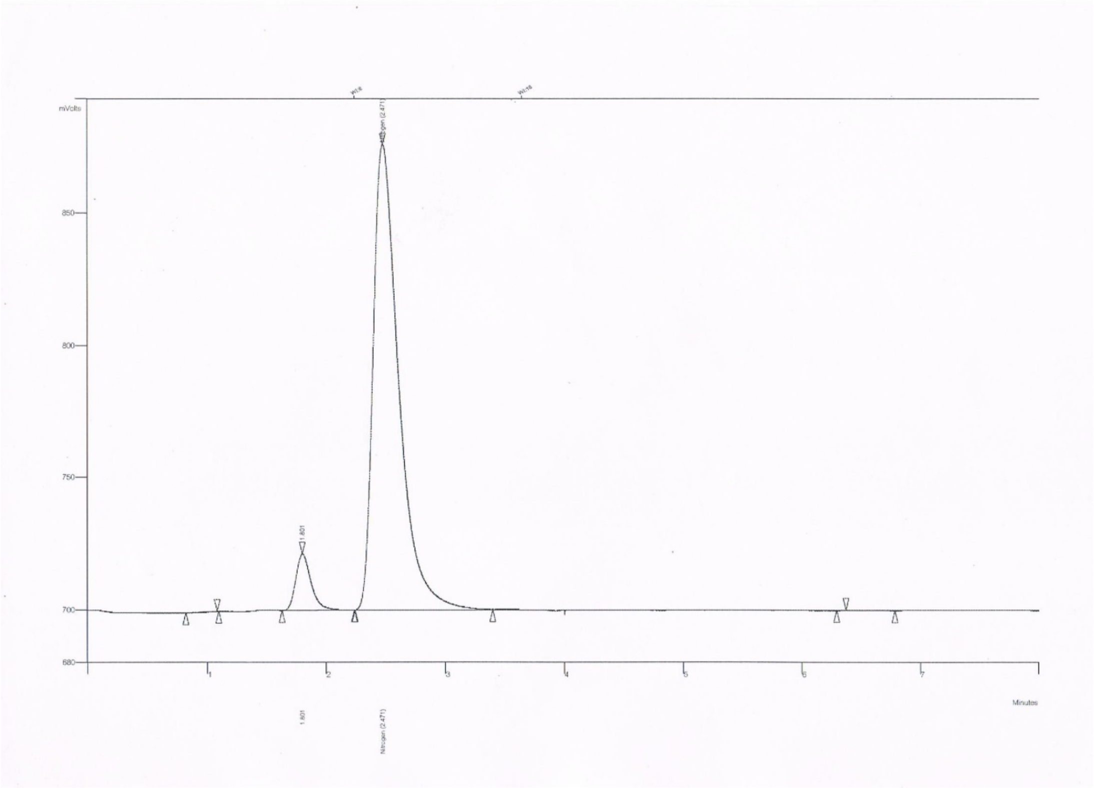

**Figure B3** Graph of standard gas (Nitrogen)

**Table B – 3** Gas chromatograph’s calibration on standard curve (99.995% Nitrogen)

| Order | % $N_2$ | Area |
|---|---|---|
| 1 | 99.995 | 317564 |
| 2 | 99.995 | 180162 |
| 3 | 99.995 | 196075 |
| 4 | 99.995 | 227666 |
| 5 | 99.995 | 260304 |
| 6 | 99.995 | 193392 |
| 7 | 99.995 | 221885 |
| 8 | 99.995 | 90977 |
| 9 | 99.995 | 84379 |
| 10 | 99.995 | 197031 |
| Average | | 196,954.50 |

# APPENDIX C
## VOLATILE FATTY ACID (VFA)

**Table C1** Gas chromatograph's calibration on standard curve for acetate

| Concentration of acetate (mg/l) | Peak area |
|---|---|
| 4 | 7004 |
| 6 | 7537 |
| 8 | 9071 |
| 10 | 9604 |
| 40 | 34604 |
| 60 | 45937 |
| 80 | 63847 |
| 100 | 78604 |

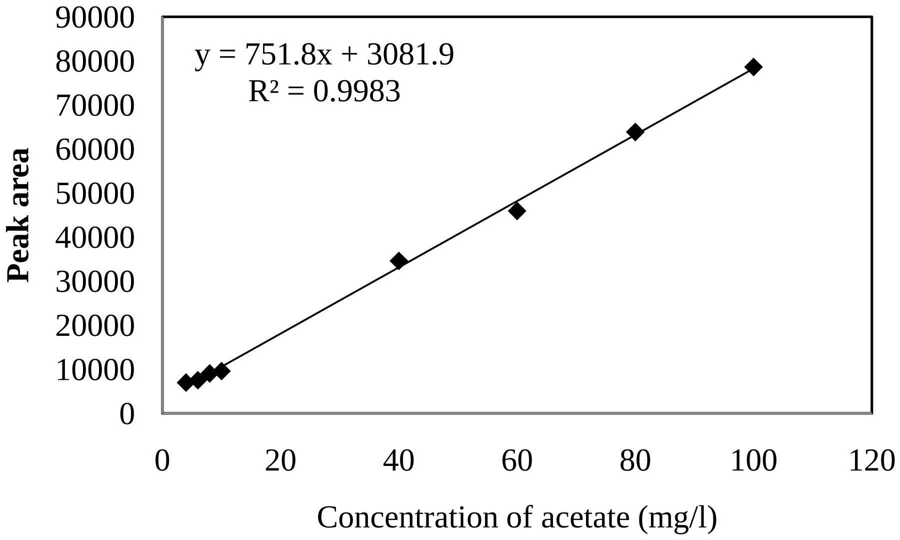

**Figure C1** The relationship between peak area and different amount of standard acetic

**Table C2** Gas chromatograph's calibration on standard curve for propionate

| Concentration of propionate(mg/l) | Peak area |
|---|---|
| 4 | 13853 |
| 6 | 16359 |
| 8 | 19780 |
| 10 | 20744 |
| 40 | 65198 |
| 60 | 94834 |
| 80 | 122836 |
| 100 | 145118 |

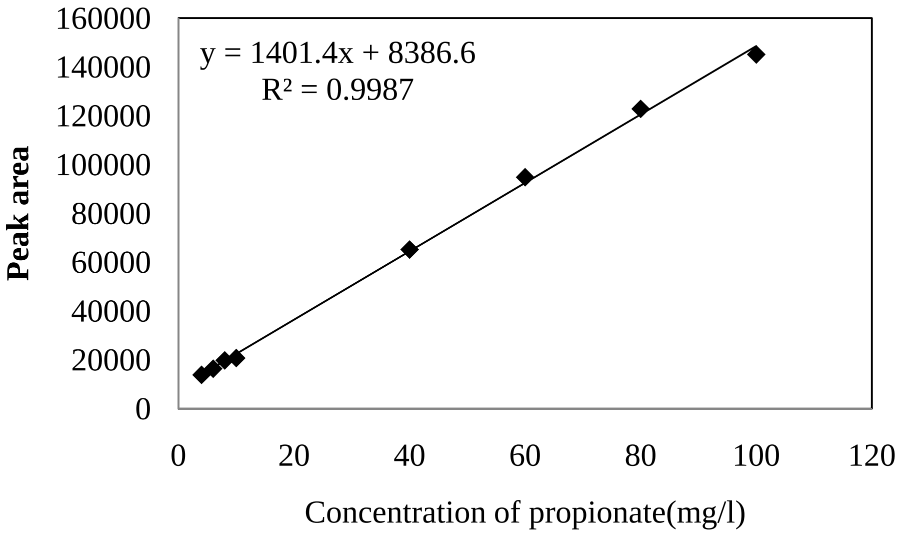

**Figure C2** The relationship between peak area and different amount of standard propionate

**Table C3** Gas chromatograph's calibration on standard curve for butyrate

| Concentration of butyrate(mg/l) | Peak area |
|---|---|
| 4 | 51890 |
| 6 | 62763 |
| 8 | 104789 |
| 10 | 122153 |
| 40 | 382619 |
| 60 | 576262 |
| 80 | 718848 |
| 100 | 893549 |

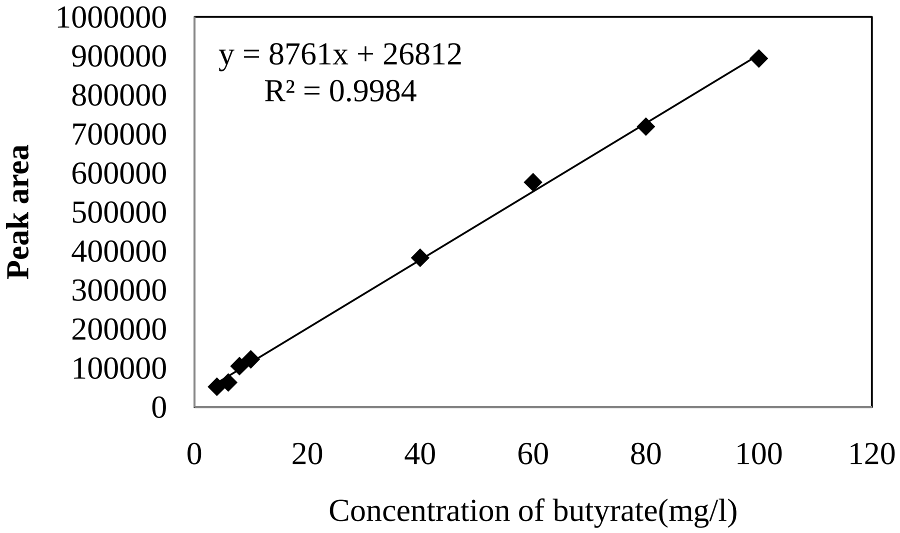

**Figure C3** The relationship between peak area and different amount of standard butyrate

# APPENDIX D
# DATA OF BIOGAS

**Appendix D 1** Raw data of hydrogen content in experiment batch reactor under mesophilic condition

| **Initial pH** | **Total biogas (ml)** | **$H_2$ (ml)** | **$N_2$ (ml)** | **$CO_2$ (ml)** | **% $H_2$** | **% $N_2$** | **% $CO_2$** |
|---|---|---|---|---|---|---|---|
| pH4 | 116.83 | 0.00 | 49.72 | 67.11 | 0.00 | 42.56 | 57.44 |
| PH5 | 167.83 | 0.10 | 53.79 | 113.94 | 0.06 | 32.05 | 67.89 |
| pH6 | 561.33 | 166.22 | 107.16 | 287.95 | 29.61 | 19.09 | 51.30 |
| pH7 | 357.42 | 122.68 | 76.34 | 158.39 | 34.32 | 21.36 | 44.32 |
| pH8 | 1531.17 | 733.49 | 71.63 | 726.05 | 47.90 | 4.68 | 47.42 |
| PH9 | 854.83 | 337.19 | 77.96 | 439.68 | 39.45 | 9.12 | 51.43 |
| pH10 | 271.67 | 14.57 | 81.01 | 176.08 | 5.36 | 29.82 | 64.82 |
| pH11 | 253.17 | 5.19 | 62.94 | 185.04 | 2.05 | 24.86 | 73.09 |
| pH12 | 240.00 | 21.61 | 71.02 | 147.38 | 9.00 | 29.59 | 61.41 |

**Appendix D2** Raw data of hydrogen content in experiment batch reactor under thermophilic condition

| **Initial pH** | **Total biogas (ml)** | **$H_2$ (ml)** | **$N_2$ (ml)** | **$CO_2$ (ml)** | **% $H_2$** | **% $N_2$** | **% $CO_2$** |
|---|---|---|---|---|---|---|---|
| pH4 | 140.00 | 0.00 | 34.80 | 105.20 | 0.00 | 24.86 | 75.14 |
| PH5 | 167.50 | 0.00 | 15.84 | 151.66 | 0.00 | 9.46 | 90.54 |
| pH6 | 797.75 | 233.42 | 61.65 | 502.68 | 29.26 | 7.73 | 63.01 |
| pH7 | 1118.00 | 356.58 | 61.34 | 700.08 | 31.89 | 5.49 | 62.62 |
| pH8 | 1550.33 | 767.64 | 34.16 | 748.53 | 49.51 | 2.20 | 48.28 |
| PH9 | 1339.67 | 423.55 | 36.98 | 879.14 | 31.62 | 2.76 | 65.62 |
| pH10 | 1388.67 | 413.55 | 141.10 | 834.02 | 29.78 | 10.16 | 60.06 |
| pH11 | 1832.17 | 280.62 | 163.12 | 1388.43 | 15.32 | 8.90 | 75.78 |
| pH12 | 1079.00 | 320.23 | 112.56 | 646.21 | 29.68 | 10.43 | 59.89 |

**Appendix D3** Raw data of hydrogen content in experiment batch reactor in different initial F/M ratios

| **F/M ratios** | **Total biogas (ml)** | **$H_2$ (ml)** | **$N_2$ (ml)** | **$CO_2$ (ml)** | **% $H_2$** | **% $N_2$** | **% $CO_2$** |
|---|---|---|---|---|---|---|---|
| 4 | 1571.67 | 648.43 | 87.54 | 835.69 | 41.26 | 5.57 | 53.17 |
| 6 | 1285.83 | 533.33 | 69.43 | 683.07 | 41.48 | 5.40 | 53.12 |
| 8 | 1164.50 | 477.94 | 60.79 | 625.77 | 41.04 | 5.22 | 53.74 |
| 10 | 1353.67 | 457.43 | 67.41 | 828.83 | 33.79 | 4.98 | 61.23 |
| 12 | 1272.00 | 426.30 | 68.43 | 777.27 | 33.51 | 5.38 | 61.11 |

**Appendix D4** Raw data of hydrogen content in experiment batch reactor in different initial iron concentration

| **Initial $Fe^{2+}$ concentration (mg $FeSO_4$/l)** | **Total biogas (ml)** | **$H_2$ (ml)** | **$N_2$ (ml)** | **$CO_2$ (ml)** | **% $H_2$** | **% $N_2$** | **% $CO_2$** |
|---|---|---|---|---|---|---|---|
| 0 | 1391.83 | 445.68 | 89.36 | 856.79 | 32.02 | 6.42 | 61.56 |
| 100 | 1532.67 | 588.25 | 89.66 | 854.76 | 38.38 | 5.85 | 55.77 |
| 200 | 1431.00 | 511.47 | 71.26 | 848.27 | 35.74 | 4.98 | 59.28 |
| 300 | 1313.00 | 405.90 | 75.23 | 831.87 | 30.91 | 5.73 | 63.36 |
| 400 | 861.00 | 367.88 | 44.86 | 448.26 | 42.73 | 5.21 | 52.06 |

**Appendix D 5** Raw data of hydrogen content in experiment batch reactor in pretreatment method

| **Pretreatment methods** | **Total biogas (ml)** | **H2 (ml)** | **N2 (ml)** | **CO2 (ml)** | **% H2** | **% N2** | **% CO2** |
|---|---|---|---|---|---|---|---|
| Heat | 1435.50 | 588.65 | 588.65 | 103.93 | 41.01 | 7.24 | 51.75 |
| Re Heat | 1468.50 | 656.57 | 656.57 | 100.89 | 44.71 | 6.87 | 48.42 |
| Chloroform | 121.67 | 0.47 | 0.47 | 57.28 | 0.39 | 47.08 | 52.53 |
| Ultrasonic | 1537.50 | 443.36 | 443.36 | 153.44 | 28.84 | 9.98 | 61.18 |

# APPENDIX E
# ANOVA STATISTIC OF HYDROGEN ANALYSIS

**Appendix E 1** Comparison of initial pH under mesophilic condition

| | Sum of squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between groups | 954955.034 | 8 | 119369.379 | 45727.510 | .000 |
| Within groups | 23.494 | 9 | 2.610 | | |
| Total | 954978.528 | 17 | | | |

| (I) Initial pH | (J) Initial pH | | | | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | Mean difference (I-J) | Std. error | Sig. | Lower bound | Upper bound |
| 4 | 5 | .00000 | 1.61569 | 1.000 | -3.6549 | 3.6549 |
| | 6 | -166.22000* | 1.61569 | .000 | -169.8749 | -162.5651 |
| | 7 | -122.68000* | 1.61569 | .000 | -126.3349 | -119.0251 |
| | 8 | -733.89000* | 1.61569 | .000 | -737.5449 | -730.2351 |
| | 9 | -337.18500* | 1.61569 | .000 | -340.8399 | -333.5301 |
| | 10 | -14.57000* | 1.61569 | .000 | -18.2249 | -10.9151 |
| | 11 | -5.19000* | 1.61569 | .011 | -8.8449 | -1.5351 |
| | 12 | -21.61000* | 1.61569 | .000 | -25.2649 | -17.9551 |

| (I) Initial pH | (J) Initial pH | Mean difference (I-J) | Std. error | Sig. | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| 5 | 4 | .00000 | 1.61569 | 1.000 | -3.6549 | 3.6549 |
| | 6 | -166.22000* | 1.61569 | .000 | -169.8749 | -162.5651 |
| | 7 | -122.68000* | 1.61569 | .000 | -126.3349 | -119.0251 |
| | 8 | -733.89000* | 1.61569 | .000 | -737.5449 | -730.2351 |
| | 9 | -337.18500* | 1.61569 | .000 | -340.8399 | -333.5301 |
| | 10 | -14.57000* | 1.61569 | .000 | -18.2249 | -10.9151 |
| | 11 | -5.19000* | 1.61569 | .011 | -8.8449 | -1.5351 |
| | 12 | -21.61000* | 1.61569 | .000 | -25.2649 | -17.9551 |
| 6 | 4 | 166.22000* | 1.61569 | .000 | 162.5651 | 169.8749 |
| | 5 | 166.22000* | 1.61569 | .000 | 162.5651 | 169.8749 |
| | 7 | 43.54000* | 1.61569 | .000 | 39.8851 | 47.1949 |
| | 8 | -567.67000* | 1.61569 | .000 | -571.3249 | -564.0151 |
| | 9 | -170.96500* | 1.61569 | .000 | -174.6199 | -167.3101 |
| | 10 | 151.65000* | 1.61569 | .000 | 147.9951 | 155.3049 |
| | 11 | 161.03000* | 1.61569 | .000 | 157.3751 | 164.6849 |
| | 12 | 144.61000* | 1.61569 | .000 | 140.9551 | 148.2649 |
| 7 | 4 | 122.68000* | 1.61569 | .000 | 119.0251 | 126.3349 |
| | 5 | 122.68000* | 1.61569 | .000 | 119.0251 | 126.3349 |

| (I) Initial pH | (J) Initial pH | Mean difference (I-J) | Std. error | Sig. | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| 7 | 6 | -43.54000* | 1.61569 | .000 | -47.1949 | -39.8851 |
| | 8 | -611.21000* | 1.61569 | .000 | -614.8649 | -607.5551 |
| | 9 | -214.50500* | 1.61569 | .000 | -218.1599 | -210.8501 |
| | 10 | 108.11000* | 1.61569 | .000 | 104.4551 | 111.7649 |
| | 11 | 117.49000* | 1.61569 | .000 | 113.8351 | 121.1449 |
| | 12 | 101.07000* | 1.61569 | .000 | 97.4151 | 104.7249 |
| 8 | 4 | 733.89000* | 1.61569 | .000 | 730.2351 | 737.5449 |
| | 5 | 733.89000* | 1.61569 | .000 | 730.2351 | 737.5449 |
| | 6 | 567.67000* | 1.61569 | .000 | 564.0151 | 571.3249 |
| | 7 | 611.21000* | 1.61569 | .000 | 607.5551 | 614.8649 |
| | 9 | 396.70500* | 1.61569 | .000 | 393.0501 | 400.3599 |
| | 10 | 719.32000* | 1.61569 | .000 | 715.6651 | 722.9749 |
| | 11 | 728.70000* | 1.61569 | .000 | 725.0451 | 732.3549 |
| | 12 | 712.28000* | 1.61569 | .000 | 708.6251 | 715.9349 |
| 9 | 4 | 337.18500* | 1.61569 | .000 | 333.5301 | 340.8399 |
| | 5 | 337.18500* | 1.61569 | .000 | 333.5301 | 340.8399 |
| | 6 | 170.96500* | 1.61569 | .000 | 167.3101 | 174.6199 |
| | 7 | 214.50500* | 1.61569 | .000 | 210.8501 | 218.1599 |

| (I) Initial pH | (J) Initial pH | Mean difference (I-J) | Std. error | Sig. | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| 9 | 8 | -396.70500* | 1.61569 | .000 | -400.3599 | -393.0501 |
| | 10 | 322.61500* | 1.61569 | .000 | 318.9601 | 326.2699 |
| | 11 | 331.99500* | 1.61569 | .000 | 328.3401 | 335.6499 |
| | 12 | 315.57500* | 1.61569 | .000 | 311.9201 | 319.2299 |
| 10 | 4 | 14.57000* | 1.61569 | .000 | 10.9151 | 18.2249 |
| | 5 | 14.57000* | 1.61569 | .000 | 10.9151 | 18.2249 |
| | 6 | -151.65000* | 1.61569 | .000 | -155.3049 | -147.9951 |
| | 7 | -108.11000* | 1.61569 | .000 | -111.7649 | -104.4551 |
| | 8 | -719.32000* | 1.61569 | .000 | -722.9749 | -715.6651 |
| | 9 | -322.61500* | 1.61569 | .000 | -326.2699 | -318.9601 |
| | 11 | 9.38000* | 1.61569 | .000 | 5.7251 | 13.0349 |
| | 12 | -7.04000* | 1.61569 | .002 | -10.6949 | -3.3851 |
| 11 | 4 | 5.19000* | 1.61569 | .011 | 1.5351 | 8.8449 |
| | 5 | 5.19000* | 1.61569 | .011 | 1.5351 | 8.8449 |
| | 6 | -161.03000* | 1.61569 | .000 | -164.6849 | -157.3751 |
| | 7 | -117.49000* | 1.61569 | .000 | -121.1449 | -113.8351 |
| | 8 | -728.70000* | 1.61569 | .000 | -732.3549 | -725.0451 |
| | 9 | -331.99500* | 1.61569 | .000 | -335.6499 | -328.3401 |
| | 10 | -9.38000* | 1.61569 | .000 | -13.0349 | -5.7251 |

| (I) Initial pH | (J) Initial pH | Mean difference (I-J) | Std. error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| 11 | 12 | -16.42000* | 1.61569 | .000 | -20.0749 | -12.7651 |
| 12 | 4 | 21.61000* | 1.61569 | .000 | 17.9551 | 25.2649 |
| | 5 | 21.61000* | 1.61569 | .000 | 17.9551 | 25.2649 |
| | 6 | -144.61000* | 1.61569 | .000 | -148.2649 | -140.9551 |
| | 7 | -101.07000* | 1.61569 | .000 | -104.7249 | -97.4151 |
| | 8 | -712.28000* | 1.61569 | .000 | -715.9349 | -708.6251 |
| | 9 | -315.57500* | 1.61569 | .000 | -319.2299 | -311.9201 |
| | 10 | 7.04000* | 1.61569 | .002 | 3.3851 | 10.6949 |
| | 11 | 16.42000* | 1.61569 | .000 | 12.7651 | 20.0749 |

*. The mean difference is significant at the 0.05 level.

**Appendix E 2** Comparison of initial pH under thermophiclic condition

| | Sum of squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between groups | 868500.104 | 8 | 108562.513 | 14887.303 | .000 |
| Within groups | 65.631 | 9 | 7.292 | | |
| Total | 868565.735 | 17 | | | |

| (I) Initial pH | (J) Initial pH | Mean difference (I-J) | Std. error | Sig. | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| 4 | 5 | .00000 | 2.70042 | 1.000 | -6.1088 | 6.1088 |
| | 6 | -233.42000* | 2.70042 | .000 | -239.5288 | -227.3112 |
| | 7 | -356.59000* | 2.70042 | .000 | -362.6988 | -350.4812 |
| | 8 | -767.64000* | 2.70042 | .000 | -773.7488 | -761.5312 |
| | 9 | -423.55000* | 2.70042 | .000 | -429.6588 | -417.4412 |
| | 10 | -413.55000* | 2.70042 | .000 | -419.6588 | -407.4412 |
| | 11 | -280.62000* | 2.70042 | .000 | -286.7288 | -274.5112 |
| | 12 | -320.23000* | 2.70042 | .000 | -326.3388 | -314.1212 |
| 5 | 4 | .00000 | 2.70042 | 1.000 | -6.1088 | 6.1088 |
| | 6 | -233.42000* | 2.70042 | .000 | -239.5288 | -227.3112 |
| | 7 | -356.59000* | 2.70042 | .000 | -362.6988 | -350.4812 |
| | 8 | -767.64000* | 2.70042 | .000 | -773.7488 | -761.5312 |
| | 9 | -423.55000* | 2.70042 | .000 | -429.6588 | -417.4412 |
| | 10 | -413.55000* | 2.70042 | .000 | -419.6588 | -407.4412 |
| | 11 | -280.62000* | 2.70042 | .000 | -286.7288 | -274.5112 |
| | 12 | -320.23000* | 2.70042 | .000 | -326.3388 | -314.1212 |
| 6 | 4 | 233.42000* | 2.70042 | .000 | 227.3112 | 239.5288 |
| | 5 | 233.42000* | 2.70042 | .000 | 227.3112 | 239.5288 |
| | 7 | -123.17000* | 2.70042 | .000 | -129.2788 | -117.0612 |

| (I) Initial pH | (J) Initial pH | Mean difference (I-J) | Std. error | Sig. | 95% Confidence Interval Lower bound | Upper bound |
|---|---|---|---|---|---|---|
| 6 | 8 | -534.22000* | 2.70042 | .000 | -540.3288 | -528.1112 |
|  | 9 | -190.13000* | 2.70042 | .000 | -196.2388 | -184.0212 |
|  | 10 | -180.13000* | 2.70042 | .000 | -186.2388 | -174.0212 |
|  | 11 | -47.20000* | 2.70042 | .000 | -53.3088 | -41.0912 |
|  | 12 | -86.81000* | 2.70042 | .000 | -92.9188 | -80.7012 |
| 7 | 4 | 356.59000* | 2.70042 | .000 | 350.4812 | 362.6988 |
|  | 5 | 356.59000* | 2.70042 | .000 | 350.4812 | 362.6988 |
|  | 6 | 123.17000* | 2.70042 | .000 | 117.0612 | 129.2788 |
|  | 8 | -411.05000* | 2.70042 | .000 | -417.1588 | -404.9412 |
|  | 9 | -66.96000* | 2.70042 | .000 | -73.0688 | -60.8512 |
|  | 10 | -56.96000* | 2.70042 | .000 | -63.0688 | -50.8512 |
|  | 11 | 75.97000* | 2.70042 | .000 | 69.8612 | 82.0788 |
|  | 12 | 36.36000* | 2.70042 | .000 | 30.2512 | 42.4688 |
| 8 | 4 | 767.64000* | 2.70042 | .000 | 761.5312 | 773.7488 |
|  | 5 | 767.64000* | 2.70042 | .000 | 761.5312 | 773.7488 |
|  | 6 | 534.22000* | 2.70042 | .000 | 528.1112 | 540.3288 |
|  | 7 | 411.05000* | 2.70042 | .000 | 404.9412 | 417.1588 |
|  | 9 | 344.09000* | 2.70042 | .000 | 337.9812 | 350.1988 |
|  | 10 | 354.09000* | 2.70042 | .000 | 347.9812 | 360.1988 |

| (I) Initial pH | (J) Initial pH | Mean difference (I-J) | Std. error | Sig. | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| 8 | 11 | 487.02000* | 2.70042 | .000 | 480.9112 | 493.1288 |
| | 12 | 447.41000* | 2.70042 | .000 | 441.3012 | 453.5188 |
| 9 | 4 | 423.55000* | 2.70042 | .000 | 417.4412 | 429.6588 |
| | 5 | 423.55000* | 2.70042 | .000 | 417.4412 | 429.6588 |
| | 6 | 190.13000* | 2.70042 | .000 | 184.0212 | 196.2388 |
| | 7 | 66.96000* | 2.70042 | .000 | 60.8512 | 73.0688 |
| | 8 | -344.09000* | 2.70042 | .000 | -350.1988 | -337.9812 |
| | 10 | 10.00000* | 2.70042 | .005 | 3.8912 | 16.1088 |
| | 11 | 142.93000* | 2.70042 | .000 | 136.8212 | 149.0388 |
| | 12 | 103.32000* | 2.70042 | .000 | 97.2112 | 109.4288 |
| 10 | 4 | 413.55000* | 2.70042 | .000 | 407.4412 | 419.6588 |
| | 5 | 413.55000* | 2.70042 | .000 | 407.4412 | 419.6588 |
| | 6 | 180.13000* | 2.70042 | .000 | 174.0212 | 186.2388 |
| | 7 | 56.96000* | 2.70042 | .000 | 50.8512 | 63.0688 |
| | 8 | -354.09000* | 2.70042 | .000 | -360.1988 | -347.9812 |
| | 9 | -10.00000* | 2.70042 | .005 | -16.1088 | -3.8912 |
| | 11 | 132.93000* | 2.70042 | .000 | 126.8212 | 139.0388 |
| | 12 | 93.32000* | 2.70042 | .000 | 87.2112 | 99.4288 |
| 11 | 4 | 280.62000* | 2.70042 | .000 | 274.5112 | 286.7288 |

| (I) Initial pH | (J) Initial pH | Mean difference (I-J) | Std. error | Sig. | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| 11 | 5 | 280.62000* | 2.70042 | .000 | 274.5112 | 286.7288 |
| | 6 | 47.20000* | 2.70042 | .000 | 41.0912 | 53.3088 |
| | 7 | -75.97000* | 2.70042 | .000 | -82.0788 | -69.8612 |
| | 8 | -487.02000* | 2.70042 | .000 | -493.1288 | -480.9112 |
| | 9 | -142.93000* | 2.70042 | .000 | -149.0388 | -136.8212 |
| | 10 | -132.93000* | 2.70042 | .000 | -139.0388 | -126.8212 |
| | 12 | -39.61000* | 2.70042 | .000 | -45.7188 | -33.5012 |
| 12 | 4 | 320.23000* | 2.70042 | .000 | 314.1212 | 326.3388 |
| | 5 | 320.23000* | 2.70042 | .000 | 314.1212 | 326.3388 |
| | 6 | 86.81000* | 2.70042 | .000 | 80.7012 | 92.9188 |
| | 7 | -36.36000* | 2.70042 | .000 | -42.4688 | -30.2512 |
| | 8 | -447.41000* | 2.70042 | .000 | -453.5188 | -441.3012 |
| | 9 | -103.32000* | 2.70042 | .000 | -109.4288 | -97.2112 |
| | 10 | -93.32000* | 2.70042 | .000 | -99.4288 | -87.2112 |
| | 11 | 39.61000* | 2.70042 | .000 | 33.5012 | 45.7188 |

*. The mean difference is significant at the 0.05 level.

**Appendix E 3** Comparison of initial pH 8 under between mesophilic with thermophilic condition

**Independent Samples Test**

| | | Levene's test for equality of variances | | t-test for equality of means | |
|---|---|---|---|---|---|
| | | F | Sig. | t | df |
| Hydrogen | Equal variances assumed | 6.685E14 | .000 | -27.599 | 2 |
| | Equal variances not assumed | | | -27.599 | 1.918 |

**Appendix E 4** Comparison of initial F/M ratios

| | Sum of squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between groups | 61029.592 | 4 | 15257.398 | 452.040 | .000 |
| Within groups | 168.761 | 5 | 33.752 | | |
| Total | 61198.353 | 9 | | | |

| (I) F/M ratio | (J) F/M ratio | Mean difference (I-J) | Std. error | Sig. | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| 4 | 6 | 115.09500$^{*}$ | 5.80967 | .000 | 100.1608 | 130.0292 |
| | 8 | 170.48500$^{*}$ | 5.80967 | .000 | 155.5508 | 185.4192 |
| | 10 | 191.19500$^{*}$ | 5.80967 | .000 | 176.2608 | 206.1292 |
| | 12 | 222.12500$^{*}$ | 5.80967 | .000 | 207.1908 | 237.0592 |
| 6 | 4 | -115.09500$^{*}$ | 5.80967 | .000 | -130.0292 | -100.1608 |
| | 8 | 55.39000$^{*}$ | 5.80967 | .000 | 40.4558 | 70.3242 |
| | 10 | 76.10000$^{*}$ | 5.80967 | .000 | 61.1658 | 91.0342 |
| | 12 | 107.03000$^{*}$ | 5.80967 | .000 | 92.0958 | 121.9642 |

| (I) F/M ratio | (J) F/M ratio | Mean difference (I-J) | Std. error | Sig. | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| 8 | 4 | -170.48500* | 5.80967 | .000 | -185.4192 | -155.5508 |
| | 6 | -55.39000* | 5.80967 | .000 | -70.3242 | -40.4558 |
| | 10 | 20.71000* | 5.80967 | .016 | 5.7758 | 35.6442 |
| | 12 | 51.64000* | 5.80967 | .000 | 36.7058 | 66.5742 |
| 10 | 4 | -191.19500* | 5.80967 | .000 | -206.1292 | -176.2608 |
| | 6 | -76.10000* | 5.80967 | .000 | -91.0342 | -61.1658 |
| | 8 | -20.71000* | 5.80967 | .016 | -35.6442 | -5.7758 |
| | 12 | 30.93000* | 5.80967 | .003 | 15.9958 | 45.8642 |
| 12 | 4 | -222.12500* | 5.80967 | .000 | -237.0592 | -207.1908 |
| | 6 | -107.03000* | 5.80967 | .000 | -121.9642 | -92.0958 |
| | 8 | -51.64000* | 5.80967 | .000 | -66.5742 | -36.7058 |
| | 10 | -30.93000* | 5.80967 | .003 | -45.8642 | -15.9958 |

*. The mean difference is significant at the 0.05 level.

**Appendix E 5** Comparison of initial iron concentration

| | Sum of squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between groups | 52404.908 | 4 | 13101.227 | 3262.445 | .000 |
| Within groups | 20.079 | 5 | 4.016 | | |
| Total | 52424.987 | 9 | | | |

| (I) Iron concentration | (J) Iron concentration | Mean difference (I-J) | Std. error | Sig. | 95% Confidence Interval | |
| --- | --- | --- | --- | --- | --- | --- |
| | | | | | Lower bound | Upper bound |
| 0 | 100 | -131.84000$^{*}$ | 2.00394 | .000 | 0 | 100 |
| | 200 | -60.84000$^{*}$ | 2.00394 | .000 | | 200 |
| | 300 | 36.78000$^{*}$ | 2.00394 | .000 | | 300 |
| | 400 | 71.94500$^{*}$ | 2.00394 | .000 | | 400 |
| 100 | 0 | 131.84000$^{*}$ | 2.00394 | .000 | 100 | 0 |
| | 200 | 71.00000$^{*}$ | 2.00394 | .000 | | 200 |
| | 300 | 168.62000$^{*}$ | 2.00394 | .000 | | 300 |
| | 400 | 203.78500$^{*}$ | 2.00394 | .000 | | 400 |
| 200 | 0 | 60.84000$^{*}$ | 2.00394 | .000 | 200 | 0 |
| | 100 | -71.00000$^{*}$ | 2.00394 | .000 | | 100 |
| | 300 | 97.62000$^{*}$ | 2.00394 | .000 | | 300 |
| | 400 | 132.78500$^{*}$ | 2.00394 | .000 | | 400 |
| 300 | 0 | -36.78000$^{*}$ | 2.00394 | .000 | 300 | 0 |
| | 100 | -168.62000$^{*}$ | 2.00394 | .000 | | 100 |
| | 200 | -97.62000$^{*}$ | 2.00394 | .000 | | 200 |
| | 400 | 35.16500$^{*}$ | 2.00394 | .000 | | 400 |
| 400 | 0 | -71.94500$^{*}$ | 2.00394 | .000 | 400 | 0 |
| | 100 | -203.78500$^{*}$ | 2.00394 | .000 | | 100 |
| | 200 | -132.78500$^{*}$ | 2.00394 | .000 | | 200 |
| | 300 | -35.16500$^{*}$ | 2.00394 | .000 | | 300 |

*. The mean difference is significant at the 0.05 level.

**Appendix E 6** Comparison of pre-treatment method

| | Sum of Squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between groups | 521872.958 | 3 | 173957.653 | 67527.196 | .000 |
| Within groups | 10.304 | 4 | 2.576 | | |
| Total | 521883.263 | 7 | | | |

| (I) pretreatment method | (J) pretreatment method | Mean Difference (I-J) | Std. error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| Heat | Re-heat | -67.91500* | 1.60503 | .000 | -72.3713 | -63.4587 |
| | Chloroform | 588.18000* | 1.60503 | .000 | 583.7237 | 592.6363 |
| | Ultrasonic | 145.29000* | 1.60503 | .000 | 140.8337 | 149.7463 |
| Re-heat | Heat | 67.91500* | 1.60503 | .000 | 63.4587 | 72.3713 |
| | Chloroform | 656.09500* | 1.60503 | .000 | 651.6387 | 660.5513 |
| | Ultrasonic | 213.20500* | 1.60503 | .000 | 208.7487 | 217.6613 |
| Chloroform | Heat | -588.18000* | 1.60503 | .000 | -592.6363 | -583.7237 |
| | Re-heat | -656.09500* | 1.60503 | .000 | -660.5513 | -651.6387 |
| | Ultrasonic | -442.89000* | 1.60503 | .000 | -447.3463 | -438.4337 |
| Ultrasonic | Heat | -145.29000* | 1.60503 | .000 | -149.7463 | -140.8337 |
| | Re-heat | -213.20500* | 1.60503 | .000 | -217.6613 | -208.7487 |
| | Chloroform | 442.89000* | 1.60503 | .000 | 438.4337 | 447.3463 |

*. The mean difference is significant at the 0.05 level.