APPENDICES

APPENDIX A
METHADOLOGY OF ANALYSIS

A1 pH Analysis

Apparatus

- Beaker 50 mL
- pH meter

Reagents

- Standard buffer solutions (pH 4, 7 and 10)

Procedure

- Rinse the electrode with distilled water and wipe with tissue paper (do this every time before soaking the electrode in a new solution).
- pH meter is calibrated with standard buffer solution at pH 4, 7 and 10, respectively.
- Measure pH of samples.

A2 Total Solids (TS) Analysis

Apparatus

- Analytical balance
- Crucible 50 mL
- Desiccator
- Drying oven 103 - 105°C
- Muffle furnace
- Water bath

Procedure

- Preparation of evaporation crucible: If volatile solids are to be measured ignite clean evaporating 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 - 105°C and then cool crucible in desiccator for 30 min or until a constant weight.
- Determine weight in an analytical balance.

Calculation

$$\text{Total solids (g/L)} = \frac{(A - B) \times 1{,}000}{\text{mL of sample}}$$

where A = Weight of residue + crucible after dry evaporation, g

B = Weight of crucible after ignition, g

A3 Volatile Solids (VS) Analysis

Apparatus

- The residue from total solids
- Analytical balance
- Desiccator
- Muffle furnace

Procedure

- The residue from total solids is ignited in the muffle furnace at 550°C for 1 h.
- Cool in desiccator to a constant temperature and weigh.
- Determine weigh in an analytical balance.

Calculation

$$\text{Volatile solids (g/L)} = \frac{(A - B) \times 1{,}000}{\text{mL of sample}}$$

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

B = Weight of residue + crucible after ignition, g

A4 Chemical Oxygen Demand (COD) Analysis (Closed reflux titration method)

Apparatus

- Analytical balance
- Beaker 250 mL
- Burette 50 mL
- Cylinder 25 and 100 mL
- Digestion vessel 16 x 100 mm
- Heating Block
- Hot air oven
- Pipettes 1 and 5 mL
- Stir glass and rubber pump
- Volumetric flasks 100 and 1,000 mL

Reagents

- 0.0167 M Potassium dichromate digestion 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 103ºC for 2 h, then add 167 mL conc.H_2SO_4 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 1,000 mL.
- Sulfuric acid reagent: Add 1% w/v into conc.H_2SO_4. The mixture was let for 1 - 2 days for completely dissolve Ag_2SO_4.

- Ferroine indicator: 1.485 g 1-10 phenanthroline monohydrate ($C_{12}H_8N_2 \cdot H_2O$) and 0.695 g $FeSO_4 \cdot 7H_2O$ dissolve into distilled water, and finally diluted to 100 mL.
- 0.1 M Ferrous ammonium sulfate (FAS) standard solution: 39.2 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ dissolve into 500 mL distilled water, then add 20 mL conc.H_2SO_4, and finally diluted to 1,000 mL.

Note: 0.1 M Ferrous ammonium sulfate standard solution compare with potassium dichromate digestion 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 M FAS standard solution. The end point change green blue to red brown.

$$\text{Normality of FAS solution} = \frac{\text{mL } K_2Cr_2O_7 \text{ x } 0.10}{\text{mL } Fe(NH_4)_2(SO_4)_2}$$

Procedure

- 2.5 mL of sample was added to digestion vessel.
- Add 1.5 mL potassium dichromate digestion standard solution, 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 heated at 150ºC for 2 h, and then left to be cooled in room temperature.
- Afterward, ferroine indicator was dropped for 1 – 2 drops, and finally titrated with 0.1 M ferrous ammonium sulfate standard solution. The end point change green blue to red brown.

Note: Distilled water was used to blank

Calculation

$$\text{COD (mg/L)} = \frac{(A - B) \text{ x } M \text{ x } 8{,}000}{\text{mL of sample}}$$

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

A5 Total Kjeldahl Nitrogen (TKN) Analysis (Kjeldahl method)

Apparatus

- Analytical balance
- Beaker 250 mL
- Burette 50 mL
- Cylinder 25 and 100 mL
- Digestor
- Digestor tube 250 mL
- Distilling unit
- Erlenmeyer flask 250 mL
- Hood
- Pipettes 5, 10, 15 and 20 mL
- Stir glass and rubber pump
- Volumetric flasks 100, 500 and 1,000 mL

Reagents

- Conc.H_2SO_4
- 30% H_2O_2
- Sodium hydroxide solution: The following reagents were added 1 kg sodium hydroxide (commercial grade) into 1,000 mL distilled water.
- Mixed catalyst: That consists of K_2SO_4 and $CuSO_4 \cdot 5H_2O$ in 100:10 ratio.
- Mixed indicator: That consists of methyl red indicator (0.2 g methyl red dissolve into 100 mL 95% ethyl alcohol) and methyl blue indicator (0.1 g methyl blue dissolve into 50 mL 95% ethyl alcohol) in 2:1 ratio. The indicator was collected in a light brown bottle.

- Boric indicator: Add 40 g boric acid into 1,000 mL volumetric flask, that have a bit distilled water in it and add 10 mL mixed indicator and then diluted to 1,000 mL.
- 0.1 N Sulfuric acid standard solution: Pipette 1.5 mL conc.H_2SO_4 transfer to 500 mL volumetric flask, fill flask to mark with distilled water. Titration certainly determines concentration of sulfuric acid standard solution with 40 mL 0.05 N sodium carbonate (2.5xx g Na_2CO_3 dissolve into 1,000 mL distilled water). Phenolphthalein was dropped 3 - 4 drops. The end point change pink to colorless.

$$\text{Normality of sulfuric acid} = \frac{A \times B}{53 \times C}$$

where A = g Na_2CO_3 into 1,000 mL volumetric flask
B = mL of Na_2CO_3 solution taken for titration
C = mL acid used

Procedure

Digestion

- Add 100 mL sample into 250 mL digestor tube and add 10.5 g mixed catalyst.
- Add 15 mL conc.H_2SO_4 and 2 mL 30% H_2O_2
- Initial in digestor machine at temperature about 250°C, when temperature increases to 420°C count time for 2 h.

Distillation

- Sample is distilled by auto distillation unit (Kjeltectm2200). 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 erlenmeyer flask which is supported from auto distillation.

Titration

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

Note: Distilled water was used to blank

Calculation

$$N\ (mg/L) = \frac{(A - B) \times N \times 14{,}000}{mL\ of\ sample}$$

where A = mL sulfuric acid used titrate sample
B = mL sulfuric acid used titrate blank
N = Normality of sulfuric acid standard

$$Protein\ (mg/L) = N \times 6.25$$

where N = Nitrogen (mg/L)

A6 Fat Oil and Grease (FOG) Analysis (Soxhlet extraction method)

Apparatus

- Analytical balance
- Buchner funnel
- Clean cotton
- Desiccator
- Electric heating mantle
- Extraction thimble
- Filter paper
- Glass beads
- Muslin remnant of cloth
- Soxhlet & distill bottle
- Vacuum pump
- Water bath

Reagents

- 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 - 105°C.
- Extraction thimble is add in soxhlet, that connect with distill bottle and soxhlet extraction for 4 h. 200 mL n–hexane is a solvent in distill bottle. Extraction ratio 20 rounds per hour.
- Afterward, distill extraction bottle until dry (n–hexane is distilled). Dry constant weight in oven at 103 - 105°C and place in desiccator until weigh constant.
- Determine weight by analytical balance.

Calculation

$$\text{Oil and grease (mg/L)} = \frac{\text{mg increase in weight of flask x 1,000}}{\text{mL of sample}}$$

A7 Carbohydrate Analysis (Colorimetric method)

Apparatus

- Micro pipette
- Spectrophotometer
- Tube
- Water bath

Reagents

- 20% Phenol: Add 20 g phenol into 80 mL deionize water at 25°C.

- conc.H_2SO_4
- 100 ppm of D-glucose standard solution: 1.485 g 1-10 phenanthroline monohydrate ($C_{12}H_8N_2 \cdot H_2O$) dissolve into distilled water, and finally diluted to 100 mL.

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 20% phenol is added. Then 5 mL conc.H_2SO_4 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 490 nm with spectrophotometer. Blank are prepared by substituting deionize water for the sugar solution.

Sample Analysis

- Add 2 mL sample, that it is diluted 1,000 times by deionize water in tube.
- Add 50 µL of 20% phenol and then add 5 mL conc.H_2SO_4 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 spectrophotometer at 490 nm. Plot graph in standard curve and calculate concentration.

A8 Ammonia-nitrogen Analysis (Titrimetric method)

Apparatus

- Burette 50 mL
- Digestor tube 800 mL
- Distilling unit
- Erlenmeyer flask 300 mL
- pH meter

Reagents

- Borate buffer solution: Add 88 mL of 0.1 sodium hydroxide solution into 500 mL of 0.025 M sodium tetra borate solution (9.5 g $Na_2B_4O_7.10H_2O$ dissolve into 1,000 mL distilled water) and then diluted to 1,000 mL with distilled water.
- 6 N Sodium hydroxide solution: Add 24 g sodium hydroxide into 1,000 mL distilled water.
- Mixed indicator: That consists of methyl red indicator (0.2 g methyl red dissolve into 100 mL 95% ethyl alcohol) and methyl blue indicator (0.1 g methyl blue dissolve into 50 mL 95% ethyl alcohol) in 1:1 ratio. The indicator was collected in a light brown bottle.
- Boric indicator: Add 20 g boric acid into 1,000 mL volumetric flask, that have a bit distilled water in it and add 10 mL mixed indicator and then diluted to 1,000 mL.
- 0.02 N Sulfuric acid standard solution: 200 mL of 1 N H_2SO_4 diluted to 1,000 mL with distilled water. Titration certainly determines concentration of sulfuric acid standard solution with 20 mL 0.05 N sodium carbonate (2.5xx g Na_2CO_3 dissolve into 1,000 mL distilled water). Phenolphthalein was dropped 3 - 4 drops. The end point change pink to colorless.

$$\text{Normality of sulfuric acid} = \frac{A \times B}{53 \times C}$$

where A = g Na_2CO_3 into 1,000 mL volumetric flask

B = mL of Na_2CO_3 solution taken for titration

C = mL acid used

Procedure

Sample preparation

- Add 500 mL sample in beaker and add 25 mL borate buffer solution. Then, adjust pH into 9.5 with 6N sodium hydroxide.

- Transfer 25 mL sample in beaker to 300 mL digestor tube.

Distillation

- Sample is distilled by auto distillation unit with rate 6 mL/min for 4 min until get 300 mL distillate and then was analyzed by titration.
- Add 50 mL boric indicator in 300 mL erlenmeyer flask which is supported from auto distillation.

Titration

- Dilute distillate to 500 mL with distilled water before titration.
- Titration with 0.02 N sulfuric acid standard solution. The end point change green to purple.

Note: Distilled water was used to blank

Calculation

$$\text{Ammonia (mg/L)} = \frac{(A - B) \times N \times 14{,}000}{\text{mL of sample}}$$

where A = mL sulfuric acid used titrate sample

B = mL sulfuric acid used titrate blank

N = Normality of sulfuric acid standard

APPENDIX B
STANDARD CURVES

Table B1 Gas chromatograph's calibration on standard curve (60% Hydrogen).

Order	% H_2	Area
1	60	1801733
2	60	1988585
3	60	1823359
4	60	1888033
5	60	1993569
6	60	1952700
7	60	1871085
8	60	1822995
9	60	1809472
10	60	1886590
	Average	1883812.10

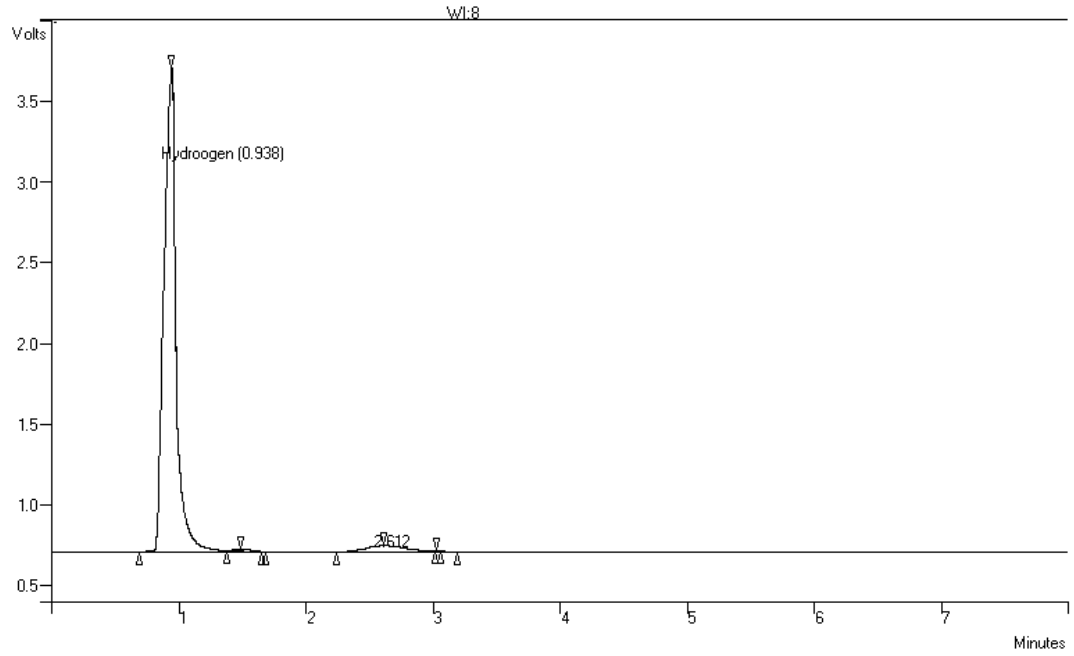

Figure B1 Graph of standard gas (60% Hydrogen).

Table B2 Gas chromatograph's calibration on standard curve (70% Methane).

Order	% CH_4	Area
1	70	335074
2	70	345505
3	70	354096
4	70	357146
5	70	341111
6	70	352485
7	70	336220
8	70	348656
9	70	342597
10	70	340077
	Average	345296.70

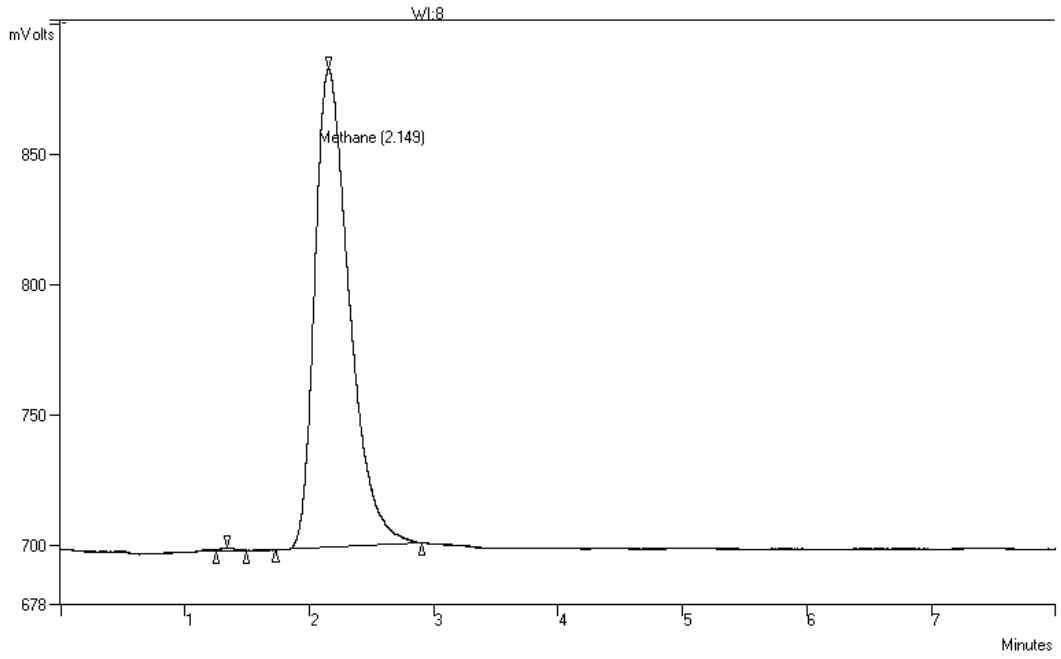

Figure B2 Graph of standard gas (70% Methane).

Table B3 Gas chromatograph's calibration on standard curve (99.99% Nitrogen).

Order	% N_2	Area
1	99.99	336600
2	99.99	323296
3	99.99	329013
4	99.99	311073
5	99.99	345449
6	99.99	335369
7	99.99	338414
8	99.99	329740
9	99.99	343954
10	99.99	350962
	Average	334387

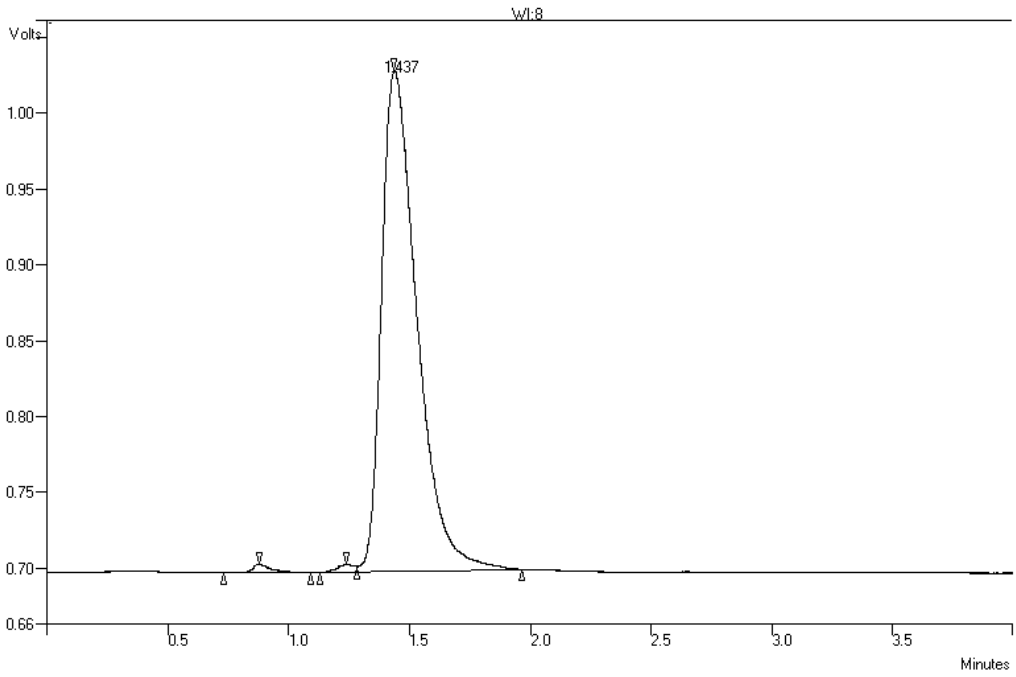

Figure B3 Graph of standard gas (99.99% Nitrogen).

Table B4 Gas chromatograph's calibration on standard curve (30% Carbon dioxide).

Order	% CO_2	Area
1	30	35657
2	30	41180
3	30	40251
4	30	38776
5	30	36728
6	30	39344
7	30	37762
8	30	38226
9	30	32137
10	30	35214
	Average	37527.50

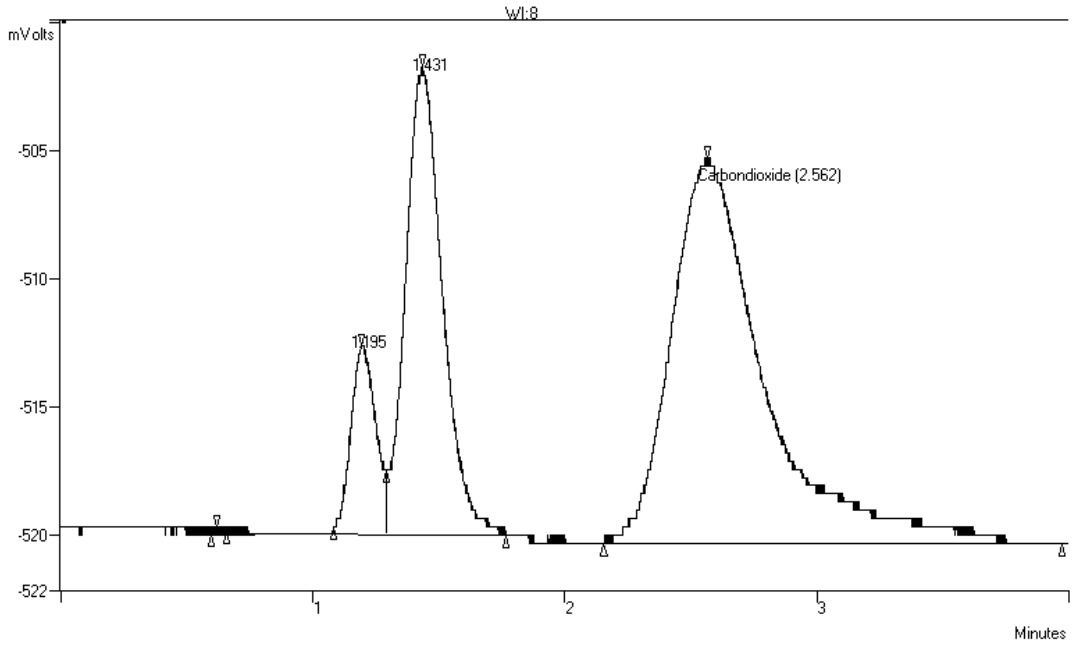

Figure B4 Graph of standard gas (30% Carbon dioxide).

Table B5 Gas chromatograph's calibration on standard curve for acetic acid.

Concentration of acetic acid (mg/L)	Peak area
400	103
600	135
800	178
1,000	237
4,000	910
6,000	1,350
8,000	1,805
10,000	2,251

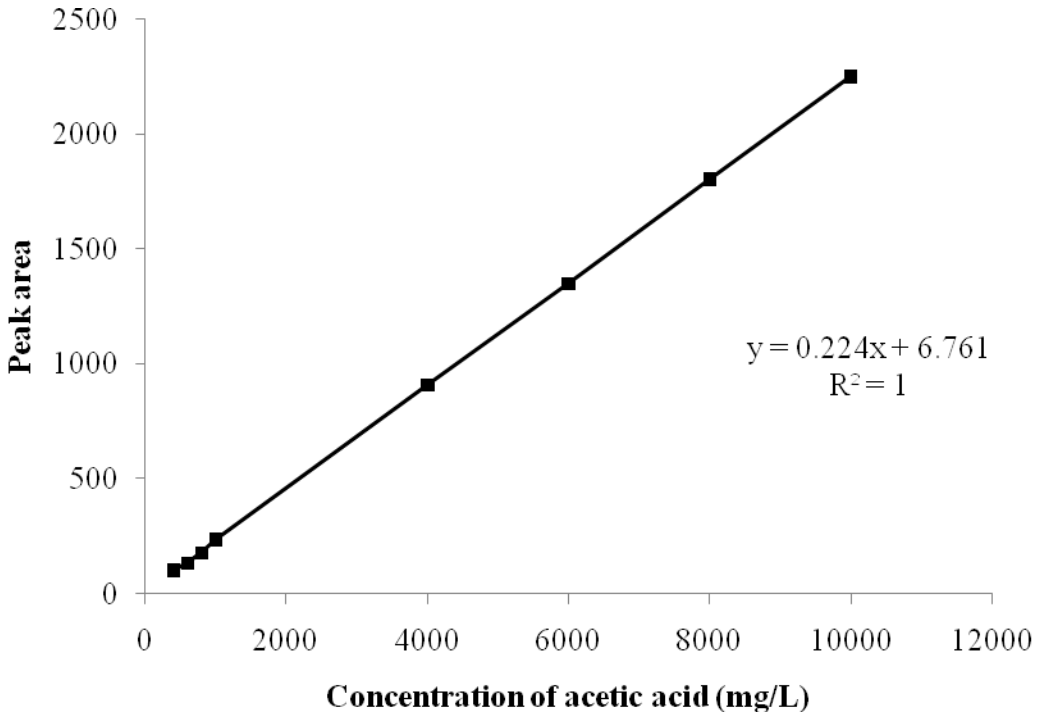

Figure B5 The relationship between peak area and various concentration of standard acetic acid.

Table B6 Gas chromatograph's calibration on standard curve for butyric acid.

Concentration of butyric acid (mg/L)	Peak area
400	137
600	193
800	255
1,000	338
4,000	1,271
6,000	1,877
8,000	2,506
10,000	3,123

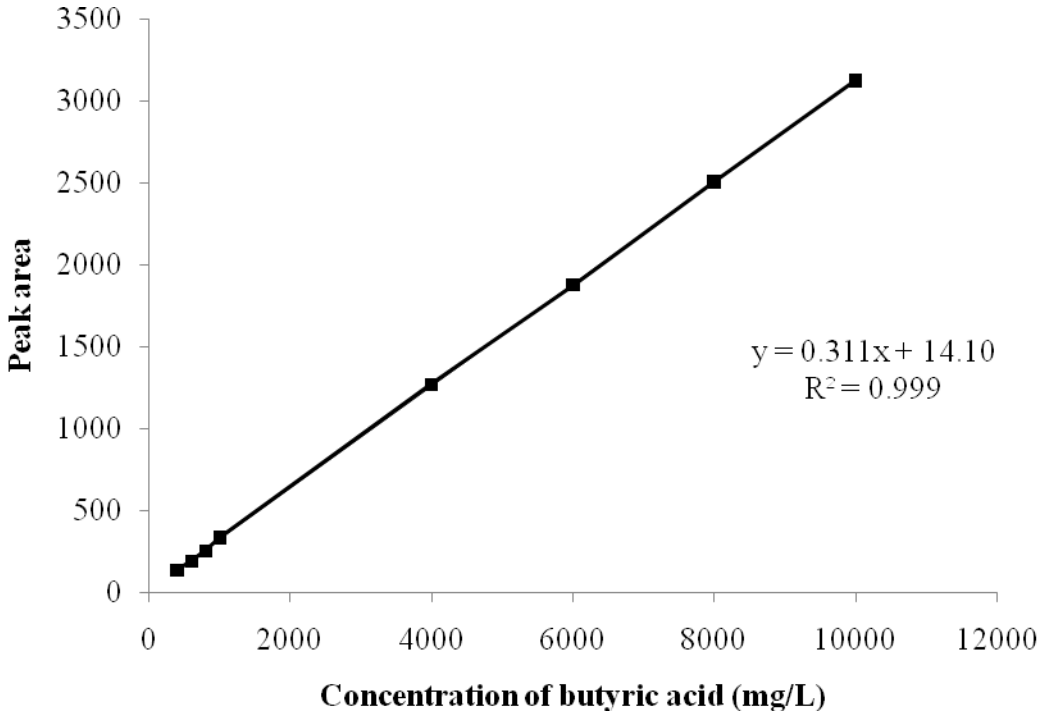

Figure B6 The relationship between peak area and various concentration of standard butyric acid.

Table B7 Gas chromatograph's calibration on standard curve for propionic acid.

Concentration of propionic acid (mg/L)	Peak area
400	158
600	209
800	276
1,000	366
4,000	1,383
6,000	2,043
8,000	2,727
10,000	3,398

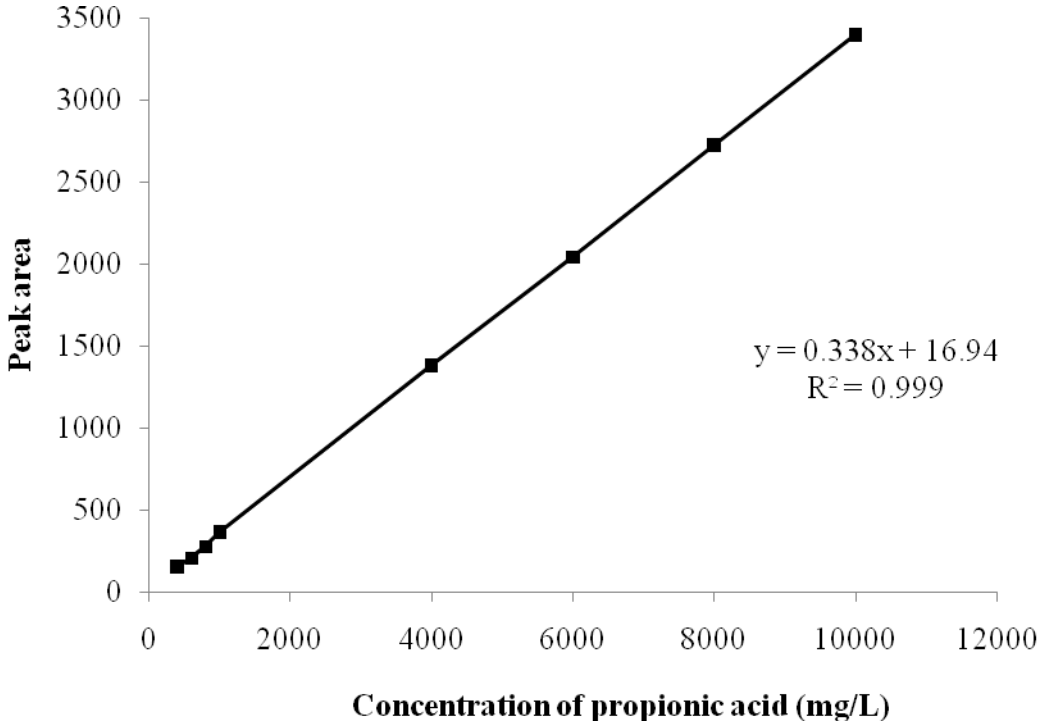

Figure B7 The relationship between peak area and various concentration of standard propionic acid.

Table B8 Gas chromatograph's calibration on standard curve for methanol.

Concentration of methanol (mg/L)	Peak area
400	77
600	96
800	128
1,000	171
4,000	653
6,000	962
8,000	1,289
10,000	1,619

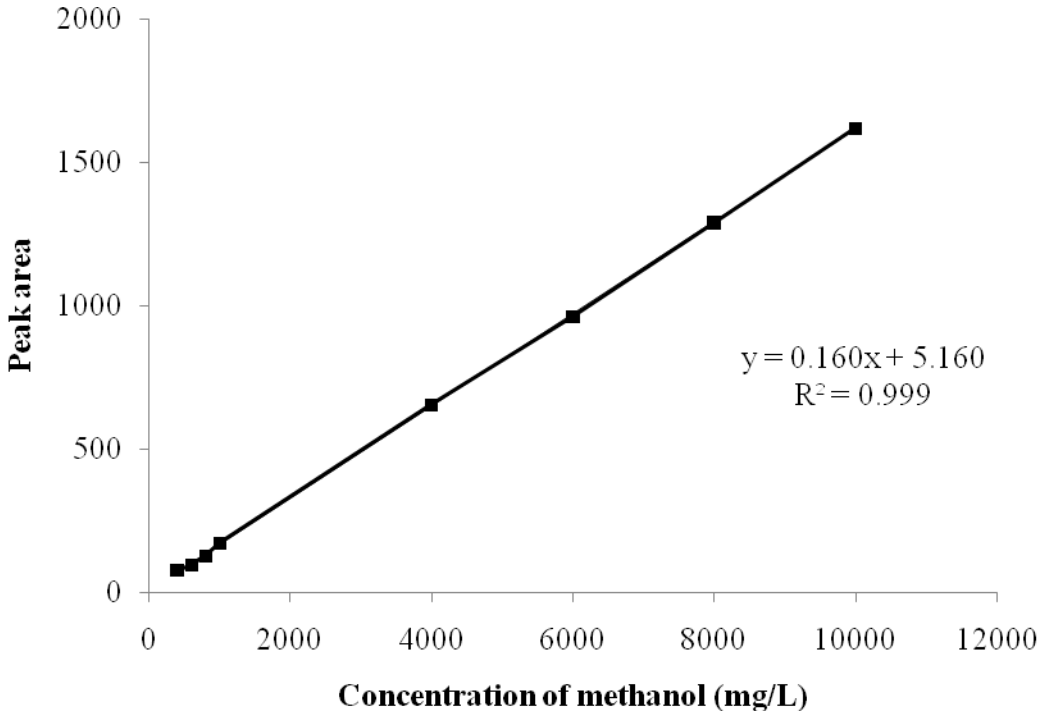

Figure B8 The relationship between peak area and various concentration of standard methanol.

APPENDIX C
EXPERIMENTAL DATA

Table C1 Raw data of biogas component from a two-stage fermentation process (Phase I).

Treatment	Total biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	CH_4 (mL)	H_2 (%)	CO_2 (%)	N_2 (%)	CH_4 (%)
Control	121.00	10.71	57.39	52.90	ND	8.85	47.43	43.72	ND
IA	848.00	285.01	373.04	189.95	ND	33.61	43.99	22.40	ND
IB	486.50	133.06	229.29	124.15	ND	27.35	47.13	25.52	ND
IC	3,161.50	269.36	1,417.30	346.18	1,128.66	8.52	44.83	10.95	35.70

Note Control: Food waste.

IA: Food waste and pretreated seed sludge.

IB: Food waste and seed sludge without pretreatment method.

IC: Food waste and pretreated seed sludge were operated in stage I. Then, seed sludge without pretreatment method was added in stage II.

ND: Not detectable.

Table C2 Raw data of biogas component at various initial pH values under mesophilic temperature (35 $\pm$ 2^{o}C) (Phase IIA).

Initial pH	Total biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	CH_4 (mL)	H_2 (%)	CO_2 (%)	N_2 (%)	CH_4 (%)
5.0	880.33	218.94	379.25	282.14	ND	24.87	43.08	32.05	ND
6.0	1,243.00	422.87	494.96	325.17	ND	34.02	39.82	26.16	ND
7.0	1,386.00	757.59	446.71	181.70	ND	54.66	32.23	13.11	ND
8.0	1,051.00	326.34	409.57	315.09	ND	31.05	38.97	29.98	ND
9.0	982.33	298.63	369.65	314.05	ND	30.40	37.63	31.97	ND

Note ND: Not detectable.

Table C3 Raw data of biogas component at various initial pH values under thermophilic temperature (55 $\pm$ 2^{o}C) (Phase IIA).

Initial pH	Total biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	CH_4 (mL)	H_2 (%)	CO_2 (%)	N_2 (%)	CH_4 (%)
5.0	1,060.67	276.83	469.77	314.07	ND	26.10	44.29	29.61	ND
6.0	2,075.33	972.91	615.34	487.08	ND	46.88	29.65	23.47	ND
7.0	2,406.00	1,430.37	519.94	455.69	ND	59.45	21.61	18.94	ND
8.0	1,803.33	728.18	601.95	473.20	ND	40.38	33.38	26.24	ND
9.0	1,438.33	588.13	463.29	386.91	ND	40.89	32.21	26.90	ND

Note ND: Not detectable.

Table C4 Raw data of biogas component at various C/N ratios (Phase IIB).

C/N ratio	Total biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	CH_4 (mL)	H_2 (%)	CO_2 (%)	N_2 (%)	CH_4 (%)
Control	2,380.67	1,202.24	565.17	613.26	ND	50.50	23.74	25.76	ND
10	1,055.67	296.96	271.62	487.09	ND	28.13	25.73	46.14	ND
20	4,265.33	1,023.68	1,285.57	1,956.08	ND	24.00	30.14	45.86	ND
30	5,066.67	1,829.07	1,413.60	1,824.00	ND	36.10	27.90	36.00	ND
40	3,111.33	1,450.81	759.79	900.73	ND	46.63	24.42	28.95	ND
50	2,457.67	1,221.22	608.03	628.42	ND	49.69	24.74	25.57	ND

Note Control: No addition of NH_4HCO_3.

ND: Not detectable.

Table C5 Raw data of biogas component at various initial pH values under mesophilic temperature (35 ± 2^oC) (Phase IIC).

Initial pH	Total biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	CH_4 (mL)	H_2 (%)	CO_2 (%)	N_2 (%)	CH_4 (%)
Control	255.00	3.65	95.22	59.71	96.42	1.43	37.34	23.42	37.81
6.0	103.67	4.43	52.71	46.53	ND	4.28	50.85	44.88	ND
7.0	256.33	4.51	97.02	59.70	95.10	1.76	37.85	23.29	37.10
8.0	153.67	1.66	63.46	44.28	44.27	1.08	41.30	28.82	28.81
9.0	54.00	0.57	31.37	15.54	6.52	1.06	58.10	28.76	12.07
10.0	33.67	0.37	16.02	17.28	ND	1.09	47.59	51.33	ND

Note Control: Initial pH 7.43.

ND: Not detectable.

Table C6 Raw data of biogas component at various initial pH values under thermophilic temperature (55 $\pm$ 2^{o}C) (Phase IIC).

Initial pH	Total biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	CH_4 (mL)	H_2 (%)	CO_2 (%)	N_2 (%)	CH_4 (%)
Control	474.00	8.06	92.90	119.69	253.35	1.70	19.60	25.25	53.45
6.0	154.67	20.18	64.63	69.86	ND	13.05	41.79	45.17	ND
7.0	486.67	7.87	112.22	117.03	249.55	1.62	23.06	24.05	51.28
8.0	362.33	6.05	109.71	111.05	135.51	1.67	30.28	30.65	37.40
9.0	102.33	1.78	48.89	32.77	18.89	1.74	47.78	32.03	18.46
10.0	55.67	0.64	22.94	23.61	8.48	1.15	41.21	42.42	15.23

Note Control: Initial pH 7.43.

ND: Not detectable.

APPENDIX D
ANOVA STATISTICAL ANALYSIS

Table D1 ANOVA analysis (α = 0.05) of the difference between hydrogen yield of biohydrogen production from a two-stage fermentation process (Phase I).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1333.003	3	444.334	138.944	.000
Within Groups	12.792	4	3.198		
Total	1345.795	7			

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	IA	-33.212200(*)	1.7882779	.000	-38.177256	-28.247144
	IB	-15.457400(*)	1.7882779	.001	-20.422456	-10.492344
	IC	-28.557250(*)	1.7882779	.000	-33.522306	-23.592194
IA	Control	33.212200(*)	1.7882779	.000	28.247144	38.177256
	IB	17.754800(*)	1.7882779	.001	12.789744	22.719856
	IC	4.654950	1.7882779	.060	-.310106	9.620006
IB	Control	15.457400(*)	1.7882779	.001	10.492344	20.422456
	IA	-17.754800(*)	1.7882779	.001	-22.719856	-12.789744
	IC	-13.099850(*)	1.7882779	.002	-18.064906	-8.134794
IC	Control	28.557250(*)	1.7882779	.000	23.592194	33.522306
	IA	-4.654950	1.7882779	.060	-9.620006	.310106
	IB	13.099850(*)	1.7882779	.002	8.134794	18.064906

* The mean difference is significant at the .05 level.

Table D2 ANOVA analysis ($\alpha = 0.05$) of the difference between hydrogen yield at various initial pH values under mesophilic temperature ($35 \pm 2^{o}C$) (Phase IIA).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7909.470	4	1977.367	8471.783	.000
Within Groups	2.334	10	.233		
Total	7911.804	14			

(I) Initial pH	(J) Initial pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
pH 5	pH 6	-23.804067(*)	.3944670	.000	-24.682994	-22.925139
	pH 7	-65.382800(*)	.3944670	.000	-66.261727	-64.503873
	pH 8	-11.913267(*)	.3944670	.000	-12.792194	-11.034339
	pH 9	-9.137767(*)	.3944670	.000	-10.016694	-8.258839
pH 6	pH 5	23.804067(*)	.3944670	.000	22.925139	24.682994
	pH 7	-41.578733(*)	.3944670	.000	-42.457661	-40.699806
	pH 8	11.890800(*)	.3944670	.000	11.011873	12.769727
	pH 9	14.666300(*)	.3944670	.000	13.787373	15.545227
pH 7	pH 5	65.382800(*)	.3944670	.000	64.503873	66.261727
	pH 6	41.578733(*)	.3944670	.000	40.699806	42.457661
	pH 8	53.469533(*)	.3944670	.000	52.590606	54.348461
	pH 9	56.245033(*)	.3944670	.000	55.366106	57.123961
pH 8	pH 5	11.913267(*)	.3944670	.000	11.034339	12.792194
	pH 6	-11.890800(*)	.3944670	.000	-12.769727	-11.011873
	pH 7	-53.469533(*)	.3944670	.000	-54.348461	-52.590606
	pH 9	2.775500(*)	.3944670	.000	1.896573	3.654427
pH 9	pH 5	9.137767(*)	.3944670	.000	8.258839	10.016694
	pH 6	-14.666300(*)	.3944670	.000	-15.545227	-13.787373
	pH 7	-56.245033(*)	.3944670	.000	-57.123961	-55.366106
	pH 8	-2.775500(*)	.3944670	.000	-3.654427	-1.896573

* The mean difference is significant at the .05 level.

Table D3 ANOVA analysis ($\alpha = 0.05$) of the difference between hydrogen yield at various initial pH values under thermophilic temperature ($55 \pm 2^{o}C$) (Phase IIA).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32832.377	4	8208.094	9612.672	.000
Within Groups	8.539	10	.854		
Total	32840.916	14			

(I) Initial pH	(J) Initial pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
pH 5	pH 6	-86.034800(*)	.7544900	.000	-87.715908	-84.353692
	pH 7	-139.489567(*)	.7544900	.000	-141.170675	-137.808458
	pH 8	-55.932033(*)	.7544900	.000	-57.613142	-54.250925
	pH 9	-39.525133(*)	.7544900	.000	-41.206242	-37.844025
pH 6	pH 5	86.034800(*)	.7544900	.000	84.353692	87.715908
	pH 7	-53.454767(*)	.7544900	.000	-55.135875	-51.773658
	pH 8	30.102767(*)	.7544900	.000	28.421658	31.783875
	pH 9	46.509667(*)	.7544900	.000	44.828558	48.190775
pH 7	pH 5	139.489567(*)	.7544900	.000	137.808458	141.170675
	pH 6	53.454767(*)	.7544900	.000	51.773658	55.135875
	pH 8	83.557533(*)	.7544900	.000	81.876425	85.238642
	pH 9	99.964433(*)	.7544900	.000	98.283325	101.645542
pH 8	pH 5	55.932033(*)	.7544900	.000	54.250925	57.613142
	pH 6	-30.102767(*)	.7544900	.000	-31.783875	-28.421658
	pH 7	-83.557533(*)	.7544900	.000	-85.238642	-81.876425
	pH 9	16.406900(*)	.7544900	.000	14.725792	18.088008
pH 9	pH 5	39.525133(*)	.7544900	.000	37.844025	41.206242
	pH 6	-46.509667(*)	.7544900	.000	-48.190775	-44.828558
	pH 7	-99.964433(*)	.7544900	.000	-101.645542	-98.283325
	pH 8	-16.406900(*)	.7544900	.000	-18.088008	-14.725792

* The mean difference is significant at the .05 level.

Table D4 ANOVA analysis (α = 0.05) of the difference between hydrogen yield at various C/N ratios (Phase IIB).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	51690.777	5	10338.155	4333.781	.000
Within Groups	28.626	12	2.385		
Total	51719.403	17			

(I) C/N ratio	(J) C/N ratio	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	C/N 10	107.590967(*)	1.2610793	.000	104.843311	110.338622
	C/N 20	25.482267(*)	1.2610793	.000	22.734611	28.229922
	C/N 30	-67.403700(*)	1.2610793	.000	-70.151356	-64.656044
	C/N 40	-26.596233(*)	1.2610793	.000	-29.343889	-23.848578
	C/N 50	-.658100	1.2610793	.611	-3.405756	2.089556
C/N 10	Control	-107.590967(*)	1.2610793	.000	-110.338622	-104.843311
	C/N 20	-82.108700(*)	1.2610793	.000	-84.856356	-79.361044
	C/N 30	-174.994667(*)	1.2610793	.000	-177.742322	-172.247011
	C/N 40	-134.187200(*)	1.2610793	.000	-136.934856	-131.439544
	C/N 50	-108.249067(*)	1.2610793	.000	-110.996722	-105.501411
C/N 20	Control	-25.482267(*)	1.2610793	.000	-28.229922	-22.734611
	C/N 10	82.108700(*)	1.2610793	.000	79.361044	84.856356
	C/N 30	-92.885967(*)	1.2610793	.000	-95.633622	-90.138311
	C/N 40	-52.078500(*)	1.2610793	.000	-54.826156	-49.330844
	C/N 50	-26.140367(*)	1.2610793	.000	-28.888022	-23.392711
C/N 30	Control	67.403700(*)	1.2610793	.000	64.656044	70.151356
	C/N 10	174.994667(*)	1.2610793	.000	172.247011	177.742322
	C/N 20	92.885967(*)	1.2610793	.000	90.138311	95.633622
	C/N 40	40.807467(*)	1.2610793	.000	38.059811	43.555122
	C/N 50	66.745600(*)	1.2610793	.000	63.997944	69.493256

* The mean difference is significant at the .05 level.

Table D4 ANOVA analysis (α = 0.05) of the difference between hydrogen yield at various C/N ratios (Phase IIB) (cont.).

(I) C/N ratio	(J) C/N ratio	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C/N 40	Control	26.596233(*)	1.2610793	.000	23.848578	29.343889
	C/N 10	134.187200(*)	1.2610793	.000	131.439544	136.934856
	C/N 20	52.078500(*)	1.2610793	.000	49.330844	54.826156
	C/N 30	-40.807467(*)	1.2610793	.000	-43.555122	-38.059811
	C/N 50	25.938133(*)	1.2610793	.000	23.190478	28.685789
C/N 50	Control	.658100	1.2610793	.611	-2.089556	3.405756
	C/N 10	108.249067(*)	1.2610793	.000	105.501411	110.996722
	C/N 20	26.140367(*)	1.2610793	.000	23.392711	28.888022
	C/N 30	-66.745600(*)	1.2610793	.000	-69.493256	-63.997944
	C/N 40	-25.938133(*)	1.2610793	.000	-28.685789	-23.190478

* The mean difference is significant at the .05 level.

Table D5 ANOVA analysis (α = 0.05) of the difference between methane yield at various initial pH values under mesophilic temperature (35 ± 2°C) (Phase IIC).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	95961.667	5	19192.333	6669.780	.000
Within Groups	34.530	12	2.878		
Total	95996.197	17			

(I) Initial pH	(J) Initial pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	pH 6	172.272267(*)	1.3850406	.000	169.254522	175.290011
	pH 7	5.620767(*)	1.3850406	.002	2.603022	8.638511
	pH 8	69.403900(*)	1.3850406	.000	66.386156	72.421644
	pH 9	143.823000(*)	1.3850406	.000	140.805256	146.840744
	pH 10	172.272267(*)	1.3850406	.000	169.254522	175.290011

* The mean difference is significant at the .05 level.

Table D5 ANOVA analysis ($\alpha = 0.05$) of the difference between methane yield at various initial pH values under mesophilic temperature ($35 \pm 2^{o}C$) (Phase IIC) (cont.).

(I) Initial pH	(J) Initial pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
pH 6	Control	-172.272267(*)	1.3850406	.000	-175.290011	-169.254522
	pH 7	-166.651500(*)	1.3850406	.000	-169.669244	-163.633756
	pH 8	-102.868367(*)	1.3850406	.000	-105.886111	-99.850622
	pH 9	-28.449267(*)	1.3850406	.000	-31.467011	-25.431522
	pH 10	.000000	1.3850406	1.000	-3.017744	3.017744
pH 7	Control	-5.620767(*)	1.3850406	.002	-8.638511	-2.603022
	pH 6	166.651500(*)	1.3850406	.000	163.633756	169.669244
	pH 8	63.783133(*)	1.3850406	.000	60.765389	66.800878
	pH 9	138.202233(*)	1.3850406	.000	135.184489	141.219978
	pH 10	166.651500(*)	1.3850406	.000	163.633756	169.669244
pH 8	Control	-69.403900(*)	1.3850406	.000	-72.421644	-66.386156
	pH 6	102.868367(*)	1.3850406	.000	99.850622	105.886111
	pH 7	-63.783133(*)	1.3850406	.000	-66.800878	-60.765389
	pH 9	74.419100(*)	1.3850406	.000	71.401356	77.436844
	pH 10	102.868367(*)	1.3850406	.000	99.850622	105.886111
pH 9	Control	-143.823000(*)	1.3850406	.000	-146.840744	-140.805256
	pH 6	28.449267(*)	1.3850406	.000	25.431522	31.467011
	pH 7	-138.202233(*)	1.3850406	.000	-141.219978	-135.184489
	pH 8	-74.419100(*)	1.3850406	.000	-77.436844	-71.401356
	pH 10	28.449267(*)	1.3850406	.000	25.431522	31.467011
pH 10	Control	-172.272267(*)	1.3850406	.000	-175.290011	-169.254522
	pH 6	.000000	1.3850406	1.000	-3.017744	3.017744
	pH 7	-166.651500(*)	1.3850406	.000	-169.669244	-163.633756
	pH 8	-102.868367(*)	1.3850406	.000	-105.886111	-99.850622
	pH 9	-28.449267(*)	1.3850406	.000	-31.467011	-25.431522

* The mean difference is significant at the .05 level.

Table D6 ANOVA analysis ($\alpha = 0.05$) of the difference between methane yield at various initial pH values under thermophilic temperature ($55 \pm 2^{o}C$) (Phase IIC).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	304842.864	5	60968.573	2052.835	.000
Within Groups	356.396	12	29.700		
Total	305199.261	17			

(I) Initial pH	(J) Initial pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	pH 6	315.249700(*)	4.4496965	.000	305.554644	324.944756
	pH 7	4.474833	4.4496965	.334	-5.220223	14.169889
	pH 8	120.111200(*)	4.4496965	.000	110.416144	129.806256
	pH 9	268.051233(*)	4.4496965	.000	258.356177	277.746289
	pH 10	280.808933(*)	4.4496965	.000	271.113877	290.503989
pH 6	Control	-315.249700(*)	4.4496965	.000	-324.944756	-305.554644
	pH 7	-310.774867(*)	4.4496965	.000	-320.469923	-301.079811
	pH 8	-195.138500(*)	4.4496965	.000	-204.833556	-185.443444
	pH 9	-47.198467(*)	4.4496965	.000	-56.893523	-37.503411
	pH 10	-34.440767(*)	4.4496965	.000	-44.135823	-24.745711
pH 7	Control	-4.474833	4.4496965	.334	-14.169889	5.220223
	pH 6	310.774867(*)	4.4496965	.000	301.079811	320.469923
	pH 8	115.636367(*)	4.4496965	.000	105.941311	125.331423
	pH 9	263.576400(*)	4.4496965	.000	253.881344	273.271456
	pH 10	276.334100(*)	4.4496965	.000	266.639044	286.029156
pH 8	Control	-120.111200(*)	4.4496965	.000	-129.806256	-110.416144
	pH 6	195.138500(*)	4.4496965	.000	185.443444	204.833556
	pH 7	-115.636367(*)	4.4496965	.000	-125.331423	-105.941311
	pH 9	147.940033(*)	4.4496965	.000	138.244977	157.635089
	pH 10	160.697733(*)	4.4496965	.000	151.002677	170.392789

* The mean difference is significant at the .05 level.

Table D6 ANOVA analysis ($\alpha = 0.05$) of the difference between methane yield at various initial pH values under thermophilic temperature ($55 \pm 2^{o}C$) (Phase IIC) (cont.)

(I) Initial pH	(J) Initial pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
pH 9	Control	-268.051233(*)	4.4496965	.000	-277.746289	-258.356177
	pH 6	47.198467(*)	4.4496965	.000	37.503411	56.893523
	pH 7	-263.576400(*)	4.4496965	.000	-273.271456	-253.881344
	pH 8	-147.940033(*)	4.4496965	.000	-157.635089	-138.244977
	pH 10	12.757700(*)	4.4496965	.014	3.062644	22.452756
pH 10	Control	-280.808933(*)	4.4496965	.000	-290.503989	-271.113877
	pH 6	34.440767(*)	4.4496965	.000	24.745711	44.135823
	pH 7	-276.334100(*)	4.4496965	.000	-286.029156	-266.639044
	pH 8	-160.697733(*)	4.4496965	.000	-170.392789	-151.002677
	pH 9	-12.757700(*)	4.4496965	.014	-22.452756	-3.062644

* The mean difference is significant at the .05 level.