APPENDICES

APPENDIX A
METHODOLOGY OF ANALYSIS

Appendix A1 Total solids (TS) dried at 103-105 °C

Apparatus

- Evaporating dish, porcelain
- Muffle furnace
- Steam bath
- Desiccator
- Drying oven
- Analytical balance
- Magnetic stirrer
- Wide-bore pipets
- Graduated cylinder
- Low-form beaker

Procedure

- Preparation of evaporating dish: If volatile solids are to be measured ignite clean evaporating dish at 550 °C for 1 hour in a muffle furnace. If only total solids are to be measured, heat clean dish to 103-105 °C for 1 hour. Store and cool dish in desiccator until needed. Weigh immediately before use.
- Choose a sample volume that will yield a residue between 2.5 and 200 mg.
- Pipet a measured volume of well-mixed sample, during mixing, to a preweighed dish. For homogeneous samples, pipet from the approximate midpoint of the container but not in the vortex. Choose a point both middepth and midway between wall and vortex. If necessary, add successive sample portions to the same dish after evaporation.
- Evaporate to dryness in a drying oven at least 1 hour under 103-105 °C.

- Cool dish in desiccator to balance temperature and weight.
- Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained, or until weight change is less than 0.5 mg of previous weight.

Calculation

$$\text{mg total solids/L} = \frac{(A-B)\times 1000}{\text{sample volume (mL)}}$$

A = weight of dried residue + dish (mg)

B = weight of dish (mg)

Appendix A2 Total suspended solids (TSS) dried at 103-105 °C

Apparatus

- Muffle furnace
- Desiccator
- Drying oven
- Analytical balance
- Magnetic stirrer
- Wide-bore pipets
- Graduated cylinder
- Low-form beaker
- Glass-fiber filter disks (without organic binder)
- Filtration apparatus
- Suction flask
- Aluminum weighing dish

Procedure

- Preparation of glass-fiber filter dish: Insert disk with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive 20-mL portions of reagent-grade water. Continue suction to remove all traces of water, turn the vacuum off, and discard washings. Remove filter from filtration apparatus and transfer to an inert aluminum weighing dish.

If volatile solids are to be measured, ignite at 550 °C for 15 minutes in a muffle furnace. Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained, or until weight change is less than 0.5 mg of previous weight.

- Assemble filtering apparatus and filter and begin suction.
- Wet filter with small volume of reagent-grade water to seat it.
- Stir sample with a magnetic stirrer at a speed to shear larger particles.
- While stirring, pipet a measured volume onto the seated glass-fiber filter. Choose a sample volume that will yield a residue between 2.5 and 200 mg. For homogeneous samples, pipet from the approximate midpoint of the container but not in the vortex. Choose a point both middepth and midway between wall and vortex.
- Wash filter with 3 successive 10-mL volumes of reagent-grade water, allowing complete drainage between washings
- Continue suction for about 3 minutes after filtration is complete.
- Carefully remove filter from filtration apparatus and transfer to an aluminum weighting dish as support.
- Dry for at least 1 hour at 103-105 °C in an oven.
- Cool in a desiccator to balance temperature, and weigh.
- Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained, or until weight change is less than 0.5 mg of previous weight.

Calculation

$$\text{mg total suspended solids/L} = \frac{(A-B)\times 1000}{\text{sample volume (mL)}}$$

A = weight of filter + dried residue (mg)

B = weight of filter (mg)

Appendix A3 Volatile solids (VS, VSS) ignited at 550 °C

Apparatus

- Evaporating dish, porcelain
- Muffle furnace
- Steam bath
- Desiccator
- Drying oven
- Analytical balance
- Magnetic stirrer
- Wide-bore pipets
- Graduated cylinder
- Low-form beaker
- Glass-fiber filter disks
- Filtration apparatus
- Suction flask
- Drying oven

Procedure

- Ignite residue produced by Appendix A1 or A2 to constant weight in a muffle furnace at a temperature of 550 °C for 15-20 minutes. Ignite a blank glass fiber filter along with samples.
- Let dish or filter disk cool partially in air until most of the heat has been dissipated.
- Transfer to desiccator.
- Weigh dish or disk as soon as it has cooled to balance temperature.
- Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained, or until weight change is less than 0.5 mg of previous weight.

Calculation

$$\text{mg volatile solids/L} = \frac{(A-B)\times 1000}{\text{sample volume (mL)}}$$

A = weight of filter + dried residue (mg)

B = weight of filter (mg)

Appendix A4 Total Kjeldahl nitrogen (TKN)

Apparatus

- Weigh balance
- Hood
- Digester
- Distilling unit
- 250 mL Digester tube
- 250 mL Erlenmeyer flask
- 100, 500, and 1000 mL volumetric flasks
- 250 mL beaker
- 50 mL burette
- 5, 10, 15, and 20 mL pipettes
- 25 and 100 mL cylinders
- Stir glass and rubber pump

Reagents

- Concentrated 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
- Mixed catalyst, that consists of K2SO4 and $CuSO4 \cdot 5H_2O$ 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 collects in bottle. 0.1 g of methyl blue 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 flask that have a bit distilled water in it and add 10 ml of indicator (mixed 10 ml methyl red and 5 ml methyl blue) 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 to determine concentration of sulfuric acid standard solution with 40 ml of 0.05 N sodium carbonate. The following calculation express:

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

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 sample 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 (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 sulfuric acid standard solution. The end-point change green to purple.

Calculation

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

A = ml acid used titrate sample

B = ml acid used titrate Blank

N = Normality of standard acid

Appendix A5 Chemical oxygen demand (COD) closed reflux, titrimetric method

Apparatus

- Digestion vessels, borosilicate culture tubes 16- x 100-mm, with TFE-lined screw caps
- Block heater or similar device to operate at 150±2 °C. Do not use an oven because of the possibility of leaking samples generating a corrosive and possibly explosive atmosphere. Also, culture tube caps may not withstand the 150 °C temperature in an oven.
- Burette
- Erlenmeyer flasks
- Test tube rack
- TFE-covered magnetic stirring bar

Reagents

- Standard potassium dichromate digestion solution, 0.01667 M

  Add to about 500 mL distilled water 4.903 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150 °C for 2 hours, 167 mL conc. H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 mL.
- Sulfuric acid reagent

  Add Ag_2SO_4, reagent or technical grade, to conc. H_2SO_4 at the rate of 5.5 g Ag_2SO_4/kg H_2SO_4. Let stand 1-2 days to dissolve.
- Ferroin indicator solution

  Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $FeSO_4.7H_2O$ in distilled water and dilute to 100 mL. Dilute this reagent again by a factor of 5 (1 + 4).
- Standard ferrous ammonium sulfate titrant (FAS), approximately 0.10 M

Dissolve 39.2 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in a distilled water. Add 20 mL conc. H_2SO_4, cool, and dilute to 1000 mL. Standardize solution daily against the digestion solution as follow:

Pipet 5.00 mL digestion solution into a small beaker. Add 10 mL reagent water to substitute for sample. Cool to room temperature. Add 1-2 drops diluted ferroin indicator and titrate with FAS titrant.

Molarity of FAS solution

$$= \frac{\text{Volume 0.01667M digestion solution (mL)}}{\text{Volume FAS used (mL)}} \times 0.100$$

- Potassium hydrogen phthalate (KHP) standard, $HOOCC_6H_4COOK$:
  Lightly crush and then dry KHP to constant weight at 110 °C. Dissolve 425 mg in distilled water and dilute to 1000 mL. This solution has a theoretical COD of 500 µg O_2/ mL. This solution is stable when refrigerated, but not indefinitely. Be alert to development of visible biological growth.
- 20% H_2SO_4

Procedure

- Wash culture tubes and caps with 20% H_2SO_4 before use to prevent contamination.
- Place sample 2.50 mL in culture tube
- Add digestion solution 1.50 mL.
- Carefully run sulfuric acid reagent 3.5 mL down inside of vessel so an acid layer is formed under the sample-digestion solution layer.
- Tightly cap tubes
- Invert several times to mix completely. These sealed vessels may be under pressure from gases generated during digestion. Wear face and hand protection when handling.
- Cool to room temperature and place vessels in test tube rack.
- Remove culture tube caps

- Transfer the culture solution into Erlenmeyer flasks. Add TFE-covered magnetic stirring bar.
- Add 1-2 drops ferroin indicator
- Stir rapidly on magnetic stirrer while titrating with standardized 0.10 M FAS.
- The end point is a sharp color change from blue-green to reddish brown, although the blue-green may reappear within minutes.
- In the same manner, reflux and titrate a blank containing the reagents and a 2.50 mL of distilled water.

Calculation

$$\text{COD (mg } O_2\text{/L)} = \frac{(A\text{-}B)\times M\times 8000}{\text{sample (mL)}}$$

A = mL FAS used for blank

B = mL FAS used for sample

M = molarity of FAS

8000 = milliequivalent weight of oxygen × 1000 mL/L

Appendix A6 Nitric acid digestion of metals for flame atomic absorption

Apparatus

- Hot plate
- 125-mL Erlenmeyer flasks, 125-mL, or 150-mL Griffin beakers, acid-washed and rinsed with water.
- 100-mL volumetric flasks
- Watch glasses, ribbed and unribbed

Reagents

- Nitric acid, HNO_3, concentrated, analytical or trace-metals grade.

Procedures

- Transfer a measured volume (100 mL recommended) of well-mixed, acid preserved sample appropriate for the expected metals concentrations to a flask or beaker.

- In a hood, add 5 mL conc HNO_3. If a beaker is used, cover with a ribbed watch glass to minimize contamination. Boiling chips, glass beads, or Hengar granules may be used to aid boiling and minimizing spatter when high concentration levels (> 10 mg/L) are being determined.
- Bring to a slow boil and evaporate on a hot plate to the lowest volume possible (about 10 – 20 mL) before precipitation occurs.
- Continue heating and adding conc HNO_3 as necessary until digestion is complete as shown by a light-colored, clear solution. Do not let sample dry during digestion.
- Wash down flask or beaker walls and watch glass cover (if used) with metal-free water and then filter if necessary.
- Transfer filtrate to a 100-mL volumetric flask with two 5-mL portions of water, adding these risings to the volumetric flask.
- Cool, dilute to mark, and mix thoroughly.
- Take portions of this solution for flame atomic absorption spectrometry (FAAS). Specify iron (wave length 248.3 nm and air-acetylene flame gases).

APPENDIX B
STANDARD CALIBRATION CURVE

Appendix B1 Calibration of gas chromatograph

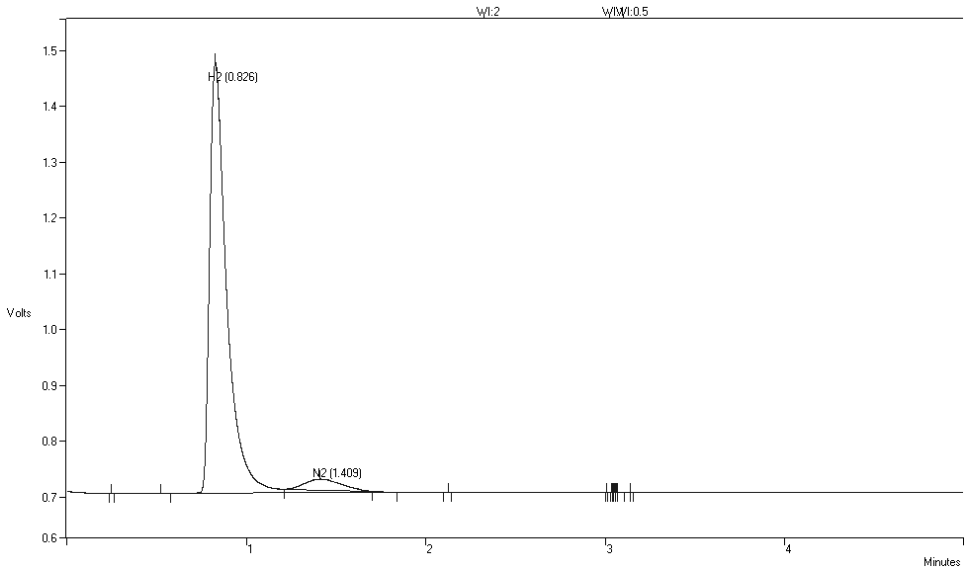

Figure B1 Gas chromatograph of standard hydrogen and nitrogen gas

Table B1 Average peak area of hydrogen (60%) and nitrogen (10%) standard gas

Order	H_2		N_2	
	$\%H_2$	Peak area	$\%N_2$	Peak area
1	60	524500	10	27747
2	60	535391	10	28974
3	60	598952	10	32420
4	60	549725	10	21388
5	60	510604	10	27291
6	60	639227	10	28534
7	60	565799	10	29754
8	60	525970	10	22581
9	60	636018	10	23670
10	60	640673	10	30722
Average	60	572686	10	27308

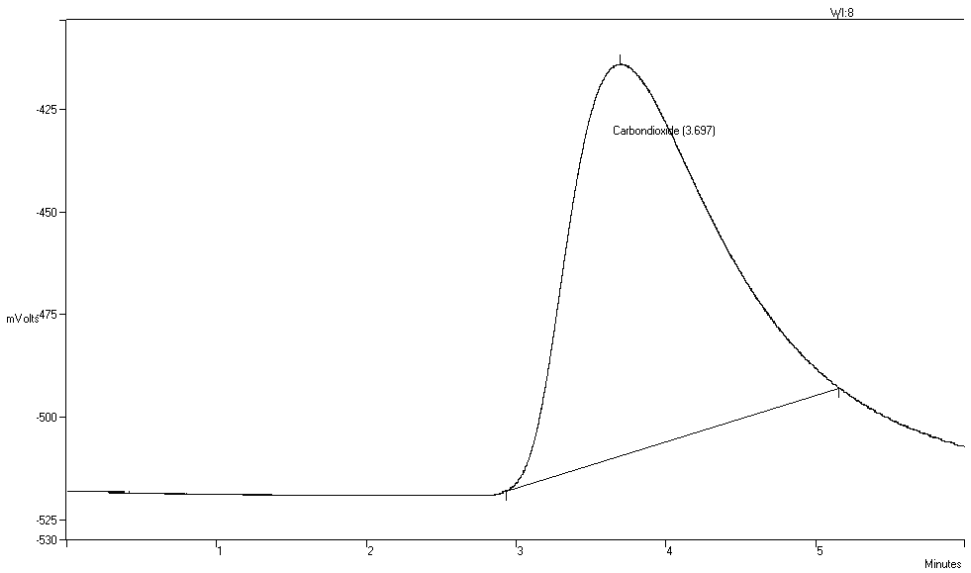

Figure B2 Gas chromatograph of standard carbon dioxide (CO_2) gas

Table B2 Average peak area of carbon dioxide (30%) standard gas

Order	%CO_2	Peak area
1	30	647590
2	30	617231
3	30	699477
4	30	598865
5	30	585022
6	30	523167
7	30	502151
8	30	453493
9	30	442731
10	30	435523
Average	30	550525

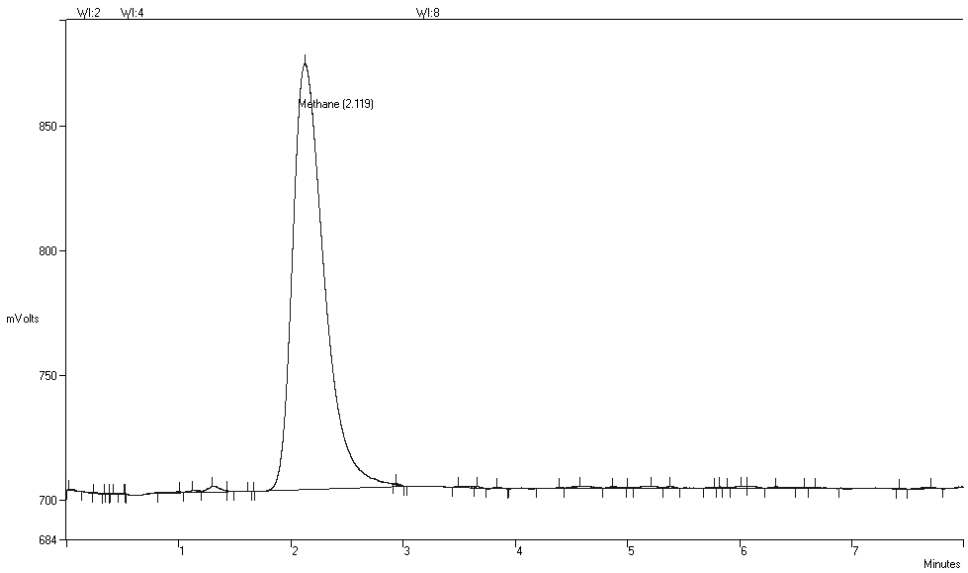

Figure B3 Gas chromatograph of standard methane (CH_4) gas

Table B3 Average peak area of methane (70%) standard gas

Order	$\%CH_4$	Peak area
1	70	342598
2	70	345506
3	70	354099
4	70	340074
5	70	335074
6	70	357147
7	70	341112
8	70	352484
9	70	336221
10	70	348655
Average	70	345197

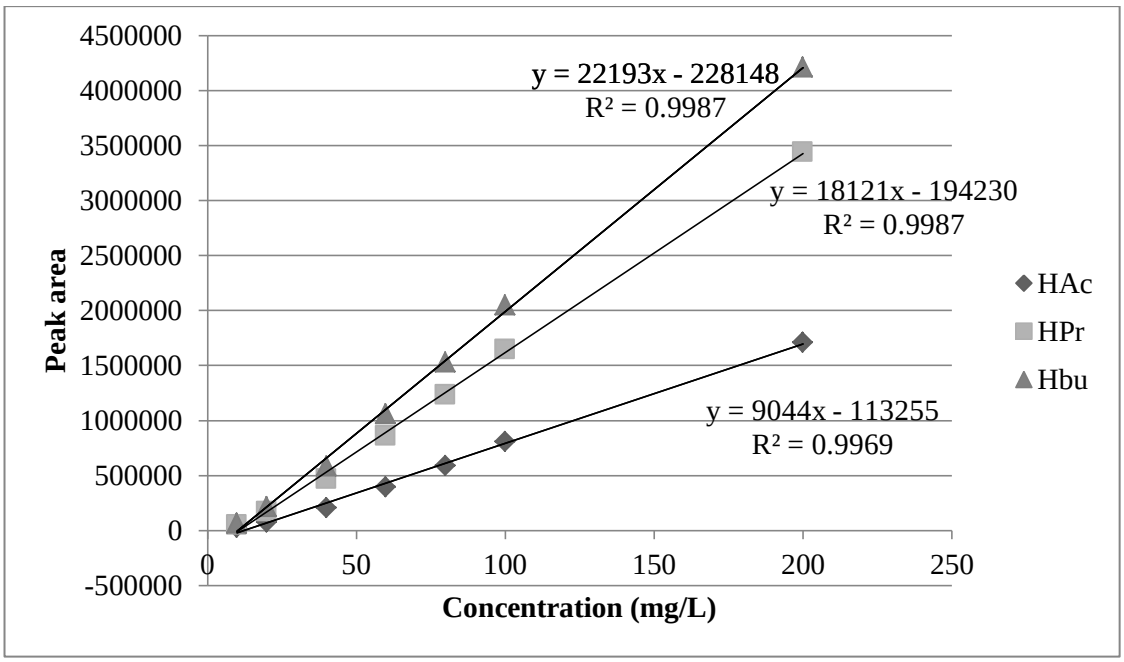

Figure B4 Calibration curve of VFA

Table B4 Gas chromatography value of VFA at various concentrations

Concentration (mg/L)	Peak area		
	HAc	HPr	HBu
10	29065	52782	65512
20	77403	174836	218856
40	207405	468372	585821
60	398468	861920	1059461
80	590760	1234600	1529465
100	807448	1649107	2050193
200	1709136	3340571	4212130

APPENDIX C
DATA OF BIOGAS

Table C1 Raw data of gas content in experimental batch reactor under mesophilic condition at various pH

Temperature	Initial pH	Biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	% H_2	% CO_2	% N_2
Mesophilic	4	864.65	26.41	247.92	590.32	3.05	28.67	68.28
	5	828.48	14.69	205.55	608.24	1.77	24.81	73.42
	6	794.65	18.73	261.60	514.32	2.36	32.92	64.72
	7	818.65	19.70	258.13	540.82	2.41	31.53	66.06
	8	851.90	27.23	241.64	583.03	3.20	28.36	68.44
Thermophilic	4	883.15	55.81	417.27	410.07	6.32	47.25	46.43
	5	865.90	59.39	478.36	328.15	6.86	55.24	37.90
	6	830.90	50.78	444.39	305.73	6.11	53.48	40.41
	7	930.90	73.95	518.57	338.38	7.94	55.71	36.35
	8	947.30	66.53	504.77	376.00	7.02	53.29	36.69

Table C2 Raw data of gas content in experimental batch reactor at various iron concentrations

Iron concentration (mg/L)	Biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	% H_2	% CO_2	% N_2
0	1118.00	52.87	563.09	502.04	4.73	50.37	44.91
200	1033.50	108.97	535.69	388.84	10.54	51.83	37.62
400	1145.75	114.23	654.05	377.47	9.97	57.08	32.95
600	1162.00	111.53	651.45	399.02	9.60	56.06	34.34
800	1212.25	121.49	640.92	449.84	10.02	52.87	37.11
1000	1113.75	98.70	592.12	442.93	8.86	53.16	37.97

Table C3 Raw data of hydrogen content in experimental batch reactor at various type of biomaterials (BM)

Type of BM	Biogas (mL)	H_2 (mL)	% H_2
Coir (CO)	280.13	37.32	13.32
Corncob (CC)	245.38	25.42	10.36
Loofa sponge (LS)	207.00	38.21	18.46
Pine tree bark (PT)	223.75	34.68	15.50
Silk cocoon (SC)	278.25	38.18	13.72
Shell (SH)	291.36	26.54	9.11
Crab exoskeleton (CE)	-	-	-

Table C4 Raw data of gas content in experimental batch reactor at various LS concentrations

Concentration of BM (%)	Biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	% H_2	% CO_2	% N_2
0	695.25	22.90	301.77	370.58	3.29	43.40	53.30
5	698.75	51.99	267.77	378.99	7.44	38.32	54.24
10	710.00	41.74	257.23	411.03	5.88	36.23	57.89
15	662.25	21.79	251.74	388.72	3.29	38.01	58.70
20	681.75	19.01	261.97	400.77	2.79	38.43	58.79

Table C5 Raw data of gas content in experimental batch reactor at various SC concentrations

Concentration of BM (%)	Biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	% H_2	% CO_2	% N_2
0	851.80	47.45	357.25	447.10	5.57	41.94	52.49
5	828.80	47.72	342.41	438.67	5.76	41.34	52.90
10	977.30	35.29	421.86	520.15	3.61	43.17	53.22
15	953.30	14.76	421.93	516.61	1.55	44.26	54.19
20	917.30	10.18	367.91	539.21	1.11	40.11	58.78

Table C6 Raw data of gas content in experimental batch reactor comparing plants (LS) and animals (SC) based BM

Type of BM	Biogas (mL)	H_2 (mL)	CO_2 (mL)	N_2 (mL)	% H_2	% CO_2	% N_2
Plants (LS)	935.50	55.14	363.65	516.71	5.89	38.87	55.23
Animal (SC)	899.50	34.90	401.22	463.38	3.88	44.60	51.52