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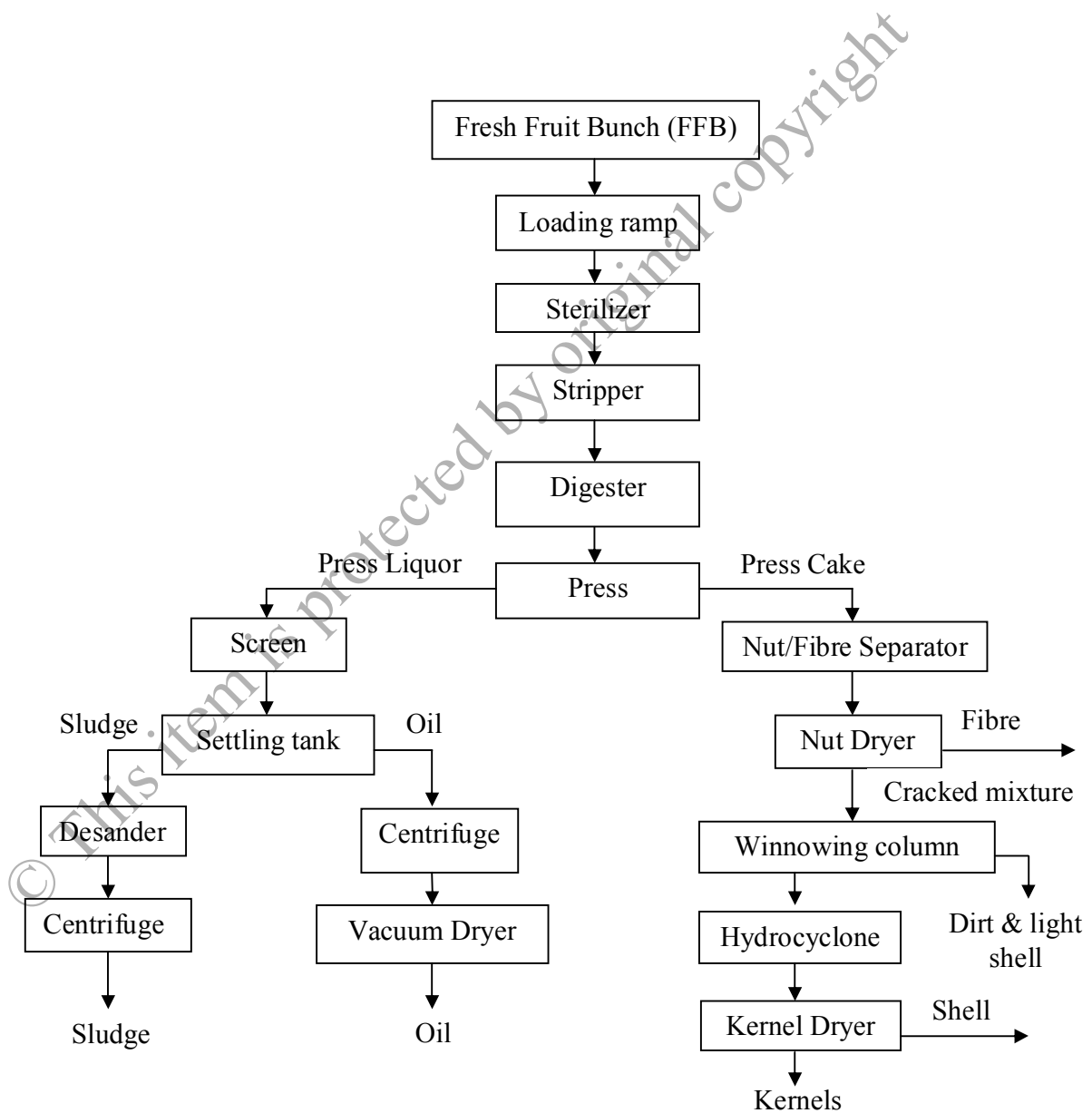
## APPENDIX A

Effluent discharge standards for crude palm oil mills (Environmental Quality Act 1974, 2005) (EQA, 1974).

<b>Parameter</b>	<b>unit</b>	<b>Parameter limits (Second schedule)</b>	<b>Remarks</b>
Biochemical Oxygen Demand (BOD; 3-Day, 30 °C)	mg/L	100	
Chemical Oxygen Demand (COD)	mg/L	*	
Total Solids	mg/L	*	
Suspended Solids	mg/L	400	
Oil and Grease	mg/L	50	
Ammoniacal Nitrogen	mg/L	150	Value of filtered sample
Total Nitrogen	mg/L	200	Value of filtered sample
pH	-	5-9	
Temperature	°C	45	

\* No discharge standard after 1984.

## APPENDIX B



Schematic flow of conventional palm oil extraction process (DOE, 2009).

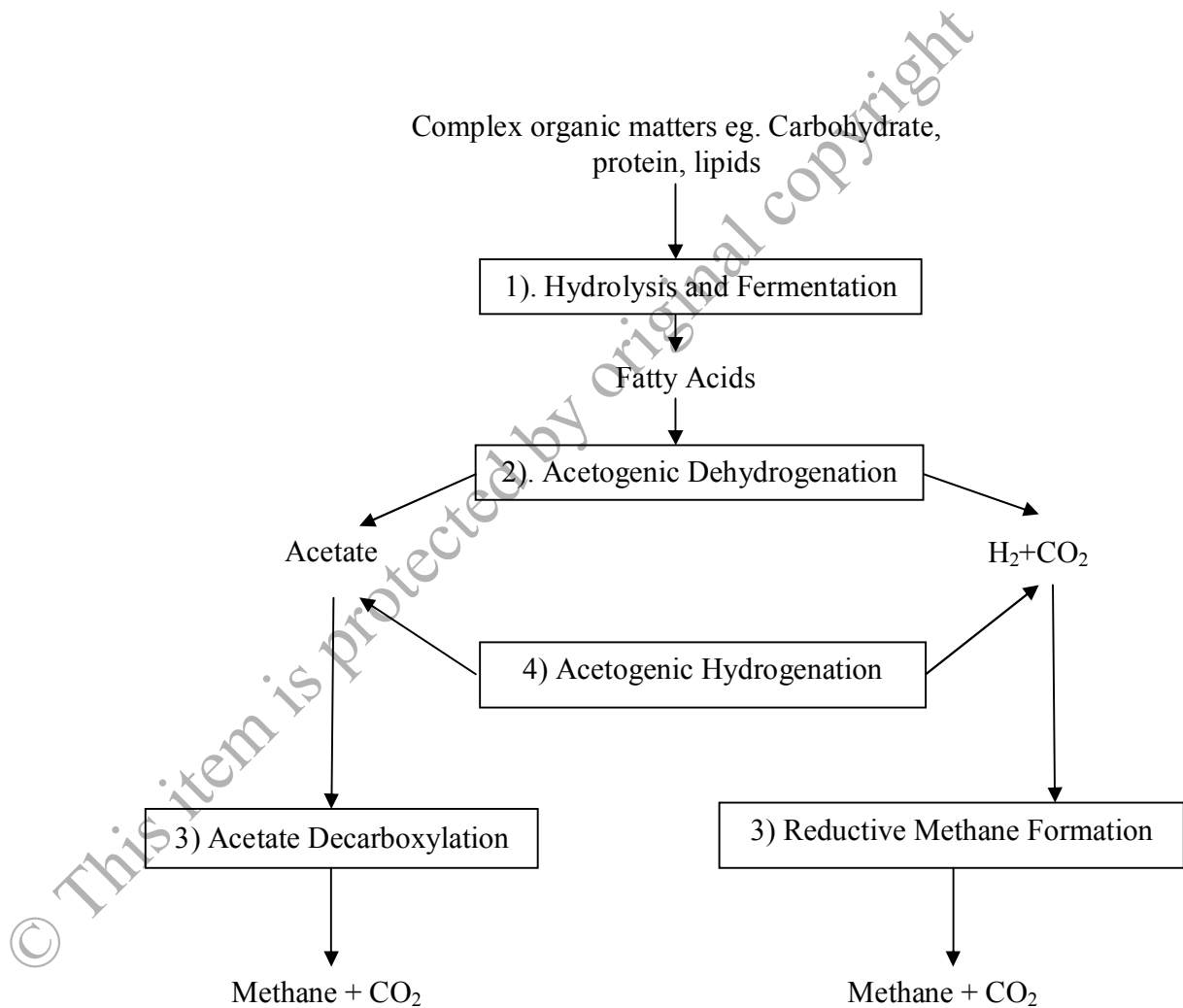
## APPENDIX C

Characteristics of untreated palm oil mill effluent (POME) (DOE, 2009).

Parameter	Concentration* (mg/L)
pH	4.7
Temperature	80 - 90
BOD 3-day, 30°C	25,000
COD	50,000
Total Solids	40,500
Suspended Solids	18,000
Total Volatile Solids	34,000
Ammoniacal-Nitrogen	35
Total Nitrogen	750
Phosphorus	18
Potassium	2,270
Magnesium	615
Calcium	439
Boron	7.6
Iron	46.5
Manganese	2.0
Copper	0.89
Zinc	2.3

\* All parameters in mg/l except pH and temperature (°C).

## APPENDIX D



Steps in anaerobic digestion involving four groups of bacterial activities (Harper, S.R. and Pohland, F.G., 1986).

## APPENDIX E

Varying composition of Biogas components mixture (Cheng, K.-J. *et al.*, 1984).

<b>Constituents</b>	<b>% Composition</b>
Methane	50-75
Nitrogen	0-1
Carbon Dioxide	30-45
Water	0-1
Hydrogen	Traces
Ammonia	Traces
Hydrogen Sulphide	Traces (ppm)

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## APPENDIX F

### pH (STANDARD METHOD 4500-H<sup>+</sup> B. ELECTROMETRIC METHOD)

pH values were measured by the HANNA Microprocessor Logging pH meter. Prior to pH measurement, calibration of the pH meter was carried out with standard buffer solutions of pH 4, pH 7 and pH 10 to make sure the accuracy of the pH value taken. The pH measurements were carried out immediately after the sample was collected. Before taken the results from the pH meter, establish equilibrium between electrodes and sample by stirring sample to insure homogeneity; stir gently to minimize carbon dioxide entrainment and make sure the electrode of the pH meter was immersed 5 cm from the surface of the sample until stable reading was obtained.

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## APPENDIX G

### TOTAL SOLIDS (STANDARD METHOD 2540-B)

In the preparation of evaporating dish, heat clean dish to 103 to 105°C for 1 hour. Stored and cooled dish in desiccator until needed. Weigh immediately before use. A well mixed sample of 10 ml was evaporated in a weighed porcelain dish with steam bath. Then dried evaporated sample for at least 1 h in a Protect Oven FAC-100 at 103 to 105 °C, cooled dish in desiccator to balance temperature, and weight. The total solids content was calculated using equation below:

$$TS(mg/l) = \frac{(A - B) \times 1000}{\text{volume\_of\_sample, ml}}$$

A = weight of dried residues + dish, mg

B = weight of dish, mg

## APPENDIX H

### VOLATILE SOLIDS/TOTAL VOLATILE SOLIDS (STANDARD METHOD 2540-E)

In the preparation of evaporating dish, heat clean dish to 103 °C to 105 °C for 1 hour. Stored and cooled dish in desiccator until needed. Weigh immediately before use. In order to determine the solids content, a well mixed sample of 10 ml was evaporated in a weighed porcelain dish with steam bath, then dried evaporated sample for at least 1 h in an Protect Oven FAC-100 at 103 to 105 °C, cooled dish in desiccator to balance temperature, and weight. After the drying process, ignited residue produced to constant weight in a Carbolite Furnace CSF 1100 at a temperature of 550 ± 50 °C. The weight loss on ignition represents the total volatile solids. The total volatile solids content was calculated by equation below:

$$TVS(mg/l) = \frac{(A - B) \times 1000}{\text{volume\_of\_sample,ml}}$$

A = weight of dried residue + dish before ignition, mg

B = weight of dried residue + dish after ignition, mg



## APPENDIX I

### MIXED LIQUOR SUSPENDED SOLIDS (STANDARD METHOD 2540-D)

The mixed liquor suspended solids content was determined by using glass microfibre filter paper, Fisherbrand FB59431. Insert the filter paper with the wrinkled side up in a filtration apparatus. Washed the filter paper with 20 ml portions of distilled water in succession and applied vacuum. Continue suction was carried out to remove all the traces of the water, turn vacuum off and to discard washings. The filter disk was taken away from the filtration apparatus. Then dried in the Carbolite Furnace CSF 1100 at a temperature of  $550 \pm 50$  °C for 20 minutes, cooled dish in desiccator to balance temperature, and weight. In order to obtain a constant weight or weight change was less than 4 % of the previous weighting or 0.5 mg, whichever was less, the cycle of drying, cooling, desiccating and weighing was repeated. Stored filter disk in a desiccator until needed.

Filtered well mixed samples with the filter disk using vacuum suction. Then dried the residual retained on the filter paper in a Protect Oven FAC-100 at 103 °C to 105 °C and cooled in desiccators before weighting. In order to obtain a constant weight or weight change was less than 4 % of the previous weighting or 0.5 mg, whichever was less, the cycle of drying, cooling, desiccating and weighing was repeated. The mixed liquor suspended solids of the sample was calculated using equation below:

$$MLSS(mg/l) = \frac{(A - B) \times 1000}{\text{volume\_of\_sample,ml}}$$

A = weight of dried residue + filter + dish, mg

B = weight of filter + dish, mg

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## APPENDIX J

### MIXED LIQUOR VOLATILE SUSPENDED SOLIDS (STANDARD METHOD 2540-B & E)

The Fisherbrand FB 59431 glass microfibre filter paper was used to filter the well mixed samples by vacuum suction. In order to determine the mixed liquor suspended solids, the residual retained on the filter paper was dried in a Protect Oven FAC-100 at 103 °C to 105 °C, cooled dish in desiccator to balance temperature, and weight. After the drying process, ignited residue produced to constant weight in a Carbolite Furnace CSF 1100 at a temperature of 550 ± 50°C for 20 minutes and cooled in desiccators before weighing. In order to obtain a constant weight or weight change was less than 4 % of the previous weighing or 0.5 mg, whichever was less, the cycle of drying, cooling, desiccating and weighing was repeated. The MLVSS of the sample was calculated using equation below:

$$MLVSS(mg/l) = \frac{(A - B) \times 1000}{\text{volume\_of\_sample, ml}}$$

A = weight of dried residue + filter + dish before ignition, mg

B = weight of dried residue + filter + dish after ignition, mg

## APPENDIX K

### CHEMICAL OXYGEN DEMAND (DICHROMATE REACTOR DIGESTION METHOD)

The samples were diluted to a concentration lower than 1500 mg/L before used for analysis. Blank sample was prepared by using 2 ml of deionised water. 800 ml of concentrated sulphuric acid (95-98 %,  $H_2SO_4$ ), 18 g of silver sulphate ( $Ag_2SO_4$ ) and 14.8 g of potassium dichromate ( $K_2Cr_2O_7$ ) were used to prepare the COD digestion reagent. In a 10 ml COD digestion vial consisted of little amount mercury sulphate,  $HgSO_4$ A.R (Bendosen with an assay of min. 99 %), 2 ml of sample and 3 ml digestion reagent were mixed and digested in a Hach COD Reactor DRB200 for 2 hours at 150 °C. The sample absorbance was read by a Hach Portable Data Logging Spectrophotometr DR/2800 at a wave length of 620 nm using program number 435 after the sample was cooled to room temperature. In this kit, the sample absorbance was converted into concentration.

## APPENDIX L

### BIOCHEMICAL OXYGEN DEMAND (STANDARD METHOD 5210-B)

Before the BOD test was done, prepare dilution water by adding 1 ml of the following per liter of distilled water and then aerated the mixture to oxygen saturation (at least 3 h) at room temperature.

- 1 ml phosphate buffer solution
- 1 ml magnesium sulphate solution
- 1 ml calcium chloride solution
- 1 ml ferric chloride solution

For a dilution purpose, the 0.05 ml well-mixed sample was added into a labeled BOD bottle using a pipette, thus the final dissolved oxygen (DO) was at least 1 mg O<sub>2</sub>/L or DO uptake was at least 2 mg O<sub>2</sub>/L. In order to avoid air bubble which can affect the DO reading of the BOD test, some dilution water were then slowly added to BOD bottle. The initial DO reading of the sample was measured by using dissolved oxygen meter, YSI model 5000 with membrane probe. The BOD bottle with sample was then filled to the top with dilution water. Stopper each BOD bottle so that no bubbles were visible in the sample. A water seal was placed on each bottles and a Para-film cap over the stopper. The BOD bottle was then placed in the Protect BOD incubator model SD-450 in the dark for 5 days at 20 °C. The BOD bottles were removed from the incubator at the end of the incubation period. The DO was determined on the BOD bottle with the DO meter. The reduction of DO concentration after the incubation period yields the BOD content of the sample. The

BOD for the sample was calculated by the following equation:

$$BOD_3, mg/l = \frac{D_1 - D_2}{P}$$

$D_1$  = DO of diluted sample immediately after preparation, mg/L

$D_2$  = DO of diluted sample after 5 d incubation at 20 °C, mg/L

P = Decimal volumetric fraction of sample used (volume of sample/volume of mixture)

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## APPENDIX M

### OIL AND GREASE (STANDARD METHOD 5520-B. PARTITION-GRAVIMETRIC METHOD)

The sample was collected in a 1 L glass bottle which pre-rinsed with n-hexane. The sample was acidified to pH 2 with 1:1 hydrochloric acid, (HCL) and transferred into a 500 ml separatory funnel using liquid funnel. By using 100 ml n-hexane, the oil was extracted from 100 ml of sample in the separator funnel. The sample was shaken vigorously for 2 minutes. After that, let two layers separated in the separator funnel. The aqueous layer and small amount of organic layer were drained into original sample container, while the solvent layer was drained through a funnel containing solvent moisture of Advantec 150 mm filter paper to a distilling flask. The extraction was repeated two times and the extracts were combined in the same distilling flask. N-Hexane was distilled in water bath at 70 °C until all of the solvent was evaporated. The sample was then dried in a Protect Oven FAC-100 at 103 to 105 °C, cooled and weighed. The oil and grease content of the sample was calculated using equation below:

$$\text{Oil \_ and \_ Grease \_ (mg / l)} = \frac{(A - B) \times 1000}{\text{volume \_ of \_ sample, ml}}$$

A = weight of oil extracted + distilling flask after dried, mg

B = weight of blank distilling flask, mg

## APPENDIX N

### AMMONIUM NITROGEN (NESSLER METHOD)

Fill a 25 ml mixing graduated cylinder to the 25 ml mark with the POME wastewater. At the same time, fill a 25 ml mixing graduated cylinder to the 25 ml mark with deionized water. Then, added three drops of Mineral Stabilizer, three drops of Polyvinyl Alcohol Dispersing Agent and 1.0 ml of Nessler Reagent into each cylinder. Both cylinders then were shaken vigorously. After a one-minute reaction period begin, poured 10 ml of each solution into a square sample cell. When the timer expires, insert the blank into the cell holder with the fill line facing right. The display will show: 0.00 mg/L NH<sub>3</sub>-N after press ZERO. Next, wipe the prepared sample and insert it into the cell holder with the fill line facing right. Results were been shown in mg/L NH<sub>3</sub>-N after press READ.



## APPENDIX O

### ALKALINITY (STANDARD METHOD 2320-B. POTENTIOMERIC TITRATION METHOD)

50 ml sample and 0.1 N standard H<sub>2</sub>SO<sub>4</sub> acid solutions were prepared. Before the titration was performed, the electrode of the HANNA Microprocessor Logging pH meter was rinsed with distilled water. 50 ml sample was then titrated with 0.1 N standard H<sub>2</sub>SO<sub>4</sub> acid solutions to the end point pH which is pH 4.5 without recording intermediate pH values and without undue delay. After each addition of acid, the sample was mixed thoroughly and gently until a constant reading was obtained. As the end point was close, make smaller additions of acid and be sure that pH equilibrium was reached before adding more titrant. The alkalinity value of the sample was calculated according to equation below:

$$\text{Alkalinity, mgCaCO}_3 / l = \frac{S \times N \times 1000}{\text{volume\_of\_sample, ml}}$$

S = ml of titrant to reach endpoint

N = equivalents H<sub>2</sub>SO<sub>4</sub> per liter titrant

## APPENDIX P

### VOLATILE ACIDITY (STANDARD METHOD 5560-C. DISTILLATION METHOD)

Prior to the measurement of the volatile acidity was carried out, the recovery factor of this study was determined for a used apparatus. First of all, an appropriate volume of acetic acid stock solution was diluted to 250 ml in a volumetric flask to approximate the expected sample concentration and distilled as for a sample. The recovery factor was calculated according to the Equation 1.

100 ml of well mixed sample was placed into a 500 ml distillation flask. 100 ml distilled water and 5 ml of concentrated H<sub>2</sub>SO<sub>4</sub> were then added into the distillation flask. The sample was mixed comprehensively to avoid acid remain on bottom of flask. The flask was then connected to the condenser and distilled at rate of about 5 ml/min. The first 15 ml of the distillate were discarded and exactly 150 ml of distillate were collected in the 250 ml beaker. The 150 ml of distillate was then titrated with 0.1 N NaOH by a HANNA Microprocessor Logging pH meter. The end point of the titration was pH 8.3. The volatile acidity as acetic acid of the sample was calculated according to Equation 2:

$$\text{Recovery\_factor, } f = \frac{A}{B} \quad (1)$$

A = volatile acid concentration recovered in distillate, mg/L

B = volatile acid concentration in standard solution used, mg/L

$$\text{Volatile\_acidity, mgCH}_3\text{COOH / l} = \frac{S \times N \times 60000}{\text{volume\_of\_sample, ml} \times f} \quad (2)$$

S = ml of titrant to reach endpoint

N = equivalents NaOH per liter titrant

F=recovery factor

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## APPENDIX Q

### BIOGAS COMPOSITION (STANDARD METHOD 2720-C. GAS CHROMATOGRAPHIC METHOD)

The biogas composition was measured by gas chromatography (Clarus 500) equipped with thermal conductivity detector (TCD). A 2 m x 1 mm ID ShinCarbon ST Micropacked GC column (100/120 mesh) was used and helium was used as a carrier gas at a flow rate of 10 ml/ min. Column head pressure was maintained at 248 kPa. The temperature of the injection port was 100 °C. The chromatography was performed using the following program: 40 °C for 3 min, 40–250 °C with a ramping of 8 °C/min, 250 °C for 10 min and the detector temperature was 200 °C.

Before the measurement of biogas composition was carried out, 1 ml of biogas standard consisting of 4 % (v/v) CH<sub>4</sub> and 5 % of CO<sub>2</sub> was injected by a 10ml syringe for GC calibration. The response for each standard gas was noted and computed as height of peak after correcting for attenuation. After that the same sample volume as that used during calibration was injected by a 10 ml syringe for biogas composition analysis. Like the calibration, the response for each gas was noted and computed as height of peak after correcting for attenuation. The sample and standard gases were injected in sequence to allow calculation of unidentified gas concentration in volume percent by direct comparison of sample and standard gas peak heights. The volume percentages of each gas were calculated by the equation below:

$$\text{Volume, \%} = \text{volume\%(std)} \times \frac{A}{B}$$

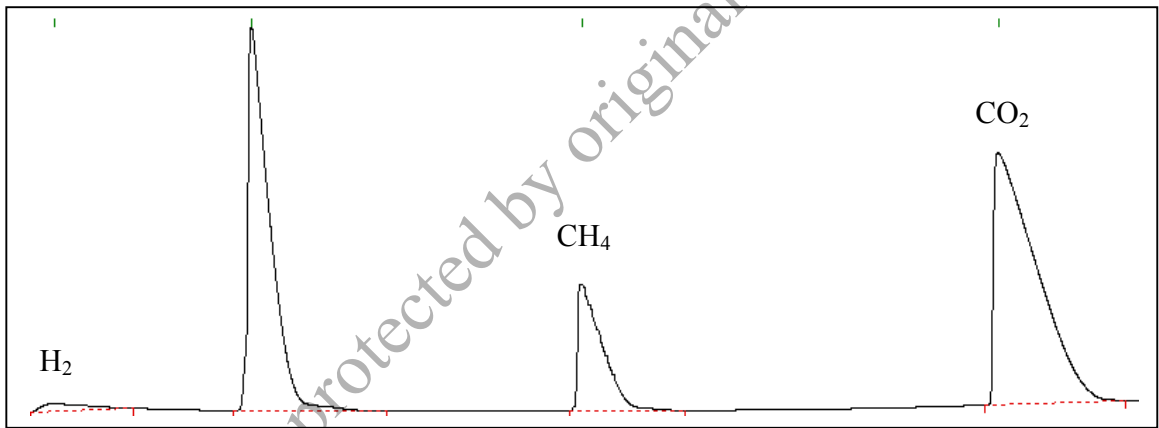
A = height of peak of sample

B = height of peak of standard

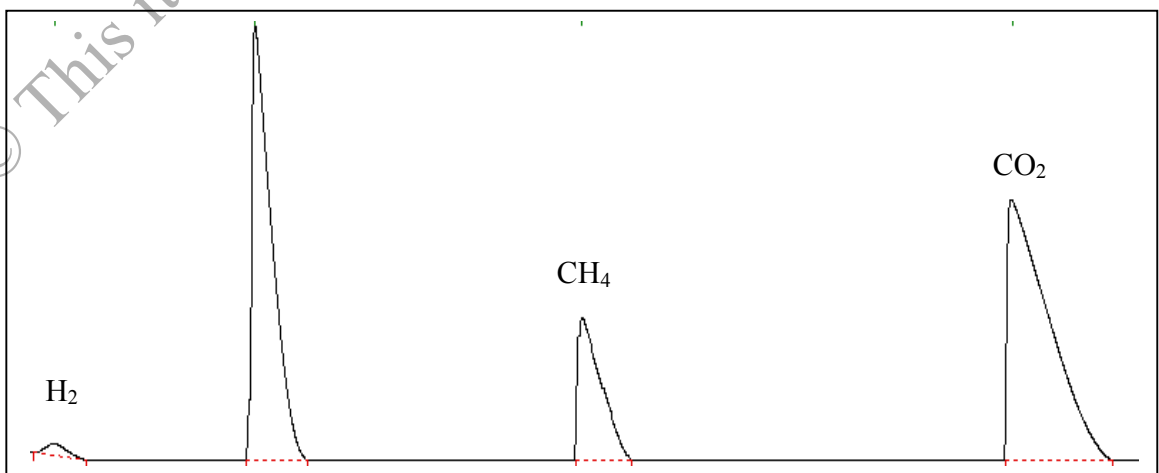
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## APPENDIX R

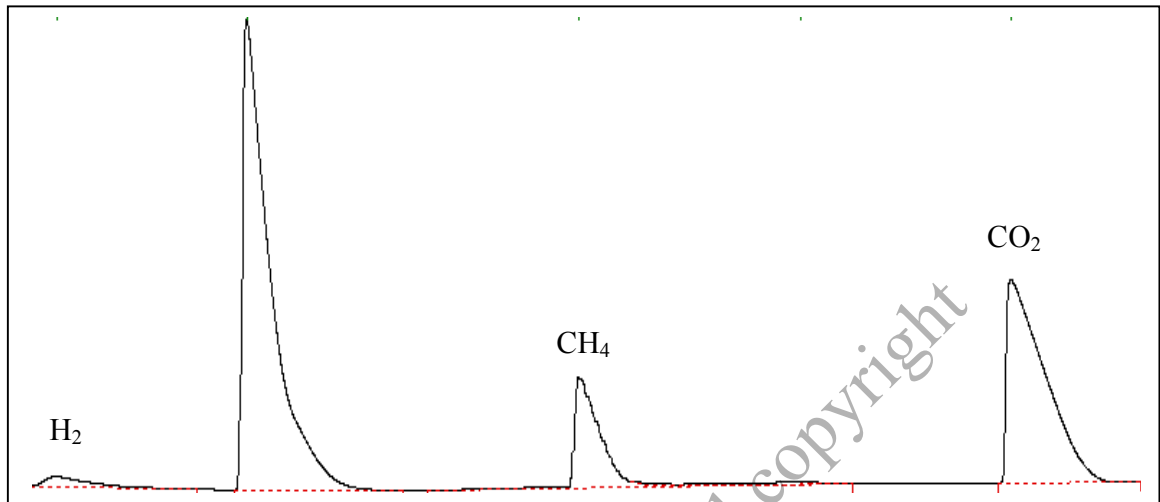
### RESPONSE OF BIOGAS (FROM GAS CHROMATOGRAPHY)



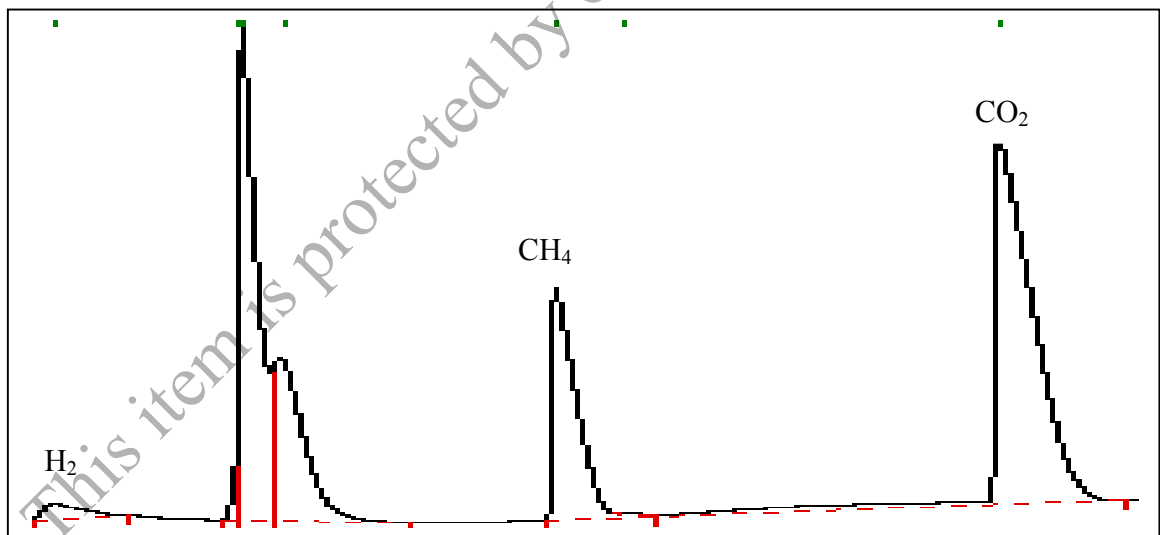
The response for each standard gas (CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>) after correcting for attenuation



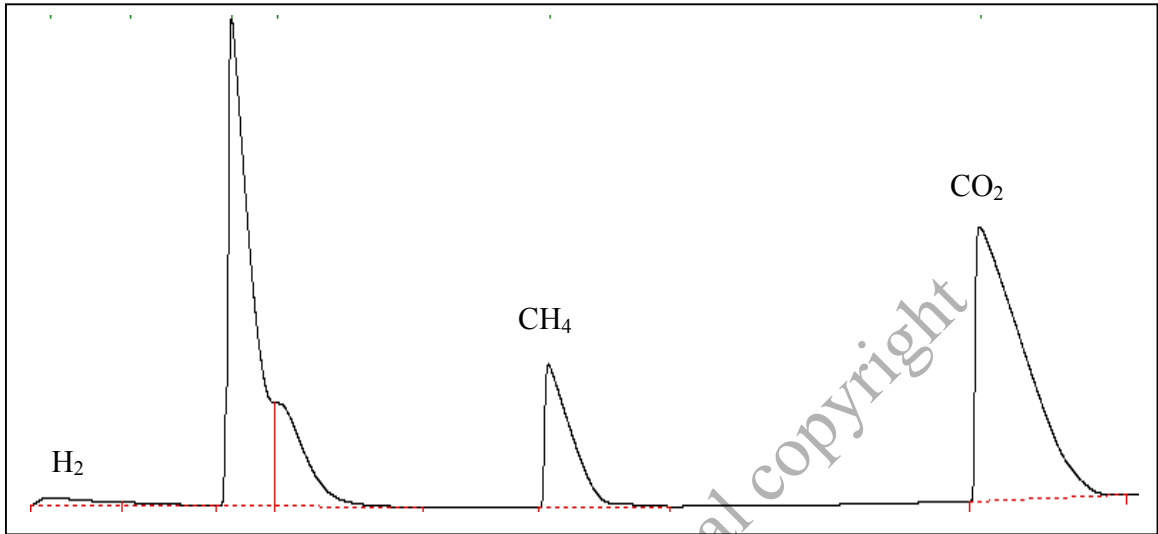
The response for each biogas (CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>) at HRT of 12 days after correcting for attenuation



The response for each biogas (CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>) at HRT of 10 days after correcting for attenuation



The response for each biogas (CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>) at HRT of 8 days after correcting for attenuation



The response for each biogas (CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>) at HRT of 6 days after correcting for attenuation

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