

Evaluation of biogas production from manure of hybrid and local breed cows fed with different types of feeding practices

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ABSTRACT

This study aimed to evaluate the biogas production from the manure of hybrid and local breed cows fed with different types of feeding practices. The feedstock and digestive composition were measured to determine their effects on biogas production. The batches were prepared from manure of hybrid and local breed cows fed with roughages and mixed ration (roughages and concentrate) adding Inoculum (I) and with a total weight of 200 g. Four experimental groups (T1, T2, T3 and T4) were set up using mixing ratios of CM: H₂O: I (25: 25: 50). The digesters were set up at ambient temperature for 40 days of Hydraulic Retention Time (HRT) using a water displacement method to monitor biogas production in the proto-type digesters. The biogas yield from the manure was found 250.90 Nml/g VS for T1, 176.50 Nml/g VS for T2, 208.25 Nml/g VS for T3 and 180.88 Nml/g VS for T4, respectively. The average CH₄ and CO₂ concentration (% vol.) in biogas were found 53% and 47% for T1, 55% and 45% for T2, 52% and 48% for T3, 53% and 47% for T4, respectively. The content of H₂S was not found in this study. The study concluded that the overall biogas production was higher in the manure of hybrid cows fed with roughages. However, the biogas production was also higher in the manure of local breed cows fed with roughages than mixed ration. The results indicated that C, N, P, K and S values were relatively lower in this study.

Keywords: Biogas, CH₄, hybrid and local breed cows, prototype digester, HRT.

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INTRODUCTION

Bangladesh is a densely populated country with an estimated about 165 million population (Worldometer, 2021). The annual Gross Domestic Product (GDP) growth rate of Bangladesh is 8.2% (Trading Economics, 2020). About 75% of people in Bangladesh live in rural areas and use traditional stoves for cooking their three meals daily and other heating purposes. About 30% of rural people have access to national grid electricity where the quality of service is unreliable and electricity is 23%. Less than 10% of households have natural gas connections via national pipelines, but unfortunately, remote and rural areas have no natural gas access

(Chakrabarty et al., 2013). Barnes et al. (2011) reported that 58% of the rural families in the country are officially "energy poor" (i.e., utilization of modern energy services per capita is very low), with the shortage of access to even necessary energy facilities. In Bangladesh, 70% of electricity is generated from natural gas (Halder et al., 2016). Bangladesh has a very insufficient energy reserve. To overcome the present situation, finding alternative sources of renewable energy is the only option. Biogas is one of the essential promising renewable energy resources for Bangladesh, mainly from animal manure and municipal waste (Uddin et al., 2018).

Anaerobic digestion (AD) is a biological process used for anaerobic digestion of organic matter like livestock manure and agriculture residues into clean, renewable energy and organic fertilizer. AD is a series of complex microbiological processes where diverse types of aerobic and anaerobic bacteria work in four different stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Gonzalez-Fernandez et al., 2015). These bacteria are susceptible to environmental conditions, and it is essential to balance a range of factors to maximize the chances of achieving optimum design and efficient operation (Mao et al., 2015).

Biogas is typically composed of 50 to 70% methane (CH₄), 30 to 50% carbon dioxide (CO₂), and trace amounts of H₂S and other gases. Biogas is a clean, efficient, and renewable source of energy that offers a multi-purpose carrier of energy that can be used as a substitute for other fuels (like firewood and cattle dung) currently used in rural areas (Bond and Templeton, 2011).

Cow manure (CM) is one of the most common substrates for biogas production in the AD process. The CM contains enormous amounts of polysaccharides, lignocelluloses, proteins, and other biomaterials (Jingura and Kamusoko, 2017). Biogas production from manure can be increased through several mechanisms. If the manure fibers are decreased, the biogas yield in manure increases by 30% (Angelidaki and Ahring, 2000).

This study's objective was evaluation of biogas production from the manure of hybrid and local breed cows fed with roughages and mixed ration and determination of the feedstock and digestive characteristics and its effects on biogas production.

MATERIALS AND METHODS

Sources of CM

The fresh CM was collected from hybrid and local breed cows which were fed with roughages and mixed ration (roughages and concentrate) from the Animal Research Farm (ARF), Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh-2202.

Sources of inoculum

CM sludge was used as inoculum collected from the outlet of floating dome-type prefabricated red mud digester (Volume = 7.5 m³, Taiwan) operated at ambient temperature and used to treat CM as a substrate for anaerobic digestion in the Biogas Research Laboratory, Department of Microbiology and Hygiene, BAU, Mymensingh-2202. The inoculum was pre-incubated for 1 to 5 days at 35°C and reduces the impact of its methane production. The methane yield (38.16 Nml CH₄/g VS) of inoculum was used in this study.

Preparation of lab-based prototype anaerobic digester

The experiment was conducted based on the water displacement

method according to the German standard procedure Verein Deutscher Ingenieure (VDI) 4630 (VDI 4630, 2006). Laboratory-based batch digesters were made using 250 ml Coca Cola plastic bottles. A hole (0.4 cm in diameter) was made in the center of each bottle's cap. A rubber hose pipe of length 2 feet and diameter 0.5 cm was inserted through the hole and sealed with M-seal adhesive glue. The hosepipe served as the gas conduit leading into a measuring cylinder of 250 ml capacity, filled with water. The cylinder was kept inverted and immersed in water in a 1.5-liter plastic water bottle. The hosepipe was placed so that its tip was in contact with the base of the cylinder. A second hose pipe was inserted into the cylinder to withdraw biogas, with the free end sealed with a clip. The biogas generated in the digesters displaced water from the graduated cylinders. The volume of the graduated cylinders' headspace represented the volume of biogas generated in the digester (Figure 1). The gas was collected from the cylinder at daily until day 40 using 100 ml plastic syringes.

Setup of experimental design and procedure

The batches were prepared from manure of hybrid and local breed cows fed with roughages and mixed ration (roughages and concentrate) adding Inoculum (I) and with a total weight of 200 g. In the batch T1, (25%) manure collected from hybrid cow fed with roughages mixed with (25%) H₂O and (50%) I. In the batch T2, (25%) manure from hybrid cow fed with mixed ration (roughages and concentrate) adding with (25%) H₂O and (50%) I. However, in the batch T3, (25%) manure collected from local breed cow fed with roughages mixed with (25%) H₂O and (50%) I. Whereas in the batch T4, (25%) manure collected from local breed cow fed with mixed ration (roughages and concentrate) adding with (25%) H₂O and (50%) I (Table 1). The digesters were set up at ambient temperature for 40 days of HRT.

Collection and measurement of biogas

Biogas generated in the digesters was transported by a rubber hose pipe into graduated cylinders and displaced the graduated cylinders' water. The volume of the headspace of the graduated cylinders represented the volume of biogas generated in the digester. The biogas was collected from the graduated cylinder using another hose pipe and a 100 ml gas-tight plastic syringe. When not in use, this tube was sealed with a clip.

Analytical methods

Total Solid (TS) and Volatile Solids (VS) were analyzed by using a muffle furnace (Model: JSMF-30T, JSR, China) according to standard methods of Drosig (2013). The feedstock and digested effluent pH were measured using an electronic pH meter (PHS-25 pH meter, Shanghai, China). The nitrogen was determined by the micro-Kjeldahl method as described in Pearson (1976) and carbon was determined by the Walkley-Black method (Walkley and Black 1934). The concentration of phosphorus and potassium was determined by Vanadomolybdo-Phosphoric yellow color method in the nitric acid and flame emission spectrophotometer methods described by Jackson (1962). The concentration of sulfur was determined by the Turbidimetric method, according to Hart (1961). The digested samples were centrifuged at 10,000 g for 10 minutes and assess the accumulation rate of Volatile fatty acids (VFA) according to Liebetrau et al. (2016). Total Volatile fatty acids of the sample's liquid portion were determined by Kapp's (1984) three-point-titration method. The temperature was recorded daily using Thermo Hygrometer (Model N° DO 2001, Conrad Electronic) and the digester temperature were also recorded using a portable

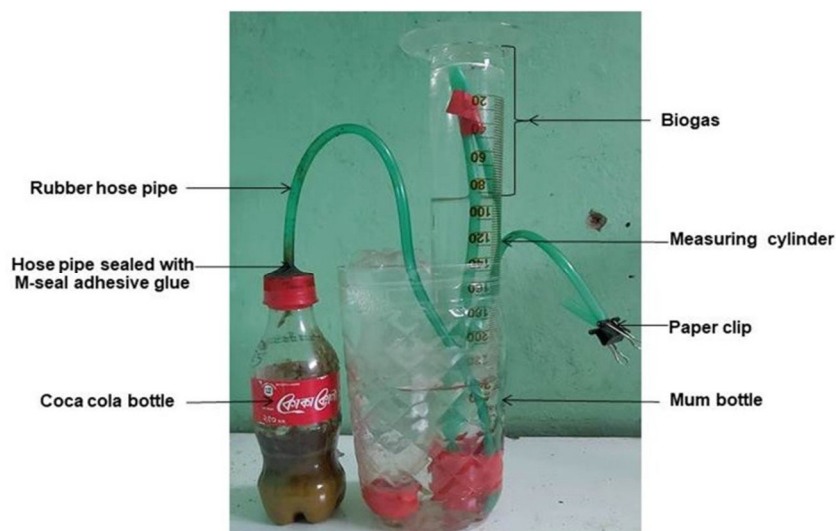


Figure 1. Experimental set up of lab-based prototype biogas digester by water displacement method with different parts.

Table 1. Mixing ratios of the substrate in hybrid and local breed CM with inoculum.

Experimental batches	Mixing ratio (% wt.)	Weight (g)
T1	CM (25%), H ₂ O (25%), I (50%)	200
T2	CM (25%), H ₂ O (25%), I (50%)	200
T3	CM (25%), H ₂ O (25%), I (50%)	200
T4	CM (25%), H ₂ O (25%), I (50%)	200

CM - Cow manure; I – inoculum.

thermometer sensor. The composition of biogas (methane and carbon dioxide, % vol.) was analyzed using a portable biogas analyzer gas board-3200P. The H₂S gas was measured using Sulphide detector tubes (Hebixinxing, China, range = 100-1000 ppm) and portable gas dragger syringes. All the data obtained from the three replications of four treatment batches in this study were recorded in MS excel sheet (MS-2019) and determined the average value, cumulative average value and graphical analysis.

RESULTS AND DISCUSSION

Feedstock characteristics of hybrid and local breed CM

The pH value for manure of Hybrid Cow fed with Roughage (HCR), Hybrid Cow fed with Mixed ration (HCM), Local Cow fed with Roughage (LCR) and Local Cow fed with Mixed ration (LCM) was measured 7.3, 7.1, 7.0 and 7.0, respectively (Table 2). The pH value was indicated within the neutral range. Jingura and Kamusoko (2017) have reported that the optimal pH range for obtaining maximal biogas yield was 6.5 to 7.5. Weinfurtner (2011) also noted that the range for CM pH was 6.2 to 8.8.

The TS content was found 12% for HCR, 20% for HCM, 15% for LCR and 24% for LCM (Table 2). The TS values were found within the reported range 12-25% for CM described by Korres et al. (2013); FNR (2010) and Bioenergy (2013). However, the VS/TS content was 83% for HCR, 70% for HCM, 80% for LCR and 83% for LCM. The finding of this study was related to other studies. Vogeli et al. (2014) reported that the accepted range of VS/TS content was 70 to 90%. Korres et al. (2013) and Bioenergy (2013) also found that the VS content of TS was 70 to 80% for CM.

The C: N ratio for HCR, HCM, LCR and LCM was found 40:1, 41:1, 41:1 and 32:1, respectively (Table 2). The C: N ratio of HCR, HCM and LCR was relatively higher than reported in the previous studies. Korres et al. (2013) and FAO (2015) reported that the C: N ratio of feedstock was found 6:1–32:1. The optimal range of C: N ratio was 10:1 to 30:1, higher C: N could mean that all the carbon cannot entirely be converted and the maximum methane yield eventually not achieved (FNR, 2010). According to Braun (1982), C:N ratios were acceptable in the range from 10:1 to 45:1, which means all substrates are within the range and it might be expected lower biogas yield but higher methane composition. The

Table 2. Feedstock characteristics of manure from hybrid and local breed cows fed with roughages and mixed ration.

Parameters	Hybrid cow fed with roughages (HCR)	Hybrid cow fed with mixed ration (HCM)	Local breed cow fed with roughages (LCR)	Local breed cow fed with mixed ration (LCM)
pH	7.3	7.1	7.0	7.0
TS (% wt.)	12	20	15	24
VS (% wt.)	10	14	12	20
VS/TS (% wt.)	83	70	80	83
C (% wt.)	37.51	38.30	37.50	38.90
N (% wt.)	0.94	0.94	0.92	1.20
C: N ratio	40:1	41:1	41:1	32:1
P (% wt.)	0.614	1.860	0.588	0.790
K (% wt.)	0.942	0.933	0.686	0.713
S (% wt.)	0.190	0.174	0.171	0.194

TS - Total solid; VS - Volatile solid; C - Carbon; N - Nitrogen; P - Phosphorus; K - Potassium; S - Sulphur.

hybrid cow fed with a concentrated mixed ration has higher carbon content than roughage where N remained similar.

The C, N, P, K and S of CM were found 37.5 to 38.90%, 0.92 to 1.20%, 0.61 to 1.9 %, 0.68 to 0.94% and 0.17 to 0.19%, respectively (Table 2). The finding of this present study is closely related to the other finding. Naher and Paul (2017) reported that the values of C, N, P, K and S of CM were 36%, 0.9 to 1.2%, 0.7 to 1%, 0.75% and 0.21 to 0.35% in Bangladesh. Islam (2006) also reported that the value of N, P, K and S in CM were 1.35%, 2.89%, 0.88% and 0.71%, respectively. The nitrogen content (2.4 to 3.5%) of CM was reported higher in Europe than Bangladesh (Korres et al., 2013).

Batch characteristics of hybrid and local breed CM

The pH values for all inoculated batches (T1, T2, T3 and T4) were measured ranging from 7.0 to 7.5 (Table 3). The pH values of all inoculated batches were laid within an acceptable range. This range of pH was ideal for methanogenic bacteria to produce a high amount of biogas. For optimal performance of microbes, the digester's pH should be kept in the range of 6.8 to 8.0 (Sreekrishnan et al., 2004). According to Liu et al. (2008) the most favorable pH range to attain maximal biogas yield in anaerobic digestion is 6.5 to 7.5.

The TS content of T1, T2, T3 and T4 batches were 7, 10, 8 and 10%, respectively (Table 3). The TS content was slightly lower than the previously reported value. Korres et al. (2013) have found that the TS content was 12 to 25% for CM. Moreover, the VS/TS content of T1, T2, T3 and T4 batches were 71, 60, 75 and 80%, respectively (Table 3). The VS/TS content was slightly lower in T2 batches than the previously reported value. Korres et al. (2013) and Bioenergy, (2013) have found the VS/TS values were 70 to 80% for CM.

For all inoculated batches, the C:N ratio was found

within the acceptable range 32:1 to 40:1 (Table 3). According to Bischofsberger et al. (2005), the acceptable range of C: N ratio was 10:1 to 45:1. The ideal range of C: N ratio was 10:1 to 30:1 for optimum and sustainable biogas production (FNR, 2010). The high content of carbon was present in the substrate that may lead to lower biogas yield.

All the batches were observed over a specified period of 40 days HRT. The batch experiment was conducted at a mesophilic temperature range between 27.3 to 31.7°C, with a median temperature of 29°C. Vogeli et al. (2014) have reported that methanogenic archaea were active at mesophilic conditions.

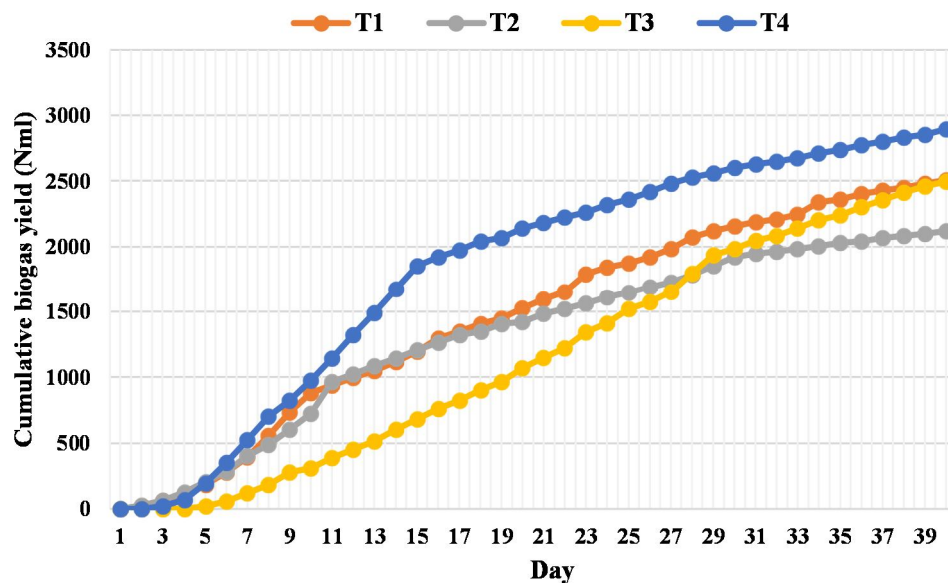
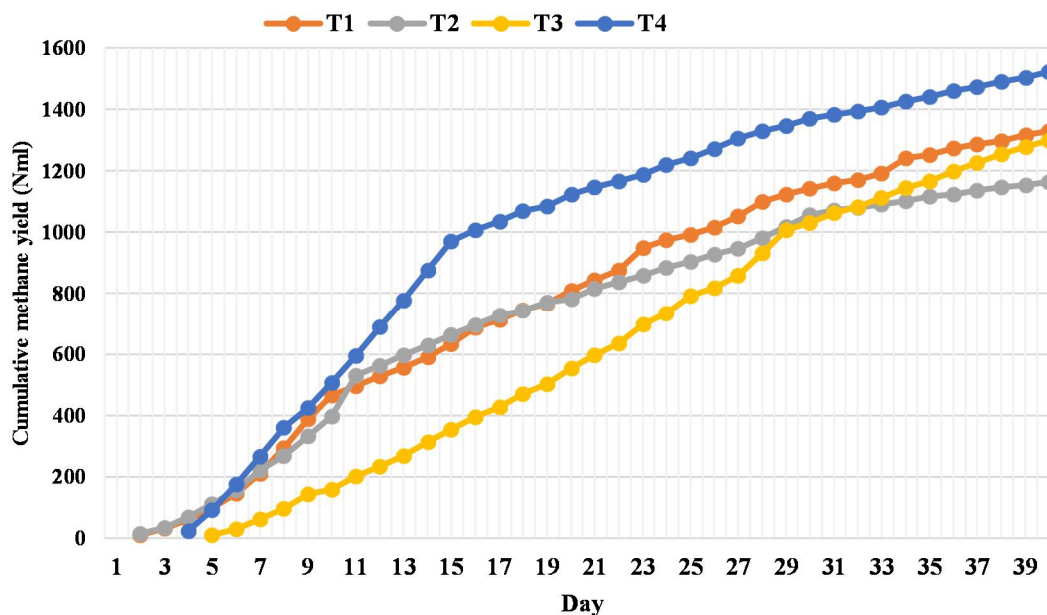
Comparison of biogas and methane yield of different experimental batches

The results of cumulative biogas and methane yield from the manure of hybrid and local breed cow fed with roughages and mixed ration during the experiment were expressed in Nml. The cumulative biogas from the manure was found 2509 Nml for T1, 2118 Nml for T2, 2499 Nml for T3 and 2894 Nml for T4, respectively (Figure 2). The biogas yield from the manure was found 250.90 Nml/g VS for T1, 176.50 Nml/g VS for T2, 208.25 Nml/g VS for T3 and 180.88 Nml/g VS for T4, respectively. The biogas yield was closely related to the previous study except in T1 batch experiment. Budiyo et al. (2010) found that the best performance for biogas production in the digester with 7.4 and 9.2% of TS i.e., give biogas yield 184.09 and 186.28 ml/g VS, respectively. The overall biogas yield was comparatively higher in T1 batch than the other three batches. The biogas yield was higher in T1 batch this might be due to the substrate contains adequate VS/TS and C: N ratio. Whereas, the cumulative methane yield was found 1330 Nml for T1, 1165 Nml for T2, 1400 Nml for T3 and 1523 Nml for T4, respectively (Figure 3). The values of

Table 3. Batch characteristics of hybrid and local breed CM.

Experimental batches	pH	TS (% wt.)	VS (% wt.)	VS/TS (% wt.)	C (% wt.)	N (% wt.)	C: N
T1	7.5	7	5	71	19.54	0.64	30:1
T2	7.0	10	6	60	19.62	0.48	40:1
T3	7.0	8	6	75	19.38	0.48	40:1
T4	7.0	10	8	80	20.34	0.59	35:1

TS - Total solid; VS - Volatile solid; C - Carbon; N - Nitrogen.

**Figure 2.** Cumulative biogas yield of different experimental batches.**Figure 3.** Cumulative methane yield of different experimental batches.

methane yield were found 133 Nml CH₄/g VS for T1, 97.08 Nml CH₄/g VS for T2, 108.29 Nml CH₄/g VS for T3 and 95.20 Nml CH₄/g VS for T4, respectively.

Comparison in biogas production between T1 and T2 experimental batches

The biogas production was started on day 2 of both T1 and T2 experimental batches. The cumulative biogas was found 2509 Nml for T1 and 2118 Nml for T2 batch (Figure 4). The volume of biogas produced was higher in T1 treatment batch than T2. The highest volume of biogas produced in batch T1 was 178 Nml on day 9 and in batch T2 240 Nml on day 11. In general, the gas volume produced by group T1 from day 2 until day 40 was higher than that of group T2. Average gas production per day in treatment group T1 was also higher (62 Nml) compared to that of group T2 (52 Nml). The biogas production was higher in T1 batch this might be due to the substrate

contains optimum C: N ratio.

Comparison in biogas production between T3 and T4 experimental batches

The biogas production was started on day 5 for T3 and day 3 for T4 experimental batches. The cumulative biogas was found 2499 Nml for T3 and 2894 Nml for T4 batch (Figure 5). The volume of biogas produced was higher in T4 batch than T3. The highest volume of biogas produced in batch T3 was 144 Nml on day 29 and in batch T4 186 Nml on day 14 and in general, the gas volume produced by group T4 from day 3 until day 40 was higher than that of group T3. Average gas production per day in treatment group T4 was also higher (72 Nml) compared to that of group T3 (62 Nml). The biogas production was higher in T4 batch this might be due to contains adequate amount of VS/TS content and C: N ratio in the substrate.

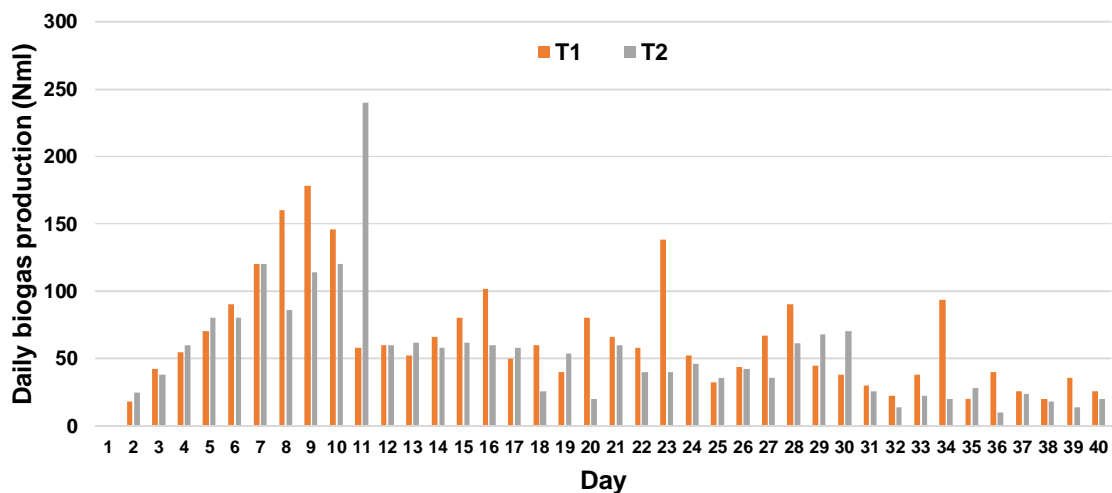


Figure 4. Biogas production between T1 and T2 experimental batches.

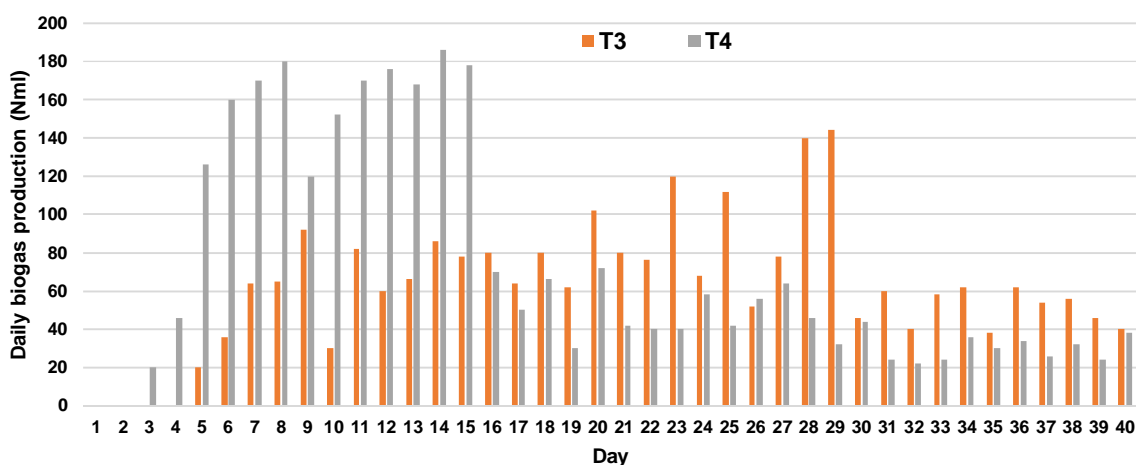


Figure 5. Biogas production between T3 and T4 experimental batches.

Biogas composition of different experimental batches

The average CH₄ and CO₂ concentration (% vol.) in biogas were found 53 and 47% for T1, 55 and 45% for T2, 52 and 48% for T3, 53 and 47% for T4, respectively. The content of H₂S was not found in this study. However, the concentration of CH₄ and CO₂ in this study's biogas was closely agreed with other studies' findings. Haryanto et al. (2018) have conducted the experiment using a lab-scale self-designed anaerobic digester of 36-L capacity with the substrate of a mixture of fresh cow dung and water at a ratio of 1:1. They have reported that the concentration of CH₄ and CO₂ was 41.73 to 57.23% and 31.13 to 39.04%, respectively.

Digestive characteristics of hybrid and local breed CM

After 40 days of digestion, the substrate was analyzed once more for determining the composition. The digestive pH value of all batches was within a suitable range (6.7 to

7.2) for producing methanogenic microorganisms (Table 4). FNR (2010) reported that the favorable range of pH was found 6.5 to 8.0 for producing methanogenic microorganisms in the digester.

The C: N ratio of T1, T2, T3 and T4 batches were found 23:1, 24:1, 41:1 and 44:1 respectively (Table 4). According to Bischofsberger et al. (2005), the acceptable range of C: N ratio was 10:1 to 45:1. The C: N ratio adjustment for batches within time closer to the ideal range (10:1 to 30:1) than the batch characteristic might indicate that the carbon was utilized faster than N.

The C, N, P, K and S content of all batches were found ranging from 1.71 to 2.29%, 0.05 to 0.08%, 0.03 to 0.07%, 0.05 to 0.09% and 0.01%, respectively (Table 4). The results indicated that C, N, P, K and S values were relatively lower than other studies. Islam (2006) reported that the N, P, K and S content in CM slurry were 1.23 to 1.35%, 2.71 to 2.89%, 0.62 to 0.88% and 0.67 to 0.71%, respectively. The VFA concentration of all batches was found within an acceptable range from 56 to 131 mg/L (Table 4). Bioenergy (2013) reported that the ideal concentration of VFA was found below 1000 mg/L stable for biogas production.

Table 4. Digestive characteristics of hybrid and local breed CM.

Experimental batches	pH	C (% wt.)	N (% wt.)	C: N ratio	P (% wt.)	K (% wt.)	S (% wt.)	VFA (mg/L)
T1	6.8	1.71	0.08	23:1	0.03	0.09	0.01	65
T2	6.7	2.04	0.08	24:1	0.05	0.08	0.01	131
T3	7.2	2.10	0.05	41:1	0.04	0.05	0.01	67
T4	6.7	2.29	0.05	44:1	0.07	0.07	0.01	56

TS - Total solid; VS - Volatile solid; C - Carbon; N - Nitrogen; P - Phosphorus; K - Potassium; S - Sulphur; VFA - Volatile fatty acid.

CONCLUSION

The finding of this study concluded that the biogas yield was higher in the manure of hybrid fed with roughages than mixed ration. However, the biogas production was higher in local breed cows fed with mixed ration. The overall finding of this study found that the biogas yield was comparatively higher in the manure of hybrid cow fed with roughages compared to that of other three treatment groups. In this study, the nutrient (N, P, K and S) concentration was found low in the digestive of hybrid and local breed cows.

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