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Co-digestion of elephant grass (*Penisetum purpuerum*) and cow manure to produce biogas using semi-continuous fed anaerobic digester

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Abstract

Novelty: Biogas is a prospective source of renewable energy in the future, and therefore the development of new substrates is important. In this research, we prove that elephant grass is a potential substrate for the biogas process. In addition, we also found that the physical treatment and addition of urea combined with the loading rate and frequency of substrate feeding had an effect on the biogas production from a mixture of elephant grass and cow manure.

Highlight: 1) Biogas production from mixture of elephant grass and com manure was assessed; 2) Physical pretreatment and urea addition enhanced the biogas and methane yield from anaerobic digestion of elephant grass; 3) Loading rate and feeding frequency influence biogas yield; 4) The highest biogas yield (average 4.608 L/d or 153.60 Ld-1m-3) with methane content 51.79% was observed from digester with feeding frequency of once in four days.

Abstract— Elephant grass is promising substrate to produce biogas. This research aimed at investigating the effect of loading rate, physical pretreatments, urea addition, and feeding frequency on the biogas production from co-digestion of elephant grass and cow manure. Experiments were conducted using semi-continuous digesters separated into two steps. Experiment-1 used chopped grass with cow manure at a total solid (TS) content 10%, working volume 25 L, loading rate of 0.625 and 1.25 L/d, and urea addition 1.25 g/L. Experiment-2 used pulped grass with TS content 5%, loading rate 0.5 L/d and five different feeding frequency. Results showed that biogas yield was influenced by urea addition, loading rate, feeding frequency, and grass physical treatment. Biogas yield increase with decreasing loading rate and urea addition and achieved 3.09 L/d or 123.72 Ld-1m-3 at loading rate 0.625 L/d and urea addition 1.25 g/L. Biogas yield was affected strongly by grass physical treatment than urea addition. Biogas generated from pulped grass had significantly higher methane content than those of chopped grass. Feeding frequency of four days produced the highest biogas yield (average 4.608 L/d or 153.60 Ld-1m-3) with methane content of 51.79%.

Keywords: Biogas yield; C/N ratio; Feeding frequency; Loading rate; Urea addition

1. Introduction

Biogas is a renewable fuel produced from anaerobic digestion process of organic materials which goes on through four steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Depending on the substrate type, the biogas generaly constitutes of methane (CH_4) in the range of 45-70%, carbon dioxide (CO_2) 30-45%, and traces of hydrogen (H_2), water vapor (H_2O), ammonia (NH_3), and hydrogen sulphide (H_2S). Biogas is smart fuel that can be explored for

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substituting fuel in many utilizations, such kitchen stove and generating power [1]. Recently, compressed biogas can also be used for transportation fuel. Biogas technology has contributed important benefits to the supply of energy in various countries.

Recently elephant grass or Napier grass (*Pennisetum purpureum* Schumach) have been identified as good substrate for biogas [2], [3], [4], [5], [6]. Cadavid-Rodríguez and Bolaños-Valencia [7] recommend that even though more research is required, fresh grass can be employed as feedstock for anaerobic digestion in a tropical countries by using an economical and simpler operation. Elephant grass (also called Napier grass) has a high posture and is one of tropical forage characteized by high yield and high photosynthetic efficiency. Elephant grass can be cut several times a year with annual prodution of fresh biomass around 500 t/ha in average [8]. In addition, the grass is very adaptive, grows rapidly, and is easy to propagate, tolerant in acidic and marginal soils and drought condition. Elephant grass produces high biomass yield with high organic matter that makes it as good substrate for biogas production.

Studies on producing biogas using elephant grass have been reported long time back in reference [9] who obtained maximum methane production levels from energy crops including Napier grass with total solid between 25% and 30%. Recently, codigestion of elephant grass with cowdung and other wastes has attracted special interests. For example the work of Sawanon with cowdung reported that biogas yield was affected by substrate composition with the maximum rate of 0.169 m^3/kg VS (volatile solid) added [6]. Other works were also reported with chicken manure [10], food waste [11], and slaughterhouse waste water [12].

Like other biomass, grass usually has high carbon to nitrogen (C/N) ratio so that external nitrogen source is required to bring C/N ratio to the ideal values for anaerobic digestion. Ekpenyong [13] reported biogas production from anaerobic digestion of elephant grass powder and concluded that biogas yield rise by 40% as a result of urea addition. For continuous fed anaerobic digesters, loading rate is important factor to be considered. Cow manure is a common substrate for most family scale semi continuous biogas digesters applied in rural areas. Therefore, our present work aims to determine biogas yield from anaerobic digestion of mixture of elephant grass and cow manure under semi continuous fed digester, especially to address some important factors like loading rate, C/N ratio, physical pretretment, and feeding frequency.

2. Material and methods

2.1. Digester and Substrate Preparation

In this research, anaerobic process to produce biogas was performed using lab scale 36-L digester. The digester preparation has been described previously [14]. The digester can be used with working volume between 25 and 30 L. Elephant grass (Figure 1) was harvested at about 60-day of age. Both grass and fresh cow manure were collected from the Animal Husbandry Department, University of Lampung. Fresh cow manure was used as inoculum source. To decrease C/N ratio, Urea fertilizer was put as additional nitrogen. Prior to anaerobic digestion process, grass and cow manure were analyzed to characterize the contents of TS (total solids), VS (volatile solids), C (carbon), and N (nitrogen). Table 1 reveals the characteristic of Elephant grass and cow manure. To address some important factors we performed experiment in two steps, namely experiment-1 and experiment-2. Experiment-1 used chopped grass, whereas experiment-2 used puped grass.

Figure 1 Elephant grass. (From left to right: fresh, chopped, and pulped)

Table 1 Fresh substrate characteristic

Parameters	Cow manure	Elephant grass
Total solid, TS (%, wb)	16.98	17.29
Volatile solid, VS (% TS)	79.17	88.24
Ash (% TS)	20.83	11.76
C (%)	39.87	55.51
N (%)	1.42	1.81
C/N ratio	28.08	30.62

2.2. Treatment (Experiment-1)

For experiment-1, fresh grass was knive-cut into pieces of 1 cm length. Four digesters with working volume of 25-L each were set up for this experiment. Initially, chopped grass and cow manure were mixed completely. Experiments were conducted with substrate mixture constituted of one part of grass and three parts of cow manure that was mixtured by adding tap water to achieve TS content of around 10%. Based on Table 1, substrate in each digester was composed of 6.73 kg cowdung, 4.96 kg fresh chopped grass, and 13.30 kg tap water. For this experiment we prepared four packages of substrate mixture. Two packages of the substrate recipe was put into the digester labeled as P1 and P2. The other two packages had the same composition, but into the water it first has been added urea at a rate of 1.25 g/L. Substrate packages with additional urea were introduced into digester with label P3 and P4.

The digesters were left for some days until stable. When a digester had produced the biogas for the first time, starting in the next day the digester was fed with matching substrate at a loading rate of 0.625 L/d for digesters P1 and P3, and a loading rate of 1.25 L/d for digesters P2 and P4. At the same time, an equal amount was removed from the digester outlet making the substrate volume in the digester remain constant 25 L. Table 2 presents designed treatments along with Urea addition, C/N ratios, loading rate (LR), organic loading rate (OLR), and hydraulic retention time (HRT). Measurement for biogas production was conducted for 47 days.

Table 2 Urea addition and loading rate treatment

Treatment	Urea (kg)	C/N ratio	LR (kg/d)	OLR (kg VS/m^3.d)	HRT (d)
P1	0	28.7	0.625	2.082	40
P2	0	28.7	1.250	4.164	20
P3	0.031	20.9	0.625	2.082	40
P4	0.031	20.9	1.250	4.164	20

2.3. Treatment (Experiment-2)

Experiment-2 was prepared after the experiment-1 finished and was designed to overcome problems observed during experiment-1. Chopped grass was pulped using a kitchen blender (Figure 2) and then mixed with cowdung at the same TS ratio as in the previous experiment (1:3). In this experiment, however, the substrate was diluted using tap water to get TS content of 5%. To maximise space in the digester, we used the digesters with working volume of 30 L. Five semi-continuous digesters, labeled as F1 to F5, were loaded with the substrate mixture of 30 L each and let them until stable. When the digesters started to produce biogas, the prepared substrate mixture was loaded into the digesters at same loading rate of 0.5 L/d but different feeding frequencies, namely 0.5 L everyday (F1), 1.0 L every another day (F2), 1.5 L every 3 days (F3), 2.0 L every 4 days (F4), and 2.5 L every 5 days (F5). The same parameters as in the experiment-1 were observed. Biogas production was observed for 20 days starting from the addition of the substrate.

2.4. Calculations and Analysis

Total solids (TS) of substrate components were determined gravimetrically using an oven (MEMMERT Universal Oven UN55) operated for a day overnight at 105 °C. Oven dry sample was then combusted into ash using a furnace (Ney VULCAN D-550) working at 550 °C for 2.5 hours to get the volatile solid (VS). Total and volatile solid are determined from the following relations:

$$TS = \frac{W_2}{W_1} \times 100 \qquad \text{.............. (1)}$$

$$VS = \frac{W_2 - \text{Ash}}{W_2} \times 100 \text{ .... (2)}$$

where W_1 and W_2 is respectively mass weight of sample before and after drying. TS is expressed in % of wet basis (wb) and VS is in % of TS.

Digester efficiency was evaluated from the VS removal (VS_r) calculated from difference of initial and final VS as the following:

$$VS_r\ (\%) = 100 \times (VS_{in} - VS_{out})/VS_{in} \text{ .... (3)}$$

Atomic elements (C and N) were determined using element analyzer (Elementar Vario EL Cube). C/N ratio of each substrate component was used to calculate C/N ratio of the substrate mixture using the following relation:

$$C/N = \frac{(C_c \times m_c) + (C_g \times m_g)}{(N_c \times m_c) + (N_g \times m_g) + 0.46 \times m_{Urea}} \text{ .... (4)}$$

with m is dry mass of each substrate component, C is carbon content (%), and N is nitrogen content (%). Subscripts c denotes cow manure and g refers to grass.

Conditions during anaerobic digestion process were evaluated daily from substrate's pH and temperature. The pH was observed using a pH meter (PHMETER PH_009-I) for fresh as well as spent substrates. Daily biogas yield was measured using water replacement method. Methane content in the biogas was analyzed using gas chromatograph (Shimadzu GC 2014). Specific biogas yield (SBY) was calculated as in the following:

$$\text{SBY} = (\text{Biogas Yield})/VS_r \qquad \text{.............. (5)}$$

where VS_r is expressed in kg.

3. Results and discussion

3.1. Substrate Acidity (pH) and Temperature

Process condition is evaluated from the substrate acidity (pH), working temperature, and degradation efficiency. Figure 2 showed average pH of the four different treatments in the experiment-1. The daily pH values actually decreased for the first four days and then increased till the day of 20. Since then the pH values were almost stable for each treatment with average values of 6.3 for P1, 6.4 for P2, and 6.6 for P3 and P4. The minimum pH value was 5.7 (in digesters P1 and P2) and the maximum value was 7.2 (P3). The range of minimum to maximum value was about one for all treatments. Treatments with urea addition (P3 and P4) resulted in a higher pH value than no urea addition treatments (P1 and P2). The addition of urea has increased the pH value. Urea contains 46% of nitrogen. The nitrogen will accumulate in form of ammonia (NH_4) that increasing pH in the digester.

Another factor affecting digester pH is loading rate. According to Babaee and Shayegan (2011), high loading rate decreases pH and biogas production, but increases CO_2 content [15]. Treatments P3 and P4 had pH that is close to the optimum pH requirement as compared to that of treatments P1 and P2. The pH values of all treatments, however, were in the range of good condition for anaerobic digestion. Anaerobic digestion processes accomplish the prerequisite for cell activities as well as cell growth at pH values between 5.5 and 8.5.

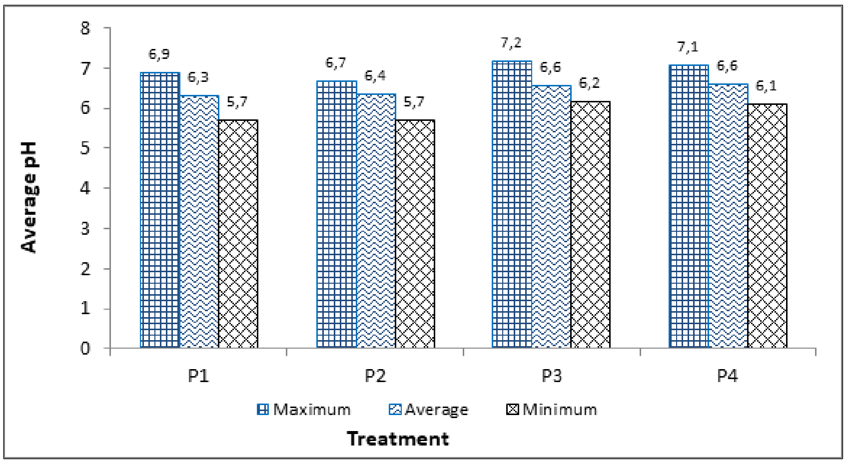

Figure 2 Average pH values for different treatments (Experiment-1)

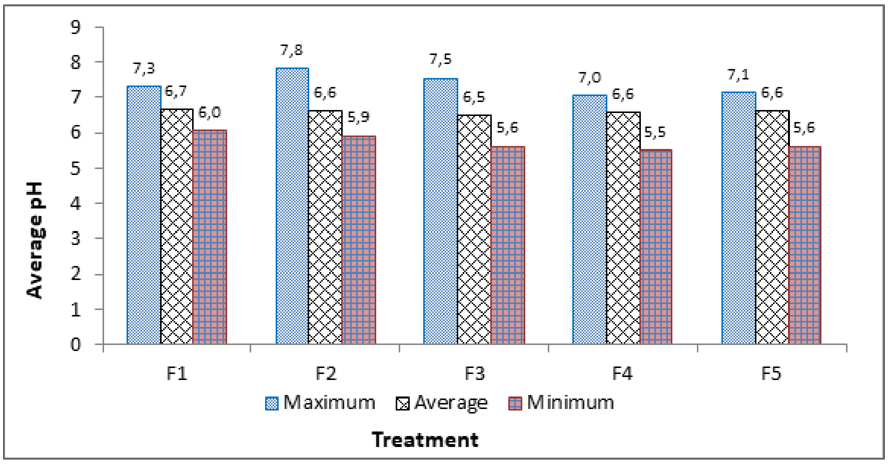

Figure 3 Average pH values for different treatments (Experiment-2)

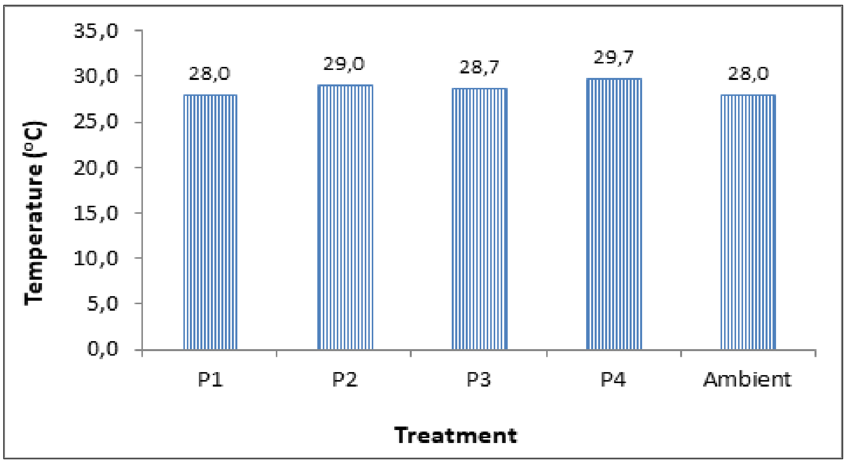

Figure 4 Average temperature for different treatments (Experiment-1)

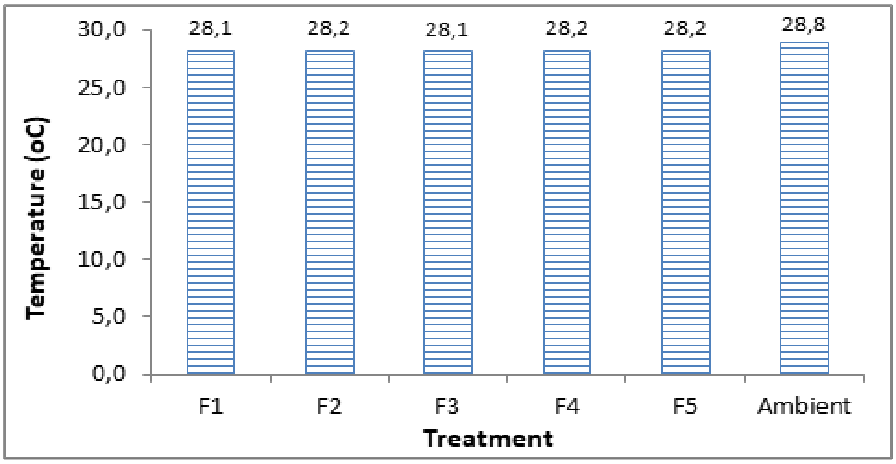

Figure 5 Average temperature for different treatments (Experiment-2)

In experiment-2 with pulped grass, the average pH value was a little higher than that of experiment-1 and almost same for all feeding frequency treatments, namely 6.5 to 6.7 (Figure 3). However, the range between minimum and maximum value was higher, 1.3 (treatment F1) to 1.9 (F2 and F3). Pulping the grass had slightly increased pH by 0.3 as compared to chopped grass. Again, the pH values of all treatments in the experiment-2 were in the range of acceptable condition for anaerobic process.

Digester temperature influences the microorganism performance during decomposition process of organic materials. Specific microorganisms will not stand at a condition where the temperature is too high or to low. For mesophilic bacteria, the ideal temperatures are in the range of 25–40°C. Figure 4 and 5 showed average temperature of digesters in the experiment-1 and experiment-2, respectively. It can be observed that all digesters in both experiments work at average temperatures close to ambient temperature. The average digester temperatures in experiment-1 (with chopped grass) were little bit higher than average ambient temperature (28.0 °C), but average digester temperature in experiment-2 were slightly lower than ambient temperature. Overall biogas reaction is slightly exothermic. With small digesters, however, this effect will immediately achieve balance with the environment. The temperature differences among digesters or between digesters and ambient air might be resulted from environmental conditions due to the position or placement of the digester.

3.2. VS Removal

Table 3 VS removal of Experiment-1 and Experiment-2

Treatment	VSout (% TS)	VSr (%)
Experiment-1		
P1	74.4	33.6
P2	74.9	31.8
P3	74.6	32.9
P4	74.6	32.9
Experiment-2		
F1	75.3	30.3
F2	76.0	27.6
F3	76.1	27.2
F4	74.2	42.6
F5	78.3	17.6

During anaerobic digestion process, substrate decomposition occurs. In this case degradation efficiency is represented by VS removal (VSr). Table 3 presents VS of the substrate and its removal efficiencies (calculated by using Equation 3) for each treatment in the two experiment packages. Compared to real family digester that is also fed semi continuously, this result is quite low. Haryanto reported that household-size digester with cow manure at a working volume of 3.5 m^3 to 6 m^3 produced biogas with removal efficiency range of 43.31% to 58.02% (average 51.32 %) [16]. The low volatile solid removal in this current works is likely resulted from the addition of grass in the substrate. Grass fibers are more difficult to be degraded biologically. Table 3 also reveals that loading rate, urea addition, pulping the grass, and feeding frequency did not improve digestion process efficiency.

3.3. Biogas Yield

Figure 6 and 7 show cumulative biogas yield, respectively for treatments in experiment-1 (with chopped grass) and experiment-2 (with macerated grass). Cumulative biogas yield in experiment-1 for 47 days was 54.14, 42.36, 130.82, and 109.32 L respectively for treatment P1, P2, P3, and P4. We also calculated average daily biogas production (L/d) and specific biogas yield (L/d/m^3 substrate) as presented in Table IV for all treatments in both experiments as well. The table also includs methane content for each treatment.

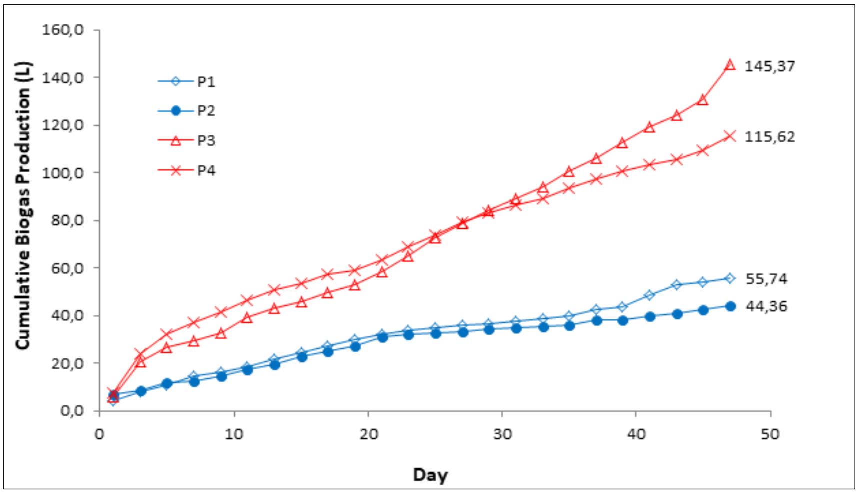

Figure 6 Accumulation of biogas yield using substrate of chopped grass from different pretreatments

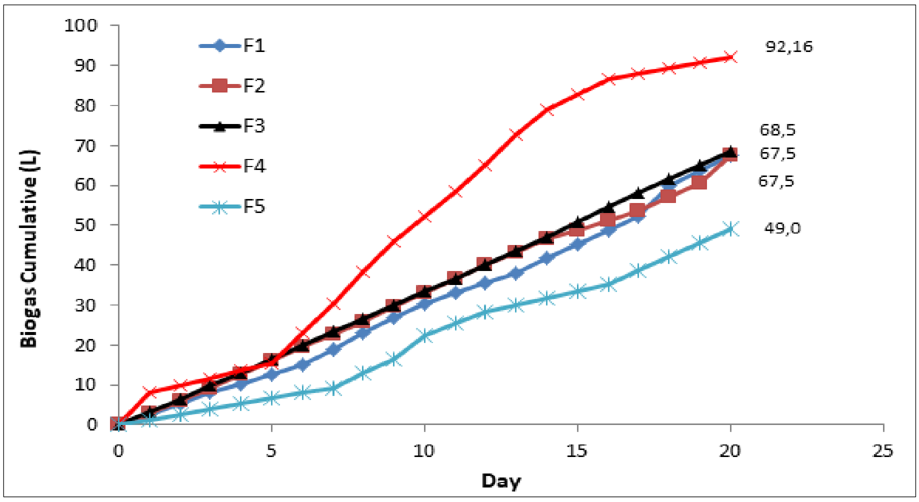

Figure 7 Accumulation of biogas yield using substrate of pulped grass from different pretreatments

We can observe from Figure 6 that both loading rate and urea addition influence the total biogas yield. Increasing loading rate has resulted in lower biogas yield. Doubling the loading rate had reduced total biogas yield from average 1.19 L/d into 0.94 L/d or from 47.44 L/d/m^3 substrate into 37.75 L/d/m^3 substrate. This is in line with the findings of other researchers who concluded as the organic loading rate increased, the degradation of organic matter and biogas yield decreased [15]. On the other side, Figure 6 also presents that urea addition has resulted in positive effect on biogas yield. Budiono [17] reported that urea addition increased biogas formation by 52.47% greater than that of treatment without urea addition. In our experiment, the biogas production increased by almost tripple with the dose of urea addition at 1.25 g/L substrate. The combination treatment of low loading rate and urea addition (P3) resulted in better effect on total biogas yield of 130.82 L or 3.09 L/d in average.

Table 4 Biogas yield and methane content from anaerobic co-digestion of Elephant grass

Treatment	Ave. Yield (L/d)	Specific Yield (L/d/m³)	CH_4 (%)
Experiment-1			
P1	1.19	47.44	10.23
P2	0.94	37.75	8.06
P3	3.09	123.72	17.77
P4	2.46	98.40	22.35
Experiment-2			
F1	3.375	112.50	41.72
F2	3.375	112.50	39.61
F3	3.425	114.17	36.75
F4	4.608	153.60	51.79
F5	2.450	81.67	41.13

We also noted in our experiment that biogas produced from barely chopped grass was low, only about 1.19 L/d in average with specific yield of 47.44 L/d per m^3 substrate (P1) at loading rate of 0.625 L/d. Even though urea addition improved the average yield into 3.09 L/d or 161.8 L/kg VS_r, it was still low. This can be resulted from from the presence of pieces of grass. This meant that chopping the grass is not enough for the optimum biogas process. Another factor could be resulted from TS content of the substrate mixture that is relatively high (10%). At high TS content, the digester experienced overloading that resulted in low biogas yield. This is confirmed by results from experiment-2 using pulped grass and lower TS content (5%) as can be observed in the lower part of Table IV.

By using pulped grass and lower TS content, average biogas yield at loading rate 0.5 L/d was 3.375 L/d, significantly higher as compared to the average biogas produced from chopped grass (1.19 L/d) at comparable loading rate (0.625 L/d). It was also revealed that feeding frequency affects biogas yield with maximum value 4.608 L/d and 153.60 L/d/m^3 substrate or 513 L/kg VS_r occured at feeding frquency of once in four days (F4).

3.4. Methane Yield

All experiments using chopped grass produced biogas with substantially low CH_4 content so that it could not be combusted. Biogas analysis on day-39 revealed that methane content was considerably low, namely 10.23%, 8.06 %, 17.77%, and 22.35%, respectively for P1, P2, P3, and P4. In experiment-1, urea addition has actually increased the methane content. Liu concluded that urea addition of 2% was able to increase cumulative methane yield from giant reed grass ensilage by 18% higher as compared to that of fresh grass [18]. The low methane content in experiment-1 could be resulted from insufficient physical treatment of the grass. According to Rekha and Aniruddha, elephant grass contains complex macromolecules that are difficult to decompose directly by microorganisms [19]. Microorganisms need longer time to degrade the grass, especially at hydrolysis phase. This has caused retardation on methane formation. Hydrolysis is an important step in anaerobic decomposition process that is restrictive for high lignocellulosic substrates like elephant grass. Therefore, pretreatments are required prior to anaerobic digestion process of elephant grass to lessen its structural strength and decrease compositional barriers of lignocellulosic component as well as to improve solubilization of the lignocellulosic biomass and subsequent enhancement in biogas productivity [19]. Putting barely chopped grass had resulted in incomplete process that resulted in low quality biogas. Another reason for low methane content might be the high TS content in the substrate mixture, which was 10%. Liotta reported that specific methane yield decrease from 1648 mL/gVS at substrate TS of 4.5% into 1117 mL/gVS at substrate TS of 12.9% [20].

It was observed, however, that when the experiment was terminated for a week, the biogas could be combusted easily and giving a blue flame. This implied that methane content increase by termination of the loading. Sawanon [6] reported on biogas production using mixture of elephant grass and cow manure at a ratio of 10%:10% (wet basis) and feeding frequency of once (0.625 L) in five days. Therefore, we conducted experiment-2 to solve problems observed during experiment-1, including pulping the grass, reduce TS content to 5% in the substrate mixture, and feeding frequencies from 1 to 5 days.

As presented in Table 4 methane content of biogas yield produced from all treatment using pulped elephant grass was significanly higher than that of biogas resulted from the grass that only being chopped. The table also reveals that feeding frequency of once in one to three days resulted in little effect on biogas production as well as methane level. This finding answers the low yield of biogas and methane resulted in experiment-1 which is caused primarily by high TS content together with insufficient physical treatment of the grass. At feeding frequency once in four days, biogas yield achieved maximum value of 4.608 L/min or average of 153.60 $Ld^{-1}m^{-3}$ substrate with the highest methane content of 51.79%. Feeding frequency, however, should not be extended to once in five days because of detrimental effect on biogas yield.

Table 5 Comparison of biogas and methane yield from co-digestion of Elephant grass

Substrate and condition	Biogas Yield (L/kg VS_r)	Methane Yield (L/kg VS_r)	References
Cowdung + elephant grass (chopped), semi continuous digester, working volume 25 L, TS ratio 75:25, total TS 10.23%, OLR 2.08 kg VS/m^3.d	161.8	28.8	This work
Cowdung + elephant grass (pulped), semi continuous digester, working volume 30 L, TS ratio 75:25, total TS 5%, OLR 1.66 kg VS/m^3.d	513	266	This work
Elephant grass + cowdung (10:10), semi continuous digester, working volume 5 L, TS 3.3%, OLR 0.70 kg VS/m^3.d	--	252	[6]
Elephant grass with inoculum from anaerobic digester, CSTR, working volume 0.97 L, TS 2.5%, working volume 5 L, OLR 0.57 kg VS/m^3.d	529	242	[3]

When we compare our result on biogas yield with other value published in journals, we find that our result was very promising. Table V presents biogas yield we collected from some references. Our result using chopped grass and high TS content (10%) was substantially lower than other works. Different organic loading rate (OLR) could be another reason. Our study used OLR of 2.08 kg VS/m^3.d, while they used only 0.5 kgVS/(m^3.d) [6] and 0.57 kgVS/(m^3.d) [3]. By using pulped grass and lower TS content (5%), however, our result was comparable to other works.

4. Conclusion

From the above discussion we can derive some conclusions that elephant grass is promising as co-substrate for producing biogas through anaerobic digestion using semi continuous digester. Loading rate as well as urea addition influence biogas and methane yield. Urea addition positively improved biogas generation from co-digestion of cow manure and elephant grass using semi continuous digester as well as its methane content. On the other side, increasing loading rate of substrate mixture produced in lower biogas yield. Loading rate of 0.625 L/d in combination with additional urea at a dose of 1.25 g/L substrate generated biogas at 161.8 L/kg VS_r and methane yield of 28.8 L CH_4/kg VS_r. Physical pretreatment by pulping chopped fresh grass affected more significantly on biogas yield and biogas quality (methane content). Co-digestion of pulped elephant grass and cowdung produced the highest biogas yield (average 153.60 L/d/m^3 substrate) and highest methane content (51.79%) at feeding frequency of once in four days.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Author's contributions

All authors contributed to this research. AH designed the overall research setup, while PAC and AJ played key roles in the operational run. UH was responsible for designing the digester. SS, WR, ST, and FKW handled operational monitoring, data analysis, and paper publication. All authors have approved the final version.

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