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Abstract

The goal of this study was to investigate utilization of Kans grass (KG) (*Saccharum spontaneum*) as a co-substrate with dairy cow manure (DCM) under mesophilic conditions (37^{o}C) on the digester biogas performance. This study used four continuous stirred tank reactors (CSTR). The treatments were the proportion of KG in the mixed substrate in term of VS: control digester (KG 0%), KG 10.18%, KG 18.60%, and KG 25.67%, respectively. The DCM was made by mixing dairy cow faeces and water in a ratio of 1/1.75. In addition, the methane production of digested slurries from each CSTR digester were also evaluated using batch digesters. The anaerobe digestion process was carried out for 66 d that correspond to 3 times hydraulic retention time. The results showed that the use of KG as a co-substrate with DCM was significantly (p<0.05) able to increase the methane production per active digester volume (CH_4/L active digester volume) and methane volume per substrate weight (L CH_4/kg substrate), but it had no significant effect (p>0.05) on the methane yield per substrate volatile solid (VS) (L CH_4/kg VS). All digesters resulted in stable methane production, low ammonia concentration and total volatile fatty acid concentrations in which they were not significantly affected (p>0.05) by the various proportion of KG in the final substrate in term of VS. The digested slurries pH in this study was in a normal level range so the anaerobic digestion (AD) process worked optimally. Therefore, KG can be used as a co-substrate to increase methane production from the AD process with DCM as the main substrate.

Keywords: *biogas, co-substrate, post digestion, mesophilic, slurry*

Introduction

The population of dairy cows that continues to increase causes the amount of dairy cow waste to also increase in Indonesia. Every dairy cow that weighs 635 kg can produce 23 tons of wet faeces annually (Rajagopal et al 2019; Arikan et al 2015). The dairy cow faeces are rich in nutrients such as organic matter, nitrogen (N), phosphorus (P) and potassium (K), so they have the potential to be used as a biofertilizer for plants and can be processed anaerobically for biogas production (Zhuang et al 2020). In general, the treatment of the faeces can be conducted under aerobic conditions through a composting process or under anaerobic conditions in digesters to produce biogas.

The biogas produced through the anaerobic digestion (AD) process can be used as a renewable energy source for personal or community needs and can reduce greenhouse gas emissions, odours and water contamination (Yen et al 2017; Li et al 2014; Masse et al 2011). AD with a substrate of livestock manure alone results in sub-optimal biogas production. The pig manure and broilers, for instance, have a high total nitrogen content so the AD of those wastes results in high concentrations of ammonia in the digester, which can result in non-optimal biogas production (Sutaryo et al 2014). Meanwhile, dairy cow manure (DCM) has a fairly low total solid (TS) concentration, usually 7-9%, and can only produce methane gas of 10-20 m^{3} CH_4/ton (Angelidaki and Ellegaard, 2003). Hence, it is necessary to add organic materials to increase nutrient and TS concentration in the manure so that methane production increases. Biomass that can be used as sources of organic matters as raw materials for biogas production must have low lignification characteristics and high content of easily degraded components such as non-structural carbohydrates, soluble carbohydrates, and soluble cell components so the AD of them can result in high methane production (Kandel et al 2013).

Kans grass (KG) (*Saccharum spontaneum*) (Photo 1) is the right choice as a feedstock for ethanol production and biogas production since the KG can grow throughout the year regardless of the season. The KG is a grass species with clumps of roots and can grow more than 3 meters so it is considered a weed because it can cover agricultural land quickly but the KG also has benefits for ecological restoration and stabilization of various wastes (Mukherjee et al 2017). Cell walls in the KG stems contain high carbohydrates of 67.9%, so they can be used as a substrate for ethanol or biogas production (Komolwanich et al 2014). The purpose of this study was to examine the effect of the KG as a co-substrate in the AD of DCM on methane gas production, pH value, volatile fatty acids (VFAs) concentration, ammonia concentration, and volatile solid (VS) reduction using digesters with the type of continuous stirred tank reactor (CSTR), and then evaluate the methane production from the digested slurries coming out from the CSTR digesters using anaerobic batch digesters.

Materials and Methods

This study used four CSTR (Photo 2) with a total capacity of 7 litres. The stirring speed of the CSTR digesters was 36 revolution per minute. The digester was placed in an incubator under a temperature of 37^{o}C and operated with an active digester volume of 5.25 litres. The CSTR digesters were made of stainless steel to avoid rust. The treatments were proportion of KG in the mixed substrate in term of VS: control digester (KG 0%), KG 10.18%, KG 18.60%, and KG 25.67%, respectively. The experiment was carried out with the following steps:

1) Adaptation period, as much as 5.25 kg of inoculant was put into the digesters. Furthermore, on the second day, as much as 238.6 g of basal substrates was fed into the digesters, in which previously the same amount of slurry was removed from the bottom of the digesters. The basal (DCM) substrate was made by mixing dairy cow faeces and water in a ratio of 1/1.75. The mixing of dairy cow faeces with water aimed to achieve a total solid (TS) in the basal substrate of about 6% so the substrate was easier to be fed into the digester. In Indonesia the livestock scale mainly is small-scale farms, which the farmers design the floor of the cowshed in the animal pens tilted towards the gutter, so that feces is separated from urine and spilled drinking water. Thus, it will be more efficient for farmers during sanitation the cowshed floor (Sutaryo et al 2021). Therefore, in this previous

experiment the feces were diluted with tap water with no urine addition. The DCM used came from lactating cows in the cowshed in the Faculty of Animal and Agricultural Sciences, Diponegoro University. The adaptation process was carried out for 22 d.

2) Collection data period, the digesters were fed KG and DCM at various KG proportion (Table 1). This period was run for 66 d.

Photo 1. Kans grass used in this experiment

Photo 2. Biodigesters configuration

The characteristics of the substrates in all digesters can be seen in Table 1. Overall, this research was carried out for 66 d or 3 times hydraulic retention time (HRT). Alepu et al (2016) stated that residence time is a determining factor for the amount of substrate to be fed into the digester. In general, the anaerobic digester is operated with HRT for 15-30 d at a mesophilic temperature of 30-35°C because a short residence time can cause a decrease in pH value.

Inoculum and KG

The inoculum was obtained from an active biogas digester in the Faculty of Animal and Agricultural Sciences, Diponegoro University. The characteristics of inoculum were TS: 4.52%, VS: 3.96% and pH value 7.57, respectively. The KG (*Saccharum spontaneum*) was obtained in Tembalang District, Semarang, Central Java Province, Indonesia. It was cutting manually and followed by sun drying for about 2-3 d. Since this experiment was used laboratory biodigester scale, in order to facilitate during feeding to the biodigester the dried KG was ground using hammer mill with 1 mm screen size. The nutrient content of the KG can be seen in Table 2.

Table 1. Substrate characteristics

Treatments	Total solid (%)	Volatile solid (%)	Protein (%)	C/N ratio	VS proportion of KG (%)
KG 0%	6.02 ± 0,11	5.34 ± 0.14	0.86 ± 0.14	21.56	0
KG 10.18%	7.13 ± 0,13	6.40 ± 0.14	1.00 ± 0.02	22.22	10.18
KG 18.60%	7.99 ± 0,54	7.21 ± 0.49	1.10 ± 0.05	22.76	18.60
KG 25.67%	8.85 ± 0,31	7.95 ± 0.33	1.18 ± 0.12	23.39	25.67

Table 2. Chemical composition of KG

Nutrient	Leaf of KG	Stem of KG	Whole plant
		(%)	
Total solid	87.19	91.10	91.32
Volatile solid	75.89	81.11	79.65
Ash	11.31	9.99	11.67
Crude protein	4.65	6.78	3.37
Crude fat	1.54	1.79	0.80
Crude fibre	32.99	31.80	34.49
Nitrogen free extract	36.70	40.74	40.99
Acid detergent fibre	59.84	50.49	55.91
Neutral detergent fibre	90.93	78.90	79.90
Lignin	32,99	26.25	42.04
Hemicellulose	31.09	28.41	23.99
Cellulose	27.76	24.57	13.94

Post digestion test

Utilization of KG can increase nutrient and TS concentration in mixed substrate. Since the HRT is limited therefore not all those nutrients and TS can be degraded by anaerobe microorganism and allow it to remain in the digested slurry. In this recent study, methane production of digested slurries coming out from the CSTR digesters was also evaluated. The slurry of each CSTR digester was collected on day 40-45 or after the main digestion process run for 2 times of HRT. The 200 g of the digested slurry was fed into a batch digester with a capacity of 500 ml. No inoculum was added in the post digestion test. There were four replications in each treatment. The digesters were closed using a rubber stopper and locked using an aluminium crimp, then flushed with nitrogen gas for 2 minutes to remove the oxygen gas in the headspace of the digesters. The digesters were put in an incubator at 37°C for 30 d. Biogas production was measured periodically by passing the biogas in a 4% NaOH solution. The methane gas was then stored in a Tedlar gas bag and the volume was measured using the water displacement method (Sutaryo et al 2020).

Analytical method

The biogas production from each CSTR digester was passed through a 500 ml bottle containing a 4% NaOH solution, then the methane gas was collected using a 5 L Tedlar gas bag (Hedetech-Dupont, China). The storage process used a 5 mm diameter Teflon hose. Methane gas was measured every day using the water displacement method described by Sutaryo et al (2020). Measurement of the pH of substrate and slurry in the digester was conducted using a pH meter (OHAUS®ST 300). TS was analysed by drying the sample using an oven at 105°C for 7 hours and followed by drying the sample at 550°C for 6 hours to determine the ash content. VS was a difference in mass between the TS content and the ash content (APHA, 1995). Ammonia concentration was analysed using the standard method (APHA, 1995). Total volatile fatty acids (VFAs) were measured using the steam distillation method. Total organic carbon can be estimated by dividing VS by 1.8 according to Haug (1993). The C/N ratio was obtained from a ratio of the total organic carbon content to the total nitrogen content (Syaichurrozi, 2018). Acid detergent fibre (ADF), neutral detergent fibre (NDF), and lignin content of sample were analysed using protocol developed by Van Soest et al (1991). The hemicellulose content of PS was measured by NDF minus ADF, while the concentration of cellulose in sample was evaluated as ADF minus acid detergent lignin (ADL), and lignin content was assumed to be equal to ADL (Møller et al 2014). The data were statistically analysed using one-way analysis of variance (ANOVA) at a significance level of 5%. If there was a significant effect (p< 0.05), the Duncan's multiple range test was then conducted (Gomez and Gomez, 2007).

Results and Discussion

Methane production in units of methane volume per active digester volume (L CH_4/L digester) and methane volume per substrate weight (L CH_4/kg substrate) is shown in Table 3, while methane production as function of proportion of KG in the mixed substrate in term of VS are presented in Figure 1, 2, and 3. There was strong positive correlation (p<0.05) between the proportion of KG in the mixed substrate and methane production both in term of L/L digester volume and L/Kg substrate, while in the unit of L/Kg VS the correlation was also strong positive correlation (p>0.05). Based on the statistical analysis, it was found that the presence of the KG as a co-substrate for DCM significantly increased methane production in a unit of L CH_4/L digester (p<0.05) as well as methane production in a unit of L CH_4/kg substrate. However, it had no significant effect on methane production in a unit of methane volume per substrate VS (L CH_4/kg VS) (p>0.05) (Table 3).

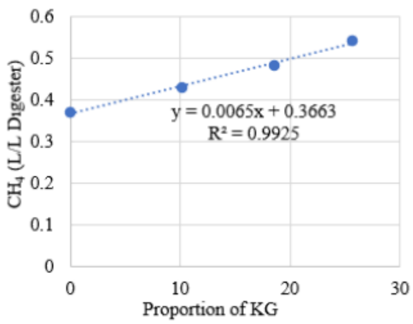

Figure 1. Methane yield (L/L digester)

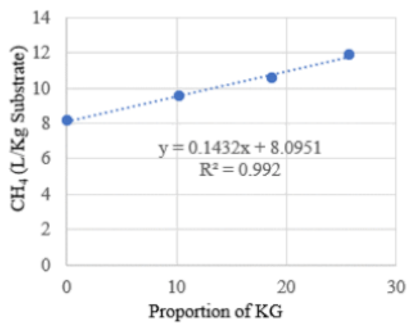

Figure 2. Methane yield (L/Kg substrate)

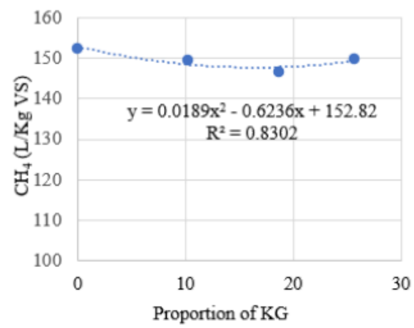

Figure 3. Methane yield (L/Kg VS)

Methane Production

Co-digestion of KG and DCM increased methane production in units of L CH_4/L digester and L CH_4/kg substrate significantly (p<0.05). It is because the use of KG as a co-substrate for the DCM increased the nutrient content in the substrates of the treatment digester (Table 1). The appropriate nutrient content can increase the activity of microorganisms for methane production. Kandel et al (2013) stated that the composition of the organic matter in substrates can affect methane production. Komolwanich et al (2014) stated that the carbohydrate content in the stem cell walls of the KG was 67.9%. The insignificant effect on methane production in a unit of L CH_4/kg VS was correlated with an increase in the organic matter content of the substrates of the treatment digester, so it led to an increase in the divisor in the calculation of methane production in a unit of L CH_4/kg VS. On the other hand, the digestibility of organic matter in the substrate containing the KG was not significantly different from the digestibility of the organic matter in the substrate without the KG (manure alone) (Table 3). This phenomenon was the same as in the study of Sutaryo et al (2012), where in the study, acidified solid fraction of DCM was used as a co-substrate for DCM and it was found that methane production in a unit of L CH_4/L digester increased significantly due to a higher organic concentration in the substrate, but that in a unit of L CH_4/kg VS was not increased. Sutaryo et al (2021) stated that easily degraded substrates can increase methane production because they can stimulate the ability of microorganisms to digest organic matter better and convert it into methane. Therefore, the methane production in the treatment digesters was higher than that in the control digester.

Variables in the digester slurry

There was no significant effect (p>0.05) of utilization the KG as a co-substrate for DCM on total VFAs concentration, total ammonia nitrogen (TAN) concentration, VS reduction and pH of the slurry from digesters. The VFAs concentration was not significantly affected by the various ratios of KG and DCM. It indicated that in general there was no interference for methanogenic bacteria in converting acetic acid into methane. Bhui et al (2018) stated that methanogenic bacteria activity requires acetic acid as a precursor for producing methane gas, and in the acetogenesis process, acetogenic bacteria convert organic acids (VFAs) into acetic acid. Vongvichiankul et al (2017) also reported that the nutrient balance in the substrate affects the liquid pH and VFAs concentration in which these conditions will affect the activity of acidogenic and methanogenic microorganisms in the formation of methane. Wahid et al (2018) stated that VFAs play a very important role in maintaining stability because they can affect pH, alkalinity and methanogenic bacteria activity.

Table 3. Methane yield, total VFA, TAN concentration, VS reduction and pH digester

Treatments	Methane Production			VS reduction %	pH
	L CH_4/L digester	L/kg substrate	ml CH_4/g VS		
KG 0%	0.37 ± 0.02[a]	8.14 ± 0.45[a]	152.53 ± 0.09	27.52 ± 7.75	7.04 ± 0.18
KG 10.18%	0.43 ± 0.02[bc]	9.57 ± 0.33[bc]	149.50 ± 0.05	27.97 ± 7.21	7.02 ± 0.08
KG 18.60%	0.48 ± 0.01[c]	10.56 ± 0.12[c]	146.50 ± 0.02	30.56 ± 8.57	7.04 ± 0.15
KG 25.67%	0.54 ± 0.04[d]	11.91 ± 0.97[d]	149.80 ± 0.12	37.17 ± 9.38	7.12 ± 0.20

[abcd]*Means in the same row without common letter are different at $p<0.05$*

Ammonia is one of the nutrients needed by microorganisms but its availability should not be excessive. Yenigün and Demirel (2013) stated that the activity of microorganisms can be inhibited if the ammonia concentration is in the range of 1700-1800 mg/L. Microorganism activity in this study was not disturbed by the ammonia because the ammonia concentration was less than the inhibition level so it was still ideal for methane production. Hao et al (2017) stated that a high total ammonia concentration can inhibit the methanogenesis process if the ammonia concentration is up to tens of thousands mg/L. Wahid et al (2018) also stated that high total ammonia results in low methane production because the total VFAs will increase and the digestion process of organic matter by microorganisms will be hampered. The VS reduction value in this study was comparable to the study of Sutaryo et al (2012), where in the study, the digestibility of organic matter in digesters having different TS concentrations with a 14 d HRT and worked at 51°C was around 27-35%. Rajput et al (2018) stated that there is a strong relationship between biogas yield and organic matter digestibility, in which the higher the methane gas is produced, the more organic substrates will be digested by microorganisms. The pH conditions in the digester were not affected statistically by the various ratios of KG and DCM. Mao et al (2015) stated that the ideal pH condition for methane production is 6.8-7.4. The pH conditions in the digester can be affected by the VFAs produced during the methane production process. This is in line with the study of Sutaryo et al (2020) which stated that the concentration of VFAs is negatively correlated with the pH conditions in the digester in which the higher the concentration of VFAs in the slurry, the lower the pH value will be.

Post digestion test

The use of KG as a co-substrate for DCM increased the organic matter contents in the mixed substrates. Therefore, this allowed the organic matter to remain in the slurry resulting from the CSTR digesters. The resulting slurry of each CSTR digester was then fermented anaerobically using batch digesters. The methane gas produced was quite high. Table 4 shows that the digested slurry resulting from the CSTR digesters still has the potential to produce methane. Based on the results of statistical analysis, the utilization of KG significantly increased methane gas production in a unit of ml CH_4/g substrate ($p<0.05$) in the post digestion test. The high methane yield resulting from the digestion of the digested slurry was influenced by the period of the HRT in the CSTR digesters and the organic matter content in the substrates used. The study of Ruile et al (2015) reported that 21 anaerobic digesters containing cow dung as the main ingredient and plants (maize silage, grass and grain) produced 24-126 ml CH_4/g VS. The yield of residual methane gas resulting from digestion with 50 d HRT was higher than that with 100 day and 150 d HRT. Thygesen et al (2014) also reported that the digested slurry coming out from seven mesophilic digesters containing animal manure and food waste with low HRT (16-25 days) can be anaerobically digested to produce 156-240 ml CH_4/g VS. According to Uludag and Demirer (2022), the advantages obtained from slurry processing are an increase in methane recovery per ton of raw materials used in the digester, reduction in slurry management costs, reduction in soil, water and air contamination, and reduction in greenhouse gas emissions.

Table 4. Residual methane yield

Treatments	Methane production	
	L/Kg substrate	L/Kg VS
KG 0%	5.41 ± 0.69[a]	143.11 ± 18.36
KG 10.18%	6.03 ± 0.67[b]	138.89 ± 15.42
KG 18.60%	6.86 ± 0.24[c]	150.36 ± 5.24
KG 25.67%	7.39 ± 0.19[d]	148.85 ± 3.80

[abcd]*Means in the same row without common letter are different at $p<0.05$*

Conclusion

- This study concluded that AD of mixed substrates of KG and DCM resulted in a higher methane production than that with DCM as a mono-substrate. Stable methane production, low total VFAs and ammonia concentrations and optimal pH levels during the digestion process occurred in all CSTR digesters. Therefore, the KG was suitable as a co-substrate for the DCM to increase methane production.

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