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The Effect of Using Acidified *Imperata cylindrica* as a Co-substrate with Dairy Cow Manure on the Digesters Performance

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ABSTRACT

The objective of the current study was to examine the effect of the utilization of acidified cogon grass (CG) (*Imperata cylindrica*) as a co-substrate for the anaerobic digestion of dairy cow manure (DCM). Four continuous stirred-tank reactor (CSTR) digesters with various substrate compositions, namely, T1 (100% DCM), T2 (95% DCM and 5% CG), T3 (95% DCM and 5% acidified CG using 4% H_2SO_4 solution), and T4 (95% DCM and 5% acidified CG using Wuluh star fruit (*Averrhoa bilimbi* L) filtrate), were operated. This study was conducted for 66 d or 3 hydraulic retention times (HRTs). Also, it evaluated the methane production of the digested slurry of each CSTR digester (batch-type) with five replications in each treatment. The results indicated that the methane production in T2, T3, and T4 in the unit of L/kg substrate increased by 35.52%, 41.95%, and 45.44%, respectively, compared with that in T1. Furthermore, the productions in T3 and T4 increased by 4.35% and 7.25%, respectively, compared with that in T2. The production from the slurries of CSTR digesters in units of L/kg substrate and L/kg volatile solid (VS) showed significantly different results ($p<0.05$). A neutral pH value was maintained so the anaerobic digestion process could occur optimally. The total ammonia nitrogen concentrations were low, and the volatile fatty acid concentrations were not significantly different ($p>0.05$). Therefore, with or without acidification, CG can be used as a co-substrate with DCM to enhance methane production.

***Keywords:** acidification; biogas; cogon grass; manure; pre-treatment*

INTRODUCTION

Dairy cow waste not properly managed can negatively impact the environment due to its high organic matter content. Dairy cow manure (DCM) can be treated using anaerobic digesters to generate renewable energy, biogas, to reduce greenhouse gas emissions (Browne *et al.,* 2015). DCM contains organic matter and nutrients that can be anaerobically processed to produce biogas. However, biogas production from DCM is not optimal; thus, other materials to be used as co-substrates are required (Tufaner & Avsar, 2016).

However, the organic matter used as a co-substrate with DCM should not compete with human food and animal feed; thus, it should not be cultivated on productive land where food and animal feed are produced (Jury *et al.,* 2022). Among the sources of organic matter, cogon grass (CG) (*Imperata cylindrica*) meets this criterion as this plant is a weed, can grow well on marginal land, and has low palatability and digestibility for a ruminant. CG is widespread in tropical and subtropical regions in Asia and has the potential to increase biogas production from DCM owing to its nutrient contents: 2.97% ash, 0.8% protein, 17.89% lignin, 44.49% cellulose, and 25.13% hemicellulose (Hidayat *et al.,* 2018). However, the main obstacle in the utilization of CG as a co-substrate is its low biodegradability owing to its high crude fiber content; thus, pre-treatment is necessary to break down the lignocellulosic complex bonds (McVoitte & Clark, 2019).

Chemical pre-treatment is effective in increasing CG biodegradability as it can break down the lignocellulosic complex bonds into relatively easy decompose components to allow anaerobic bacteria to access cellulose more easily (Sarto *et al.,* 2019). During anaerobic digestion (AD), acidity is one of the important factors in the methanogenic process. In acetogenesis, acidogenic bacteria convert sugars and amino acids produced in the hydrolysis process into carbon dioxide, hydrogen, ammonia, and organic acids. Then, bacteria convert these resulting organic acids into acetic acids, along with ammonia, hydrogen, and carbon dioxide.

A previous Syaichurrozi *et al*. (2019) study demonstrated that soaking in 4% sulfuric acid (H_2SO_4) pre-treatment can effectively increase *Salvinia molesta* biogas production. Sarto *et al*. (2019) reported that the best level of H_2SO_4 for pre-treatment to improve the methane production of water hyacinth is 5%, with a residence time of 60 min. Goshadrou (2019) evaluated the chemical pre-treatment of CG using 2% sodium hydroxide for bioethanol production and found that enzymatic hydrolysis can increase from 24.8% to 90.8%. However, to the best of our knowledge, there has been a lack of information on the application of acid pre-treatment using H_2SO_4 and Wuluh star fruit (*Averrhoa bilimbi* L.) (AB) filtrate on CG and subsequently using them in anaerobic co-digestion with DCM to produce biogas. Therefore, the current study used an inorganic acid (4% H_2SO_4 solution) and an organic acid AB filtrate in the pre-treatment of CG. The pre-treatment of CG is expected to increase biogas production from the mixed substrate of DCM and CG.

AB belongs to the Oxalidaceae family. It is a tropical tree with many bioactive compounds, such as amino acids, citric acids, and phenolics. Thus, its filtrate is expected to break down the lignocellulosic complex bonds in CG and increase the availability of cellulose and hemicellulose for microorganisms during AD (Muhammad *et al*., 2014). This study aimed to examine the effect of acid pre-treatment of CG using an organic acid (AB filtrate) and an inorganic acid (H_2SO_4) on the performance of biogas digesters with a mixed substrate of DCM and CG.

MATERIALS AND METHODS

Experimental Setup

This study used four continuous stirred-tank reactor (CSTR) digesters with a total volume of 7000 mL and made of stainless steel with an active volume of 5250 mL (Figure 1) and batch-type digesters with a capacity of 500 mL were used. The temperature of each digester was kept constant at 35 °C using an incubator. The basal substrate (DCM) was prepared by mixing dairy cow dung and water at a ratio of 1:1.75. The cow dung was collected from Friesian Holstein cows during the lactation period in the stables of the Faculty of Animal and Agricultural Sciences, University of Diponegoro. The adaptation phase lasted 3 weeks, starting with filling the digester with a 5250 mL starter. The input substrate was calculated as 5250 mL divided by 22 d, equivalent to 1 hydraulic retention time (HRT); thus, the calculated input substrate per day was 238.6 g. The four CSTR digesters were operated containing various mixed substrates: T1 (100% DCM), T2 (95% DCM and 5% CG), T3 (95% DCM and 5% acidified CG using 4% H_2SO_4 solution), and T4 (95% DCM and 5% acidified CG using AB filtrate).

Inoculum and Cogon Grass

In this study, the inoculant was a slurry collected from an active digester at the Faculty of Animal and Agricultural Sciences, University of Diponegoro, with a total solid (TS) content of 4.02%, volatile solid (VS) content of 3.10%, and a pH of 7.41. CG was obtained from the Meteseh area, Semarang, Central Java Province, Indonesia. The CG was cut and dried under direct sunlight. The dried CG was then crushed using a grinder to easily put it into the digester, considering that the digesters used were on a laboratory scale. The CG meal was then stored in a plastic clip container, put in a jar and closed tightly, and then kept in a dry place without direct exposure to sunlight. The nutrient contents of CG and acidified CG meal are presented in Table 1 and those of the mixed substrates for CSTR digesters in Table 2.

Figure 1. Continuously stirred tank reactor configuration. The biogas digester was placed in the incubator, and the biogas flowed into a bottle filled with NaOH solution and the methane from the bottle was collected using a tedlar gas bag (Oje-Adetule *et al*., 2023).

Table 1. Nutrient contents of non-acidified and acidified cogon grass

Nutrient contents	CG (%)	Acidified CG using 4% H_2SO_4 (%)	Acidified CG using AB filtrate (%)
Total solid (TS)	84.40	88.60	88.95
Volatile solid (VS)	80.42	84.70	84.97
Proteins	2.13	2.20	2.31
Crude fat	9.34	9.34	9.47
Crude fiber	48.29	44.50	47.45
Neutral detergent fiber (NDF)	90.32	86.23	84.86
Acid detergent fiber (ADF)	56.07	54.56	54.36
Lignin	26.77	24.62	24.33
Hemicellulose	34.25	31.67	30.50
Cellulose	28.86	29.50	28.54

Note: CG= cogon grass, AB= *Averrhoa bilimbi* L.

Table 2. Nutrient content of mixed substrate in each continuous stirred-tank reactor with various treatments

Treatments	Nutrients				
	TS (%)	VS (%)	Crude proteins (%)	VS proportions of CG in the mixed substrates (%)	C/N
T1	7.11 ± 1.16	6.12 ± 1.24	1.07 ± 0.09	0.00	19.86
T2	8.63 ± 0.42	7.78 ± 0.32	1.22 ± 0.07	42.15	23.26
T3	8.56 ± 1.41	7.63 ± 1.17	1.23 ± 0.11	41.94	21.61
T4	8.50 ± 1.10	7.48 ± 0.94	1.25 ± 0.14	40.89	20.89

Note: Data are presented as means ± SD. TS= total solid (TS), VS= volatile solid; C/N= carbon/nitrogen. T1= 100% digestion of dairy cow manure (DCM), T2= 95% DCM and 5% cogon grass (CG, *Imperata cylindrica*), T3= 95% DCM and 5% acidified CG using 4% H2SO4 solution, T4= 95% DCM and 5% acidified CG using Wuluh star fruit (*Averrhoa bilimbi* L) filtrate.

AB Filtrate

Wuluh star fruits were collected from around the University of Diponegoro to obtain AB filtrate. The fruits were washed thoroughly with running water and then drained and blended (Miyako, PT. Kencana Gemilang, Banten-Indonesia) at a medium speed for 5 min. The resulting juice was then filtered using a chiffon cloth to obtain the filtrate. In the acid pre-treatment, the CG meal was mixed with (1) 4% H_2SO_4 solution or (2) AB filtrate at a ratio of 1:7 until all parts of the CG were well soaked. The CG was soaked for 3 × 24 h (Syaichurrozi *et al.*, 2019) and then washed with running water for 5 min to clean the acidic solution from the CG. After being washed, the CG was then sun-dried.

Evaluation of the Methane Production of Digested Slurries

The methane production of slurries collected from the CSTR digesters (T1, T2, T3, and T4) on days 40–44 was evaluated. As much as 200 g of the slurries was put into the batch-type digesters with a volume of 500 mL. The evaluation was conducted with five replications for each slurry. The digesters were closed using a rubber stopper and locked using an aluminium crimp; then, flushing was carried out using nitrogen gas for 2 min to release the oxygen gas from the digesters. The digesters were placed in an incubator at 35 °C for 30 d. The methane gas volume was measured by first periodically passing biogas through a 4% NaOH solution. Then, the methane gas was collected in a Tedlar bag, and the methane volume was measured using the water displacement method (Sutaryo *et al.*, 2020).

Analysis Methods

The methane volume was measured by first passing biogas from the digesters through a bottle (500 mL) containing 4% NaOH (Merck®, cat no: 1064981000) solution. Then, methane gas was collected in a Tedlar bag (Hedetech-Dupont, China) with a capacity of 5 L for the CSTR digesters and 1 L for the batch-type digesters. A Teflon hose with a 5 mm diameter was used in the storage process (Figure 1). The methane volume was measured daily for the CSTR digesters and periodically for the batch-type digesters. Analysis of total ammonia nitrogen (TAN) was conducted *via* spectrophotometry (HACH DR 3900) with a 10 mL nitrogen-ammonia reagent set (Catalog No. 2668000). Volatile fatty acid (VFAs) analysis was conducted *via* gas chromatography (GC BUKER 436). The pH values of the substrate and slurry were measured using a digital pH meter (OHAUS®ST 300). TS was measured by heating the sample in an oven at 105 °C for 6 h and heating in a furnace at 550 °C for 6 h to determine the ash content (APHA, 1995). VS was determined by subtracting the ash content from TS. Analyses of the neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were conducted according to the method described by Van Soest *et al*. (1991). Cellulose was calculated by subtracting ADL from ADF, whereas hemicellulose was calculated by subtracting ADF from NDF; ADL was assumed to be lignin (Møller *et al.,* 2014). Protein content was determined using the Kjeldahl method. The carbon/nitrogen (C/N) ratio was determined from total organic carbon per total nitrogen. The total organic carbon was determined by dividing the organic matter (VS) by 1.8 (Haug, 1993), and then the result was divided by the total N in the substrate to obtain the C/N ratio (Syaichurrozi, 2018). Experimental data were statistically analyzed using one-way analysis of variance with a significance level of 5%. If a significant treatment effect ($p<0.05$) was observed, Duncan's multiple range test was conducted.

RESULTS

Methane Production of Continuous Experiment

The trend of methane production in this experiment is presented in Figure 2. The mean methane yields in terms of L/kg substrate, L/L digester volume, and L/kg VS were 11.18, 15.15, 15.87, and 16.26; 0.51, 0.69, 0.72, and 0.74; and 182.64, 194.69, 208.01, and 217.41 for T1, T2, T3, and T4, respectively. The utilization of CG with or without acidification can significantly increase methane production ($p<0.05$) compared with the control (100% DCM) (Table 3).

Variables in the Liquid Phase

In the slurries of T1, T2, T3, and T4 (Table 3), the VFA concentrations were 4.99 mM, 5.03 mM, 3.41 mM, and 4.47 mM; the TAN concentrations were 164.00 mg/L, 70.00 mg/L, 71.44 mg/L, and 72.44 mg/L; the

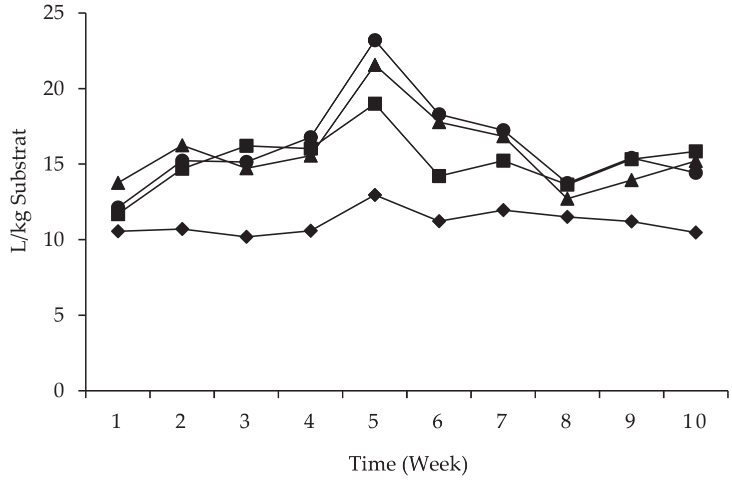

Figure 2. Trend of methane production of continuous bio-digester for three hydraulic retention times (♦: T1, ■: T2, ▲: T3, ●: T4). T1= 100% digestion of dairy cow manure (DCM), T2= 95% DCM and 5% cogon grass (CG, *Imperata cylindrica*), T3= 95% DCM and 5% acidified CG using 4% H_2SO_4 solution, T4= 95% DCM and 5% acidified CG using Wuluh star fruit (*Averrhoa bilimbi* L) filtrate.

Table 3. Production of methane gas, VFA concentrations, TAN concentrations, VS reduction, and pH value of digested slurry from each continuous bio-digester with various treatments

Treatments	Methane production			VFA (mM)	TAN (mg/L)	VS reduction (%)	pH
	L/kg substrate	L/L digester	L/kg VS				
T1	11.18 ± 1.52[a]	0.51 ± 0.07[a]	182.64 ± 24.86[a]	4.99 ± 0.97	164.00 ± 54.58[b]	35.05 ± 3.10	7.29 ± 0.22
T2	15.15 ± 3.52[b]	0.69 ± 0.16[b]	194.69 ± 45.19[ab]	5.30 ± 2.18	70.00 ± 16.64[a]	35.07 ± 2.45	7.29 ± 0.18
T3	15.87 ± 3.87[b]	0.72 ± 0.18[b]	208.01 ± 50.66[bc]	3.41 ± 1.28	71.44 ± 29.07[a]	34.81 ± 3.11	7.15 ± 0.10
T4	16.26 ± 4.24[b]	0.74 ± 0.19[b]	217.41 ± 56.74[c]	4.47 ± 2.01	72.44 ± 14.10[a]	34.69 ± 2.95	7.20 ± 0.11

Note: Means in the same column with different superscripts differ significantly ($p<0.05$). VFA= volatile fatty acid; TAN= total ammonia nitrogen; VS= volatile solid. T1= 100% digestion of dairy cow manure (DCM), T2= 95% DCM and 5% cogon grass (CG, *Imperata cylindrica*), T3= 95% DCM and 5% acidified CG using 4% H_2SO_4 solution, T4= 95% DCM and 5% acidified CG using Wuluh star fruit (*Averrhoa bilimbi* L) filtrate.

VS reduction rates were 35.05%, 35.07%, 34.81%, and 34.69%; and the pH values were 7.29, 7.29, 7.15, and 7.20, respectively. Statistical analysis revealed that the presence of CG with or without acidification had no significant effect ($p>0.05$) on the VFA concentrations, VS reduction, and slurry pH values but had a significant effect ($p<0.05$) on the TAN concentrations compared with the control (100% DCM).

Methane Production of Batch Experiment

The methane yields of the digested slurry in the units of L/kg substrate and L/kg VS were 5.25, 8.15, 8.42, and 7.78 and 141.72, 163.76, 196.46, and 171.91 for T1, T2, T3, and T4, respectively. The statistical analysis revealed that there was a significant effect on the methane production of slurry in the units of L/kg substrate and L/kg VS ($p<0.05$) (Table 4). The utilization of CG, with or without acidification, as a co-substrate with DCM can increase the organic matter content in the mixed substrate. Therefore, the slurry from the CSTR digester still contains organic matter that could be digested to produce methane gas again. The strategy to gain the residual methane of digested slurry is commonly known as post-digestion of digested slurry.

Table 4. Methane production of digested slurry from each treatment using batch digester

Treatments	Methane production	
	L/kg substrate	L/kg VS
T1	5.25 ± 0.35[a]	141.72 ± 9.35[a]
T2	8.15 ± 0.09[b]	163.76 ± 1.74[b]
T3	8.42 ± 0.61[b]	196.46 ± 14.26[c]
T4	7.78 ± 0.13[b]	171.91 ± 2.75[b]

Note: Means in the same column with different superscripts differ significantly ($p<0.05$). VS= volatile solid. T1= 100% digestion of dairy cow manure (DCM), T2= 95% DCM and 5% cogon grass (CG, *Imperata cylindrica*), T3= 95% DCM and 5% acidified CG using 4% H_2SO_4 solution, T4= 95% DCM and 5% acidified CG using Wuluh star fruit (*Averrhoa bilimbi* L) filtrate.

DISCUSSION

Methane Production

The results indicated (Table 3) that methane yield in the unit of L/kg VS, only acid pre-treatment using AB filtrate (T4), can significantly increase methane production ($p<0.05$) than that in non-acidification (T2). AB contains many bioactive compounds, such as amino acids, citric acids, and phenolics, rendering its filtrate capable of breaking down the lignocellulosic complex bonds and increasing the availability of cellulose and hemicellulose for microorganisms (Muhammad *et al.*, 2014). On the other hand, in the other two units, the acid pre-treatment (using 4% H_2SO_4 and AB filtrate) was not significantly different (p>0.05) from the non-acidification. This was probably because the use of crushed CG caused the chemical pre-treatment to exert no significant effect on the increase in the digestibility of the organic matter of the substrate (Table 3). According to Zheng *et al*. (2014), reducing the size of the substrate was an effective physical pre-treatment method to increase methane production.

The methane production in T2, T3, and T4 in the unit of L/kg substrate increased by 35.52%, 41.95%, and 45.44% compared with that in T1, whereas the methane production in T3 and T4 in the unit of L/kg substrate increased by 4.35% and 7.25% compared with that in T2. The methane production in the units of L/kg substrate, L/L digester volume, and L/kg VS substrate in T2, T3, and T4 significantly increased (p<0.05) compared with that in control (T1 with 100% DCM). This phenomenon indicated that the utilization of CG with or without acidification as co-substrate with DCM can increase the content and quality of organic matter in the final mixed substrates of DCM and CG (Table 3). Bołkowska *et al*. (2022) reported that using co-substrates has a synergistic effect on anaerobic bacteria, increasing their biogas production.

Variables in the Digesters

Substrate characteristics and microbial inoculums can affect biogas production and VFA concentrations (Tampio *et al.*, 2018). The VFA concentrations in all digesters were not significantly different (p>0.05). This indicated that acidogenic and methanogenic bacteria were able to convert organic matter into VFA and process VFA into biogas in all CSTR digesters. Begum *et al*. (2018) reported that the formation of VFA was influenced by acidogenic bacteria and the pH value in the digester because acidogenic bacteria cannot survive in an acidic or alkaline environment; hence, the maintenance of acidogenic pH is very important in maximizing VFA production.

Ammonia is an important nutrient for the growth of microorganisms, but it can inhibit the activity and growth of anaerobic microorganisms at high concentrations (Sutaryo *et al.,* 2020). Yellezuome *et al*. (2022) stated that TAN could act as an inhibitor at a concentration of 1700–1800 mg/L and decrease the growth of microorganisms. In this study, the TAN concentrations were below the threshold; thus, the activity of microorganisms was not disturbed. The TAN concentration in T1 was higher (p<0.05) than in T2–T4. This could be explained by the lower C/N ratio in the substrate in T1 than that in the substrates in T2–T4. During hydrolysis, organic nitrogen in the form of proteins, amino acids, nucleic acids, and urea will decompose into nitrogen-ammonia (Yellezuome *et al.*, 2022).

The digestibility of organic matter is important in evaluating microorganism performance in digesting organic matter in the substrate (Orhorhoro *et al.,* 2017). No significant difference in organic matter digestibility was observed among all digesters. This indicates that the ability of the microorganisms in each digester was not much different in digesting the organic matter in the substrate. Rajput & Visvanathan (2018) demonstrated that the higher the amount of organic matter digested, the higher the methane gas produced.

The difference in the substrate compositions did not significantly affect the slurry's pH value. The pH value in all digesters was within the neutral pH range; thus, it did not interfere with the activity of microorganisms. Mao *et al*. (2015) stated that the neutral pH range in methane production is 6.8–7.4. Furthermore, Begum *et al*. (2018) reported that pH value plays an important role in the biogas formation process as the performance of microorganisms is not tolerant to very low or very high pH levels. Zhou *et al*. (2016) stated that the pH value affects total VFA production and the acetate conversion to biogas.

Evaluation of the Methane Production of Digested Slurry

A study by Thygesen *et al.* (2014) found that slurry digested by seven mesophilic biogas digesters used for the treatment of animal manure and food waste with 16–25 d HRT can produce 156–240-L CH_4/kg VS. This finding is consistent with the result of the present study. The post-digestion strategy is performed to optimally obtain methane production potential from the substrate and reduce methane emissions from the resulting slurry. Noorollahi *et al*. (2015) stated that post-digestion of slurry from the main digester could reduce environmental pollution and greenhouse gas emissions.

CONCLUSION

The utilization of CG with (using H_2SO_4 or AB filtrate) or without acidification as a co-substrate with DCM can increase methane production compared with the control (DCM only, without CG addition). Since all digester can run smoothly, therefore CG with or without acidification can be used as a co-substrate with DCM to increase methane production of the mixed substrate. Thus, the AD application of DCM is more attractive.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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