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# Utilization of Papaya seed in anaerobic co-digestion with dairy cow manure on the bio-digester performance

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**ABSTRACT**

This study examined utilization of papaya seed (PS) and the effect of pre-treatment in the form germination of PS and used it as a co-substrate with dairy cow manure (DCM) on the biogas digester performance. There were three digesters, namely, R1 (100% DCM), R2 (95% DCM and 5% non-germinated meal of PS), and R3 (95% DCM and 5% germinated meal of PS), which were run for three hydraulic retention times. The presence of PS as co-substrates of DCM can increase ($p<0.05$) methane production (L/kg substrate) by 195.01% at R2 and 211.08% at R3 compared to that in R1, and R3 resulted in 5.45% more methane production than that in R2. This enhancement was a significant increase in the biogas industry. Total ammonia nitrogen concentrations, pH values were close to the neutral levels, and the concentrations of volatile fatty acids were not significantly different ($p>0.05$) so that the digester conditions were optimum for the anaerobic digestion process. Therefore, germinated and non-germinated PS can be used as co-substrates with DCM to increase the methane yield, and germination can be considered as a method that can increase the methane yield of PS.

## Introduction

A lactating Holstein Friesian (HF) dairy cow with a body weight of 387–854 kg can produce an average of 25.6 kg feces/d (Appuhamy *et al*., 2014). The anaerobic digestion (AD) of animal manure to produce biogas helps reduce the negative impact of dairy cow manure (DCM). However, the high contents of water, ash, and crude fiber in DCM cause the methane production per ton of DCM to be relatively less (Li *et al*., 2021). One of the efforts to increase methane production from DCM is to co-digest it with other materials having improved nutrient contents.

The limitations of biomass sources that can be used as co-substrates with animal manure have prompted the search for novel biomass sources. Unconsumed fruit seed, e.g., papaya seeds (PS), is a good prospect for a co-substrate for animal manure. Papaya production ranks third worldwide after mango and pineapple, with a total production of ~11.22 Mt in 2014 (Evans and Ballens 2018). The annual papaya production is showing an increasing trend owing to improved cultivation management and plant breeding to produce virus-resistant papayas (Mishra *et al*., 2016). The PS proportion is about 16% of fresh fruit weight (Sugiharto, 2020).

The high proportion and high nutrient content of PS make it a suitable as a co-substrate with DCM in AD treatment. As per Sugiharto *et al*. (2021), the moisture, crude protein, crude fat, fiber, and ash contents of PS meal are 7.2%, 21.7%, 22.9%, 38.7%, and 10.8%, respectively. However, the crude fiber content of PS is somewhat high. Li *et al*. (2021) reported that the high crude fiber content caused low digestibility and leads to suboptimal methane production. Therefore, efforts are required to overcome this constraint. Seed germination is known as a pre-treatment to improve its nutritional value, and certain experiments demonstrated satisfactory results in terms of this phenomenon (Cornejo *et al*., 2015; Joshi and Varma, 2016; Cornejo *et al*., 2019). This current study aimed to evaluate the utilization of non-germinated and germinated PS as a co-substrate with DCM on the bio-digester performance.

## Materials and methods

*Experimental set up*

This study used three digesters of a continuous stirred tank reactor (CSTR) having a capacity of 7 L and an active volume of 5.25 L (Figure 1). The digesters were placed in an incubator at 35°C. DCM as a basal substrate was prepared by mixing dairy cow dung and tap water (1:1.75) to obtain a final total solid (TS) content of about 7%. Total solid (TS) content of cow manure is in the range of 5-12% (Song *et al*., 2023). Feces were obtained from lactating HF cows at the stables of the Department of Animal Science, Diponegoro University.

The adaptation period was determined by filling the starter in the digester to 75% of the digester volume or 5250 mL. The amount of input substrate was determined from the active volume of the digester, i.e., 5250 mL, divided by 22 d, which is one hydraulic retention time (HRT) period. Therefore, the substrate added daily was 238.6 g. The adaptation period was three weeks or 21 d until methane was stably produced. Furthermore, the substrates with various compositions were placed in three digesters: digester R1 (100% DCM), digester R2 (95% DCM and 5% non-germinated meal PS (NGPS)), and digester R3 (95% DCM and 5% germinated meal PS (GPS)). Tables 1 and 2 show the PS, NGPS, GPS meal, and substrate properties in digesters R1-R3.

Continuous stirred tank reactor used in this experiment (during experiment bio-reactor was placed in an incubator at 35°C while glass bottle and tedlar gas bag were kept outside incubator) (Fig.1).

*Inoculum and germination of PS*

The inoculant used in this previous experiment was the digested slurry of a fixed-dome biogas digester at the Department of Animal Science, Diponegoro University, with a TS content of 4.52%, volatile solid (VS) con-

Table 1. Chemical composition of germinated and non-germinated papaya seed meal used in this experiment.

| Nutritional content (% Dry matter) | Treatments | |
|---|---|---|
| | Germinated | Non-germinated |
| Volatile solid | 95.25±0.09 | 94.92±0.13 |
| Crude protein | 26.99±0.10 | 24.55±0.03 |
| Crude fat | 21.23±0.24 | 23.33±0.04 |
| Crude fiber | 43.92±0.35 | 42.46±0.16 |
| Ash | 4.75±0.09 | 5.08±0.13 |
| Hemicellulose | 18.42±0.97 | 19.71±1.90 |
| Cellulose | 19.71±1.22 | 18.75±0.49 |

Table 2. Characteristics of substrates in the bio-digesters.

| Digesters | Total solid (%) | Volatile solid (%) | Crude protein (%) | Volatile solid proportion of PS in the mixed substrate (%) | C/N ratio |
|---|---|---|---|---|---|
| R1 | 6.57±1.42 | 5.87±1.28 | 1.07±0.09 | 0 | 18.97 |
| R2 | 9.89±1.31 | 8.77±1.13 | 1.37±0.02 | 38.07 | 22.15 |
| R3 | 9.94±1.40 | 8.89±1.14 | 1.42±0.01 | 38.27 | 21.69 |

tent of 3.96%, and pH of 7.57.

In this study, the germination was performed as per a method by Sugiharto *et al.* (2021). The PS used were the seeds of the Bangkok variety papaya (*Carica papaya* L.) that were obtained from a traditional fruit market in Semarang, Central Java. The NGPS and GPS were dried by sun drying for 2–3 days and were mashed for 5 min using a kitchen blender (Miyako). The PS milling step was performed to make NGPS and GPS easier to fill in the digesters because the biogas digesters used were on a laboratory scale (Fig. 1), and then placed NGPS and GPS in a plastic zip and followed by kept it in a plastic jar and closed tightly and stored in a dry place.

Fig. 1. Continuous stirred tank reactor.

*Analytical method*

The methane production of continuous experiment was measured by passing biogas from the continuous stirred tank reactor (CSTR) through a 500-mL-sized bottle containing 4% NaOH (Merck®, cat no: 1064981000) solution (Gelegenis *et al.*, 2007). The solution was then changed using a fresh one on a weekly basis, and then methane gas was collected using a Tedlar gas bag with a capacity of 5 L (Hedetech-Dupont, China). The shelter process used a 5-mm-sized Teflon hose. Methane was measured daily using the water displacement method as described by Sutaryo *et al.* (2020). The total volatile fatty acid (VFA) concentration was measured using gas chromatography (GC BRUKER 436). The total ammonia nitrogen (TAN) concentration in the slurry was analyzed using the following steps: the slurry was diluted 1000 times, 10 mL of the diluted slurry was placed in a test tube, and then ammonia salicylate was added and waited for 15 min. The sample was then inserted in a spectrophotometer (HACH DR3900, ammonia test kit cat no. 2606945, USA) until the resultant value was observed. The resultant value was then multiplied by the dilution factor to obtain the TAN concentration data. Sample acidity was measured using a digital pH meter (OHAUS®ST 300). TS was then determined by heating the sample in an oven at 105°C for 7 h, followed by heating the sample at 550°C for 7 h to analyze the ash content (APHA 1995). VS was the difference between the TS content and ash concentration. Total organic carbon content was then determined as per the method used by Syaichurrozi (2018). Total nitrogen analysis was performed using the Kjeldahl method. C/N ratio was obtained by dividing the total organic carbon content by the total nitrogen content in the substrate. Crude fat analysis was then performed using the Soxhlet method. The collected data were tabulated and analyzed using one-way analysis of variance (ANOVA) with a 5% of confidence level. Duncan's multiple range test was used in the post-ANOVA analysis (Gomez and Gomez, 2007).

## Results

*Methane production*

The presence of NGPS (R2) and GPS (R3) as co-substrates of DCM can considerably increase methane production in units of L/kg substrate, L/L digester volume, and L/kg VS ($p<0.05$) compared to that without PS (R1) (Table 3). Fig. 2 shows the weekly average methane production from each digester.

Methane production at digester R1 was 174.68 L/kg VS (Table 3). The

Table 3. Methane production, concentrations of VFA and TAN, VS reduction and pH values.

| Digester | Methane Production | | | VFA (Mm) | TAN (mg/L) | VS reduction (%) | pH |
|---|---|---|---|---|---|---|---|
| | L/L digester | L/kg substrate | L/kg VS | | | | |
| R1 | 0.46±0.09[a] | 10.02±2.06[a] | 174.68±35.93[a] | 5.94±8.24 | 195.50± 53.66[a] | 35.10±3.83 | 6.90±0.07[a] |
| R2 | 1.34±0.37[b] | 29.56±8.17[b] | 333.96±92.26[b] | 4.56±2.28 | 568.38±261.15[b] | 37.02±3.02 | 7.19±0.14[b] |
| R3 | 1.42±0.41[b] | 31.17±9.13[b] | 348.20±101.97[b] | 5.07±4.31 | 610.50±274.94[b] | 38.03±1.85 | 7.15±0.15[b] |

[ab] Difference in superscripts in the same column indicates a significant difference ($p<0.05$)

result was not different from that of the study by Sutaryo *et al.* (2014), which reported that methane production in a digester with DCM substrates under mesophilic conditions (35°C) was 172 L/kg VS.

The presence of PS can increase methane production (in L/kg substrate) by 195.01% at R2 and 211.08% at R3 compared to the control (R1), and R3 resulted in 5.45% more methane production than that in R2.

The C/N ratios of the substrates used in digesters R1, R2, and R3 were 18.97, 22.15, and 21.69, respectively. These values were in the normal range, and the optimum C/N ratio was in R2 and R3.

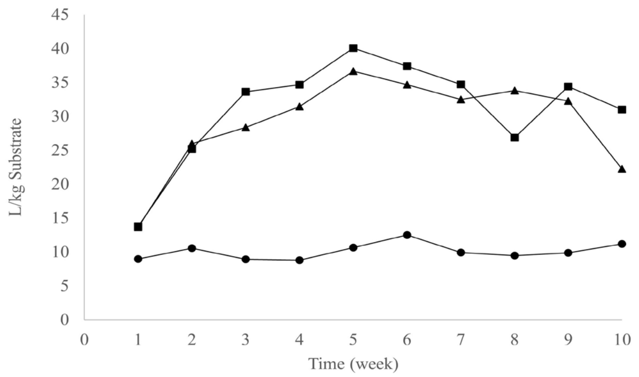


Fig. 2. Trend of methane production in CSTR (●: R1, ▲: R2, ■: R3)

*Variables in liquid phase*

Based on statistical analysis (Table 3), there was no significant effect ($p>0.05$) of the presence of NGPS and GPS in the mixture on the VFA concentrations and VS reduction percentages. Moreover, the presence of NGPS and GPS had significant effects on the TAN concentrations and pH values ($p<0.05$).

The VFA concentrations, which were 5.94, 4.56, and 5.07 mM in digesters R1, R2, and R3, respectively, after the AD process were not significantly different ($p>0.05$).

In this study, the TAN concentrations were significantly different ($p<0.05$) among the digesters, that is, 195.50, 568.38, and 610.50 mg/L in digesters R1, R2, and R3, respectively. The digester R3 (95% DCM + 5% GPS) resulted in the highest TAN concentrations, followed by digester R2 (95% DCM + 5% NGPS); moreover, the lowest TAN value was obtained in digester R1 (100% DCM).

The average pH values of the slurry in digesters R1, R2, and R3 were 6.90, 7.19, and 7.15, respectively, which were significantly different ($p<0.05$). These values were in the normal pH range for the AD process. The VS reduction values were not significantly affected ($p>0.05$) by the difference in substrate compositions in digesters R1, R2, and R3, which were 35.64%, 37.57%, and 37.91%, respectively.

## Discussion

Increased methane production ($p<0.05$) is observed because the presence of PS can increase the contents of organic matter and the nutritional quality of the substrate in digesters R2 and R3 (Tables 1 and 2) to meet the nutritional requirements of microorganisms to optimize the digestibility of manure. As per Guares *et al.* (2021), methane production was affected by manure digestibility and additional substrates.

The presence of GPS (R3) increased methane production by 5.45% compared to the presence of NGPS (R2); however, it was not significantly different ($p>0.05$) owing to the following reasons: The two PS were in the form of meal, which was in accordance with Scherzinger and Kaltschmitt (2021) that particle reduction in substrates was an effective pre-treatment method to increase methane production. However, this particle reduction may reduce the germination effect of GPS compared to NGPS. The VS proportion of PS in the mixed substrate both in R2 and R3 was not high enough to make the methane production considerably different. The PS used in this experiment were dry, which caused the enzymes in PS to be dormant. Digester R3 resulted in a higher methane production than R2 because the germination of PS (R3) can increase the enzymatic process of the protease enzyme, which hydrolyzes protein into amino acids, thus making it easier to digest. This agrees with Di *et al.* (2022), who reported that germination increases enzymatic activity, i.e., protease enzyme hydrolyzing proteins into peptides and amino acids.

The increase in methane production at digesters R2 and R3 compared to R1 was attributed to the stimulation of the activity of microorganisms during the bioconversion of organic matter into biogas because of the increased nutritional values of the substrate (Sutaryo *et al*., 2022). The improved quality of organic compounds in R2 and R3 enhanced hydrolysis and acidogenesis, leading to higher production of VFA, H2, and CO2, which were later converted into acetic acid and finally into methane by methanogenic bacteria (Dobre *et al*., 2014).

The performance of biogas digesters was significantly influenced by the C/N ratio of the substrates. The optimum C/N ratio in R2 and R3 (22.15 and 21.69, respectively) was within the ideal range of 20–30 as reported by Wang *et al.* (2015), which supports efficient anaerobic digestion.

Although TAN concentration increased in R2 and R3 due to higher protein content, the values (568.38–610.50 mg/L) were still within the acceptable range and did not inhibit methanogenesis, since inhibition is reported to occur at much higher levels (~5000 mg/L; Yenigün and Demirel, 2013). The associated increase in pH values in R2 and R3 was consistent with the alkaline nature of ammonia (Garcia-González and Vanotti, 2015) and remained within the optimal range 6.6–7.8 (Alfa *et al*., 2014) for anaerobic digestion.

The nonsignificant differences in VFA concentration and VS reduction among the digesters indicate that although substrate quality improved in R2 and R3, microorganisms were still able to efficiently convert the organic matter into biogas across all treatments. This is in line with previous findings (Pap *et al*., 2015; Rajput and Visvanathan, 2018).

## Conclusion

The use of GPS and NGPS as co-substrates with DCM can increase methane production compared to only DCM. Because the presence of PS either with germination or without germination increased methane production, resulted in almost the same total VFA concentrations, resulted in TAN concentrations that were still within the normal range, and resulted in pH values in the optimum level range, the use of PS either with germination or without germination as co-substrates can increase methane production from DCM. The use of 5% PS in the mixture can increase methane production by 5.45% more than that in NGPS. Therefore, germination can be considered a method used to increase the methane production of PS.

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## Conflict of interest

The authors have no conflict of interest to declare.

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