

# Enhancing methane production of dairy cow manure by co- digestion with modified cassava flour waste water

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# Enhancing methane production of dairy cow manure by co-digestion with modified cassava flour waste water

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## Abstract

The aim of this study was to evaluate the utilization of modified cassava flour wastewater (MCW) as a co-substrate with dairy cow manure (DCM) on the methane production in the mesophilic (37 °C) anaerobic digestion process. The experiment was performed using two identical continuously stirred tank reactors. The DCM was diluted with tap water for digester 1 (R1) and DCM was diluted with MCW at the same ratio, 1/1.7 (R2). The experiment was run for 75 d, corresponding to three times the hydraulic retention time. The result showed that co-digestion of MCW and DCM can increase ( $p < 0.05$ ) methane yield by 79.03% in terms of L/kg VS added compared to the control reactor. This treatment also can increase income by 0.92 \$ USA/ton substrate when methane production was used as a fuel in a combined and heat power plant and the produced electricity connected to high voltage grid. R1 and R2 stably indicated by stable methane production, low total VFA, and TAN concentration and pH value of the digested slurry was in the normal range for the anaerobic digestion process. Therefore, MCW can be used as a co-substrate to increase methane production of DCM in mesophilic anaerobic digestion.

**Keywords:** biogas, co-digestion, manure, methane, modified cassava flour

## Introduction

Modified cassava flour (mocaf) is prepared by fermentation of cassava chips using lactic acid bacteria. This process, changes its physical, chemical, and microbiological properties. Mocaf has characteristics similar to wheat flour but contains no gluten, therefore it is widely used as a wheat flour substitute wheat flour. Mocaf also has the potential to be further processed for the production of resistant starch which can act as a prebiotic (Firdaus et al 2018).

The production process of mocaf consists of stripping of cassava tubers, washing, slicing, fermentation, draining, drying, milling, and packaging (Kurniadi and Khasanah 2020). This process produces a large amount of wastewater since the fermentation process is performed by soaking the cassava chips in water that contains a microbial inoculum. The fermentation process can take 72 h (Kurniadi and Khasanah 2020), hence some cassava nutrients will be dissolved into the liquid waste. The discharge of food industrial wastewater directly into the environment without any treatment has a high potential for pollution including oxygen depletion in receiving water bodies, eutrophication, global warming, and toxicity-related impacts (Zang et al 2015). Due to the growth of the mocaf industry in Indonesia, mocaf wastewater (MCW) can therefore cause serious environmental problems if it is not managed properly.

Wastewater management can be performed by aerobic treatment or anaerobic digestion to produce biogas. Since the anaerobic digestion process has dual benefits i.e. waste diversion from direct disposal into the environment and bioenergy recovery, this treatment has received significant attention in recent years (Yen et al 2017; Ryue et al 2020). Anaerobic digestion of wastewater can be in a single substrate or co-digestion with other substrates. One waste that has a big potential as a substrate for anaerobic digestion system is dairy cattle manure (DCM). It is very abundant in Indonesia and according to Noorollahi et al (2015) dairy cattle can produce 2226.5 kg/year per 610 kg of body weight of wet feces plus urine. Most farms in Indonesia are mainly smallholdings and only a few farms are considered large-scale (Sutaryo et al 2019). In small-holder farms, farmers design the cowshed floor of the animal house sloping towards a gutter, hence feces are separated from urine and spilled drinking water. Thus, it will be more efficient for the farmer during cleaning the cowshed floor. Therefore, for farmers who want to treat DCM anaerobically the manure should be diluted using tap water.

There are several biogas digester designs, nevertheless, the continuously stirred tank reactors design is the most commonly applied bioreactor for treating agricultural waste (Linke et al 2015). The aim of this study was to evaluate the process performance of the anaerobic digestion treating co-digestion of MCW and DCM.

[Go to Top](#)

## Material and methods

### Experimental set up

This experiment was performed using two identical continuously stirred tank reactors (R1 and R2) with a 7 L total capacity. The mixing systems were operated at 36 revolutions per minute using propeller mixer. The reactors were maintained at 37 °C by placing them in an incubator. R1 and R2 had 5.25 L active working volume and 25 d hydraulic retention time. Both digesters were made in a double layer of stainless steel. Mao et al (2015) reported that under mesophilic conditions an average hydraulic retention time in the range of 15-30 d is required for effective operation.

The experiment was started by filling R1 and R2 with 5.25 kg of inoculum. On the second day, all digesters were fed 210 g of the substrate (after first removing the same amount of digested slurry from a port at the base of the digesters), which continued for the next 21 d as an adaptation period. The substrate was made by mixing DCM with tap water at a ratio of 1/1.7. The digesters were fed through a tube, the outlet of which was submerged under the substrate level to avoid air ingress during the feeding process.

Data were collected after this 21 d adaptation period. During the data collection period, R1 was fed DCM diluted with tap water with a ratio of 1/1.7, while R2 was fed DCM diluted with MCW at a ratio of 1/1.7. The experiment was run for a period of three hydraulic retention times corresponding to 75 d in total.

### Inoculum and substrate

The inoculum was sourced from the fixed dome active biogas digester at the Faculty of Animal and Agricultural Sciences, Diponegoro University. The digester treats dairy cow manure and operates at a tropical ambient temperature. The digested slurry from the digester was transferred directly to the laboratory scale digester.

Substrate for this experiment was DCM in the lactation period and was collected from the farm in the Faculty of Animal and Agricultural Sciences, Diponegoro University. DCM was diluted with tap water at a ratio of 1/1.7 to reach final total solid (TS) of ca 8% which is similar to that reported by Angelidaki et al (2003) where dairy cattle manure had TS: 6-9%. DCM was collected once a week, diluted with tap water directly and kept in the refrigerator. MCW was sourced in the local mocaf industry in Semarang. MCW was collected two times and was kept in the freezer till use. Inoculum and substrate properties can be seen in Table 1.

Table 1. Inoculum and substrate properties

Inoculum/ Substrate	TS (%)	VS (%)	Protein (%)	Ether extract (%)	TAN (mg L <sup>-1</sup> )	pH	C/N ratio
Inoculum	2.85	2.34	-	-	100	7.33	-
Substrate R1	7.91	6.91	0.66	-	60	7.30	36.35
Substrate R2	9.15	8.07	0.94	-	140	6.55	29.81
MCW	1.94	1.80	0.46	1.11	120	4.24	13.59

### Analytical methods

Methane production was analysed by routing the produced biogas through 0.5 L infusion bottles that contained 4% NaOH (Merck®, cat no: 1064981000) solution (Gelegenis et al 2007) using a 5 mm Teflon tube. The NaOH solution was changed with fresh solution periodically. The gas was collected using a 5 L Tedlar gasbag (Hedetech-Dupont, China). Gas volume was measured on a daily basis using the water displacement method as described by Sutaryo et al (2020).

The substrate and digested slurry pH values were measured using a pH meter (Ohaus® ST300 pH meter). TS was determined by drying samples at 105 °C for 7 h followed by combusting the dried sample at 550 °C for 6 h to analyse the ash. VS was calculated by subtracting the ash weight from the TS (APHA, 1995). Total ammonia nitrogen (TAN) concentration was measured photometrically using a HACH® spectrophotometer (DR3000, ammonia kit test cat. no. 2606945, USA). Total volatile fatty acids concentration (VFA) was determined using the titration method. Total nitrogen was measured using the Kjeldahl method while ether extract was analysed with the Soxhlet extraction method. Total organic carbon was estimated through the relationship total organic carbon = VS/1.8 according to Haug (1993). The C/N ratio was obtained from total organic carbon/total nitrogen (Syaichurrozi, 2018). The collected data were statistically analysed using a t-test with a 95% confidence level according to Gomez and Gomez (2007).

## Results and discussions

Go to Top : [production](#)

The methane production in terms of L/kg VS added is given in Fig. 1A, while in terms of digester volume per day can be seen in Fig. 1B. There was a significant effect ( $p < 0.05$ ) of co-digestion of MCW and DCM on methane production in term L/kg substrate added, L/kg VS added, and in terms L/L digester volume (Table 2). Methane production of R1 was 10.48; 168.17 and 0.42 in term of L/kg substrate added, L/kg VS added, and L/L digester volume, respectively, while methane yields of R2 were 21.85; 301.08, and 0.87 in terms of L/kg substrate added, L/kg VS added and L/L digester volume, respectively. Angelidaki et al (2003) reported that methane production in term substrate weight of animal manure is about 10-20 m<sup>3</sup> per ton, while Dong et al (2019) reported that methane production of dairy cattle manure in a plug flow reactor that working at 25 d HRT and 37-40 °C is ranging from 0.15-0.23 m<sup>3</sup>/kg VS. Therefore, the methane yield of control reactor (R1) in this study is in accordance with those previous results.

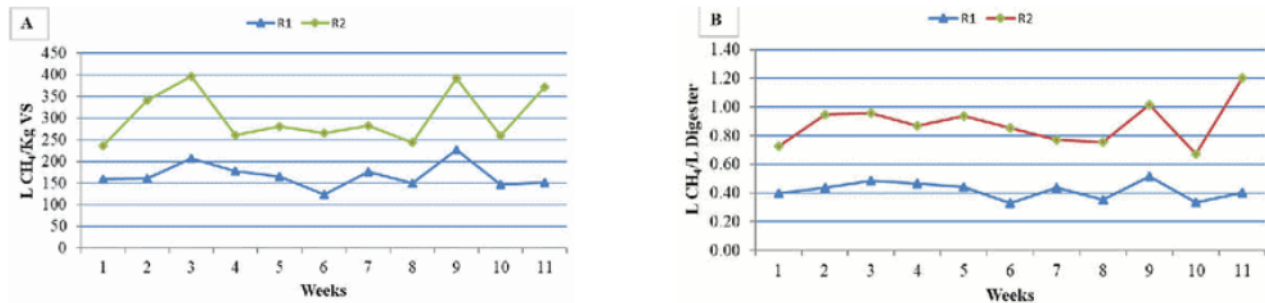


Figure 1. A. Trends in methane production per Kg VS added. B. Methane production per digester volume per day (R1: DCM diluted with tap water, R2: DCM diluted with MCW)

Table 2. Methane yield, total VFA, TAN concentration, VS reduction and pH value of digested slurry

	Methane production		VFA mg L <sup>-1</sup>	TAN mg L <sup>-1</sup>	VS reduction %	pH
	L kg VS <sup>-1</sup>	L L digester volume <sup>-1</sup>				
R1	168.17 ± 32.60 <sup>a</sup>	0.42 ± 0.07 <sup>a</sup>	226.25 ± 91.49	401.25 ± 306.15	33.56 ± 5.49	6.88 ± 0.12
R2	301.08 ± 71.07 <sup>b</sup>	0.87 ± 0.18 <sup>b</sup>	238.75 ± 24.75	487.50 ± 463.49	35.29 ± 10.56	6.98 ± 0.12

The methane production in terms of substrate weight of the co-digested MCW and DCM (R2) in this study can be already categorized a profitable substrate at industrial scale. Angelidaki and Ellegard (2003) suggested that the economic balance utilization of organic waste in a biogas plant can be achieved when the average biogas production of organic waste is higher than 30 m<sup>3</sup> of biogas per m<sup>3</sup> of biomass and approximately 20 m<sup>3</sup> of CH<sub>4</sub> per m<sup>3</sup> of biomass.

Utilization of MCW as a co-substrate with DCM (R2) in this experiment increased methane production by 79.03%; 119.15%, and 108.52% in terms of L/kg VS added, kg substrate added, and L/L active digester volume, respectively, when compared to R1. The percentage increase of methane yield in terms of L/kg VS added in this study was the lower than the measurements in terms of L/kg substrate added and L/L active digester volume. This phenomenon can be explained by the utilization of MCW as co-substrate with DCM increasing VS concentration of the combined substrate, therefore the denominator of L/kg VS added in R2 was higher than that in R1, while in term of L/kg substrate added and L/L active digester volume the denominator value in R1 and R2 was equal. A significant effect ( $p < 0.05$ ) of methane production in R2 compared to that of R1 in this study can be caused by the utilization of MCW can improve the nutritional content of substrate in R2. This fact therefore can stimulate the microorganism's ability in R2 to digest the organic material better and convert it to methane. This fact is confirmed by the protein content of the substrate in R2 being higher than that of substrate for R1, the VS reduction value of digested slurry in R2 also tended to be higher than that in R1 (Table 2). The biogas and methane yields of MCW alone were not determined in this study, but the data obtained and literature suggests that MCW is a highly degradable substrate. Borup and Muchmore (1992) reported that a typical characteristic of industrial food wastewater is a high organic matter content, most of it being composed of easily biodegradable compounds such as carbohydrates, proteins, and, in some cases, smaller contents of lipids.

In addition, co-digestion of MCW and DCM in this experiment lowered the C/N ratio of substrate into the ideal range for the AD process. The C/N ratio substrate of R1 was 36.35 while R2 was 29.81 (Table 1). Angelidaki et al (2003) reported that the ideal C/N ratio in AD process is in the range of 25 to 32. This fact therefore can also explain the higher methane production of R2 than that of R1.

### Economic calculation

A simple economic calculation of the utilization of MCW as co-substrate with DCM is presented in Table 3. For this calculation, this study only took into account transportation costs of MCW when utilizing MCW as co-substrate with DCM. The produced methane in the calculation was converted to electricity in a combined heat and power plant. It can be seen that co-digestion of DCM can increase revenue amounting to Rp 594.56 (0.04 \$ USA) per ton of substrate when electricity from combine

Go to Top



head and power plant was connected to a medium voltage grid and Rp 13013.12 (0.92 \$ USA) per ton of substrate for a high voltage grid.

**Table 3.** Simple economic analysis utilization of MCW as co-substrate with DCM

Digester	Methane yield/ton substrate	$\Delta$ methane production of R1 and R2	Electricity production (kwh)*	Revenue from selling electricity (Rp)**	Net revenue from selling electricity ton <sup>-1</sup> substrate (Rp)***; (\$ USA)***
R1	10.48	-	-	-	-
R2	21.85	11.37/10.24 N CH <sub>4</sub>	35.48	34594.56 <sup>(a)</sup> 47013.12 <sup>(b)</sup>	594.56; 0.04 13013.12; 0.92

\*1 m<sup>3</sup> N methane equal to 9.9 kwh, this paper use 35% for electricity generator efficiency \*\*Based on energy and natural resources minister regulation No. 4 of 2012 of Republic Indonesia that the price of electricity produced from biogas plant in the Java, Bali, Madura and Sumatra areas by state electricity company is valued at Rp. 975.00 if it is connected to medium voltage<sup>(a)</sup> and Rp. 1,325.00 if it is connected to a low voltage <sup>(b)</sup>\*\*\*Per ton substrate required 850 kg MCW, the transport cost for a truck of 5 m<sup>3</sup> capacity is Rp 200.000,00 (personal information), this study uses the currency exchange rate was a \$ USA equal to Rp 14100.00

### Variables in the liquid phase

10 There was no significant effect ( $p > 0.05$ ) of co-digestion of MCW and DCM on VFA and TAN concentration, VS reduction, and pH of digested slurry from R1 and R2. The VFA and TAN concentrations were stable and at low concentrations throughout the experiment. The total VFA concentration of digested slurry of R1 and R2 was 226.25 and 238.75 mg/L respectively, while TAN concentration of R1 and R2 digested slurry was 401.25 and 487.50 mg/L respectively. The TAN concentration is below the inhibitory threshold as reported by Yenigün and Demirel, (2013) that for un-acclimated inoculum and under mesophilic conditions (35 °C), the TAN inhibitory threshold is in the concentrations of around 1700–1800 mg/L. The higher TAN concentration of R2 digested slurry than that in R1 was in line with a higher protein content of substrate for R2 than that in R1. Jiang et al (2019) reported that ammonia is produced through the degradation of the nitrogenous matter in the substrate of AD, primarily in the form of proteins.

The VS reduction in this experiment was 33.56% and 35.29% for R1 and R2 respectively. The VS reduction in R2 tended to be higher than that in R1. As explained previously, a better nutrient concentration of a substrate for R2 than that of substrate for R1 can better stimulate the activity of anaerobic microorganisms in R2, and can therefore degrade more organic matter. Brown and Li (2013) reported a VS reduction ranging from 27% to 33% for batch digestion of yard waste and a combination of 90% yard waste and 10% food waste respectively. Those reactors were maintained at 36 °C for 30 d (a similar condition with a condition in this experiment). In another study, Sutaryo et al (2012) reported a VS reduction in the range of 27-35% for a thermophilic reactor that working at 52 °C and 14 d hydraulic retention time with dairy cattle manure in different TS concentration as a substrate. Hence the result of this experiment is in accordance with the result of the previous study.

Even though the pH value of substrate for R2 was lower than that for substrate for R1 (Tabel 1), however, pH value of R2 digested slurry tended to be higher than that of R1 digested slurry, although not significantly different between the two reactors. The higher TAN concentration of R2 digested slurry than that in R1 digested slurry perhaps can explain this phenomenon. The pH values of R1 and R2 digested slurries in this study were in the range of a stable anaerobic digestion process as reported by Mao et al (2015) that the ideal pH value for an anaerobic digestion process is in the range of 6.8 to 7.4.

The liquid phase variables suggest that co-digestion of MCW with DCM at the mix ratio and process parameters used in this study had no deleterious effects on the process when compared to digestion of DCM alone.

### Conclusion

- Co-digestion of MCW and DCM gave a positive effect by increasing the methane yield by 79.03% in terms of kg/Vs added, compared to that in the control.
- Both digesters can run properly indicated by stable gas production, low total VFA, TAN concentration, and pH value of digested slurry that was in the range of an optimal anaerobic digestion process.
- Therefore MCW is suitable as a co-substrate of DCM in the ratio tested here in order to increase methane production.

### 7 Acknowledgement

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Go to Top

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