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ANAEROBIC DIGESTION CO-SUBSTRATE OF DAIRY COW MANURE AND TOFU CAKE: FOCUSING ON MIXING ORGANIC RATIO

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Highlights

- This study evaluates co-digestion of tofu cake (TC) and dairy cow manure (DCM).
- Digester substituting 15% of manure with TC showed a stable biogas production.
- 52.51% more methane yield was achieved when 15% of DCM was substituted with TC.
- TC is a suitable biomass as co-substrate with DCM in AD at ratios up to 15%.

Abstract. Anaerobic digestion of dairy cow manure (DCM) is constrained by a low methane production of animal manure. A method to overcome that is by co-digestion DCM and food industry by-product. This study investigated the process performance anaerobic co-digestion of DCM and tofu cake (TC) at different volatile solid (VS) mixing ratios. The treatments were partial substitutions of DCM with TC by 5%, 10%, and 15% (w/w) in reactors T2, T3, and T4 respectively, while T1 was served as control. Co-substrate of DCM and TC gave a positive effect ($P < 0.05$) on methane production by 24.23, 34.74, and 52.51% respectively for T2, T3, and T4 compared to the control reactor. Low total volatile fatty acids, ammonia nitrogen concentration, stable methane production and neutral pH values of all digested slurries indicate that TC is suitable to increase methane production of DCM up to a DCM/TC ratio of 2.92:1, in terms of VS.

Keywords: biogas, manure, co-digestion, post digestion test, tofu cake, methane.

Introduction

Anaerobic digestion (AD) of animal manure can give dual advantages by reducing greenhouse gas emissions caused by unwanted fermentation of organic material and through controlled AD in a biogas digester where biogas is captured and can be used as renewable energy (Møller et al., 2004; Misevičius & Baltrėnas, 2011). However, AD of livestock manure is constrained by a low methane production of animal manure. Angelidaki and Ellegaard (2003) reported that the methane production of animal manure is in the range of 10–20 m^3/t and to achieve economic balance methane production of biomass has to be higher than 20 m^3/t of waste. In the case of dairy cow manure (DCM), low methane production is due to high moisture and ash concentration, and a great part of the lignocellulosic component (equal to or more than 50%) (Li et al., 2021). Therefore, some efforts are needed to increase methane production from biogas digester based on livestock manure, so that it can compete with fossil-based energy sources and can attract more investors to implement manure management through AD to produce biogas.

Strategies to overcome the shortcomings of AD of DCM including co-digestion with other substrates that have higher methane production, substrate pre-treatment, additives utilization (trace metals, carbon-based materials, low-cost composites, nanomaterials, and microbial cultures), and innovative systems (bio-electrochemical fields and laser irradiation) (Li et al., 2021). Furthermore, co-substrate of DCM and organic industrial waste can not only increase methane production but also can improve

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the bio-digester stabilization if it is utilized in a controlled fashion (Angelidaki & Ellegaard, 2003), better nutritional balance of the combined substrate (proper carbon-to-nitrogen (C/N) ratio and trace element supplementation), increased buffer capacity, dilution of toxic compounds and increases the bioavailability of nutrients in the digested slurry (Karki et al., 2021).

Among organic industrial by-products that have higher methane production than DCM is tofu cake (TC)/soybean curd residue, which are very abundant in Indonesia as tofu is a very popular soybean processed product. According to the Ministry of Agriculture of Republic Indonesia (2021), tofu consumption in Indonesia per capita in 2019 and 2020 was 7.915 and 7.957 kg/person respectively. During tofu production, a kg of soybean can produce 0.7 kg tofu (Wiyono & Baksh, 2015) and 1.2 kg of fresh TC (Guimarães et al., 2018). The utilization of this tofu by-product in Indonesia is mainly for animal feed. However, along with the green energy demand, TC is a promising substrate to boost methane production of DCM since it has high organic matter content and better nutrient composition than that in DCM. Raju et al. (2013) reported that methane production of DCM at 90 d incubation period at 35 °C was 281 L CH_4/kg volatile solid (VS). In another study Sutaryo et al. (2012) stated that methane production of DCM after incubated at 35 °C for 90 d was 281 L CH4/kg VS, both studies showed the same result. However, study from Zhou et al. (2011) showed that after very short AD period (19 d) at mesophilic condition 36 °C the methane yield of TC was 495 L/kg VS. Based on the previous study results, it was found that in the same weight unit the methane production of TC was much higher than that of DCM. Thus, it is expected that anaerobic co-substrate of TC and DCM can improve methane production of biogas digester based on livestock manure.

Although previous studies have evaluated methane production of TC as a single substrate in anaerobic batch digestion tests (Zhou et al., 2011) and using a semi-continuous digester (Zhang et al., 2019) to the best of our knowledge there has been a lack of information regarding anaerobic co-digestion process performance of TC and DCM in different levels of organic mixing ratio using a continuous digester. Angelidaki and Ellegaard (2003), reported that there is an important concern during co-digestion of manure and organic industrial by-product that it should be under well-controlled conditions. Therefore, the purpose of this research was to assess the process performance of mesophilic anaerobic co-substrate of DCM and TC in different levels of organic matter mixing ratio. In addition, this study also evaluates post digestion exams of digested slurry from a biogas digester that treating those combined substrates.

1. Materials and methods

1.1. Experimental set up

Two experimental set ups were tested in this study: a continuous experiment and post digestion tests. The continuous experiment was implemented to evaluate the process performance of a biogas digester treating different mixed organic ratios of DCM and TC, while the post digestion test was to measure the residual methane potential of digested slurry from the continuous experiment.

1.2. Continuous experiment

The continuous experiment was performed using four identical continuously stirred tank reactors (CSTR) (Figure 1). Each reactor was made of double-layer of stainless steel of 7 L total volume, 5.25 L active working volume and was maintained at 37 °C by placing each reactor in an incubator, and working at 25 d hydraulic retention time (HRT). According to Mao et al. (2015) in mesophilic temperature, the period of HRT about 15–30 d is required to effectively digest waste in the AD process. The mixing system of substrate in the digester was operated at 36 revolutions per minute using a propeller mixer (Figure 1).

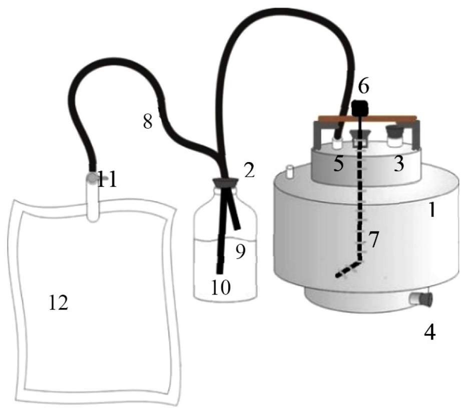


Figure 1. Continuous experimental set up (Saputra et al., 2018). (1 – CSTR bio-digester, 2 – rubber stopper, 3 – substrate inlet, 4 – digested slurry outlet, 5 – biogas outlet, 6 – dynamo, 7 – stirrer, 8 – teflon tube, 9 – 500 ml infusion bottle, 10 – 4% NaOH solution, 11 – valve, 12 – tedlar gas bag). During the study, the digesters were maintained at 37 °C by placing reactors in an incubator, while the infusion bottle and Tedlar gas bag were kept at room temperature outside of the incubator

The continuous study was started by filling each digester with 5250 g of inoculum on the first d followed by feeding all reactors with 210 g of DCM on the second day onward up to 21 d, as a start-up period. The feeding process was performed using a tube, the outlet of which was submerged below the substrate level to avoid air ingress during the feeding process. The data collection was started following this start-up period.

The treatments were substitutions of DCM with TC by 5%, 10%, and 15% (w/w) in reactors T2, T3, and T4 respectively. T1 was continued to be fed using DCM solely and served as a control reactor. The ratio of DCM and TC in terms of volatile solids (VS) was 9.80; 4.64, and 2.92 for T2, T3, and T4 respectively. The continuous experiment was performed for 75 d that corresponded to 3 HRT.

1.3. Post digestion test

The post digestion test was performed using 0.5 L infusion bottles in which was placed 200 g of digested slurry from each digester, collected during d of 50–54 of the continuous experiment. To ensure anaerobic conditions, each batch digester was closed using a black rubber stopper and flushing with nitrogen for two minutes. Each reactor was maintained at 37 °C by placing the reactors in an incubator. Each test was done in triplicate and run for 30 d.

1.4. Starter and substrate

The starter for the continuous study was the digested slurry from the fixed dome bio-digester at the Department of Animal Science, Diponegoro University, Indonesia, which operates at tropical ambient temperature. This reactor treats DCM solely. It was relocated immediately from the slurry outlet of the fixed dome reactor to the laboratory scale reactors on the first day of the experiment. The properties of the inoculum and substrate for the continuous experiment can be seen in Table 1.

DCM was collected from the research farm at the Department of Animal Science, Diponegoro University, during the lactation period. TC was obtained from the local tofu industry in the city of Semarang, Indonesia. DCM was prepared by diluting dairy cow faeces with tap water at the ratio of 1/1.7 to reach a total solid (TS) of ca 7%. Angelidaki et al. (2003) reported that that DCM has TS: 6–9%. Substrate for the continuous experiment and TC were prepared once a week and kept in the refrigerator.

1.5. Analytical methods

Methane production of the post digestion experiment was measured by releasing biogas from each batch digester to a 0.5 L infusion bottle that contained 4% NaOH solution (Merck®, cat no: 1064981000) using a 5 mm Teflon tube, to remove CO_2 (Gelegenis et al., 2007). Methane from the NaOH bottle was collected using a 1 L Tedlar gas bag (Hedetech-Dupont, China). Gas volume was measured periodically using a liquid displacement method according to Sutaryo et al. (2020). Similarly, methane volume measurement in the continuous experiment was conducted with the same method described above except that methane was collected using a 5 L Tedlar gas bag and measured on daily basis. The NaOH solution was changed once a week. TS and VS of sample were determined according to American Public Health Association (1995). The pH was measured using a pH meter (Ohaus® ST300 pH meter). The total ammonia nitrogen (TAN) concentration was determined photometrically at 655 nm using a HACH® spectrophotometer (DR3000, ammonia kit test cat. no. 2606945, USA). Total volatile fatty acids (VFA) concentration was analyzed using a titration method. Total Kjeldahl nitrogen (TKN) was determined using the Kjeldahl method. Total organic carbon (TOC) was determined using formula VS/1.8 according to Haug (1993) and the C/N ratio was determined by TOC/TKN. Subsequent data tabulation was statistically analysed using analysis of variance at the 5% confidence level according to Gomez and Gomez (2007). A Duncan multiple range test was applied when there was a significant effect of the treatment on the observed variables.

2. Result and discussions

2.1. Methane production

The methane yield in terms of L/kg VS added is presented in Figure 2a, while in terms of L/kg substrate is given in Figure 2b. Methane production in terms of L/kg VS added in the control (T1), T2, T3, and T4 reactor were 206.53; 256.59; 278.29, and 314.97 respectively (Table 2). Study of Dong et al. (2019) showed that methane yield of cattle manure from plug flow bio-digester operating at 25 d of HRT and 37–40 °C was 220 L/kg VS. Sutaryo et al. (2012) evaluated methane production of DCM using a thermophilic (51 °C) bio-reactor and working at 14 d HRT and obtained a methane yield of 196.7 L/kg VS added. Therefore the methane production of DCM from T1 bio-digester in this experiment was comparable with previous studies.

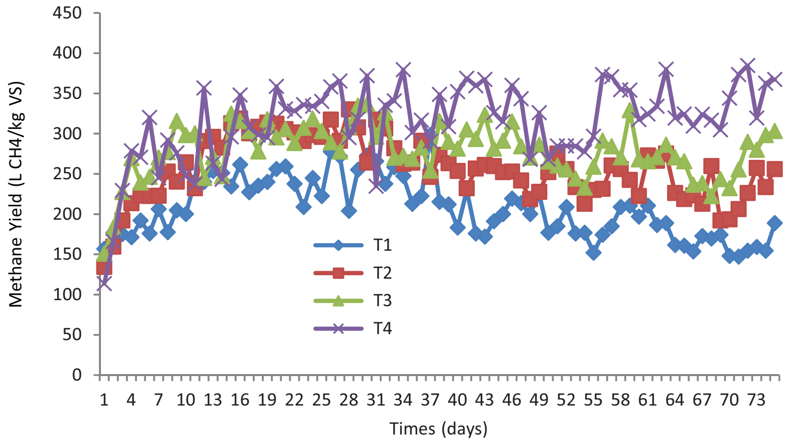
Application of TC as a co-substrate with DCM in this experiment was proven capable to increase significantly ($P < 0.05$) methane production at all ratios (Table 2) compared to that of the control digester. However, according to Angelidaki and Ellegaard (2003), only treatment T3 (which had a ratio of 85% DCM to 15% TC in terms of substrate weight, equal to 0.29 in term of VS substrate),

Table 1. Substrate and inoculum properties

Inoculum/Substrate	TS (%)	VS (%)	Protein (%)	TAN (mg/L)	pH	C/N Ratio	Organic loading rate (kg VS/m^3 d)	TOC (%)
Inoculum	2.85	2.34	na	100	7.33	Na	na	na
Substrate T1	6.89	5.95	0.85	60	7.30	24.42	2.38	3.31
Substrate T2	7.37	6.42	0.97	130	7.27	22.98	2.57	3.57
Substrate T3	7.69	6.79	1.18	100	7.26	19.98	2.72	3.77
Substrate T4	8.05	7.14	1.28	70	7.26	19.38	2.86	3.97
Tofu cake	11.89	11.54	2.37	na	na	16.91	na	na

Note: na: not available.

a)



b)

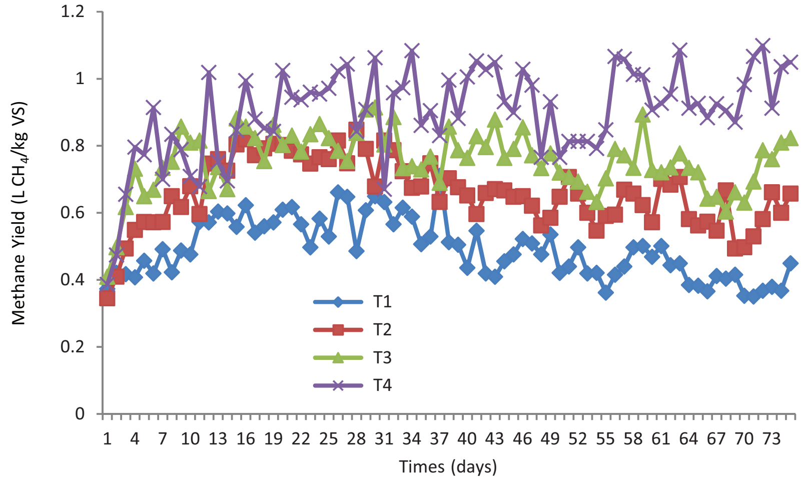


Figure 2. a) Methane yield per kg VS added; b) Methane yield per digester volume per day

can be categorized as a profitable substrate at industrial scale of biogas plant. The guidelines given by Angelidaki and Ellegaard (2003) suggest an economic balance can only be accomplished when the average biogas yield of the substrate in the biogas plant is higher than 30 m^3 of biogas per m^3 of organic waste and about 20 m^3 of methane per m^3 of biomass.

Methane yield (in terms of L/kg VS added) of T1, T2, and T3 was increased by 24.23%; 34.74% and 52.51% compared to the control reactor, while in terms of L/kg substrate weight and L/L active digester volume it was increased by 34.04%; 53.62%, and 83.11%, respectively. The study result showed that the percentage enhancement of methane yield in term of L/kg substrate added and L/L active digester volume was higher than that in term of L/kg VS added. This is due to the co-digestion of DCM increasing the organic matter of the combined substrate. This fact caused the denominator in the calculation of methane yield that presented in form of L kg/VS added in T2, T3, and T4 was higher than that in T1, while both in term of L/kg added and L/L active reactor volume, the denominator value in T2, T3, and T4 was stable (Sutaryo et al., 2021).

The increase in methane production of all treatments compared to the control reactor cannot be separated from the fact that substrate in those digesters having a better nutrient composition (Table 1). Guimarães et al. (2018) reported that the chemical composition of TC is 19.75% TS; 7.91% protein; 6.22% ether extract; 2.44% carbohydrate and 0.86% ash. This fact, therefore can stimulate anaerobic microorganisms' growth and activities in those digesters and subsequently can digest the organic matter in the substrate better and transform it to the gas. Thus, the concentration of intermediate product such as VFA, CO_2 and hydrogen in T2, T3, and T4 will be higher than that in T1 (Table 2). A higher concentration of these intermediate products in the treatment digesters, therefore allow the methanogenic microorganisms to produce a higher amount of methane than that in the control biodigester. Chandra et al. (2012) reported that bioconversion of organic material into biogas take place in four steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The methanogenesis process including: 1) acetoclastic methanogenesis (convert acetate in to CH_4 and CO_2, 2) hydogenotrophic methanogenesis (H_2+CO_2 in to CH_4), and 3) methyltrophic methanogenesis (methanol in to CH_4+H_2O). This result was supported by the methane production in the post digestion tests. Even though the substrate for T2, T3, and T4 had a higher VS concentration than that in T1, methane production of T3 and T4 digested slurry was not significantly different (P > 0.05) than that of T1. Indeed, the methane yield of T2 digested slurry was significantly lower (P < 0.05) than T1 digested slurry.

Utilization of TC as a co-substrate of DCM in this recent study actually reduce the C/N ratio of the combined substrate compare to that in control reactor (Table 1). The C/N ratio of substrate in this study were 24.42, 22.98, 19.98, and 19.38 for the T1, T2, T3, and T4 respectively. This fact was attributed by a higher crude protein content of TC than that in DCM (Table 1). However, C/N ratio in all treatments seem not gave negative affect on the microorganism activities yet. Mao et al. (2015) reported that the optimal C/N ratio in AD process is in the range of 20–30 with a ratio of 25 being the most commonly used. In addition, study of Milner et al. (2014) showed that C/N ratio of cow manure was 23 while in MaciasCorra et al. (2019) study showed that C/N ratio of cattle manure in this study was 21. Therefore, C/N ratio of DCM used in this study was comparable with the previous study results.

TC used in this study had lower TS and protein content than reported by Guimarães et al. (2018). This study used fresh TC from the local tofu industry in the city of Semarang. The difference in TC composition can be caused by different TC pressing methods; the pressing method of TC in Indonesia is by manual pressing, therefore there is still a lot of moisture that has not been removed.

2.2. Variables in the liquid phase

Application of TC as co-substrate with DCM in this study gave no significant (P > 0.05) on the observed variables (Table 2). VFA is an intermediate product in anaerobic digestion, the result of the process of decomposition of monomers and oligomers of organic matter in the acidogenesis stages, which are then converted into methane at the methanogenesis step (Bharathiraja et al., 2018).

A fairly high concentration of acetic acid suggests that there is methanogenic activity while the accumulation of total VFA concentration indicates there is substrate inhibition of acetogenic microorganisms. The mean total VFA concentrations of T1, T2, T3 and T4 digested slurry were 178.75; 206.25; 211.25, and 232.50 mM respectively. Total VFA concentration in this study was stable and low throughout the experimental period.

Similar to VFA, the TAN concentrations of digested slurry from T1, T2, T3, and T4 in this experiment were also low. The concentrations were 210.00; 263.33; 395.00, and 300.00 mg/L for T1, T2, T3, and T4 respectively. The measured values are below the ammonia inhibitory threshold according to Yenigün and Demirel (2013) that for unacclimated starter and in mesophilic temperature (35 °C), the TAN inhibitory threshold is in the concentrations of around 1700–1800 mg/L. Sutaryo et al. (2014) reported that total VFA concentration of digested slurry from a thermophilic bio-reactor (50 °C, 14 d HRT) treating DCM, with a TAN concentration of 2150 mg/L, was 282 mg/L. However, this increased sharply to 2726 mg/L when TAN concentration increased to 3620 mg/L due to urea addition up to 0.35%. Although higher TAN concentrations were measured when more TC was added (Table 2), they were much lower than the previous study and were therefore not expected to cause VFA accumulation.

The pH values of digested slurries in all digesters in this experiment were in the normal-stable normal range. Mao et al. (2015) stated that the ideal pH value for an AD process is in the range of 6.8 to 7.4, while Siddique and Wahid (2018) reported that maintaining pH in between 6.8 and 7.2 is preferable for optimum anaerobic microorganism activities. The mean pH values of digested slurries in this study were 6.88, 6.94, 6.98, and 7.12 for T1, T2, T3, T4, respectively. Wu et al. (2021) reported that various variables can be use as state indicators for process monitoring during AD process including pH value, biogas composition, VFA concentration, alkalinity, and coupled indicators. While Boe et al. (2010) reported that a combination of acetate, propionate and biogas production is an effective combination to monitor a manure digester exposed to different types of disturbances effectively.

VS reduction of T1, T2, T3, and T4 of digested slurries in this study were 30.35, 34.29, 33.24, and 35.38% respectively. There was no negative effect (P > 0.05) of the utilization of TC as co-substrate with DCM on TAN and VS reduction in this study suggesting that TC is more digestible than DCM.

2.3. Post digestion test

Methane production of digested slurry in terms of L/kg VS can be seen in Table 2. Methane production of digested slurry from T2 was significantly (P < 0.05) lower than all other treatments. This suggests that anaerobic microorganisms can decompose organic matter in the substrate to biogas better at this mix ratio than the other treatments.

Table 2. Methane yield, total VFA, TAN concentration, VS reduction and pH value of digested slurry from reactor T1, T2, T3, and T4

Treatments	Methane production			VFA	TAN	VS reduction	pH	Post digestion
	L/kg VS	L/kg substrate	L/L digester volume	mM	mg/L	%		L/kg VS
Control (T1)	206.53^a	12.29^a	0.49^a	178.75	210.00	30.35	6.88	121.70^a
5% TC (T2)	256.58^b	16.48^b	0.66^b	206.25	263.33	34.29	6.94	93.06^b
10% TC (T3)	278.29^c	18.89^c	0.76^c	211.25	395.00	33.24	6.98	124.99^a
15% TC (T4)	314.97^d	22.51^d	0.90^d	232.50	300.00	35.38	7.12	117.18^a

Note: ^{abcd} Values in each column followed by the different letter are significantly different (P < 0.05).

This phenomenon was supported by the fact that the increased methane production per g utilization of TC for this treatment was the highest of all treatments. Per g of TC in the treatment, T1 gave an increase in methane production of 4.85%, while the T2 and T3 treatments were 3.47% and 3.50%, compared to the control reactor. Wu et al. (2021) reported that various variables can be use as state indicators for process monitoring during AD process including pH value, biogas composition, VFA concentration, alkalinity, and coupled indicators. While Boe et al. (2010) reported that a combination of acetate, propionate and biogas production is an effective combination to monitor a manure digester exposed to different types of disturbances effectively. In addition, this result indicates that in order to prevent high methane emissions and to gain the full methane potential of these substrates in a biogas plant, post digestion or a longer HRT is needed.

Conclusions

Utilization of TC with DCM at the levels of 5, 10, and 15 % in terms of substrate weight corresponding to 9.80, 4.64, and 2.92 in terms of VS gave a positive effect on methane production both in terms of kg/VS added and active digester volume. Methane production was increased by 24.23, 34.74, and 52.52% respectively in terms of kg/VS added, while in terms of active digester volume it was increased by 34.04, 53.62, and 83.11% respectively. All digesters were stable in operation, indicated by low VFA and TAN concentration, stable methane production, and normal pH values of digested slurries. Therefore TC can be used to enhance methane production of DCM at least at the ratio of 2.92 in terms of VS.

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Conflict of interest

The authors certify that there is no conflict of interest regarding the material discussed in the manuscript.

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