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by Sutaryo Sutaryo

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Anaerobic co-digestion of tempe wastewater and dairy cow dung

Sutaryo Sutaryo, Solichul Huda, Gideon Arista Toba, Akhid Sofiyull Izza and Edy Rianto

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia 50275
soeta@lecturer.undip.ac.id

Abstract

One of the steps in making tempe is boiling the soybean. During this step, tempe wastewater (TWW), which contains high-quality and digestible nutrients, is produced. The aims of this study were to evaluate the methane production of a digester treating dairy cow manure (DCM) (dairy cow dung (DCD) diluted with tap water) (T0) and another digester treating the mixed substrate of DCD and TWW (T1) and to evaluate the process performance of these two digesters. Compared with the methane production of T0 that of T1 was increased by 200% ($p<0.05$) in terms of L/kg substrate and by 194.29% in terms of L/L digester volume. Volatile fatty acid concentration in both digesters were not significantly different ($p>0.05$), whereas pH, volatile solids reduction and total ammonia nitrogen (TAN) concentration in T1 were significantly higher ($p<0.05$) than those in T0. However, pH values of both digesters were in the ideal range for microorganisms in AD conditions and TAN concentrations were below the TAN inhibition threshold. In this present study, methane was used as fuel in a combine heat and power plant (CHP) and the generated electricity was sold to Indonesia's state electricity company. Therefore, TWW is a suitable as co-substrate of DCD to increase methane production in the AD process.

Keywords: *biogas, co-digestion, dairy manure, methane, tempe wastewater*

Introduction

Tempe (Photo 1) is a traditional fermentation product made from soybean it is originates in Indonesia. It is very popular because of its attractive flavor, texture and high digestibility. In Indonesia, tempe also plays a very important role as an affordable and cheap plant-origin protein source (Romulo and Surya 2021; Aoki et al 2023). According to the Indonesian Ministry of Agriculture (2022), the consumption of tempe in Indonesia was 3.80 and 3.70 kg per capita per year in 2021 and 2022, respectively.

The process of making tempe involves several steps including dehulling, soaking, boiling, inoculating and incubating. The starter used to inoculate the soybean is *Rhizopus* sp. and the incubating step is performed in ambient temperature (Romulo and Surya 2021). In the boiling step (Photo 1), water is heated until it boils and then the soybeans are heated. This step is carried out for 3 h (Ameliya and Falakh 2020). Because the soybean boiling step is performed at high temperature and for a long period, some of the nutrients of the soybean will dissolve in the wastewater. According to Ameliya and Falakh (2020), boiling 1 kg of soybean results in 0.86 L of tempe wastewater (TWW). Tempe production in Indonesia for 2012 was 2.4 million tons per year (Badan Standarisasi Nasional, 2012) and per kg tempe need 0.89 kg of soybean (Ameliya and Falakh, 2020), therefore in 2012, TWW production in Indonesia was 1.84 million m^3. Utilization and handling of TWW that have currently been carried out in Indonesia including being used as mono substrate in AD to produce biogas (Pereiz et al 2023), used it as drinking water for cattle or mixture it with concentrate for cattle feed and as organic fertilizer (Krisnadianto, 2019). However, considering that TWW is easily to undergo in rotting processes, therefore once TWW is produced it must be immediately given to livestock. Otherwise, TWW will undergoing a decay process which can have a negative impact on livestock health.

In AD, TWW can be used as a single substrate or as a co-substrate with another biomass. One of the abundant sources of biomass is livestock manure. The use of TWW as a co-substrate with dairy cow dung (DCD) can increase the nutrient composition and therefore methane production of the mixed substrate. This condition can thus solve the problem of the low biogas production of dairy cow manure (DCM). Thus far, it is well known that biogas production per ton of DCM is relatively low owing to DCM's high water, ash, and crude fiber content that causes low degradability (Li et al 2021). Hence, this imbalance in the nutrient content of DCM should be improved by utilizing another biomass that has better nutrient content. The application of TWW as a co-substrate with DCD is also in accordance with conditions in Indonesia, where the stable floor is tilted toward the gutter so that the livestock dung is separated from the urine. Farmers who intend to handle waste anaerobically must dilute it with tap water (Sutaryo et al 2021). Therefore, the aims of the present study were to evaluate the methane production of digesters treating DCM (DCD diluted with tap water) and the mixed substrate of TWW and DCD in mesophilic conditions and to determine the performance variables of the two biogas digesters.

Materials and methods

This experiment was conducted using two continuous stirred tank reactors (CSTRs) with a 7 L total volume and a 5.25 L working volume. Stirring was performed using a propeller stirrer at a speed of 36 revolutions per minute. The digesters were made of stainless steel and operated at 36°C–37°C and kept in the incubator. Treatments were as follows: T0 (CSTR treating DCM [DCD diluted with tap water with a mixing ratio of 1:1.75]) and T1 (CSTR treating DCD diluted with TWW in the same ratio with the previous digester). The total solids (TS) of treatment T0, which is a control treatment, in this experiment was 5.83%. Song et al (2023) stated that the TS concentration of cattle manure is in the range of 5% to 12%.

Adaptation period

The experiment was started by filling all digesters with 5.250 kg of starter. The starter was digested slurry from an active biogas digester at Diponegoro University Teaching Farm, Semarang, Central Java. Table 1 presents the starter, substrate and TWW characteristics. During the adaptation period, all digesters were fed 238.6 g of basal substrate (DCM) per day after removing the same amount of digested slurry. The DCM came from a lactating dairy cow and was collected from the same farm described previously. The adaptation period was run for 3 weeks, followed by the data collection period for three hydraulic retention times (HRTs), which correspond to 66 days, since in this study, an HRT was equal to 22 days. Mao et al (2015) stated that for the AD process treating agricultural waste and performed in mesophilic conditions, the required HRT is in the range of 15–30 days. At the collection data period, the two reactors were fed according to treatments described previously.

Photo 1. Tempe and the boiling process of soybean in the making process of tempe

Table 1. Starter, tempe wastewater and substrates characteristics

Items	Samples			
	Starter	Tempe wastewater	Substrate T0	Substrate T1
Total solid (%)	5.83	3.55	6.03	9.47
Volatile solid (%)	5.08	2.35	5.30	8.12
Crude protein (%)	na	0.45	0.88	1.26
pH	7.33	6.68	7.33	6.98
C/N ratio	na	18.13	20.91	22.38
Crude fat (%)	na	1.90	na	na

na: not available

Analytical method

During data collection, the biogas produced from each continuous bioreactor was passed through a 500 ml bottle containing a 4% NaOH (Merck®, Cat No. 1064981000, Merck KGaA, Darmstadt, Germany) solution and the remaining methane was collected using a 5 L Tedlar gas bag (Hedetech-Dupont, China) using a 5 mm diameter Teflon hose. The gas volume was measured daily using the liquid displacement method (Sutaryo et al 2020). The pH value of the sample was detected using a digital pH meter (OHAUS®ST 300). TS concentration of the substrate and digested slurry was analyzed by drying the sample at 105°C for 7 h, whereas the ash concentration was calculated by drying TS at 550°C for 7 h to determine the ash content. Volatile solids (VS) concentration was the difference between TS and ash concentrations (APHA, 1995). Total ammonia nitrogen (TAN) content was analyzed via spectrophotometry (HACH DR 3900) using a 10 ml nitrogen–ammonia reagent set (Cat. No. 2668000). Total volatile fatty acids (VFA) concentrations of the digested slurry were analyzed using the steam distillation method. The C/N ratio of the sample was calculated as the ratio of total organic carbon and total nitrogen content. Total organic carbon was calculated by dividing VS sample content by 1.8 (Haug, 1993).

Total nitrogen was determined using the Kjeldahl standard method, whereas crude fat was analyzed using the Soxhlet extraction method. The data were statistically analyzed using the *t*-test with a 95% confidence level.

Results and discussion

The chemical composition of TWW was as follows: 3.55% TS, 2.35% VS, 0.45% crude protein and 1.90% crude fat (Table 1). The results of the chemical analysis indicate that TWW contains some nutrients that can cause environmental pollution if not processed properly. Therefore, to minimize the negative impact of food wastewater on the environment, this waste must be handled properly prior to being discharged into water bodies. Lee and Stuckey (2022) reported that food wastewater contains relatively high concentrations of organic materials such as protein, carbohydrates and lipid. A high concentration of organic materials in wastewater caused it has high chemical oxygen demand and biological oxygen demand properties. In addition, when this wastewater is not treated and directly disposed of to water bodies, the decomposition process of organic matter in this wastewater will run aerobically; however, along with the increasing amount of waste and the reduced availability of dissolved oxygen, the decomposition of organic matter will switch to anaerobic conditions. In these conditions, the greenhouse gas emission will increase more since methane is produced (Nehra and Jain 2023).

Methane yields

Methane production of T0 and T1 and its weekly trend are presented in Table 2 and Figure 1. Methane production in terms of L/kg substrate was 7.59 ± 1.08 and 22.73 ± 6.02 for T0 and T1, respectively, whereas methane production in terms of L/L digester active volume was 0.35 ± 0.05 and 1.03 ± 0.27 for T0 and T1, respectively.

The use of TWW as a co-substrate with DCD was proven to be able to increase ($p<0.05$) methane production in T1 by 200% in terms L/kg substrate and by 194.29% in terms of L/L digester volume. The increase in methane production in T1 treatment compared with that in T0 treatment can be due to the following. First, the use of TWW was able to increase the nutrient content of the final substrate (Table 1) and thus it was able to increase the growth and activity of microorganisms to digest VS in the final substrate. Second, the use of TWW improved the C/N ratio from the final substrate (Table 1). Ajayi-Banji and Rahman (2022) reported that a C/N ratio in the range of 20–30 is optimal for the AD process. In addition, anaerobic co-digestion using multiple substrates has better performance than mono-digestion by increasing substrate digestibility due to synergistic effects of co-substrate, better process stability and can produce better nutrient content in the digested slurry (Karki et al 2021). In addition, Meyer et al (2018) stated that a high crude fiber and water concentration of animal manure caused it is hard for biogas plant to be economically feasible when treating solely of animal manure.

Base on the result of this recent study, that utilization of TWW as co-substrate of DCD can increase methane production of the final substrate by 200% compare to the control and from an economic perspective (Table 3) it was also more profitable, thus it can encourage more dairy farming industry to use TWW as co-substrate with DCD in AD to produce biogas. Therefore, this strategy can be an effort to create an environmentally friendly and sustainable dairy farming industry.

Table 2. Methane yield, total ammonia nitrogen dan volatile fatty acid concentration, volatile solid reduction and pH value

Reactors	Methane production		TAN concentration	VFA concentration	VS reduction	pH value
	L/kg substrate	L/L digester volume	mg/L	mM	%	
T0	7.59 ± 1,08[a]	0.35 ± 0.05[a]	82.25 ± 20.24[a]	104 ± 15.06	34.62 ± 2.27[a]	7.10 ± 0.41[a]
T1	22.73 ± 6.02[b]	1.03 ± 0.27[b]	109.25 ± 27.17[b]	106 ± 14.30	43.20 ± 7.21[b]	7.48 ± 0.32[b]

Means in the same column with different superscripts differ significantly ($p<0.05$)

Process variables

The TAN concentrations of the digested slurry of T0 and T1 were 82.25 ± 25.24 and 109.25 ± 27.17 mg/L, respectively, whereas the pH values were 7.10 ± 0.41 and 7.48 ± 0.32 for T0 and T1, respectively. The VFA concentrations were 104 ± 15.06 and 106 ± 14.30 mM for T0 and T1, respectively, whereas VS reductions were 34.62 ± 2.37 and 43.20 ± 7.21% for T0 and T1, respectively.

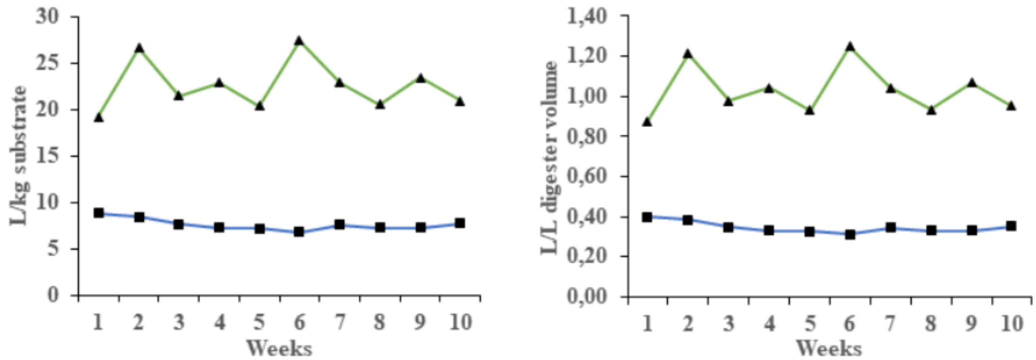

Figure 1. Methane production in term of L/kg substrate and L/L digester volume (■: T0, ▲: T1)

The TAN concentrations of the digested slurry from both digesters are presented in Table 2. The use of TMW as a co-substrate with DCD gave significant effect ($p < 0.05$) as it can increase the TAN content of the digested slurry in T1 compared with that in T0. In anaerobic conditions, ammonia is one of the nutrients needed by an anaerobe microorganism as a source of nitrogen; however, it should not be in high concentration. In the present study, even though the TAN concentration in T1 was higher than that in T0, both values were still below the TAN inhibition threshold in the AD process, which is in the range of 1,500–7,000 mg/L, as reported by Jiang et al (2019). Ammonia is one of the product decompositions of the crude protein in the substrate. A higher TAN content of the digested slurry in T1 corresponds to a higher crude protein content of the T1 substrate than that of the T0 substrate (Table 1). Moreover, compared with the TAN concentration of T0, the higher TAN concentration of T1 has correlation with the pH value of the digested slurry. In this study, the pH value of T1 was also significantly ($p < 0.05$) higher than that of T0. As stated by Liu et al (2021), ammonia has alkaline properties. In anaerobic conditions, ammonia not only has a crucial role as a source of nutrition for anaerobe microorganisms but also has the buffer capacity to maintain pH stability (Li et al 2023). The pH values of the digested slurry from both digesters were appropriate for the AD process. Mao et al (2015) stated that the ideal pH value for anaerobic microorganism activity is in the range of 6.8–7.4.

The VFA concentrations of the digested slurry of the two reactors in the present study are presented in Table 2. Even though the VFA concentration in T1 was higher than that in T0, it was not significantly different ($p > 0.05$). This indicated that microorganisms in both reactors can use VS in the substrate smoothly. The excess nutrient content in the substrate of T1 treatment can still be used and well converted into methane by microorganisms without causing VFA accumulation in the T1 digested slurry. During acidogenesis, the second step of the AD process, VFA, CO_2, ammonia, hydrogen sulfide and other by-products are produced (Mustikasari et al 2023). Furthermore, these products are metabolized by acetogens and generates acetic acid, CO_2 and hydrogen, which will be used by methanogens in the final step of AD to produce methane, CO_2 and water (Paranjpe et al 2023). Increasing the nutrient concentration in the substrate for T1 was able to increase bacterial activity in digesting organic materials; therefore, VS reduction in T1 was significantly higher ($p < 0.05$) than that in T0 (Table 2). Lee and Stuckey (2022) reported that food industry wastewater is rich in organic materials.

Conclusion

The use of TWW as a co-substrate with DCD has been proven to be able to increase methane production by 200% in terms of L/kg substrate compared with that in the reactor treating only DCM. Variables in the AD process were in the ideal range for the activities of anaerobic microorganisms. Thus, TWW can be used as a co-substrate with DCD in the AD process.

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