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Performance Comparison of Single and Two-Phase Biogas Digesters Treating Dairy Cattle Manure at Tropical Ambient Temperature

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ABSTRACT

The biodegradation process of organic waste in anaerobic digestion can be in a single or two-phase bio-reactor. This study examined the effect of different biogas digester configurations (single and two-phase) on methane production of dairy cattle manure (DCM) at tropical ambient temperature. Three identical reactors were used in this study (R1, R2, and R3). The two-phase digesters consisted of reactors R1 and R2. R1 had a 2.1 L working volume and 3 d hydraulic retention time (HRT), while R2 had 5.25 L working volume and 22 d HRT (R1 and R2 had a 25 d HRT). The digested slurry of R1 was used to feed R2. R3 served as the single-phase digester and had 5.25 L working volume and 25 d HRT. Methane production were 14.31, 132.82, and 146 L/kg VS for R1, R2, and R3, respectively. The results showed that there was no positive effect of the application of a two-phase digester configuration on the specific methane yield of DCM per kg volatile solids added than that in the single-reactor. Methane production was detected in the first reactor of the two-phase digester configuration and the total methane production of the two-phase digester was found to be 29.98% higher ($p<0.05$) than that of the single reactor in terms of digester volume (0.41 VS 0.31 L/L/d). Both digester configurations performed well, indicated by a stable methane production and low volatile fatty acids and total ammonia concentrations. The two-phase bio-digester configuration can significantly increase methane production in terms of digester volume.

Keywords: biogas; manure; tropical ambient temperature; two-phase digester biogas

INTRODUCTION

The dairy cattle industry produces large amounts of waste in the form of manure that can cause environmental pollution if it is not managed properly. Daily dairy cattle manure (DCM) (wet feces plus urine) excretion is 2226.5 kg/year per 610 kg of body weight (Noorollahi *et al*., 2015). Generally, animal waste management can take place in aerobic conditions through a composting process or by anaerobic digestion (AD) to produce biogas.

Manure management through the AD process results in numerous advantages, including the generation of renewable energy in the form of biogas. Biogas is the most efficient and effective among the various alternative sources of energy currently available, it needs less capital investment per unit production cost compared to the other renewable energy sources, and it is available as a domestic resource in the rural areas. Therefore, it is not subject to world price fluctuations (Rao *et al*., 2010). In addition, biogas production from animal manure can create new enterprises and increases the income in a rural area since it requires labor for production, collection and transport of AD substrates, manufacture of technical equipment and the construction, operation, and maintenance of biogas plants (Adekunle & Okolie, 2015).

Technically, the AD process can take place in three different temperature ranges: (1) psychrophilic (cryophilic) temperature from 10°C to 20°C; (2) mesophilic temperature from 20°C to 40°C; and (3) thermophilic temperature from 40°C to 60°C (Burton & Turner, 2003). Based on those temperature-range criteria, the AD process can be implemented at tropical ambient temperatures. Moreover, the operation of AD at tropical ambient temperatures offers advantages compared to the operation of AD under mesophilic or thermophilic temperatures since AD operation at higher temperatures requires a significant amount of energy to maintain bioreactor temperature (Bandara *et al*., 2012).

Among the other biogas-digester designs, the continuously stirred-tank reactor (CSTR) design is the most commonly applied bioreactor for treating agricultural waste (Linke *et al*., 2015). While in operation, the process of biodegradation of organic waste can be in single or two-phases. The two-phase AD process has several advantages compared to a single phase. These include the selection and enrichment of different bacteria in each digester, increasing the stability of the process by control-

ing the acidification stage, therefore reducing the risk of overloading and the build up of toxic material. The first stage in the two-phase configuration can act as a metabolic buffer preventing pH shock to the methanogenic microorganisms and low pH in the first stage since a high organic loading rate favors the establishment of the acidogenic phase (Sinbuathong *et al.*, 2012). On the other hand, single-phase biodigester has also advantageous as it is a simple and straightforward operation and for an easier degradable substrate such as fruit and vegetable waste, single-phase process could be the preferred choice rather than two-phase reactor (Ganesh *et al.*, 2014). Although previous studies have evaluated the AD process at ambient temperature (Minale & Worku, 2014; Wei *et al.*, 2014; Murrugan & Appa u, 2018) and two-phase AD (Baldi *et al.*, 2019; Tsigkou *et al.*, 2020), to the best of our knowledge there has been a lack of information regarding to a direct comparison of single and -phase AD of DCM in tropical ambient temperature. Therefore, the aim of this current study was to evaluate the process performance of single and two-phase biodigesters treating DCM and working in this specific area.

MATERIALS AND METHODS

Experimental Set-Up

Evaluation of single and two-phase processes was conducted using three identical digesters, namely R1, R2, and R3. The reactors were made from stainless steel, and in order to minimize temperature fluctuations between day and night times, all digesters were made with double layers. The two-phase digesters consisted of reactors R1 and R2. Reactor 1 had a 2.1 L working volume (the minimum volume that can be applied in the reactor) and 3 d hydraulic retention (HRT), while R2 had 5.25 L working volume and 22 d HRT. Therefore in total, R1 and R2 had a 25 d HRT. R3 served as the single-phase bioreactor and had 5.25 L working volume and 25 d HRT. Mao *et al.* (2015) report that under mesophilic conditions, an average HRT in the range of 15-30 d is required to treat waste.

The experiment was started by filling R1 with 1.4 kg inoculum and 0.7 kg DCM, R2 with 5.011 kg inoculum and 0.239 kg DCM, and R3 with 5.040 kg inoculum and 0.210 kg DCM. From the second day, all digesters were fed as follows: 0.7 kg, 0.239kg, and 0.210 kg DCM for R1, R2, and R3, respectively (after the first removing of the same amount of digestate from a port at the base of the digesters) which continued for the following 21 d adaptation period. The digesters were fed through 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 startup period. During the data collection period, R1 was fed 0.7 kg DCM. Effluent from this digester (0.239 kg) was used to feed R2, while R3 was fed 0.210 kg DCM. Digesters were kept at ambient temperature, and the experiment was run for a period of three HRT corresponding to 75 d in total.

Inoculum and Substrate

Inoculum in this study was obtained from the active biogas digester at the Faculty of Animal and Agricultural Sciences, Diponegoro University. The digester treats DCM and operates at ambient temperature. The digested slurry from the digester was transferred directly to the laboratory scale digesters.

The substrate was taken from dairy cows in the lactation period and was collected from the farm in the Faculty of Animal and Agricultural Sciences, Diponegoro University. Manure was diluted with tap water in the ratio of 1:1.5. Manure was collected once per week and diluted with tap water directly and kept refrigerated. The pH value, volatile solids (VS), and total ammonia nitrogen (TAN) concentration in the inoculum were 7.11, 7.33%, and 265.18 mg/L, respectively, while pH value, VS, TAN, and volatile fatty acids (VFA) (C2-C5) concentrations of DCM were 6.77, 7.40%, 97.98 mg/L, and 142.93 mg/L respectively.

Analytical Methods

Biogas from the laboratory scale bio-digesters was passed up through 0.5 L infusion bottles that contained 4% NaOH solution in order to absorb CO_2 using 5 mL diameter Teflon tubing. Methane production was measured on a daily basis by collecting the gas using 5 L Tedlar gas bags using a water displacement method (Figure 1). The procedures to quantify gas production consisted of 6 steps. 1) The valve to pump was in an open position. 2) The water pump was switched on, therefore, air in the measuring glass headspace was re-

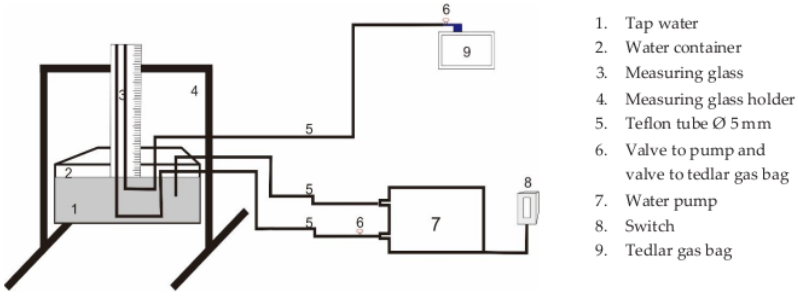

Figure 1. Apparatus for measuring gas production

moved, and the headspace was filled up with tap water. 3) The valve to pump was closed. 4) The water pump was switched off. 5) The valve to Tedlar gas bag was opened therefore the methane in the Tedlar gas bag will move to the headspace of the measuring glass. 6) The gas volume was read in the measuring glass scale. When the gas volume in the Tedlar gas bag exceeded the measuring glass volume, then the steps 1-6 were repeated. The net gas production was corrected to STP conditions.

Daily maximum and minimum ambient temperatures were recorded using a digital hygrometer thermometer HTC-2 (Taiwan). The sample pH value was measured using a pH meter (Hanna® pH meter). Dry matter (DM) contents of samples were analyzed by drying at 105°C for 7 h. Ash was determined by combusting the dried samples at 550°C for 6 h, and VS was calculated by subtracting the ash weight from the DM (APHA,1995). TAN concentration was measured using photometric kits (HACH® USA: DOC316.53.01077) at 655 nm. VFA were determined using gas chromatography (Shimadzu GC-8). The collected data were statistically analyzed manually using ANOVA with 95% confidence level. Duncan's multiple range tests were used in post ANOVA analysis when differences were found to be significant (Gomez & Gomez, 2007).

RESULTS

Ambient Temperature Variation

Average daily maximum-minimum ambient temperatures throughout the experiment were 36.55°C and 20.93°C, respectively (Figure 2). There was 15.63°C temperature difference between the maximum temperature in day time and minimum temperature in the night in this study.

Methane Production

The methane productions of the three bio-digesters throughout the experiment are presented in Figure 7. The mean methane yields were 14.31 L/kg VS, 132.82 L/kg VS, and 146 L/kg VS for R1, R2, and R3 respectively. The total methane yield of R1 and R2 (R_{TS}) was 147.13 L/kg VS (Table 1).

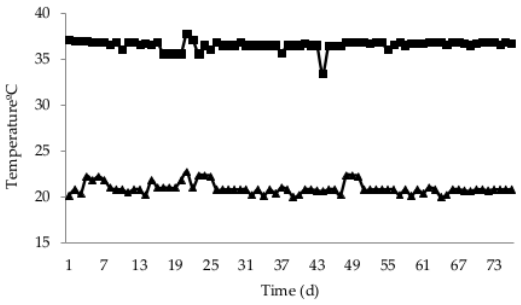

Figure 2. Maximum-minimum ambient temperatures during experiment. ■: maximum, ▲: minimum.

Variables in the Liquid Phase

Total VFA concentration and pH value of digested slurry are presented in Table 1. The mean total VFA concentration was 160.74; 48.23; 39.19 mg/L for R1, R2, and R3, respectively. Total VFA concentration of digested slurry in R1 was significantly higher ($p<0.05$) than that in R2 and R3 (Table 1). TAN concentrations of digested slurry in this study were 137.85; 178.96; and 185.86 mg/L for R1, R2, and R3, respectively (Table 1). Volatile solid reductions in this study were 29.85 and 28.03% for R2 and R3, respectively.

DISCUSSION

Ambient Temperature Variation

This study was performed in July-September, and in Indonesia that period is considered to be in the dry season. A large variation of temperatures in AD operation were found during the course of this study that eventually had an adverse impact on the microorganism activity. Mao *et al*. (2015) report that the AD process is carried out by a prime balanced population of various microorganisms. These microorganisms are very sensitive to environmental condition changes including temperature. Therefore, the ambient temperature in this study (Figure 2) falls into the mesophilic category (Burton & Turner, 2003).

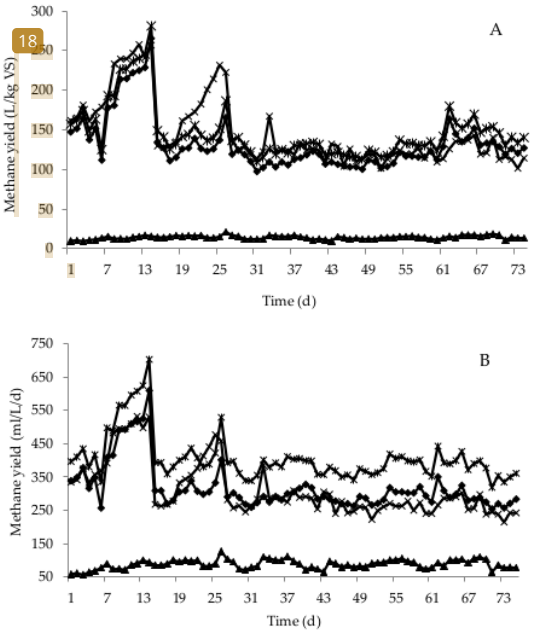

Figure 3. A. Methane yield per kg VS added. B. Methane yield per digester volume per day. ▲: R1, ♦: R2, ×: R3, *: RTS.

Table 1. Methane yield, total VFA, TAN, VS reduction, and pH of some reactors

Reactors	Variables					
	Methane yield		Total VFA	TAN	VS reduction	pH
	(L/kg VS)	(L/L/d)	(mg/L)	(mg/L)	(%)	
R1	14.31±2.29	0.08±0.01	160.74±58.95[a]	137.84±45.32[a]		6.46±0.17[a]
R2	132.82±33.92	0.32±0.07	48.23±23.73[b]	178.96±23.61[b]	29.85±6.76	6.84±0.17[b]
R3	146.65±42.47	0.31±0.08[a]	39.19±23.23[b]	185.86±23.68[b]	28.03±3.19	6.90±0.28[b]
RTS	147.13±34.29	0.41±0.07[b]				

Note: VFA= Volatile fatty acid; TAN= Total ammonia nitrogen; VS= Volatile solid; R1= First reactor of the two-phase digester; R2= Second reactor of the two-phase bio-degester; R3= Single-phase reactor, RTS: Total sum methane yield of R1 and R2. Means in the same column with different superscripts differ significantly ($p<0.05$).

Methane Production

There was no significant effect of the application of a two-phase bio-digester on specific methane yield in terms of kg VS of substrate added when compared to that from the single digester configuration (Table 1). However, methane production of R_{TS} was significantly higher ($p<0.05$) than that in R3 in terms of L/L digester volume (methane production/volume active). The non significant effect of the application of the two-stage digester than single digester on specific methane yield in this study can be due to the activities of anaerobic microorganisms in both reactor configurations operate efficiently. This study used digested slurry from an active digester that operated at a tropical ambient temperature, the same condition used in this study. This fact, along with the three weeks adaptation period, contributed to the efficient microorganism's activity in both reactor configurations in this study even though there was a large temperature difference between day and night time. A study by Chae *et al*. (2008) found that using batch digesters and treating swine manure, the methane production at 30 and 35°C were quite similar, but it was higher by more than 15%-17% than that at 25°C. Temperature shocks caused a reduction in the methane production rate compared to that of the control, but it recovered rapidly. Once adapted, no significant effect on methane production was observed between the control and the temperature shock bio-digester. This fact therefore indicates that, even though methanogenic archaea are quite sensitive to temperature shock, they have considerable abilities to adapt to temperature changes (Chae *et al*., 2008).

A study from Beneragama *et al*. (2013) using batch digesters with 16 d incubation period at 55°C showed that methane production of DCM was 145.03 L/kg VS while the study from Sutaryo *et al*. (2014) using continuous digesters with 20 d HRT at 35°C found that methane production of DCM was 177 L/kg VS. Both studies were performed at a constant mesophilic temperature while the study presented here was performed at ambient variable mesophilic temperatures. However, the result of this study is similar to those of previous results.

Methane production in terms of digester volume of R_{TS} was 29.98% higher than that in R3. The positive effect ($p<0.05$) of the application of two-phase digestion on the methane production compared to that in the single-phase reactor can be attributed to a shorter HRT period in R1 and R2 than that in R3, therefore the amounts of substrate added to R1 and R2 were higher than that in R3. Since the amount of substrate added to R2 (0.239 kg) was higher than that in R3 (0.210 kg) and in the same time, the active volumes in both digester configurations were equal (5.25 L) therefore methane production in term of digester volume R2 was higher than that in R3. In fact, methane production in R_{TS} was the summation of methane yields in R1 and in R2. In this present study, HRT in R1, R2, and R3 were 3 d, 22 d, and 25 d, respectively. Sinbuathong *et al*. (2012) reported that one of the advantages of phase separation is the ability to handle a higher organic loading rate than that in a single reactor. A similar study from Tsigkou *et al*. (2020) found the same phenomenon, that the application of a two-stage digester treating co-digestion of used disposable nappies and expired food product at 60:40 (v/v) ratio, working at mesophilic condition (37±0.5°C) and 15 d HRT, the energy production was 18.5% higher than that in the single reactor.

Variables in the Liquid Phase

During the bioconversion of organic matter in AD system, there are four steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In a two-stage digester configuration, the first digester serves as the acidogenic phase (Sinbuatong *et al*., 2012), therefore the VFA concentration will be higher than that in the second digester. A higher total VFA concentration in R1 than that on the other reactor in this study is in accordance with the report of Baldi *et al*. (2019), who found that the total VFA concentration of digested slurry in a fermentative digester was significantly higher than that of digested slurry from methanogenic digester.

The higher VFA concentration of R1-digested slurry gave consequences on the lower pH value ($p<0.05$) than those in R2 and R3-digested slurry. The mean pH values of digested slurry in this recent study were 6.46; 6.84, and 6.89 for R1, R2, and R3, respectively. The pH values of R2 and R3 in this recent study were in the range of a stable AD process. Mao *et al*. (2015) report that the ideal pH value for AD process is in the range of 6.8 to 7.4.

Ammonia is one of the essential nutrients for the growth microorganisms, however, it can inhibit the AD process if it is available at high concentrations

(Yenigün & Demirel, 2013). Under mesophilic conditions (35°C), the TAN inhibitory threshold was in the concentrations of around 1700–1800 mg/L for unacclimated inoculum (Yenigün & Demirel, 2013). The TAN concentration of digested slurry in R1 was significantly lower ($p<0.05$) than those in R2 and R3. This fact can be attributed to a shorter HRT in R1 than those in R2 and R3 therefore, microorganisms in R2 and R3 can degrade more protein in the substrate, subsequently producing more ammonia. However, TAN concentrations of digested slurry from all digester in this study were below the inhibitory level reported by Yenigün & Demirel (2013).

There was no significant effect of the application of two stages compared to single stage digester on the VS reduction. No significant effect of phase separation on volatile solid reduction in this study suggests that microorganisms in both reactor configurations can work well. Brown and Li (2013) found VS reductions of 27% and 33% for the batch of AD, treating yard waste and combination of 90% yard waste and 10% food waste, respectively, and maintained at 36°C for 30 d. Meanwhile, a study from Sutaryo *et al.* (2012) found a VS reduction in the range of 27-35% for a reactor treating DCM with different TS concentrations. Therefore, the result of this study is in accordance with the result of the previous study.

CONCLUSION

It has been demonstrated that the application of a two-phase digester treating DCM working at a tropical ambient temperature significantly increased methane production by 29.98% compared to the single stage reactor in terms of digester volume. However, there was no positive effect of this digester configuration on specific methane yield in terms of VS. Both digester configurations can run properly with stable methane production, low VFA, and TAN concentrations. Therefore the two-phase digester configuration in tropical ambient temperature can be applied to increase methane production in terms of digester volume.

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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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6