BUKTI KORESPONDENSI

No.	Kegiatan	Tanggal
1.	Pengiriman draft publikasi pertama (Bukti penerimaan draft publikasi dari team editor)	14-4-2020
2.	Permintaan revisi untuk draft yang dikirim ke jurnal	11-5-2020
3.	Perbaikan draft publikasi dari penulis	17-5-2020
4.	Permintaan revisi dari editor terhadap hasil perbaikan pertama	29-6-2020
5.	Perbaikan draft publikasi atas koreksi dari editor	7-7-2020
6.	Draft publikasi dinyatakan diterima untuk dipublikasikan	15-7-2020
7.	Permintaan copyediting dari editor	26-10-2020
8.	Pengiriman draft hasil copyediting dari penulis	30-10-2020
9.	Penerimaan draft hasil copyediting oleh editor	30-10-2020
10.	Permintaan proofreading draft sebelum naskah terbit	24-11-2020
11.	Penulis menyetujui versi proofreading	24-11-2020
12.	Draft memasuki proses produksi untuk penerbitan	25-11-2020
13.	Naskah publikasi terbit	1-12-2020

1. **Pengiriman draft publikasi pertama (Bukti penerimaan draft publikasi dari team editor)**

P

Prof. Dr. Ir. Komang G. Wiryawan<jurnal@apps.ipb.ac.id>

To: sutaryo

Tue 4/14/2020 3:23 PM

sutaryo sutaryo:

Thank you for submitting the manuscript, "A Performance Comparison of Single and Two Phase Biogas Digesters Treating Dairy Cattle Manure at Tropical Ambient Temperature" to Tropical Animal Science Journal. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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Cover letter 1 dan draft versi pertama kali dikirim

The Editor(s), 14th April, 2020

Tropical Animal Science Journal,

Dear Sir/Madam,

Subject: Submission of manuscript titled '**Performance Comparison of Single and Two Phase Biogas Digesters treating Dairy Cattle Manure at Tropical Ambient Temperature'**

- authored by Sutaryo Sutaryo, Aldila Nugrahaini Sempana, Cristina Maria Sri Lestari, Alastair James Ward

We herewith submit our paper with the title **Performance Comparison of Single and Two Phase Biogas Digesters treating Dairy Cattle Manure at Tropical Ambient Temperature**. The manuscript is an original research based on experiments carried out during 2018 at Diponegoro University, Indonesia and has not been previously submitted to any other journal.

The objective of this experiment has been to evaluate the effect of different biogas digester configurations (single and two-phase) on methane production of dairy cattle manure (DCM) at tropical ambient temperature. It has been demonstrated that there was no positive effect with the application of a two-phase digester configuration on specific methane yield of DCM per kg volatile solids added. Methane production was detected in the first reactor of the two-phase digester configuration and the total sum methane production of the two-phase digester was found to be 29.98% higher than that of the single reactor in terms of digester volume. Both digester configurations performed well, indicated by stable methane production, low volatile fatty acids and total ammonia concentrations.

We agree on the contents of the manuscript and would like to submit our manuscript to **'Tropical Animal Science Journal'**

Thank you and best regards,

On behalf of all the authors,

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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

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. The results showed that there was no positive effect with the application of a two-phase digester configuration on specific methane yield of DCM per kg volatile solids added. Methane production was detected in the first reactor of the two-phase digester configuration and the total sum 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. Both digester configurations performed well, indicated by stable methane production, low volatile fatty acids and total ammonia concentrations.

Key words: 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 not managed properly. Daily dairy cattle manure (DCM) (wet feces plus urine) excretion is 89.0; 65.9; 34.8 kg/d per 1000 kg of body weight for those that produce 29 and 14 kg/d of milk and for non-lactating dairy cows, respectively (Wilkerson *et al*., 1997). Generally, animal waste management can take place in aerobic condition 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 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 creates new enterprises and increases the income in rural area since it requires labour for production, collection and transport of AD substrates, manufacture of technical equipment and the construction, operation and maintenance of biogas plants (Adekunle and Okolie, 2015).

Technically, the AD process can take places in three different temperature ranges: (1) psychrophilic (cryophilic) temperature from 10 to 20°C; (2) mesophilic temperature from 20 to 40°C; and (3) thermophilic temperature from 40 to 60°C (Burton and Turner, 2003). Based on those temperature range criteria, the AD process can be implemented at tropical ambient temperatures. Moreover, 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 require significant energy to maintain bioreactor temperature (Bandara *et al*., 2012).

Among 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 single phase. These include the selection and enrichment of different bacteria in each digester, increasing the stability of the process by controlling the acidification stage therefore reducing the risk of overloading and the buildup of toxic material. The first stage in the two-phase configuration can act as metabolic buffer preventing pH shock to the methanogenic microorganisms and low pH in the first stage due to a high organic loading rate favours the establishment of the acidogenic phase (Sinbuathong *et al*., 2012). Although previous studies have evaluated the AD process at ambient temperature (Minale and Worku, 2014; Wei *et al*., 2014; Murrugan and Appavu, 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 direct comparison of single and two phase AD of DCM at tropical ambient temperature. Therefore the aim of this current study was to further knowledge in this specific area.

MATERIALS AND METHODS

Experimental Set Up

Evaluation of single and two phase processes was conducted using three identical digesters (R1, R2, R3). The reactors were made from stainless steel and in order to minimize temperature fluctuations between day and night time, 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) reported 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, 0.239 and 0.210 kg DCM for R1, R2 and R3 respectively (after first removing 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 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 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 sourced 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 digestate slurry from the digester was transferred directly to the laboratory scale digesters.

Substrate was taken from dairy cows in the lactation period and was collected from the farm in Faculty of Animal and Agricultural Sciences, Diponegoro University. Manure was diluted with tap water in the ratio 1:1.5. Manure was collected once per week and diluted with tap water directly and kept refrigerated. 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) concentration 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 were: 1) valve to pump was in open position. 2) water pump was switched on, therefore air in the measuring glass head space was removed and the head space was filled up with tap water. 3) valve to pump was closed. 4) water pump was switched off. 5) valve to tedlar gas bag was opened therefore the methane in the tedlar gas bag will move to the head space of the measuring glass. 6) gas volume was read in the measuring glass scale. When the gas volume in the tedlar gas bag exceeded the measuring glass volume, steps 1-6 were repeated. The net gas production was corrected to STP conditions.

Daily maximum and minimum ambient temperature was recorded using a digital hygrometer thermometer HTC-2 (Taiwan). 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 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 and Gomez, 2007).

RESULTS

Ambient Temperature Variation

Average daily maximum-minimum ambient temperature throughout the experiment was 36.55°C and 20.93°C respectively (Figure 2). There was 15.63°C temperature difference between maximum temperature in day time and minimum temperature in the night time and the ambient temperature in this study therefore falls into the mesophilic category (Burton and Turner, 2033).

Methane production

The methane production of the three bio-digesters throughout the experiment is presented in Figure 3. 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 Total methane yield of R1 and R2 (R_{TS}) was 147.13 L kg/VS (Table 2). A study from Amon *et al*. (2007) using batch digesters with 60 d incubation period at 38°C showed that methane production of DCM from cows with medium milk production was 166.3 N 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 those studies were performed at constant mesophilic temperature while the study presented here was performed at ambient variable mesophilic temperatures, however the result of this study is similar with those previous results.

Parameters 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 concentration concentrations of digested slurry in this study were 137.85; 178.96; 185.86 mg/L for R1, R2 and R3 respectively (Table 2).

Volatile solid reductions in this study were 29.85 and 28.03% for R2 and R3 respectively. Study from Bhattacharya *et al*. (1996) found a VS reduction of 26% and 30% for conventional and two-phase digester respectively treating sludges from conventional activated sludge treatment plants and maintained at 35°C. Meanwhile a study from Sutaryo *et al*. (2012) found a VS reduction in 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.

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 temperature in AD operation in the course of this study therefore has an adverse impact on the microorganism activity. Sakar *et al*. (2009) reported 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.

Methane production

There was no significant effect ($p>0.05$) 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 RTS was significantly higher ($p<0.05$) than that in R3 in term of L/L digester volume (methane production/volume active). No significant effect ($p>0.05$) of the application of the two-stage digester than single digester on specific methane yield in this study can be due to anaerobic microorganisms activities in both reactors configuration operation 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 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 13-17% 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 the 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 ability to adapt to temperature changes (Chae *et al*., 2008).

Methane production in term of digester volume of RTS 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 amount of substrate added to R1 and R2 was 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 volume in both digester configurations was equal (5.25 L) therefore methane production in term of digester volume R2 was higher than that in R3. In fact methane production in RTS was methane yield in R1 and in R2. In this present study, HRT in R1, R2 and R3 were 3 d, 22 d and 25 d respectively. Bhattacharya *et al*. (1996) 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, in 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.

Parameters in the Liquid Phase

During 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 other reactor in this study is in accordance with Baldi *et al*. (2019) who found that 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 that 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 value of R2 and R3 in this recent study was in the range of a stable AD process. Mao *et al*. (2015) reported 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 of microorganisms, however it can inhibit the AD process if it is available at high concentrations (Yenigün and Demirel, 2013). Under mesophilic conditions (35°C), the TAN inhibitory threshold was in the concentrations of around 1700–1800 mg L-1 for unacclimated inoculum (Yenigün and Demirel, 2013). TAN concentration of digested slurry in R1 was significantly lower ($p<0.05$) than that in R2 and R3. This fact can be attributed to a shorter HRT in R1 than that 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 as reported by Yenigün and Demirel (2013).

There was no significant effect ($p>0.05$) of the application two stage compared to single stage digesters 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.

CONCLUSION

It has been demonstrated that the application of a two-phase digester treating DCM working at tropical ambient temperature significantly increased methane production by 29.98% compare to the single stage reactor in term of digester volume. However, no significant 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 term 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 organization related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

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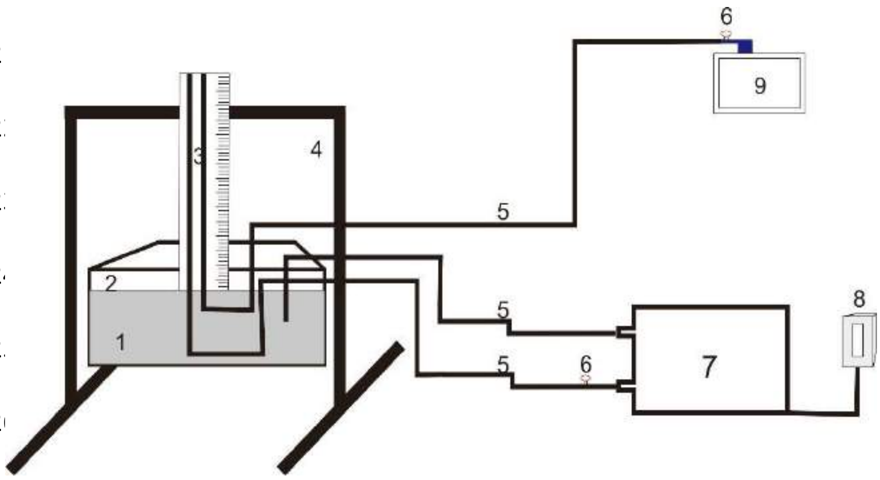
Figure caption



1. Tap water
2. Water container
3. Measuring glass
4. Measuring glass holder
5. Teflon tube Ø 5 mm
6. Valve to pump and valve to tedlar gas bag
7. Water pump
8. Switch
9. Tedlar gas bag

Figure 1. Apparatus for measuring gas production

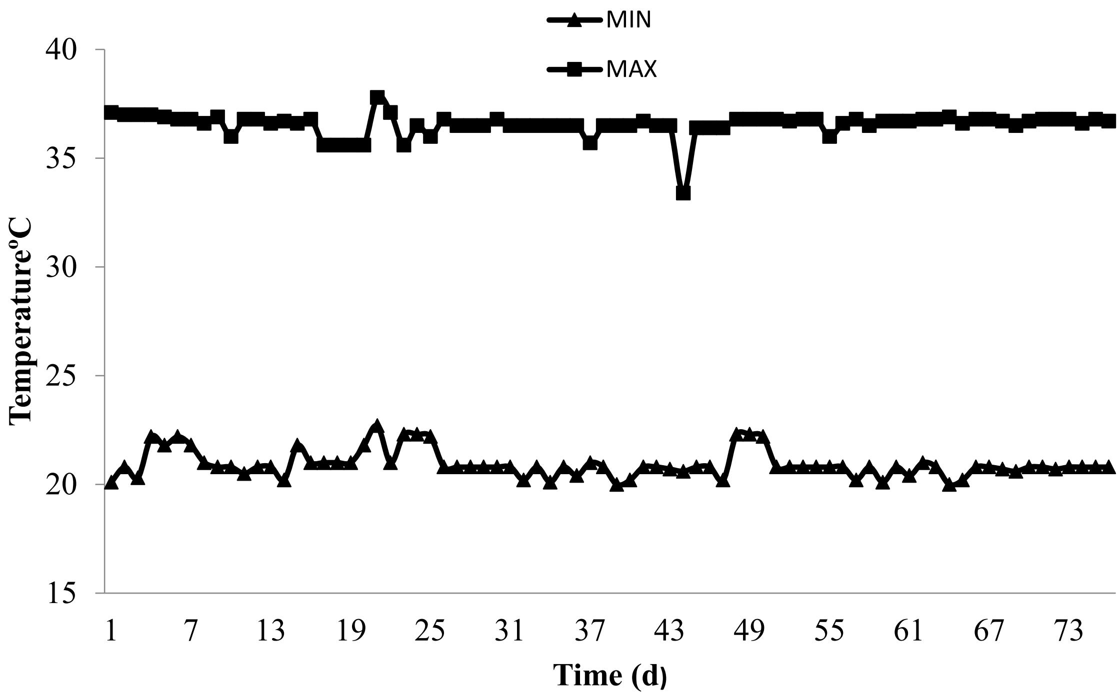


Figure 2. Maximum-minimum ambient temperature during experiment

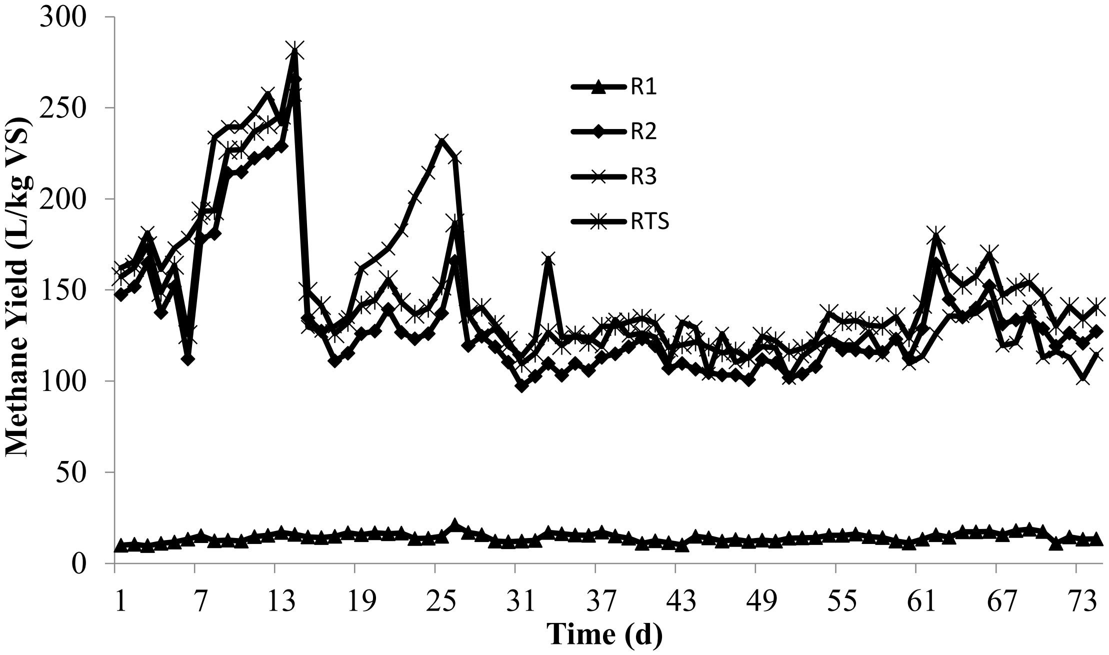


Figure 3. A. Methane yield per kg VS added.

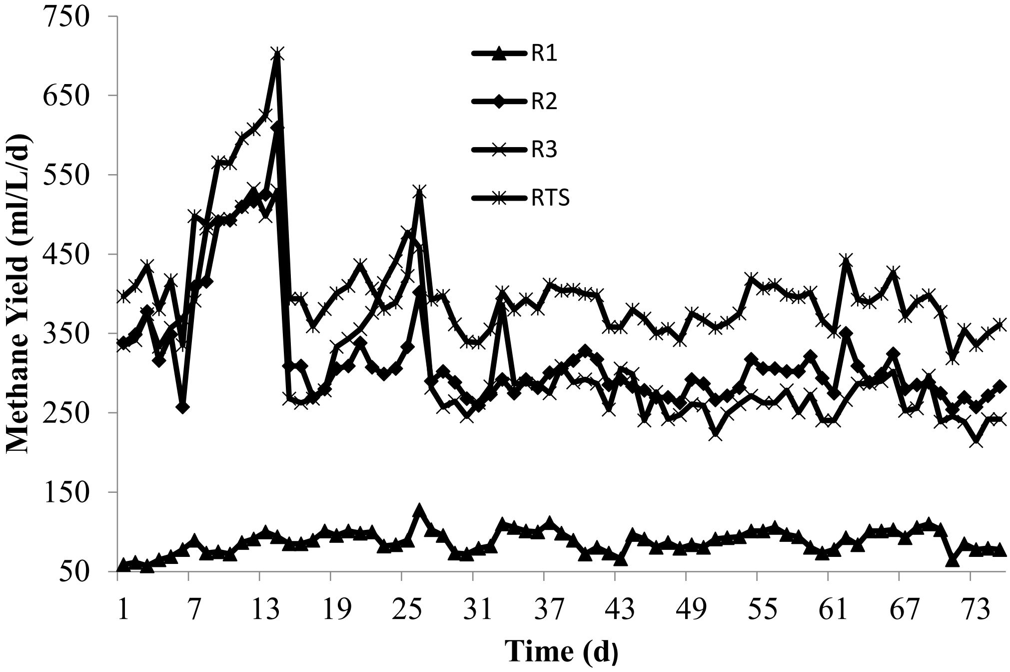


Figure 3. B. Methane yield per digester volume per day

Table caption

Table 1. Process parameters. Values in each column followed by the same letter are not significantly different ($p > 0.05$).

	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^{a}±58.95	137.84^{a}±45.32		6.46^{a}±0.17
R2	132.82±33.92	0.32±0.07	48.23^{b}±23.73	178.96^{b}±23.61	29.85^{a}±6.76	6.84^{b}±0.17
R3	146.65^{a}±42.47	0.31^{a}±0.08	39.19^{b}±23.23	185.86^{b}±23.68	28.03^{a}±3.19	6.90^{b}±0.28
R_{TS}	147.13^{a}±34.29	0.41^{b}±0.07				

2. Permintaan revisi untuk draft yang dikirim ke jurnal

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Dear sutaryo sutaryo, Aldila Sempana, Cristina Lestari, Alastair Ward:

It is my pleasure to inform you that your submission to Tropical Animal Science Journal, "A Performance Comparison of Single and Two Phase Biogas Digesters Treating Dairy Cattle Manure at Tropical Ambient Temperature" had been examined by peer reviewers. Please find the comments and suggestions:

Submission URL: http://journal.ipb.ac.id/index.php/tasj/authorDashboard/submission/30343

If you decide to revise the manuscript, please give a response or rebuttal against each point which are suggested by peer reviewers. The revised document should include revision note file in table form and revised manuscript in MS Word file. Please return back the documents to the editor within 14 days via OJS, we would be glad if you submit your revised manuscript as soon as possible.

If you have any questions, please contact me.

Prof. Dr. Komang G Wiryawan

Chief Editor

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PAPER EVALUATION

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

Comments (please use additional paper if more space is needed)

No	Comments	Author's response
A	**Reviewer I (MB1)**	
1	The abstract should contain statements of introduction, objective, methods, results, and conclusion. Please revise it.	
2	Please state the novelty of the research in the Introduction before resuming the objectives.	
3	In conclusion, you should state the the prospect of your research and novelty. The conclusion should be written briefly in a single paragraph but reflects the experimental results obtained and in sync with the objectives.	
B	**Reviewer II (MB2)**	
1	This research has described the Comparison of Single and Two-Phase Biogas Digesters performance using Dairy Cattle Manure as a substrate in Tropical Ambient Temperature. Several research indicators, including methane production, Total Volatile Fatty Acid, Total Ammonia Nitrogen, etc. has been presented in this paper. The manuscript has been written in good English and also showing good scientific merit. However, I consider these several matters:	
2	The abstract should describe research design, methods, results, statistical analysis of research parameters, and conclusion, which is unsown in the manuscript.	
3	In the Introduction section, since this research wants to compare single and two-phase of biodigester equally, this section should describe the advantage of a single-phase bioreactor also.	

4	In the Material and Methods section, especially in Experimental Set-Up, the information about R1, R2, R3, and RTS is unclear. How many replications used in this study? What is RTS, which is shown in several figures and tables? There is insufficient information about this abbreviation.	
5	In Figure 1, the resources of biogas should be shown in this design to indicate the one and two-phase of the biodigester.	
6	Table 1 should have a clear title, and sufficient information about treatment initial (what is R1, R2, R3, and RTS). The information on statistical analysis should not be the title of the table.	
7	References a) Please ensure that every reference cited in the text is also present in the reference list (and vice versa). We suggest authors to use reference manager applications, such as EndNote, Mendeley, etc., to prepare citations and the list of references. b) References should be the last 10 year publications, with minimum 80% of journals.	
8	Please see other comments in the text	

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

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ABSTRACT

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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. The results showed that there was no positive effect with the application of a two-phase digester configuration on specific methane yield of DCM per kg volatile solids added. Methane production was detected in the first reactor of the two-phase digester configuration, and the total sum 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. Both digester configurations performed well, indicated by stable methane production, low volatile fatty acids, and total ammonia concentrations.

Keywords: biogas, manure, tropical ambient temperature, two-phase digester biogas.

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INTRODUCTION

The dairy cattle industry produces large amounts of waste in the form of manure that can cause environmental pollution if not managed properly. Daily dairy cattle manure (DCM) (wet feces plus urine) excretion is 89.0; 65.9; 34.8 kg/d per 1000 kg of body weight for those that produce 29 and 14 kg/d of milk and for non-lactating dairy cows, respectively (Wilkerson *et al*., 1997). 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 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 and Okolie, 2015).

Technically, the AD process can take places in three different temperature ranges: (1) psychrophilic (cryophilic) temperature from 10 to 20°C; (2) mesophilic temperature from 20 to 40°C; and (3) thermophilic temperature from 40 to 60°C (Burton and Turner, 2003). Based on those temperature range criteria, the AD process can be implemented at tropical ambient temperatures. Moreover, operation of AD at tropical ambient temperatures offers advantages compared to the operation of AD under

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mesophilic or thermophilic temperatures since AD operation at higher temperatures requires significant energy to maintain bioreactor temperature (Bandara *et al*., 2012).

Among 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 controlling the acidification stage therefore reducing the risk of overloading and the buildup of toxic material. The first stage in the two-phase configuration can act as metabolic buffer preventing pH shock to the methanogenic microorganisms and low pH in the first stage due to a high organic loading rate favors the establishment of the acidogenic phase (Sinbuathong *et al*., 2012). Although previous studies have evaluated the AD process at ambient temperature (Minale and Worku, 2014; Wei *et al*., 2014; Murrugan and Appavu, 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 direct comparison of single and two-phase AD of DCM at tropical ambient temperature. Therefore the aim of this current study was to further knowledge in this specific area.

MATERIALS AND METHODS

Experimental Set-Up

Evaluation of single and two-phase processes was conducted using three identical digesters (R1, R2, R3). The reactors were made from stainless steel, and in order to minimize temperature fluctuations between day and night time, all digesters

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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) reported 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, 0.239, and 0.210 kg DCM for R1, R2 and R3 respectively (after first removing 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 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 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 sourced 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.

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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 1:1.5. Manure was collected once per week and diluted with tap water directly and kept refrigerated. 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) concentration 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 were: 1) valve to pump was in open position. 2) water pump was switched on. Therefore air in the measuring glass headspace was removed, and the headspace was filled up with tap water. 3) valve to pump was closed. 4) water pump was switched off. 5) 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) gas volume was read in the measuring glass scale. When the gas volume in the Tedlar gas bag exceeded the measuring glass volume, steps 1-6 were repeated. The net gas production was corrected to STP conditions.

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Daily maximum and minimum ambient temperature was recorded using a digital hygrometer thermometer HTC-2 (Taiwan). 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 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 and Gomez, 2007).

RESULTS

Ambient Temperature Variation

Average daily maximum-minimum ambient temperature throughout the experiment was 36.55°C and 20.93°C, respectively (Figure 2). There was 15.63°C temperature difference between maximum temperature in day time and minimum temperature in the night time and the ambient temperature in this study therefore falls into the mesophilic category (Burton and Turner, 2033).

Methane production

The methane production of the three bio-digesters throughout the experiment is presented in Figure 3. 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 Total methane yield of R1 and R2 (R_{TS}) was 147.13 L kg/VS (Table 2). A study from Amon *et al.* (2007) using batch digesters with 60 d incubation period at 38°C showed that methane production of DCM from cows with medium milk production was 166.3 N L kg/VS while the study from

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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 those studies were performed at 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 previous results.

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Parameters 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 concentration concentrations of digested slurry in this study were 137.85; 178.96; 185.86 mg/L for R1, R2, and R3, respectively (Table 2).

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Volatile solid reductions in this study were 29.85 and 28.03% for R2 and R3 respectively. Study from Bhattacharya *et al*. (1996) found a VS reduction of 26% and 30% for conventional and two-phase digester respectively, treating sludges from conventional activated sludge treatment plants and maintained at 35°C. Meanwhile, a study from Sutaryo *et al*. (2012) found a VS reduction in 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.

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 temperature in AD operation in the course of this study therefore has an adverse impact on the microorganism activity. Sakar *et al*. (2009) reported that the AD process is carried out by a balanced prime population of various microorganisms. These microorganisms are very sensitive to environmental condition changes including temperature.

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Methane production

There was no significant effect ($p>0.05$) 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 RTS was significantly higher ($p<0.05$) than that in R3 in term of L/L digester volume (methane production/volume active). No significant effect ($p>0.05$) of the application of the two-stage digester than single digester on specific methane yield in this study can be due to anaerobic microorganisms activities in both reactors configuration operation 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 13-17% than that at 25°C.

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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 the 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 ability to adapt to temperature changes (Chae *et al*., 2008).

Methane production in term of digester volume of RTS 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 amount of substrate added to R1 and R2 was 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 volume in both digester configurations was equal (5.25 L) therefore methane production in term of digester volume R2 was higher than that in R3. In fact methane production in RTS was methane yield in R1 and in R2. In this present study, HRT in R1, R2 and R3 were 3 d, 22 d and 25 d respectively. Bhattacharya *et al.* (1996) 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, in 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.

Parameters in the Liquid Phase

During 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 other reactor in this study is in accordance with Baldi *et al*. (2019) who found that 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 that 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 value of R2 and R3 in this recent study was in the range of a stable AD process. Mao *et al*. (2015) reported 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 of microorganisms, however it can inhibit the AD process if it is available at high concentrations (Yenigün and Demirel, 2013). Under mesophilic conditions (35°C), the TAN inhibitory threshold was in the concentrations of around 1700–1800 mg L-1 for unacclimated inoculum (Yenigün and Demirel, 2013). TAN concentration of digested slurry in R1 was significantly lower ($p<0.05$) than that in R2 and R3. This fact can be attributed to a shorter HRT in R1 than that 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 as reported by Yenigün and Demirel (2013).

There was no significant effect ($p>0.05$) of the application two stage compared to single stage digesters 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.

CONCLUSION

It has been demonstrated that the application of a two-phase digester treating DCM working at tropical ambient temperature significantly increased methane production by 29.98% compare to the single stage reactor in term of digester volume. However, no significant 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 term 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 organization related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

The authors would like to thank Diponegoro University (grant number: 109/UN7.5.5/PP/2018) for financing this study.

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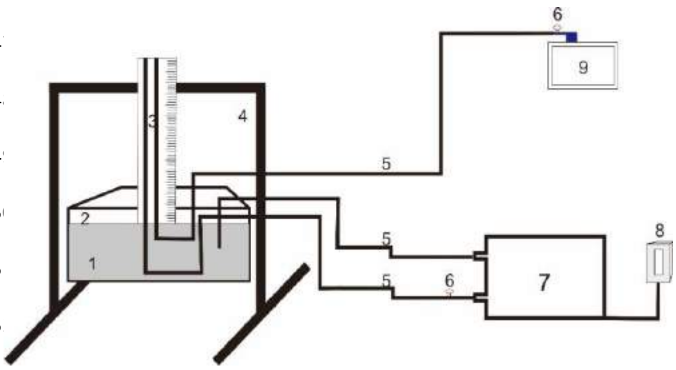
Figure caption



1. Tap water
2. Water container
3. Measuring glass
4. Measuring glass holder
5. Teflon tube Ø 5 mm
6. Valve to pump and valve to tedlar gas bag
7. Water pump
8. Switch
9. Tedlar gas bag

Figure 1. Apparatus for measuring gas production

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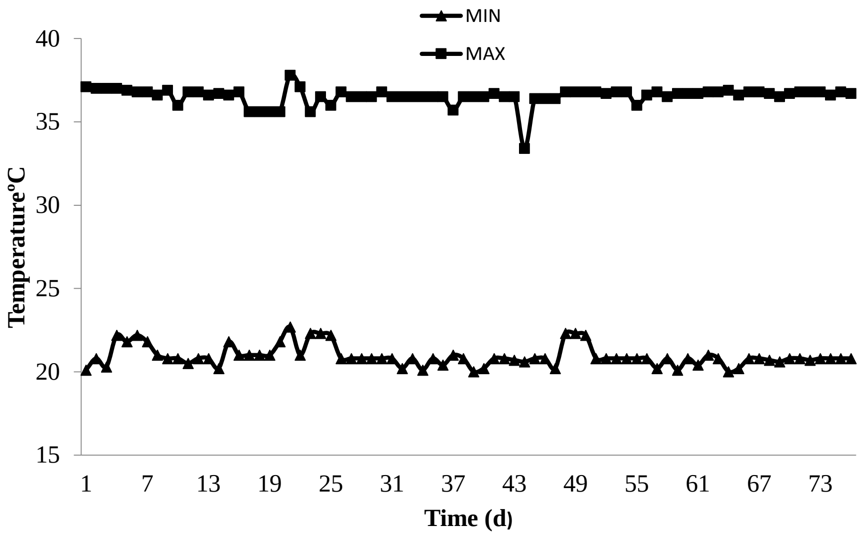

Figure 2. Maximum-minimum ambient temperature during experiment

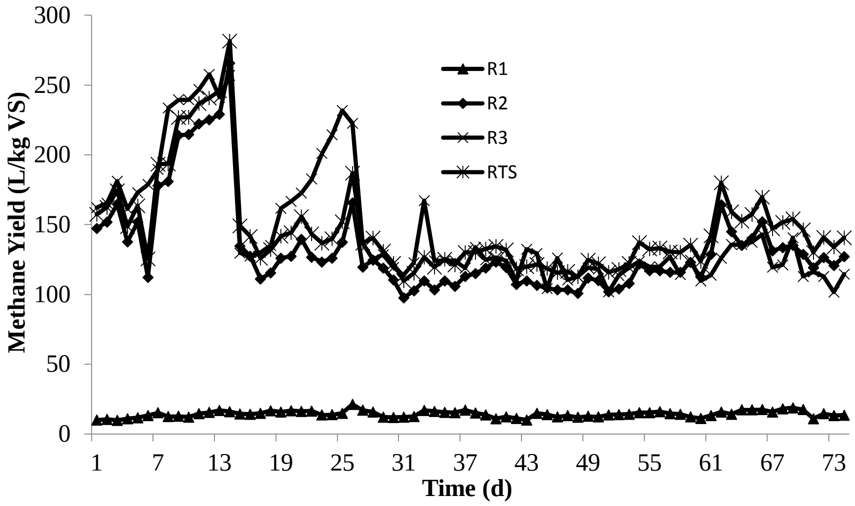

Figure 3. A. Methane yield per kg VS added.

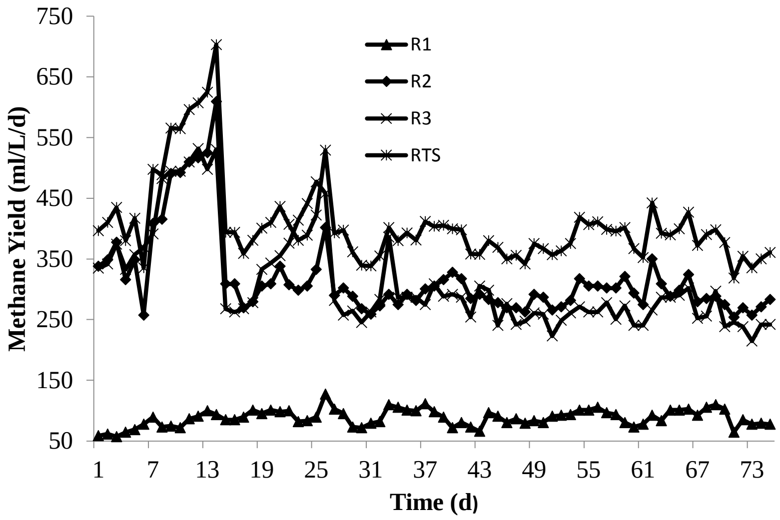

Figure 3. B. Methane yield per digester volume per day

Table caption

Table 1. Process parameters. Values in each column followed by the same letter are not significantly different ($p > 0.05$).

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

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3. **Perbaikan draft publikasi dari penulis**

Surat pengantar, respon penulis dan draft publikasi versi revisi

Dear Prof. Dr. Komang G. Wiryawan 17th May 2020
The Editor,
Tropical Animal Science Journal,

Subject: Resubmission of manuscript titled '**Performance Comparison of Single and Two-Phase Biogas Digester Treating Dairy Cattle Manure at Tropical Ambient Temperature'** **(**manuscript number: TASJ_30343**)**
- authored by Sutaryo Sutaryo, A. N. Sempana, C. M. S. Lestari, Alastair James Ward

We herewith resubmit our paper with the title **Performance Comparison of Single and Two-Phase Biogas Digesters Treating Dairy Cattle Manure at Tropical Ambient Temperature**. The manuscript is an original article based on the experiments carried out during 2018 at Faculty Animal and Agricultural Sciences, Diponegoro University.

Thank you for the opportunity you provide us to resubmit our manuscript. The authors greatly appreciate the constructive comments given by both the reviewers. In the attached new version of the manuscript (revised manuscript), changes in response to the reviewers' comments have been highlighted using blue text.

I thank you for your time and consideration, and look forward to hearing from you.

Thank you and best regards,

On behalf of all the authors,

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PAPER EVALUATION

Paper Title: Performance Comparison of Single and Two Phase Biogas Digester Treating Dairy Cattle Manure at Tropical Ambient Temperature

Comments (please use additional paper if more space is needed)

No	Comments	Author's response
A	**Reviewer I (MB1)**	The authors tanks for the valuable and constructive comments given by reviewers. The manuscript has been revised carefully. All comments have been addressed by either accepting them or giving more information and discussion and those are highlighted with dark blue colour.
1	The abstract should contain statements of introduction, objective, methods, results, and conclusion. Please revise it.	Abstract has been revised according reviewer suggestion: The biodegradation process of organic waste in anaerobic digestion can be in 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 (p>0.05) with the application of a two-phase digester configuration on

		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 sum 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 stable methane production, low volatile fatty acids, and total ammonia concentrations. The two-phase bio-digester configuration can increase significantly methane production in terms of digester volume.
2	Please state the novelty of the research in the Introduction before resuming the objectives.	This information has been written by the author in the introduction section. There has been a lack of information regarding direct comparison of single and two-phase AD of DCM at tropical ambient temperature
3	In conclusion, you should state the the prospect of your research and novelty. The conclusion should be written briefly in a single paragraph but reflects the experimental results obtained and in sync with the objectives.	Conclusion has been revised according reviewer suggestion. 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, no significant 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. However, to be implemented on an industrial scale, further study is needed related to the addition of infrastructure to the two-phase digester rather than the single reactor.
B	**Reviewer II (MB2)**	
1	This research has described the Comparison of Single and Two-Phase Biogas Digesters performance using Dairy Cattle Manure as a substrate in Tropical Ambient Temperature.	

	Several research indicators, including methane production, Total Volatile Fatty Acid, Total Ammonia Nitrogen, etc. has been presented in this paper. The manuscript has been written in good English and also showing good scientific merit. However, I consider these several matters:	
2	The abstract should describe research design, methods, results, statistical analysis of research parameters, and conclusion, which is unsown in the manuscript.	Abstract has been revised according reviewer suggestion: The biodegradation process of organic waste in anaerobic digestion can be in 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 ($p>0.05$) with the application of a two-phase digester configuration on 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 sum 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 stable methane production, low volatile fatty acids, and total ammonia concentrations. The two-phase bio-digester configuration can increase significantly methane production in terms of digester volume.

3	In the Introduction section, since this research wants to compare single and two-phase of biodigester equally, this section should describe the advantage of a single-phase bioreactor also.	The advantage of a single-phase bioreactor has been added in the introduction section. On the other hand, single-phase bio-digester has also advantageous as it is a simple and straightforward operation and for easier degradable substrate such as fruit and vegetables waste single-phase process could be the preferred rather than two-phase reactor (Ganesh *et al*., 2014).
4	In the Material and Methods section, especially in Experimental Set-Up, the information about R1, R2, R3, and RTS is unclear. How many replications used in this study? What is RTS, which is shown in several figures and tables? There is insufficient information about this abbreviation.	Thank you very much for the corrections. - R1, R2 and R3 is the name of digester, this information has been added. Evaluation of single and two-phase processes was conducted using three identical digesters, namely R1, R2, R3. - R_{TS} is total sum methane production of R1 and R2. This information is in L153-154 P 8. Total methane yield of R1 and R2 (R_{TS}). - The replication in this study is the data collection period for three hydraulic retention time (HRT). It is common in biogas study therefore more than 95% of inoculum has been replaced by substrate rather than using replication in the number of the digester. This information is in materials and methods section L102-103 P5. Digesters were kept at ambient temperature, and the experiment was run for a period of three HRT corresponding to 75 d in total.
5	In Figure 1, the resources of biogas should be shown in this design to indicate the one and two-phase of the biodigester.	Methane production from both digesters configuration is collected using a Tedlar gas bag. When measuring the gas volume, the Tedlar gas bag is removed from the digester series after the valve is closed first. Then the gas bag is connected to the apparatus. Thus the circuit is only used to measure the production of gas in the gas bag. Therefore, it is not connected to the gas resource in all time.
6	Table 1 should have a clear title, and sufficient information about treatment initial (what is R1, R2, R3, and RTS). The information on statistical analysis should not be the title of the table.	Thank you very much for the correction. Table 1 has been revised according reviewer suggestion.

<table>
<tr><td>7</td><td>References

c) Please ensure that every reference cited in the text is also present in the reference list (and vice versa). We suggest authors to use reference manager applications, such as EndNote, Mendeley, etc., to prepare citations and the list of references.
d) References should be the last 10 year publications, with minimum 80% of journals.</td><td>- Thank you for the reviewer suggestion. Reference has been check.
- Some old references have been replaced with new ones.
1. Amon, T., B. Amon, V. Kryvoruchko, W. Zollitsch, K. Mayer, & L. Gruber. 2007. Biogas production from maize and dairy cattle manure-Influence of biomass composition on the methane yield. Agric Ecosyst Environ. 118:173-182.
Replaced by:

Beneragama, N., S. A. Lateef, M. Iwasaki, T. Yamashiro, & K. Umetsu. 2013. The combined effect of cefazolin and oxytertracycline on biogas production from thermophilic anaerobic digestion of dairy manure. Bioresource Biotechnol. 133:23-30.

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Replaced by:

Brown, D. & Y. Li. 2013. Solid state anaerobic co-digestion of yard waste and food waste for biogas production. Bioresour. Technol. 127:275-280.

4. Sakar, S., K. Yetilmezsoy, & E. Kocak. 2009. Anaerobic digestion technology in poultry and livestock waste treatment – a literature review. Waste Manag. Res. 27:3-18.
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Mao, C., Y. Feng, X. Wang, & G. Ren. 2015. Review on research achievements of biogas from anaerobic digestion. Renew. Sust. Energ. Rev. 45:540-555.</td></tr>
<tr><td>8</td><td>Please see other comments in the text</td><td>Thank you very much for the valuable comments. The manuscript has been revised according reviewer comments.</td></tr>
</table>

Performance Comparison of Single and Two-Phase Biogas Digester 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 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 ($p>0.05$) with the application of a two-phase digester configuration on 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 sum 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 stable methane production, low volatile fatty acids, and total ammonia concentrations. The two-phase bio-digester configuration can increase significantly 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 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 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 and Okolie, 2015).

Technically, the AD process can take places in three different temperature ranges: (1) psychrophilic (cryophilic) temperature from 10 to 20°C; (2) mesophilic temperature from 20 to 40°C; and (3) thermophilic temperature from 40 to 60°C (Burton and Turner, 2003). Based on those temperature range criteria, the AD process can be implemented at tropical ambient temperatures. Moreover, 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 significant energy to maintain bioreactor temperature (Bandara *et al*., 2012).

Among 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 controlling the acidification stage therefore reducing the risk of overloading and the buildup of toxic material. The first stage in the two-phase configuration can act as metabolic buffer preventing pH shock to the methanogenic microorganisms and low pH in the first stage due to a high organic loading rate favors the establishment of the acidogenic phase (Sinbuathong *et al*., 2012). On the other hand, single-phase bio-digester has also advantageous as it is a simple and straightforward operation and for easier degradable substrate such as fruit and vegetables waste single-phase process could be the preferred rather than two-phase reactor (Ganesh *et al*., 2014). Although previous studies have evaluated the AD process at ambient temperature (Minale and Worku, 2014; Wei *et al*., 2014; Murrugan and Appavu, 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 direct comparison of single and two-phase AD of DCM at tropical ambient temperature. Therefore the aim of this current study was to further knowledge 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, R3. The reactors were made from stainless steel, and in order to minimize temperature fluctuations between day and night time, 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) reported 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, 0.239, and 0.210 kg DCM for R1, R2 and R3 respectively (after first removing 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 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 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 sourced 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.

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 1:1.5. Manure was collected once per week and diluted with tap water directly and kept refrigerated. 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) concentration 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 were: 1) valve to pump was in open position. 2) water pump was switched on. Therefore air in the measuring glass head space was removed, and the headspace was filled up with tap water. 3) valve to pump was closed. 4) water pump was switched off. 5) valve to Tedlar gas bag was opened therefore the methane in the Tedlar gas bag will move to the head space of the measuring glass. 6) gas volume was read in the measuring glass scale. When the gas volume in the Tedlar gas bag exceeded the measuring glass volume, steps 1-6 were repeated. The net gas production was corrected to STP conditions.

Daily maximum and minimum ambient temperature was recorded using a digital hygrometer thermometer HTC-2 (Taiwan). 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 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 and Gomez, 2007).

RESULTS

Ambient Temperature Variation

Average daily maximum-minimum ambient temperature throughout the experiment was 36.55°C and 20.93°C, respectively (Figure 2). There was 15.63°C temperature difference between maximum temperature in day time and minimum temperature in the night time and the ambient temperature in this study therefore falls into the mesophilic category (Burton and Turner, 2003).

Methane production

The methane production of the three bio-digesters throughout the experiment is presented in Figure 3. 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. Total methane yield of R1 and R2 (R_{TS}) was 147.13 L/kg VS (Table 1). 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 those studies were performed at 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 previous results.

Parameters 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 concentration concentrations of digested slurry in this study were 137.85; 178.96; 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. Brown and Li (2013) found a VS reduction of 27% and 33% for 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 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.

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 temperature in AD operation in the course of this study therefore has an adverse impact on the microorganism activity. Mao *et al*. (2015) reported 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.

Methane production

There was no significant effect ($p>0.05$) 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 term of L/L digester volume (methane production/volume active). No significant effect ($p>0.05$) of the application of the two-stage digester than single-digester on specific methane yield in this study can be due to anaerobic microorganisms activities in both reactors configuration operation 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 13-17% 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 the 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 ability to adapt to temperature changes (Chae *et al.*, 2008).

Methane production in term 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 amount of substrate added to R1 and R2 was 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 volume in both digester configurations was 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 methane yield 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, in 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.

Parameters in the Liquid Phase

During 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 other reactor in this study is in accordance with Baldi *et al*. (2019) who found that 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 that 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 value of R2 and R3 in this recent study was in the range of a stable AD process. Mao *et al*. (2015) reported 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 of microorganisms, however it can inhibit the AD process if it is available at high concentrations (Yenigün and 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 and Demirel, 2013). TAN concentration of digested slurry in R1 was significantly lower ($p<0.05$) than that in R2 and R3. This fact can be attributed to a shorter HRT in R1 than that 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 as reported by Yenigün and Demirel (2013).

There was no significant effect ($p>0.05$) of the application two stage compared to single stage digesters 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.

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, no significant 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. However, to be implemented on an industrial scale, further study is needed related to the addition of infrastructure to the two-phase digester rather than the single reactor.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

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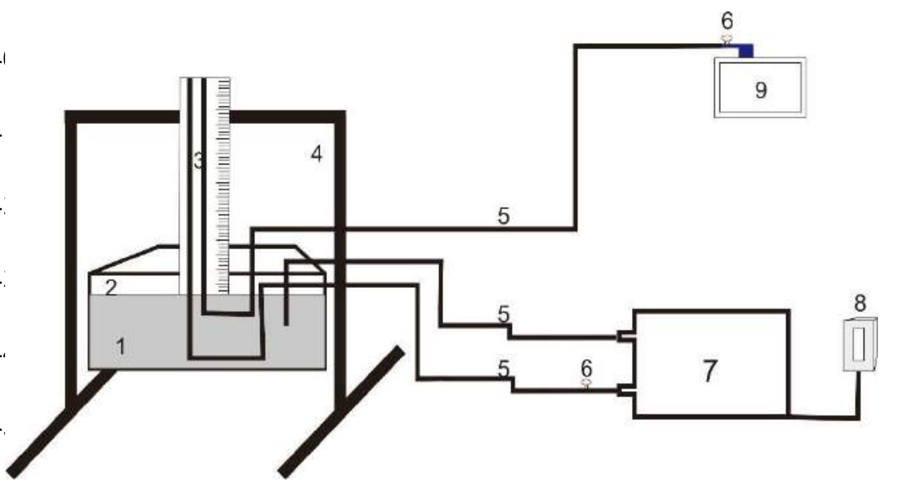
Figure caption



1. Tap water
2. Water container
3. Measuring glass
4. Measuring glass holder
5. Teflon tube Ø 5 mm
6. Valve to pump and valve to tedlar gas bag
7. Water pump
8. Switch
9. Tedlar gas bag

Figure 1. Apparatus for measuring gas production

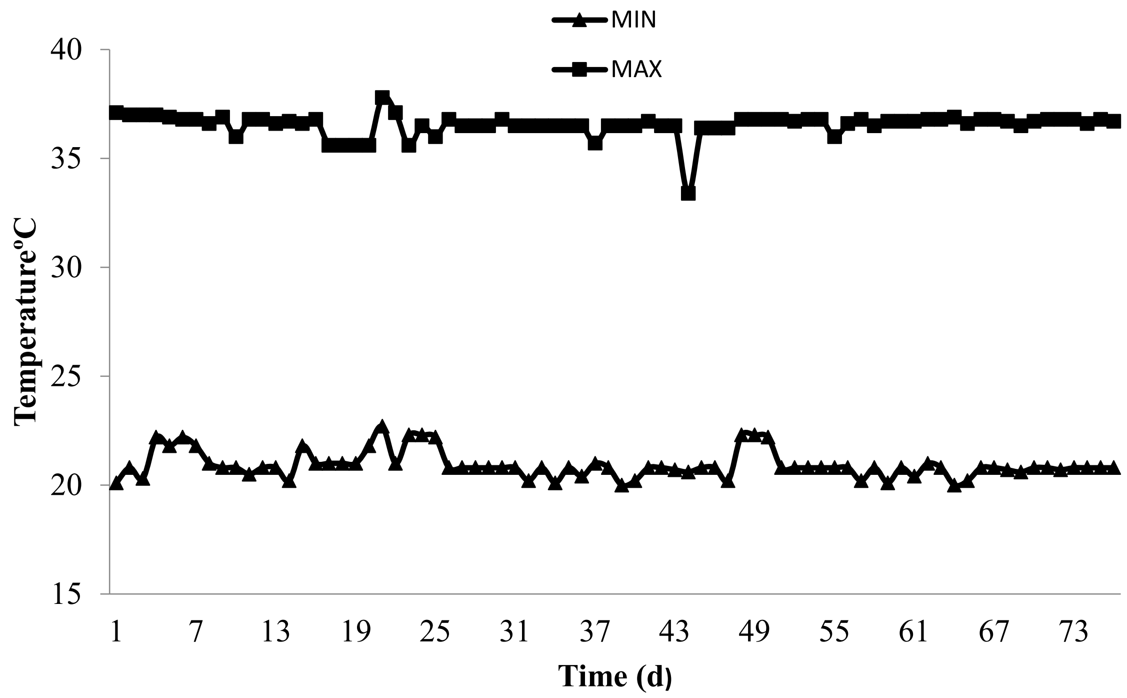
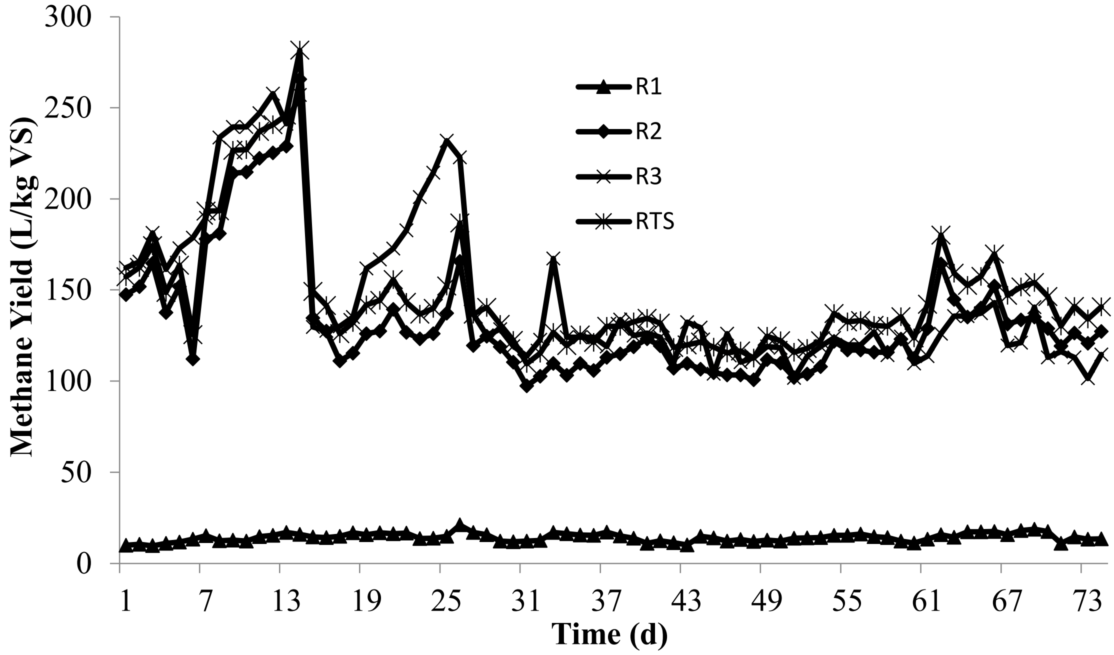


Figure 2. Maximum-minimum ambient temperature during experiment

352



353 Figure 3. A. Methane yield per kg VS added.

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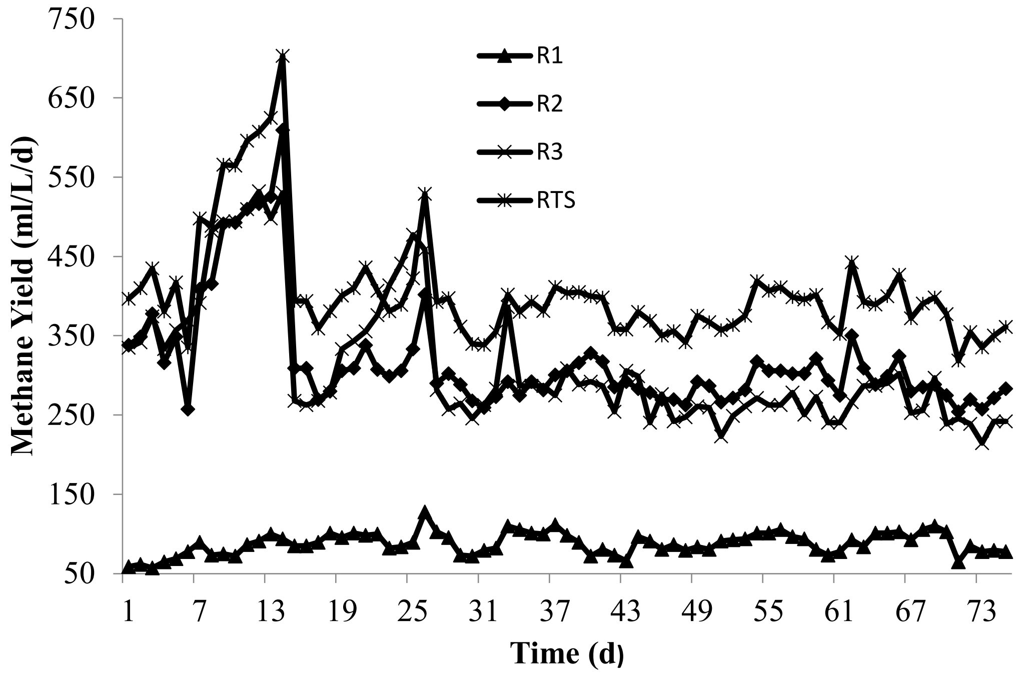

356 Figure 3. B. Methane yield per digester volume per day

357

Table caption

Table 1. Process parameters.

	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^{a}±58.95	137.84^{a}±45.32		6.46^{a}±0.17
R2	132.82±33.92	0.32±0.07	48.23^{b}±23.73	178.96^{b}±23.61	29.85^{a}±6.76	6.84^{b}±0.17
R3	146.65^{a}±42.47	0.31^{a}±0.08	39.19^{b}±23.23	185.86^{b}±23.68	28.03^{a}±3.19	6.90^{b}±0.28
R_{TS}	147.13^{a}±34.29	0.41^{b}±0.07				

^{a,b} : Values in each column followed by the same letter are not significantly different ($p>0.05$).

R1: First reactor of the two-phase digester

R2: Second reactor of the two-phase bio-degester

R3: Single-phase reactor

$R_{TS:}$ Total sum methane yield of R1 and R2

4. **Permintaan revisi dari editor terhadap hasil perbaikan pertama**

Tropical Animal Science Journal<mediapeternakan@apps.ipb.ac.id>

To: sutaryo

Mon 6/29/2020 2:44 PM

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Dear Sutaryo Sutaryo, Aldila Sempana, Cristina Lestari, Alastair Ward:

It is my pleasure to inform you that your submission to Tropical Animal Science Journal, "A Performance Comparison of Single and Two Phase Biogas Digesters Treating Dairy Cattle Manure at Tropical Ambient Temperature" had been examined by Editor. Please find the comments and suggestions:

Submission URL: http://journal.ipb.ac.id/index.php/tasj/authorDashboard/submission/30343

Username: {$authorUsername}

If you decide to revise the manuscript, please give a response or rebuttal against each point which are suggested by Editor. The revised document should include revision note file in table form and revised manuscript in MS Word file. Please return back the documents to the editor within 14 days via OJS, we would be glad if you submit your revised manuscript as soon as possible.

If you have any questions, please contact me.

Prof. Dr. Komang G Wiryawan

Chief Editor

FORM D

Tropical Animal Science Journal

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THE REVIEW RESULT

Paper Title: Performance Comparison of Single and Two-Phase Biogas Digester Treating Dairy Cattle Manure at Tropical Ambient Temperature

Comments (please use additional paper if more space is needed)

No	Comments	Author's response
1	Authors have revised some corrections following reviewer suggestions, but there are still some matters that have to be revised as follow:	
2	The objective of study has to state or explain clearly in the end of introduction	
3	Please state between results and discussions clearly (see corrected manuscript). The Results should only explain or elaborate the tabular data, but numbers should not be repeated extensively within the text, without references/discussion. Please see the journal's guidelines on how to write Results and Discussions section	
4	Use variable term for research design using inference statistic (samples) (See corrected manuscript)	
5	Please avoid statistic term in the conclusion	
6	Figure and Table have to revised following TASJ guideline	
7	Other correction please see corrected manuscripts	

5. Perbaikan draft publikasi atas koreksi dari editor

Surat pengantar, form revisi, dan draft revisi kedua

Dear Prof. Dr. Komang G. Wiryawan 7th July 2020
The Editor,
Tropical Animal Science Journal,

Subject: Resubmission of manuscript titled '**Performance Comparison of Single and Two-Phase Biogas Digester Treating Dairy Cattle Manure at Tropical Ambient Temperature'** **(**manuscript number: TASJ_30343**)**
- authored by Sutaryo Sutaryo, A. N. Sempana, C. M. S. Lestari, Alastair James Ward

We herewith resubmit our paper with the title **Performance Comparison of Single and Two-Phase Biogas Digesters Treating Dairy Cattle Manure at Tropical Ambient Temperature**. The manuscript is an original article based on the experiments carried out during 2018 at Faculty Animal and Agricultural Sciences, Diponegoro University.

Thank you for the opportunity you provide us to resubmit our manuscript. The authors greatly appreciate the corrections given by the editor. In the attached new version of the manuscript (revised manuscript), changes in response to the editors' comments have been highlighted using blue text.

I thank you for your time and consideration, and look forward to hearing from you.

Thank you and best regards,

On behalf of all the authors,

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FORM D

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Paper Title: Performance Comparison of Single and Two-Phase Biogas Digester Treating Dairy Cattle Manure at Tropical Ambient Temperature

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1	Authors have revised some corrections following reviewer suggestions, but there are still some matters that have to be revised as follow:	The authors tanks for the valuable corrections given by editor. The manuscript has been revised carefully.
2	The objective of study has to state or explain clearly in the end of introduction	The objective has been revised according editor suggestion Therefore the aim of this current study was to evaluate the process performance of single and two-phase biodigester treating DCM and working in this specific area.
3	Please state between results and discussions clearly (see corrected manuscript). The Results should only explain or elaborate the tabular data, but numbers should not be repeated extensively within the text, without references/discussion. Please see the journal's guidelines on how to write Results and Discussions section	The result section has been revised, no reference in the result section anymore.
4	Use variable term for research design using inference statistic (samples) (See corrected manuscript)	Thank you very much for the correction Parameters have been revised to be variables
5	Please avoid statistic term in the conclusion	The statistic term in the conclusion section has been revised to be However, there was no positive effect of this digester configuration on specific methane yield in terms of VS
6	Figure and Table have to revised following TASJ guideline	Figure and Table have been revised according editor suggestion

7	Other correction please see corrected manuscripts	Thank you for the corrections. Why there is no superscripcts in the methane yield of R1 and R2? This experiment did not compare methane production in R1, R2, and R3 because direct comparison of R1, R2 and R3 is not possible due to the difference in retention time

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

ABSTRACT

The biodegradation process of organic waste in anaerobic digestion can be in 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 ($p>0.05$) with the application of a two-phase digester configuration on 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 sum 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 stable methane production, low volatile fatty acids, and total ammonia concentrations. The two-phase bio-digester configuration can increase significantly 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 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 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 and Okolie, 2015).

Technically, the AD process can take places in three different temperature ranges: (1) psychrophilic (cryophilic) temperature from 10 to 20°C; (2) mesophilic temperature from 20 to 40°C; and (3) thermophilic temperature from 40 to 60°C (Burton and Turner, 2003). Based on those temperature range criteria, the AD process can be implemented at tropical ambient temperatures. Moreover, 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 significant energy to maintain bioreactor temperature (Bandara *et al*., 2012).

Among 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 controlling the acidification stage therefore reducing the risk of overloading and the buildup of toxic material. The first stage in the two-phase configuration can act as metabolic buffer preventing pH shock to the methanogenic microorganisms and low pH in the first stage due to a high organic loading rate favors the establishment of the acidogenic phase (Sinbuathong *et al*., 2012). On the other hand, single-phase bio-digester has also advantageous as it is a simple and straightforward operation and for easier degradable substrate such as fruit and vegetables waste single-phase process could be the preferred rather than two-phase reactor (Ganesh *et al*., 2014). Although previous studies have evaluated the AD process at ambient temperature (Minale and Worku, 2014; Wei *et al*., 2014; Murrugan and Appavu, 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 direct comparison of single and two-phase AD of DCM at tropical ambient temperature. Therefore the aim of this current study was to evaluate the process performance of single and two-phase biodigester 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, R3. The reactors were made from stainless steel, and in order to minimize temperature fluctuations between day and night time, 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) reported 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, 0.239, and 0.210 kg DCM for R1, R2 and R3 respectively (after first removing 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 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 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 sourced 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.

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 1:1.5. Manure was collected once per week and diluted with tap water directly and kept refrigerated. 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) concentration 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 were: 1) valve to pump was in open position. 2) water pump was switched on. Therefore air in the measuring glass head space was removed, and the headspace was filled up with tap water. 3) valve to pump was closed. 4) water pump was switched off. 5) valve to Tedlar gas bag was opened therefore the methane in the Tedlar gas bag will move to the head space of the measuring glass. 6) gas volume was read in the measuring glass scale. When the gas volume in the Tedlar gas bag exceeded the measuring glass volume, steps 1-6 were repeated. The net gas production was corrected to STP conditions.

Daily maximum and minimum ambient temperature was recorded using a digital hygrometer thermometer HTC-2 (Taiwan). 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 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 and Gomez, 2007).

RESULTS

Ambient Temperature Variation

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

Methane production

The methane production of the three bio-digesters throughout the experiment is presented in Figure 3. 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. Total methane yield of R1 and R2 (R_{TS}) was 147.13 L/kg VS (Table 1).

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 concentration concentrations of digested slurry in this study were 137.85; 178.96; 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 temperature in AD operation in the course of this study therefore has an adverse impact on the microorganism activity. Mao *et al*. (2015) reported 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. The ambient temperature in this study (Figure 2) therefore falls into the mesophilic category (Burton and Turner, 2003).

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 term of L/L digester volume (methane production/volume active). No significant effect of the application of the two-stage digester than single-digester on specific methane yield in this study can be due to anaerobic microorganisms activities in both reactors configuration operation 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 13%-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 the 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 ability 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 those studies were performed at 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 previous results.

Methane production in term 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 amount of substrate added to R1 and R2 was 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 volume in both digester configurations was 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 methane yield 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, in 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 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 other reactor in this study is in accordance with Baldi *et al*. (2019) who found that 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 that 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 value of R2 and R3 in this recent study was in the range of a stable AD process. Mao *et al*. (2015) reported 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 of microorganisms, however it can inhibit the AD process if it is available at high concentrations (Yenigün and 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 and Demirel, 2013). TAN concentration of digested slurry in R1 was significantly lower ($p<0.05$) than that in R2 and R3. This fact can be attributed to a shorter HRT in R1 than that 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 as reported by Yenigün and Demirel (2013).

There was no significant effect of the application two stage compared to single stage digesters 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 a VS reduction of 27% and 33% for 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 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. However, to be implemented on an industrial scale, further study is needed related to the addition of infrastructure to the two-phase digester rather than the single reactor.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

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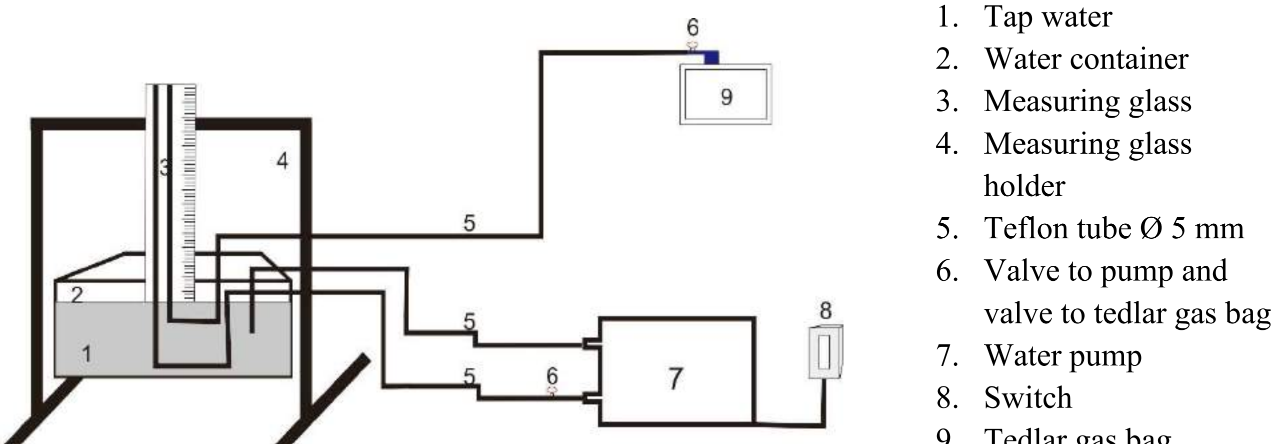


Figure 1. Apparatus for measuring gas production

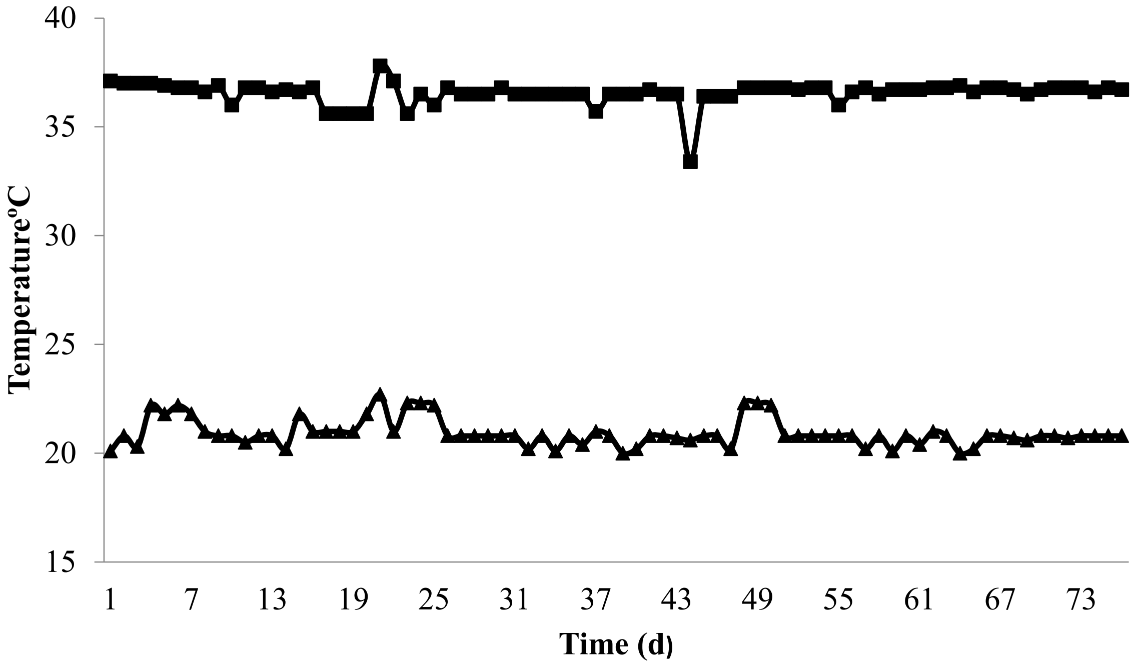
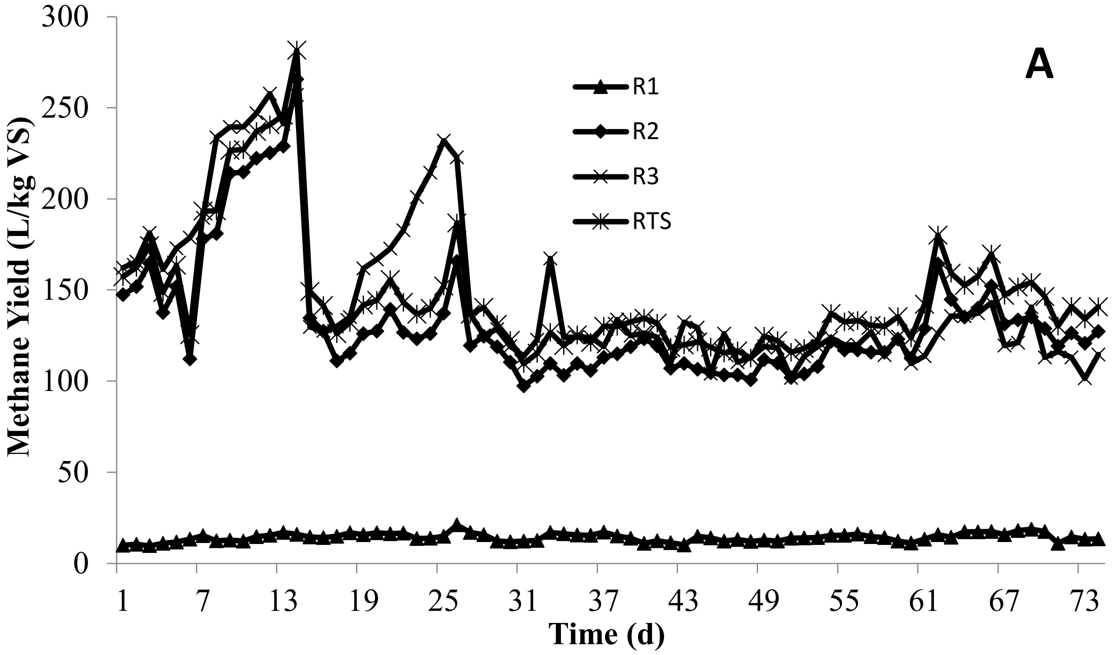


Figure 2. Maximum-minimum ambient temperature during experiment. ■: maximum, ▲: minimum.



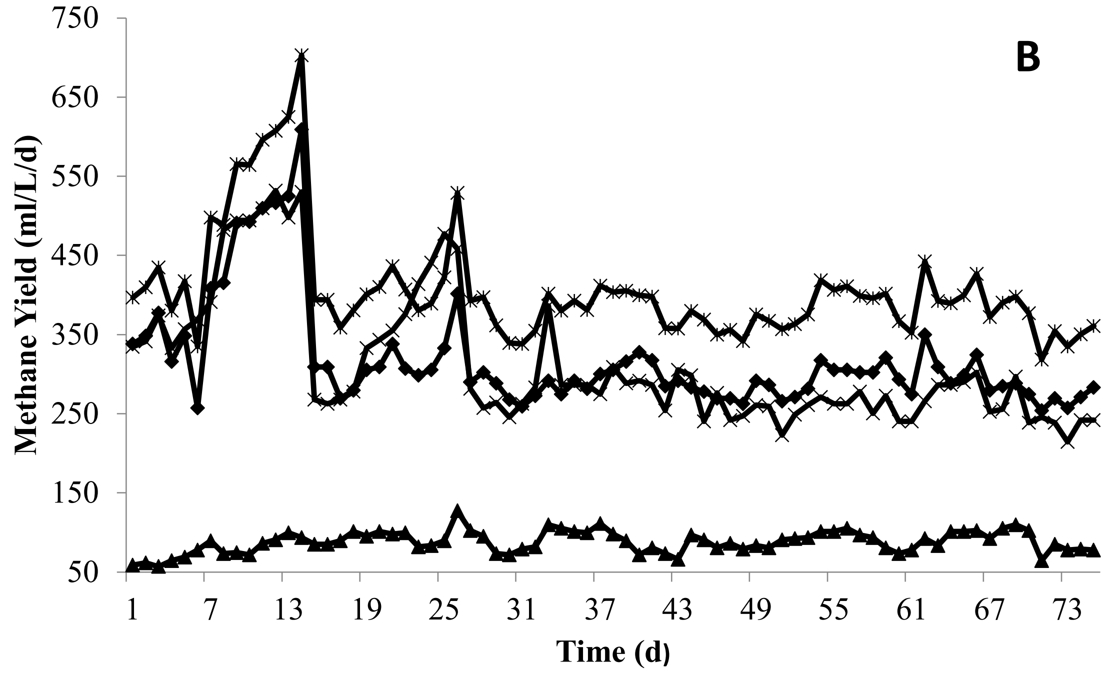


Figure 3. A. Methane yield per kg VS added. B. Methane yield per digester volume per day. ▲: R1, ♦: R2, ×: R3, *:R_{TS}.

Table 1. Methan 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	$160.74^{a}\pm58.95$	$137.84^{a}\pm45.32$		6.46 ± 0.17^{a}
R2	132.82±33.92	0.32±0.07	$48.23^{b}\pm23.73$	$178.96^{b}\pm23.61$	$29.85^{a}\pm6.76$	6.84 ± 0.17^{b}
R3	146.65±42.47	0.31 ± 0.08^{a}	$39.19^{b}\pm23.23$	$185.86^{b}\pm23.68$	$28.03^{a}\pm3.19$	6.90 ± 0.28^{b}
R_{TS}	147.13±34.29	0.41 ± 0.07^{b}				

Note: ^{a,b}: Values in the same column followed by the different superscript are significantly different ($p< 0.05$). 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, $R_{TS:}$ Total sum methane yield of R1 and R2.

6. Draft publikasi dinyatakan diterima untuk dipublikasikan

Prof. Dr. Komang G Wiryawan <jurnal@apps.ipb.ac.id>

To: sutaryo; Aldila Sempana <aldilanugrahaini22@gmail.com>; Cristina Lestari <cmslest@yahoo.co.id>; +1 other

Wed 7/15/2020 10:35 AM

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Bogor, July 15, 2020

Dear Sutaryo, Aldila Sempana, Cristina Lestari, Alastair Ward:

I am pleased to inform you that your article submitted to Tropical Animal Science Journal, entitled "Performance Comparison of Single and Two-Phase Biogas Digester Treating Dairy Cattle Manure at Tropical Ambient Temperature" has been accepted for publication in this journal. We will send you the COPYEDITING of your manuscript as we will ask you for some correction of the typesetting.

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Thank you for your article submission, and we are looking forward to receive your incoming articles.

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Dear Prof. Dr. Komang G. Wiryawan
The Editor,
Tropical Animal Science Journal,

Subject: Resubmission of manuscript titled '**Performance Comparison of Single and Two-Phase Biogas Digester Treating Dairy Cattle Manure at Tropical Ambient Temperature'** (manuscript number: TASJ_30343)
- authored by Sutaryo Sutaryo, A. N. Sempana, C. M. S. Lestari, Alastair James Ward

We herewith resubmit our paper with the title **Performance Comparison of Single and Two-Phase Biogas Digesters Treating Dairy Cattle Manure at Tropical Ambient Temperature**. The manuscript is an original article based on the experiments carried out during 2018 at Faculty Animal and Agricultural Sciences, Diponegoro University.

Thank you for the opportunity you provide us to resubmit our manuscript. The authors greatly appreciate the corrections given by the editor.

L22, P:1 : (p>0.05) has been deleted, method for ANOVA has been added in L142, P:7, Gomez and Gomez has been changed to be Gomez & Gomez (L144, P:7), L:266-268,P:12 has been deleted, L:380, P:20 row has been changed to be column.

I thank you for your time and consideration, and look forward to hearing from you.

Thank you and best regards,

On behalf of all the authors,

Sutaryo Sutaryo

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 controlling 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 and Appavu, 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 two-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 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 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 removed, 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 3. 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).

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 and Turner, 2003).

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 13%-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, in 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 of microorganisms, however, it can inhibit the AD process if it is available at high concentrations (Yenigün and 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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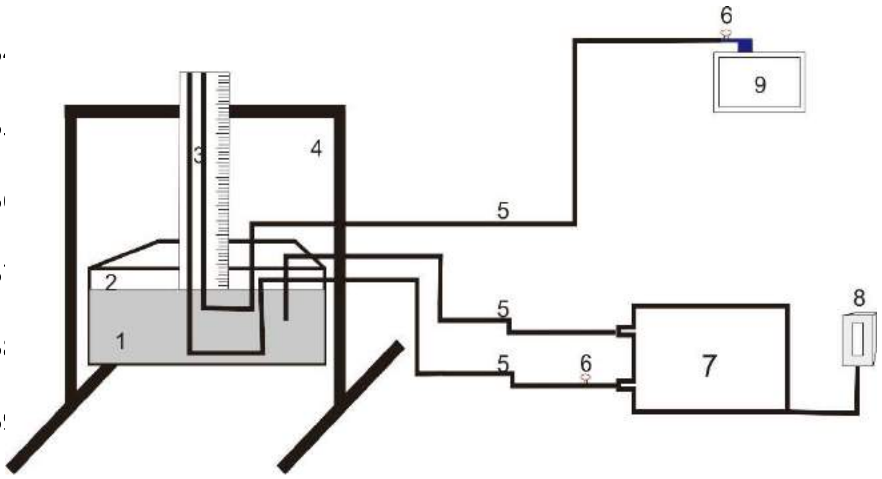

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Figure 1. Apparatus for measuring gas production

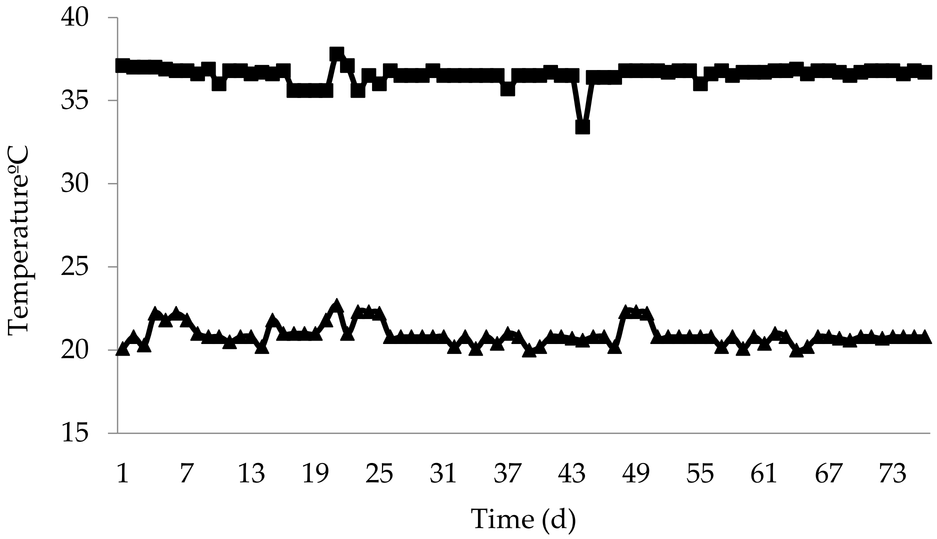
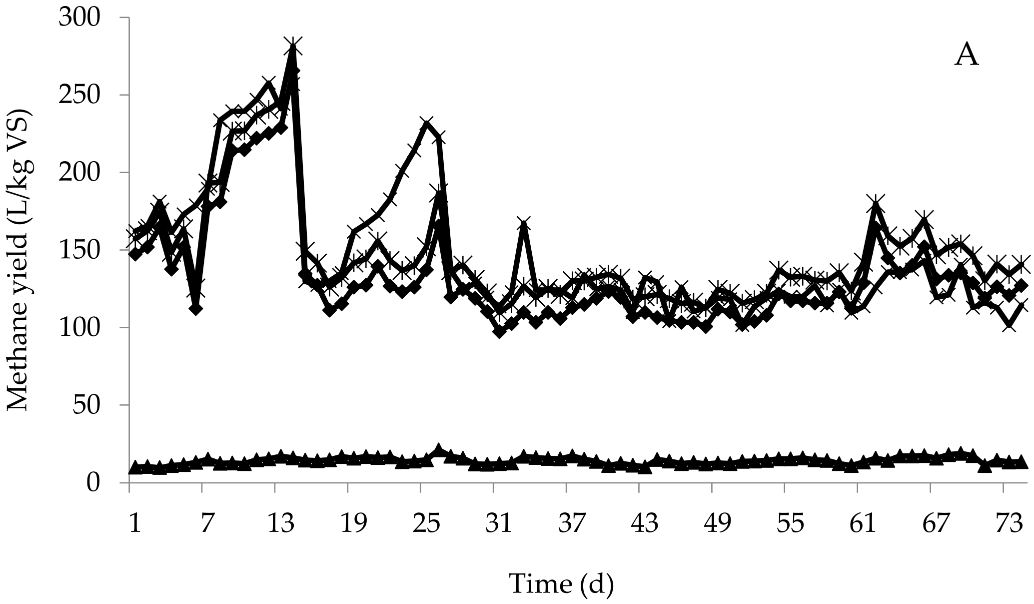


Figure 2. Maximum-minimum ambient temperatures during experiment. ■: maximum, ▲: minimum.



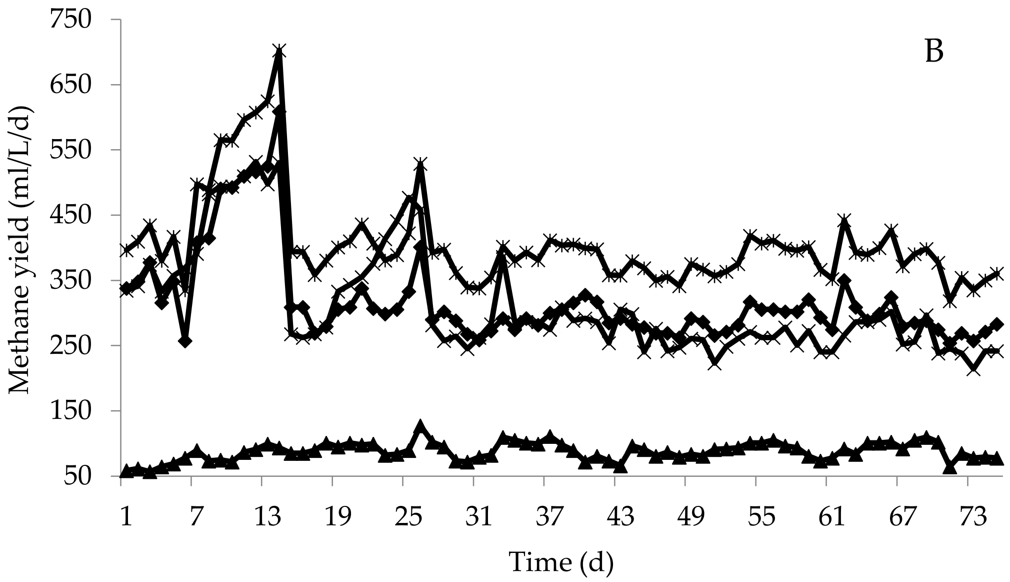


Figure 3. A. Methane yield per kg VS added. B. Methane yield per digester volume per day. ▲: R1, ♦: R2, ×: R3, *:R_{TS}.

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

Reactors	Methane yield (L/kg VS)	Methane yield (L/L/d)	Total VFA (mg/L)	TAN (mg/L)	VS reduction (%)	pH
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[a]	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[a]	6.90±0.28[b]
R_{TS}	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-digester; R3= Single-phase reactor, $R_{TS:}$ Total sum methane yield of R1 and R2. Means in the same column with different superscripts differ significantly ($p<0.05$).

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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 controlling 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 & Appavu, 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 two-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 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 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.

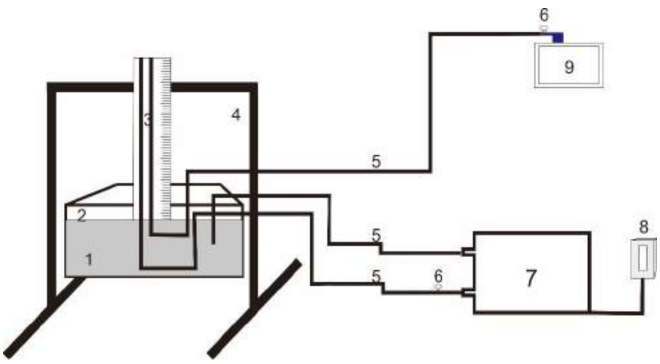
Inoculum and Substrate

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-



1. Tap water
2. Water container
3. Measuring glass
4. Measuring glass holder
5. Teflon tube Ø 5 mm
6. Valve to pump and valve to tedlar gas bag
7. Water pump
8. Switch
9. Tedlar gas bag

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 3. 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).

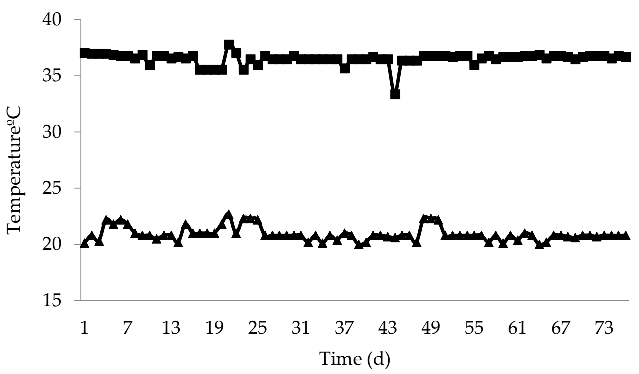


Figure 2. Maximum-minimum ambient temperatures during experiment. ■: maximum, ▲: minimum.

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).

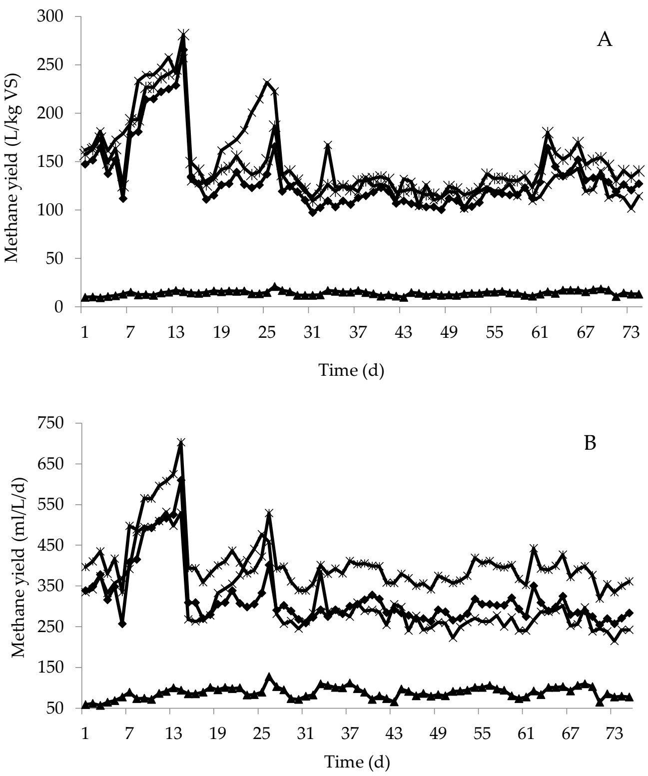


Figure 3. A. Methane yield per kg VS added. B. Methane yield per digester volume per day. ▲: R1, ♦: R2, ×: R3, *: RTS.

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 13%-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, in 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 of 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.

ACKNOWLEDGEMENT

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