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 Prof. Dr. Ir. Komang G. Wiryawan <jurnal@apps.ipb.ac.id>  Tue 4/14/2020 3:23 PM


To: sutaryo

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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Prof. Dr. Ir. Komang G. Wiryawan  Tropical Animal Science Journal
<http://journal.ipb.ac.id/index.php/tasj>

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

3
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11 **ABSTRACT**

12 This study examined the effect of different biogas digester configurations (single
13 and two-phase) on methane production of dairy cattle manure (DCM) at tropical ambient
14 temperature. The results showed that there was no positive effect with the application of
15 a two-phase digester configuration on specific methane yield of DCM per kg volatile
16 solids added. Methane production was detected in the first reactor of the two-phase
17 digester configuration and the total sum methane production of the two-phase digester
18 was found to be 29.98% higher ($p < 0.05$) than that of the single reactor in terms of digester
19 volume. Both digester configurations performed well, indicated by stable methane
20 production, low volatile fatty acids and total ammonia concentrations.

21 **Key words:** biogas, manure, tropical ambient temperature, two-phase digester biogas.

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INTRODUCTION

25

26 The dairy cattle industry produces large amounts of waste in the form of manure
27 that can cause environmental pollution if not managed properly. Daily dairy cattle manure
28 (DCM) (wet feces plus urine) excretion is 89.0; 65.9; 34.8 kg/d per 1000 kg of body
29 weight for those that produce 29 and 14 kg/d of milk and for non-lactating dairy cows,
30 respectively (Wilkerson *et al.*, 1997). Generally, animal waste management can take
31 place in aerobic condition through a composting process or by anaerobic digestion (AD)
32 to produce biogas.

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

43 Technically, the AD process can take place in three different temperature ranges:
44 (1) psychrophilic (cryophilic) temperature from 10 to 20°C; (2) mesophilic temperature
45 from 20 to 40°C; and (3) thermophilic temperature from 40 to 60°C (Burton and Turner,
46 2003). Based on those temperature range criteria, the AD process can be implemented at
47 tropical ambient temperatures. Moreover, operation of AD at tropical ambient
48 temperatures offers advantages compared to the operation of AD under mesophilic or

49 thermophilic temperatures, since AD operation at higher temperatures require significant
50 energy to maintain bioreactor temperature (Bandara *et al.*, 2012).

51 Among other biogas digester designs, the continuously stirred tank reactor
52 (CSTR) design is the most commonly applied bioreactor for treating agricultural waste
53 (Linke *et al.*, 2015). While in operation, the process of biodegradation of organic waste
54 can be in single or two-phases. The two-phase AD process has several advantages
55 compared to single phase. These include the selection and enrichment of different bacteria
56 in each digester, increasing the stability of the process by controlling the acidification
57 stage therefore reducing the risk of overloading and the buildup of toxic material. The
58 first stage in the two-phase configuration can act as metabolic buffer preventing pH shock
59 to the methanogenic microorganisms and low pH in the first stage due to a high organic
60 loading rate favours the establishment of the acidogenic phase (Sinbuathong *et al.*, 2012).
61 Although previous studies have evaluated the AD process at ambient temperature (Minale
62 and Worku, 2014; Wei *et al.*, 2014; Murrugan and Appavu, 2018) and two phase AD
63 (Baldi *et al.*, 2019; Tsigkou *et al.*, 2020), to the best of our knowledge there has been a
64 lack of information regarding direct comparison of single and two phase AD of DCM at
65 tropical ambient temperature. Therefore the aim of this current study was to further
66 knowledge in this specific area.

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68 MATERIALS AND METHODS

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Experimental Set Up

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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

73 with double layers. The two phase digesters consisted of reactors R1 and R2. Reactor 1
74 had a 2.1 L working volume (the minimum volume that can be applied in the reactor) and
75 3 d hydraulic retention (HRT) while R2 had 5.25 L working volume and 22 d HRT.
76 Therefore in total R1 and R2 had a 25 d HRT. R3 served as the single phase bioreactor
77 and had 5.25 L working volume and 25 d HRT. Mao *et al.* (2015) reported that under
78 mesophilic conditions an average HRT in the range of 15-30 d is required to treat waste.

79 The experiment was started by filling R1 with 1.4 kg inoculum and 0.7 kg DCM,
80 R2 with 5.011 kg inoculum and 0.239 kg DCM and R3 with 5.040 kg inoculum and 0.210
81 kg DCM. From the second day, all digesters were fed as follows: 0.7, 0.239 and 0.210 kg
82 DCM for R1, R2 and R3 respectively (after first removing the same amount of digestate
83 from a port at the base of the digesters) which continued for the following 21 d adaptation
84 period. The digesters were fed through a tube, the outlet of which was submerged under
85 the substrate level to avoid air ingress during the feeding process. Data were collected
86 after this 21 d startup period. During the data collection period, R1 was fed 0.7 kg DCM.
87 Effluent from this digester (0.239 kg) was used to feed R2, while R3 was fed 0.210 kg
88 DCM. Digesters were kept at ambient temperature and the experiment was run for a
89 period of three HRT corresponding to 75 d in total.

90

91 **Inoculum and Substrate**

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

96 Substrate was taken from dairy cows in the lactation period and was collected
97 from the farm in Faculty of Animal and Agricultural Sciences, Diponegoro University.
98 Manure was diluted with tap water in the ratio 1:1.5. Manure was collected once per week
99 and diluted with tap water directly and kept refrigerated. pH value, volatile solids (VS)
100 and total ammonia nitrogen (TAN) concentration in the inoculum were 7.11, 7.33% and
101 265.18 mg/L respectively, while pH value, VS, TAN and volatile fatty acids (VFA) (C2-
102 C5) concentration of DCM were 6.77, 7.40%, 97.98 mg/L and 142.93 mg/L respectively.

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Analytical Methods

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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 CO₂ 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.

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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

120 at 105°C for 7 h. Ash was determined by combusting the dried samples at 550°C for 6 h
121 and VS was calculated by subtracting the ash weight from the DM (APHA,1995). TAN
122 concentration was measured using photometric kits (HACH® USA: DOC316.53.01077)
123 at 655 nm. VFA were determined using gas chromatography (Shimadzu GC-8). The
124 collected data were statistically analyzed using ANOVA with 95% confidence level.
125 Duncan's multiple range tests were used in post ANOVA analysis when differences were
126 found to be significant (Gomez and Gomez, 2007).

127

128 **RESULTS**

129

Ambient Temperature Variation

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

135

136

Methane production

137 The methane production of the three bio-digesters throughout the experiment is
138 presented in Figure 3. The mean methane yields were 14.31 L kg/VS, 132.82 L kg/VS,
139 and 146 L kg/VS for R1, R2 and R3 respectively Total methane yield of R1 and R2 (R_{TS})
140 was 147.13 L kg/VS (Table 2). A study from Amon *et al.* (2007) using batch digesters
141 with 60 d incubation period at 38°C showed that methane production of DCM from cows
142 with medium milk production was 166.3 N L kg/VS while the study from Sutaryo *et al.*
143 (2014) using continuous digesters with 20 d HRT at 35°C found that methane production

144 of DCM was 177 L kg/VS. Both those studies were performed at constant mesophilic
145 temperature while the study presented here was performed at ambient variable mesophilic
146 temperatures, however the result of this study is similar with those previous results.

147

148 **Parameters in the Liquid Phase**

149 Total VFA concentration and pH value of digested slurry are presented in Table
150 1. The mean total VFA concentration was 160.74; 48.23; 39.19 mg/L for R1, R2 and R3
151 respectively. Total VFA concentration of digested slurry in R1 was significantly higher
152 ($p<0.05$) than that in R2 and R3 (Table 1). TAN concentration concentrations of digested
153 slurry in this study were 137.85; 178.96; 185.86 mg/L for R1, R2 and R3 respectively
154 (Table 2).

155 Volatile solid reductions in this study were 29.85 and 28.03% for R2 and R3
156 respectively. Study from Bhattacharya *et al.* (1996) found a VS reduction of 26% and
157 30% for conventional and two-phase digester respectively treating sludges from
158 conventional activated sludge treatment plants and maintained at 35°C. Meanwhile a
159 study from Sutaryo *et al.* (2012) found a VS reduction in range of 27-35% for a reactor
160 treating DCM with different TS concentrations. Therefore the result of this study is in
161 accordance with the result of the previous study.

162

163 **DISCUSSION**

164 **Ambient Temperature Variation**

165 This study was performed in July-September and in Indonesia that period is
166 considered to be in the dry season. A large variation temperature in AD operation in the
167 course of this study therefore has an adverse impact on the microorganism activity. Sakar

168 *et al.* (2009) reported that the AD process is carried out by a prime balanced population
169 of various microorganisms. These microorganisms are very sensitive to environmental
170 condition changes including temperature.

171

172 **Methane production**

173 There was no significant effect ($p>0.05$) of the application of a two phase bio-
174 digester on specific methane yield in terms of kg VS of substrate added when compared
175 to that from the single digester configuration (Table 1). However, methane production of
176 RTS was significantly higher ($p<0.05$) than that in R3 in term of L/L digester volume
177 (methane production/volume active). No significant effect ($p>0.05$) of the application of
178 the two-stage digester than single digester on specific methane yield in this study can be
179 due to anaerobic microorganisms activities in both reactors configuration operation
180 efficiently. This study used digested slurry from an active digester that operated at a
181 tropical ambient temperature, the same condition used in this study. This fact, along with
182 the three weeks adaptation period contributed to the efficient microorganism's activity in
183 both reactor configurations in this study even though there was large temperature
184 difference between day and night time. A study by Chae *et al.* (2008) found that using
185 batch digesters and treating swine manure, the methane production at 30 and 35°C were
186 quite similar, but it was higher by more than 13-17% than that at 25°C. Temperature
187 shocks caused a reduction in the methane production rate compared to that of the control,
188 but it recovered rapidly. Once adapted, no significant effect on the methane production
189 was observed between the control and the temperature shock bio-digester. This fact
190 therefore indicates that, even though methanogenic archaea are quite sensitive to

191 temperature shock they have considerable ability to adapt to temperature changes (Chae
192 *et al.*, 2008).

193 Methane production in term of digester volume of RTS was 29.98% higher than
194 that in R3. The positive effect ($p < 0.05$) of the application of two phase digestion on the
195 methane production compared to that in the single phase reactor can be attributed to a
196 shorter HRT period in R1 and R2 than that in R3, therefore the amount of substrate added
197 to R1 and R2 was higher than that in R3. Since the amount of substrate added to R2 (0.239
198 kg) was higher than that in R3 (0.210 kg) and in the same time the active volume in both
199 digester configurations was equal (5.25 L) therefore methane production in term of
200 digester volume R2 was higher than that in R3. In fact methane production in RTS was
201 methane yield in R1 and in R2. In this present study, HRT in R1, R2 and R3 were 3 d, 22
202 d and 25 d respectively. Bhattacharya *et al.* (1996) reported that one of the advantages of
203 phase separation is the ability to handle a higher organic loading rate than that in a single
204 reactor. A similar study from Tsigkou *et al.* (2020) found the same phenomenon, in that
205 the application of a two-stage digester treating co-digestion of used disposable nappies
206 and expired food product at 60:40 (v/v) ratio, working at mesophilic condition ($37 \pm 0.5^\circ\text{C}$)
207 and 15 d HRT the energy production was 18.5% higher than that in the single reactor.

208

209 **Parameters in the Liquid Phase**

210 During bioconversion of organic matter in AD system there are four steps, namely
211 hydrolysis, acidogenesis, acetogenesis and methanogenesis. In a two stage digester
212 configuration, the first digester serves as the acidogenic phase (Sinbuatong *et al.*, 2012),
213 therefore the VFA concentration will be higher than that in the second digester. A higher
214 total VFA concentration in R1 than that on other reactor in this study is in accordance

215 with Baldi *et al.* (2019) who found that total VFA concentration of digested slurry in a
216 fermentative digester was significantly higher than that of digested slurry from
217 methanogenic digester.

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

224 Ammonia is one of the essential nutrients for the growth of microorganisms,
225 however it can inhibit the AD process if it is available at high concentrations (Yenigün
226 and Demirel, 2013). Under mesophilic conditions (35°C), the TAN inhibitory threshold
227 was in the concentrations of around 1700–1800 mg L⁻¹ for unacclimated inoculum
228 (Yenigün and Demirel, 2013). TAN concentration of digested slurry in R1 was
229 significantly lower ($p < 0.05$) than that in R2 and R3. This fact can be attributed to a shorter
230 HRT in R1 than that in R2 and R3 therefore microorganisms in R2 and R3 can degrade
231 more protein in the substrate, subsequently producing more ammonia. However TAN
232 concentrations of digested slurry from all digester in this study were below the inhibitory
233 level as reported by Yenigün and Demirel (2013).

234 There was no significant effect ($p > 0.05$) of the application two stage compared to
235 single stage digesters on the VS reduction. No significant effect of phase separation on
236 volatile solid reduction in this study suggests that microorganisms in both reactor
237 configurations can work well.

238

239 **CONCLUSION**

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

248

249 **CONFLICT OF INTEREST**

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

253

254 **ACKNOWLEDGEMENT**

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257

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- 318

319 **Figure caption**

320

321

322

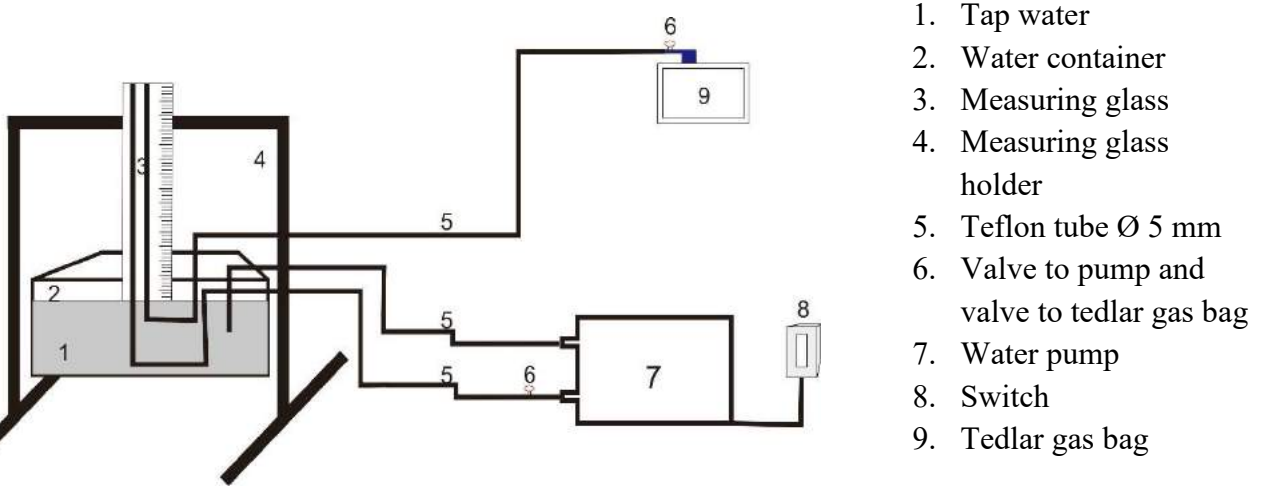
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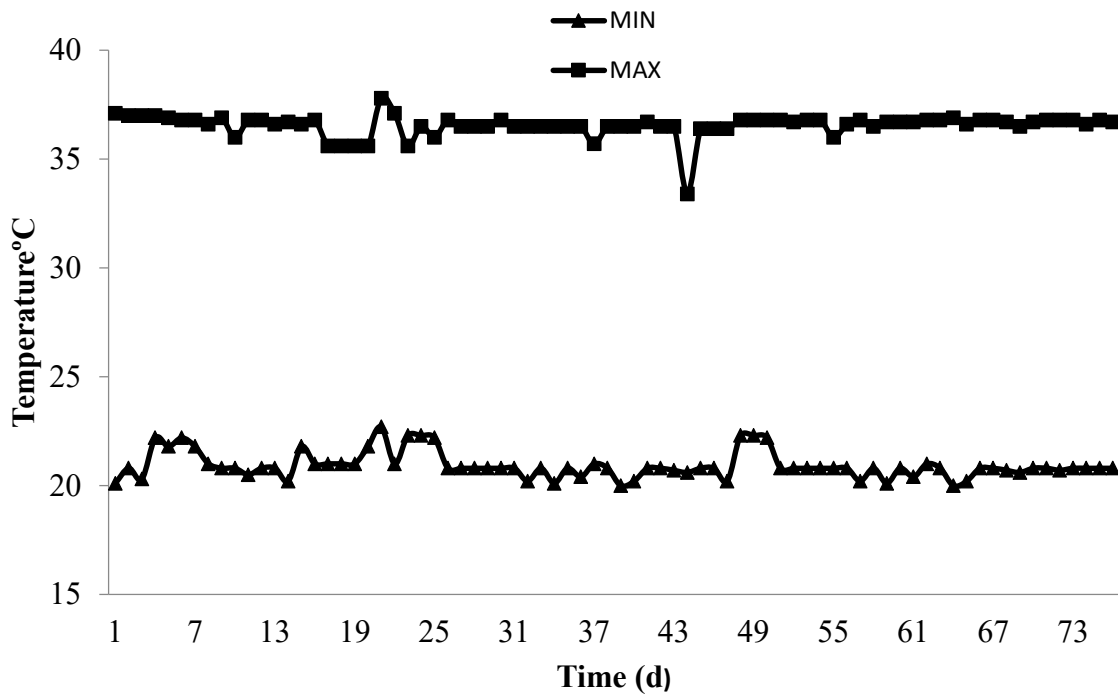
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328 Figure 1. Apparatus for measuring gas production

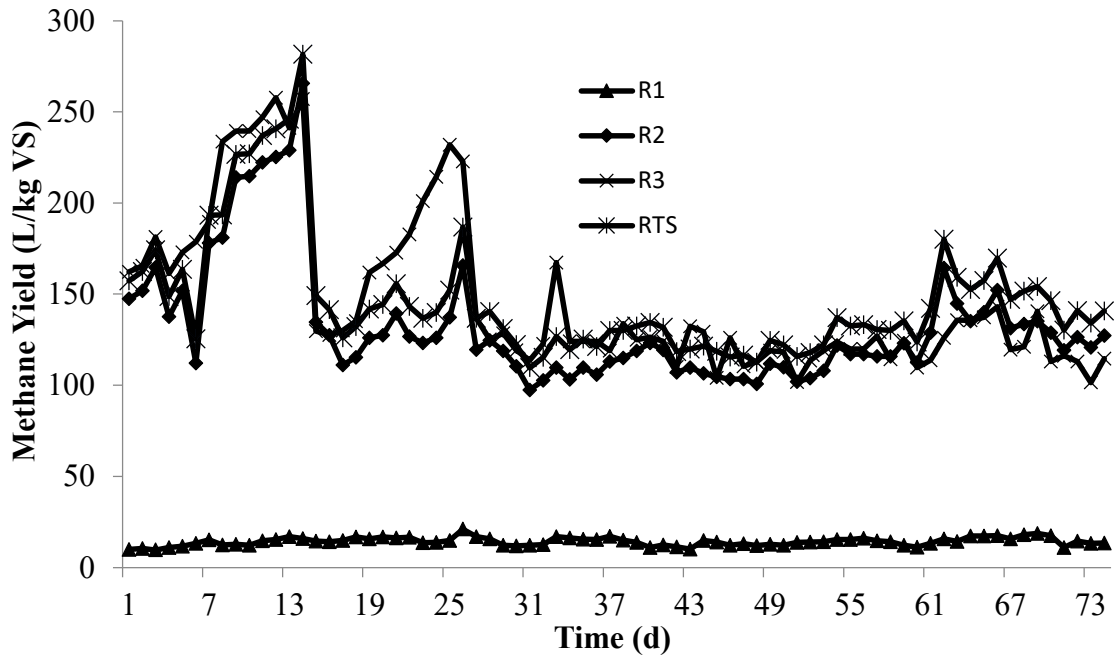
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331 Figure 2. Maximum-minimum ambient temperature during experiment

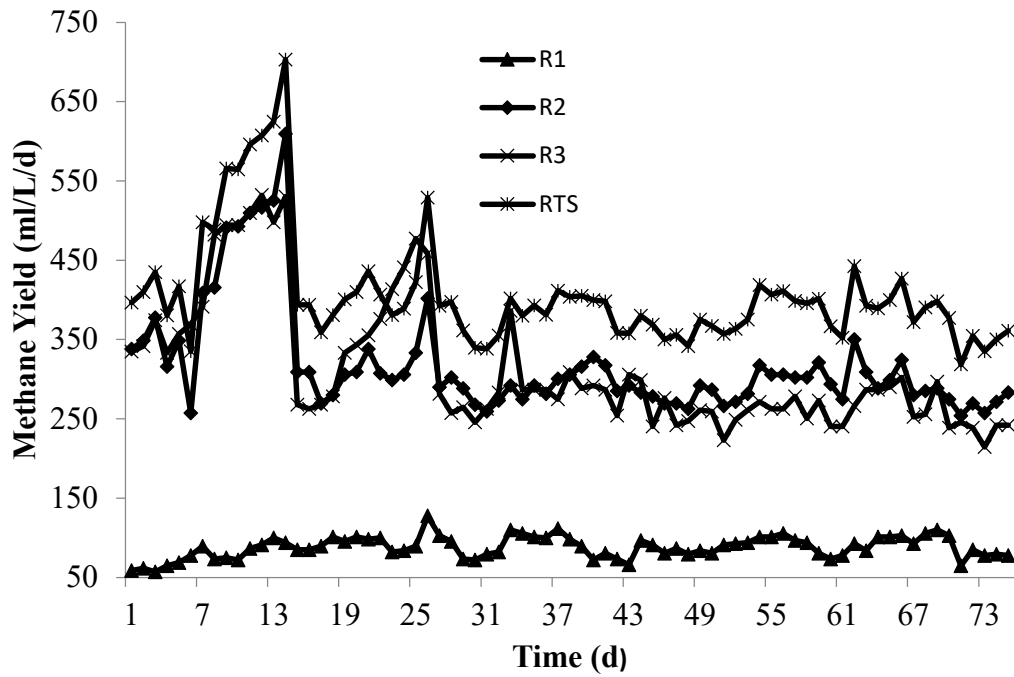
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334 Figure 3. A. Methane yield per kg VS added.

335



336

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

338

339

340 **Table caption**

341 Table 1. Process parameters. Values in each column followed by the same letter are not
342 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				

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2. Permintaan revisi untuk draft yang dikirim ke jurnal

Tropical Animal Science Journal <mediapaternakan@apps.ipb.ac.id> Mon 5/11/2020 3:25 PM

To: sutaryo

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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	<p>References</p> <ul style="list-style-type: none"> 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	

1 **Performance Comparison of Single and Two-Phase Biogas Digesters Treating**
2 **Dairy Cattle Manure at Tropical Ambient Temperature**
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5 **ABSTRACT**

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6 This study examined the effect of different biogas digester configurations (single
7 and two-phase) on methane production of dairy cattle manure (DCM) at tropical
8 ambient temperature. The results showed that there was no positive effect with the
9 application of a two-phase digester configuration on specific methane yield of DCM per
10 kg volatile solids added. Methane production was detected in the first reactor of the
11 two-phase digester configuration, and the total sum methane production of the two-
12 phase digester was found to be 29.98% higher ($p < 0.05$) than that of the single reactor in
13 terms of digester volume. Both digester configurations performed well, indicated by
14 stable methane production, low volatile fatty acids, and total ammonia concentrations.

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

22 The dairy cattle industry produces large amounts of waste in the form of manure
 23 that can cause environmental pollution if not managed properly. Daily dairy cattle
 24 manure (DCM) (wet feces plus urine) excretion is 89.0; 65.9; 34.8 kg/d per 1000 kg of
 25 body weight for those that produce 29 and 14 kg/d of milk and for non-lactating dairy
 26 cows, respectively (Wilkerson *et al.*, 1997). Generally, animal waste management can
 27 take place in aerobic conditions through a composting process or by anaerobic digestion
 28 (AD) to produce biogas.

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

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

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

50 Among other biogas digester designs, the continuously stirred tank reactor
51 (CSTR) design is the most commonly applied bioreactor for treating agricultural waste
52 (Linke *et al.*, 2015). While in operation, the process of biodegradation of organic waste
53 can be in single or two-phases. The two-phase AD process has several advantages

54 compared to a single phase. These include the selection and enrichment of different
55 bacteria in each digester, increasing the stability of the process by controlling the
56 acidification stage therefore reducing the risk of overloading and the buildup of toxic
57 material. The first stage in the two-phase configuration can act as metabolic buffer
58 preventing pH shock to the methanogenic microorganisms and low pH in the first stage
59 due to a high organic loading rate favors the establishment of the acidogenic phase

60 (Sinbuathong *et al.*, 2012). Although previous studies have evaluated the AD process at
61 ambient temperature (Minale and Worku, 2014; Wei *et al.*, 2014; Murrugan and
62 Appavu, 2018) and two-phase AD (Baldi *et al.*, 2019; Tsigkou *et al.*, 2020), to the best
63 of our knowledge there has been a lack of information regarding direct comparison of
64 single and two-phase AD of DCM at tropical ambient temperature. Therefore the aim of
65 this current study was to further knowledge in this specific area.

66

67

MATERIALS AND METHODS

68

Experimental Set-Up

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

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Commented [A2]: Since this research want to compare single and two phase of biodigester equally, in the introduction section have to describe the advantage of single phase bioreactor also.

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Commented [A3]: Is this replication or treatment? How many replication used in this study? What is RTS which is shown in several figures and table? There is insufficient information about this initial.

78 were made with double layers. The two-phase digesters consisted of reactors R1 and
79 R2. Reactor 1 had a 2.1 L working volume (the minimum volume that can be applied in
80 the reactor) and 3 d hydraulic retention (HRT)₂ while R2 had 5.25 L working volume
81 and 22 d HRT. Therefore in total, R1 and R2 had a 25 d HRT. R3 served as the single-
82 phase bioreactor and had 5.25 L working volume and 25 d HRT. Mao *et al.* (2015)
83 reported that under mesophilic conditions, an average HRT in the range of 15-30 d is
84 required to treat waste.

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85 The experiment was started by filling R1 with 1.4 kg inoculum and 0.7 kg DCM,
86 R2 with 5.011 kg inoculum₂ and 0.239 kg DCM and R3 with 5.040 kg inoculum and
87 0.210 kg DCM. From the second day, all digesters were fed as follows: 0.7, 0.239₂ and
88 0.210 kg DCM for R1, R2 and R3 respectively (after first removing the same amount of
89 digestate from a port at the base of the digesters) which continued for the following 21 d
90 adaptation period. The digesters were fed through a tube, the outlet of which was
91 submerged under the substrate level to avoid air ingress during the feeding process.
92 Data were collected after this 21 d startup period. During the data collection period, R1
93 was fed 0.7 kg DCM. Effluent from this digester (0.239 kg) was used to feed R2, while
94 R3 was fed 0.210 kg DCM. Digesters were kept at ambient temperature₂ and the
95 experiment was run for a period of three HRT corresponding to 75 d in total.

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97 Inoculum and Substrate

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

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106 Substrate was taken from dairy cows in the lactation period and was collected
 107 from the farm in [the](#) Faculty of Animal and Agricultural Sciences, Diponegoro
 108 University. Manure was diluted with tap water in the ratio 1:1.5. Manure was collected
 109 once per week and diluted with tap water directly and kept refrigerated. pH value,
 110 volatile solids (VS) and total ammonia nitrogen (TAN) concentration in the inoculum
 111 were 7.11, 7.33% and 265.18 mg/L respectively, while pH value, VS, TAN and volatile
 112 fatty acids (VFA) (C2-C5) concentration of DCM were 6.77, 7.40%, 97.98 mg/L and
 113 142.93 mg/L respectively.

114

115 Analytical Methods

116 Biogas from the laboratory scale bio-digesters was passed up through 0.5 L
 117 infusion bottles that contained 4% NaOH solution in order to absorb CO₂ using 5 ml
 118 diameter Teflon tubing. Methane production was measured on a daily basis by
 119 collecting the gas using 5 L [Tedlar](#) gas bags using a water displacement method (Figure
 120 1). The procedures to quantify gas production were: 1) valve to pump was in open
 121 position. 2) water pump was switched on, [Therefore](#) air in the measuring glass
 122 headspace was removed, and the headspace was filled up with tap water. 3) valve to
 123 pump was closed. 4) water pump was switched off. 5) valve to [Tedlar](#) gas bag was
 124 opened; therefore the methane in the [Tedlar](#) gas bag will move to the headspace of the
 125 measuring glass. 6) gas volume was read in the measuring glass scale. When the gas
 126 volume in the [Tedlar](#) gas bag exceeded the measuring glass volume, steps 1-6 were
 127 repeated. The net gas production was corrected to STP conditions.

128 Daily maximum and minimum ambient temperature was recorded using a digital
 129 hygrometer thermometer HTC-2 (Taiwan). Sample pH value was measured using a pH

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138 meter (Hanna® pH meter). Dry matter (DM) contents of samples were analyzed by
 139 drying at 105°C for 7 h. Ash was determined by combusting the dried samples at 550°C
 140 for 6 h, and VS was calculated by subtracting the ash weight from the DM
 141 (APHA,1995). TAN concentration was measured using photometric kits (HACH®
 142 USA: DOC316.53.01077) at 655 nm. VFA were determined using gas chromatography
 143 (Shimadzu GC-8). The collected data were statistically analyzed using ANOVA with
 144 95% confidence level. Duncan's multiple range tests were used in post ANOVA
 145 analysis when differences were found to be significant (Gomez and Gomez, 2007).

146

147

RESULTS

148

Ambient Temperature Variation

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

154

155

Methane production

156 The methane production of the three bio-digesters throughout the experiment is
 157 presented in Figure 3. The mean methane yields were 14.31 L kg/VS, 132.82 L kg/VS,
 158 and 146 L kg/VS for R1, R2 and R3 respectively Total methane yield of R1 and R2
 159 (R_{TS}) was 147.13 L kg/VS (Table 2). A study from Amon *et al.* (2007) using batch
 160 digesters with 60 d incubation period at 38°C showed that methane production of DCM
 161 from cows with medium milk production was 166.3 N L kg/VS while the study from

Commented [A4]: Where is Table 2?

162 Sutaryo *et al.* (2014) using continuous digesters with 20 d HRT at 35°C found that
 163 methane production of DCM was 177 L kg/VS. Both those studies were performed at
 164 constant mesophilic temperature while the study presented here was performed at
 165 ambient variable mesophilic temperatures. However the result of this study is similar to
 166 those previous results.

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168 Parameters in the Liquid Phase

169 Total VFA concentration and pH value of digested slurry are presented in Table
 170 1. The mean total VFA concentration was 160.74; 48.23; 39.19 mg/L for R1, R2, and
 171 R3, respectively. Total VFA concentration of digested slurry in R1 was significantly
 172 higher ($p < 0.05$) than that in R2 and R3 (Table 1). TAN concentration concentrations of
 173 digested slurry in this study were 137.85; 178.96; 185.86 mg/L for R1, R2, and R3,
 174 respectively (Table 2).

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175 Volatile solid reductions in this study were 29.85 and 28.03% for R2 and R3
 176 respectively. Study from Bhattacharya *et al.* (1996) found a VS reduction of 26% and
 177 30% for conventional and two-phase digester respectively, treating sludges from
 178 conventional activated sludge treatment plants and maintained at 35°C. Meanwhile, a
 179 study from Sutaryo *et al.* (2012) found a VS reduction in range of 27-35% for a reactor
 180 treating DCM with different TS concentrations. Therefore the result of this study is in
 181 accordance with the result of the previous study.

182

183

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](#) biogas 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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212 Temperature shocks caused a reduction in the methane production rate compared to that
213 of the control, but it recovered rapidly. Once adapted, no significant effect on the
214 methane production was observed between the control and the temperature shock bio-
215 digester. This fact therefore indicates that, even though methanogenic archaea are quite
216 sensitive to temperature shock they have considerable ability to adapt to temperature
217 changes (Chae *et al.*, 2008).

218 Methane production in term of digester volume of RTS was 29.98% higher than
219 that in R3. The positive effect ($p<0.05$) of the application of two phase digestion on the
220 methane production compared to that in the single phase reactor can be attributed to a
221 shorter HRT period in R1 and R2 than that in R3, therefore the amount of substrate
222 added to R1 and R2 was higher than that in R3. Since the amount of substrate added to
223 R2 (0.239 kg) was higher than that in R3 (0.210 kg) and in the same time the active
224 volume in both digester configurations was equal (5.25 L) therefore methane production
225 in term of digester volume R2 was higher than that in R3. In fact methane production in
226 RTS was methane yield in R1 and in R2. In this present study, HRT in R1, R2 and R3
227 were 3 d, 22 d and 25 d respectively. Bhattacharya *et al.* (1996) reported that one of the
228 advantages of phase separation is the ability to handle a higher organic loading rate than
229 that in a single reactor. A similar study from Tsigkou *et al.* (2020) found the same
230 phenomenon, in that the application of a two-stage digester treating co-digestion of used
231 disposable nappies and expired food product at 60:40 (v/v) ratio, working at mesophilic
232 condition ($37\pm 0.5^{\circ}\text{C}$) and 15 d HRT the energy production was 18.5% higher than that
233 in the single reactor.

234

235 **Parameters in the Liquid Phase**

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

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

250 Ammonia is one of the essential nutrients for the growth of microorganisms,
251 however it can inhibit the AD process if it is available at high concentrations (Yenigün
252 and Demirel, 2013). Under mesophilic conditions (35°C), the TAN inhibitory threshold
253 was in the concentrations of around 1700–1800 mg L⁻¹ for unacclimated inoculum
254 (Yenigün and Demirel, 2013). TAN concentration of digested slurry in R1 was
255 significantly lower ($p < 0.05$) than that in R2 and R3. This fact can be attributed to a
256 shorter HRT in R1 than that in R2 and R3 therefore microorganisms in R2 and R3 can
257 degrade more protein in the substrate, subsequently producing more ammonia. However
258 TAN concentrations of digested slurry from all digester in this study were below the
259 inhibitory level as reported by Yenigün and Demirel (2013).

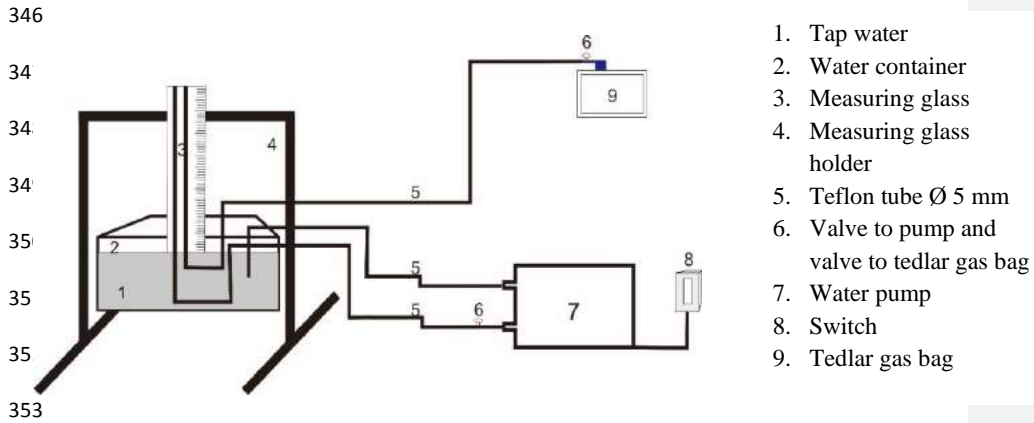
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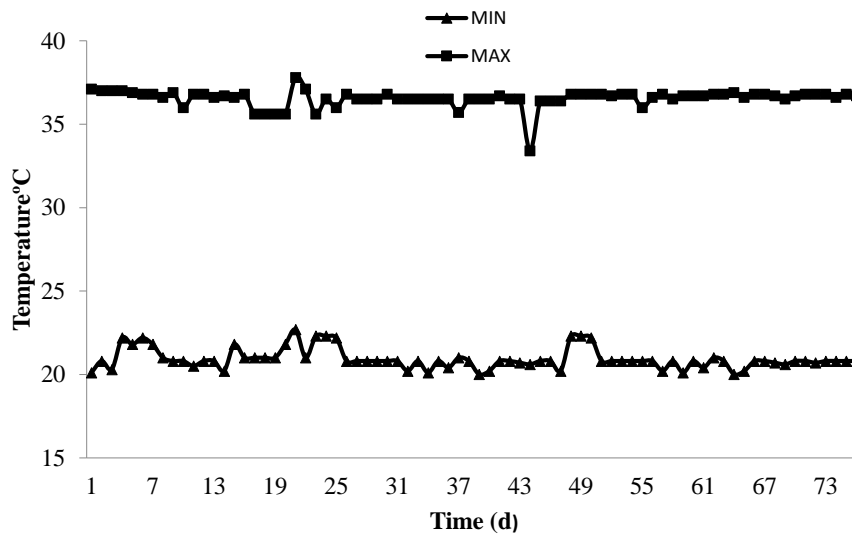
345 **Figure caption**



354 **Figure 1. Apparatus for measuring gas production**

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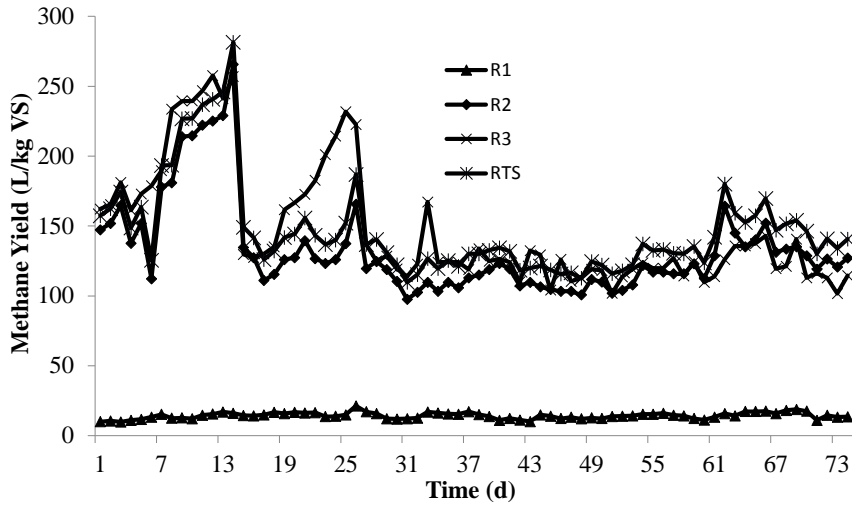
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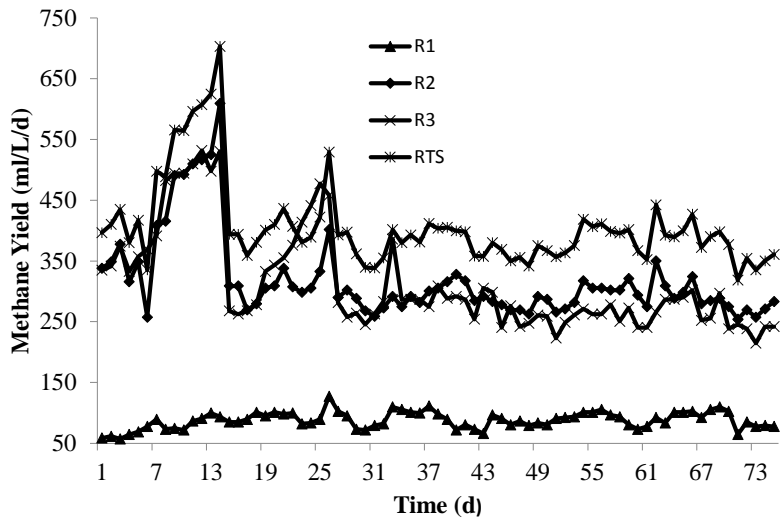
357 **Figure 2. Maximum-minimum ambient temperature during experiment**

358



359
360 Figure 3. A. Methane yield per kg VS added.

361



362
363 Figure 3. B. Methane yield per digester volume per day

364

365

366 **Table caption**

367 Table 1. Process parameters. Values in each column followed by the same letter are not
 368 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				

Commented [A7]: The Table should have clear title, and sufficient information about treatment initial (what is R1, R2, R3, and RTS). The information of statistical analysis should not to be a title of the table.

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3. Perbaiki draft publikasi dari penulis

Surat pengantar, respon penulis dan draft publikasi versi revisi

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

17th May 2020

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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Tropical Animal Science Journal



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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

		<p>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.</p>
2	<p>Please state the novelty of the research in the Introduction before resuming the objectives.</p>	<p>This information has been written by the author in the introduction section.</p> <p>There has been a lack of information regarding direct comparison of single and two-phase AD of DCM at tropical ambient temperature</p>
3	<p>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.</p>	<p>Conclusion has been revised according reviewer suggestion.</p> <p>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.</p>
B	Reviewer II (MB2)	
1	<p>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.</p>	

	<p>Several research indicators, including methane production, Total Volatile Fatty Acid, Total Ammonia Nitrogen, etc. has been presented in this paper.</p> <p>The manuscript has been written in good English and also showing good scientific merit. However, I consider these several matters:</p>	
2	<p>The abstract should describe research design, methods, results, statistical analysis of research parameters, and conclusion, which is unsown in the manuscript.</p>	<p>Abstract has been revised according reviewer suggestion:</p> <p>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.</p>

3	<p>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.</p>	<p>The advantage of a single-phase bioreactor has been added in the introduction section.</p> <p>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 <i>et al.</i>, 2014).</p>
4	<p>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.</p>	<p>Thank you very much for the corrections.</p> <ul style="list-style-type: none"> - 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	<p>In Figure 1, the resources of biogas should be shown in this design to indicate the one and two-phase of the biodigester.</p>	<p>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.</p>
6	<p>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.</p>	<p>Thank you very much for the correction. Table 1 has been revised according reviewer suggestion.</p>

7	<p>References</p> <p>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.</p> <p>d) References should be the last 10 year publications, with minimum 80% of journals.</p>	<p>- Thank you for the reviewer suggestion. Reference has been check.</p> <p>- Some old references have been replaced with new ones.</p> <p>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. <i>Agric Ecosyst Environ.</i> 118:173-182.</p> <p>Replaced by:</p> <p>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. <i>Bioresource Biotechnol.</i> 133:23-30.</p> <p>2. Wilkerson, V. A., D. R. Mertens, & D. P. Casper. 1997. Prediction of Excretion of Manure and Nitrogen by Holstein Dairy Cattle. <i>J. Dairy Sci.</i> 80:3193-3204.</p> <p>Replaced by:</p> <p>3. Bhattacharya, S. K., R. L. Madura, D. A. Walling, & J. B. Farrell. 1996. Volatile solids reduction in two-phase and conventional anaerobic sludge digestion. <i>Water Res.</i> 30(5):1041-1048.</p> <p>Replaced by:</p> <p>Brown, D. & Y. Li. 2013. Solid state anaerobic co-digestion of yard waste and food waste for biogas production. <i>Bioresour. Technol.</i> 127:275-280.</p> <p>4. Sakar, S., K. Yetilmezsoy, & E. Kocak. 2009. Anaerobic digestion technology in poultry and livestock waste treatment – a literature review. <i>Waste Manag. Res.</i> 27:3-18.</p> <p>Replaced by:</p> <p>Mao, C., Y. Feng, X. Wang, & G. Ren. 2015. Review on research achievements of biogas from anaerobic digestion. <i>Renew. Sust. Energ. Rev.</i> 45:540-555.</p>
8	Please see other comments in the text	Thank you very much for the valuable comments. The manuscript has been revised according reviewer comments.

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

3
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5
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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

34

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

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

51 Technically, the AD process can take places in three different temperature ranges:
52 (1) psychrophilic (cryophilic) temperature from 10 to 20°C; (2) mesophilic temperature
53 from 20 to 40°C; and (3) thermophilic temperature from 40 to 60°C (Burton and Turner,
54 2003). Based on those temperature range criteria, the AD process can be implemented at
55 tropical ambient temperatures. Moreover, operation of AD at tropical ambient
56 temperatures offers advantages compared to the operation of AD under mesophilic or

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

59 Among other biogas digester designs, the continuously stirred tank reactor
60 (CSTR) design is the most commonly applied bioreactor for treating agricultural waste
61 (Linke *et al.*, 2015). While in operation, the process of biodegradation of organic waste
62 can be in single or two-phases. The two-phase AD process has several advantages
63 compared to a single phase. These include the selection and enrichment of different
64 bacteria in each digester, increasing the stability of the process by controlling the
65 acidification stage therefore reducing the risk of overloading and the buildup of toxic
66 material. The first stage in the two-phase configuration can act as metabolic buffer
67 preventing pH shock to the methanogenic microorganisms and low pH in the first stage
68 due to a high organic loading rate favors the establishment of the acidogenic phase
69 (Sinbuathong *et al.*, 2012). On the other hand, single-phase bio-digester has also
70 advantageous as it is a simple and straightforward operation and for easier degradable
71 substrate such as fruit and vegetables waste single-phase process could be the preferred
72 rather than two-phase reactor (Ganesh *et al.*, 2014). Although previous studies have
73 evaluated the AD process at ambient temperature (Minale and Worku, 2014; Wei *et al.*,
74 2014; Murrugan and Appavu, 2018) and two-phase AD (Baldi *et al.*, 2019; Tsigkou *et*
75 *al.*, 2020), to the best of our knowledge there has been a lack of information regarding
76 direct comparison of single and two-phase AD of DCM at tropical ambient temperature.
77 Therefore the aim of this current study was to further knowledge in this specific area.

78

79

MATERIALS AND METHODS

80

Experimental Set-Up

81 Evaluation of single and two-phase processes was conducted using three identical
82 digesters, namely R1, R2, R3. The reactors were made from stainless steel, and in order
83 to minimize temperature fluctuations between day and night time, all digesters were made
84 with double layers. The two-phase digesters consisted of reactors R1 and R2. Reactor 1
85 had a 2.1 L working volume (the minimum volume that can be applied in the reactor) and
86 3 d hydraulic retention (HRT), while R2 had 5.25 L working volume and 22 d HRT.
87 Therefore in total, R1 and R2 had a 25 d HRT. R3 served as the single-phase bioreactor
88 and had 5.25 L working volume and 25 d HRT. Mao *et al.* (2015) reported that under
89 mesophilic conditions, an average HRT in the range of 15-30 d is required to treat waste.

90 The experiment was started by filling R1 with 1.4 kg inoculum and 0.7 kg DCM,
91 R2 with 5.011 kg inoculum, and 0.239 kg DCM and R3 with 5.040 kg inoculum and
92 0.210 kg DCM. From the second day, all digesters were fed as follows: 0.7, 0.239, and
93 0.210 kg DCM for R1, R2 and R3 respectively (after first removing the same amount of
94 digestate from a port at the base of the digesters) which continued for the following 21 d
95 adaptation period. The digesters were fed through a tube, the outlet of which was
96 submerged under the substrate level to avoid air ingress during the feeding process. Data
97 were collected after this 21 d startup period. During the data collection period, R1 was
98 fed 0.7 kg DCM. Effluent from this digester (0.239 kg) was used to feed R2, while R3
99 was fed 0.210 kg DCM. Digesters were kept at ambient temperature, and the experiment
100 was run for a period of three HRT corresponding to 75 d in total.

101

102 **Inoculum and Substrate**

103 Inoculum in this study was sourced from the active biogas digester at the Faculty
104 of Animal and Agricultural Sciences, Diponegoro University. The digester treats DCM

105 and operates at ambient temperature. The digested slurry from the digester was transferred
106 directly to the laboratory scale digesters.

107 Substrate was taken from dairy cows in the lactation period and was collected
108 from the farm in the Faculty of Animal and Agricultural Sciences, Diponegoro
109 University. Manure was diluted with tap water in the ratio 1:1.5. Manure was collected
110 once per week and diluted with tap water directly and kept refrigerated. pH value, volatile
111 solids (VS) and total ammonia nitrogen (TAN) concentration in the inoculum were 7.11,
112 7.33% and 265.18 mg/L respectively, while pH value, VS, TAN and volatile fatty acids
113 (VFA) (C2-C5) concentration of DCM were 6.77, 7.40%, 97.98 mg/L and 142.93 mg/L
114 respectively.

115

116 **Analytical Methods**

117 Biogas from the laboratory scale bio-digesters was passed up through 0.5 L
118 infusion bottles that contained 4% NaOH solution in order to absorb CO₂ using 5 ml
119 diameter Teflon tubing. Methane production was measured on a daily basis by collecting
120 the gas using 5 L Tedlar gas bags using a water displacement method (Figure 1). The
121 procedures to quantify gas production were: 1) valve to pump was in open position. 2)
122 water pump was switched on. Therefore air in the measuring glass head space was
123 removed, and the headspace was filled up with tap water. 3) valve to pump was closed.
124 4) water pump was switched off. 5) valve to Tedlar gas bag was opened therefore the
125 methane in the Tedlar gas bag will move to the head space of the measuring glass. 6) gas
126 volume was read in the measuring glass scale. When the gas volume in the Tedlar gas bag
127 exceeded the measuring glass volume, steps 1-6 were repeated. The net gas production
128 was corrected to STP conditions.

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

139

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RESULTS

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Ambient Temperature Variation

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Methane production

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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).

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

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

159

160

Parameters in the Liquid Phase

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

167 Volatile solid reductions in this study were 29.85 and 28.03% for R2 and R3
168 respectively. Brown and Li (2013) found a VS reduction of 27% and 33% for batch of
169 AD, treating yard waste and combination of 90% yard waste and 10% food waste
170 respectively, and maintained at 36°C for 30 d. Meanwhile, a study from Sutaryo *et al.*
171 (2012) found a VS reduction in range of 27-35% for a reactor treating DCM with different
172 TS concentrations. Therefore the result of this study is in accordance with the result of
173 the previous study.

174

DISCUSSION

175

Ambient Temperature Variation

176 This study was performed in July-September, and in Indonesia that period is
177 considered to be in the dry season. A large variation temperature in AD operation in the
178 course of this study therefore has an adverse impact on the microorganism activity. Mao
179 *et al.* (2015) reported that the AD process is carried out by a **prime balanced** population
180 of various microorganisms. These microorganisms are very sensitive to environmental
181 condition changes including temperature.

182

183 **Methane production**

184 There was no significant effect ($p>0.05$) of the application of a **two-phase** bio-
185 digester on specific methane yield in terms of kg VS of substrate added when compared
186 to that from the single digester configuration (Table 1). However, methane production of
187 R_{TS} was significantly higher ($p<0.05$) than that in R3 in term of L/L digester volume
188 (methane production/volume active). No significant effect ($p>0.05$) of the application of
189 the two-stage digester than single-digester on specific methane yield in this study can be
190 due to anaerobic microorganisms activities in both reactors configuration operation
191 efficiently. This study used digested slurry from an active digester that operated at a
192 tropical ambient temperature, the same condition used in this study. This fact, along with
193 the three weeks adaptation **period**, contributed to the efficient microorganism's activity
194 in both reactor configurations in this study even though there **was a** large temperature
195 difference between day and night time. A study by Chae *et al.* (2008) found that using
196 batch digesters and treating swine manure, the methane production at 30 and 35°C were
197 quite similar, but it was higher by more than 13-17% than that at 25°C. Temperature
198 shocks caused a reduction in the methane production rate compared to that of the control,
199 but it recovered rapidly. Once adapted, no significant effect on the methane production

200 was observed between the control and the temperature shock bio-digester. This fact
201 therefore indicates that, even though methanogenic archaea are quite sensitive to
202 temperature shock they have considerable ability to adapt to temperature changes (Chae
203 *et al.*, 2008).

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

219

220 **Parameters in the Liquid Phase**

221 During bioconversion of organic matter in AD system there are four steps, namely
222 hydrolysis, acidogenesis, acetogenesis and methanogenesis. In a two stage digester
223 configuration, the first digester serves as the acidogenic phase (Sinbuatong *et al.*, 2012),

224 therefore the VFA concentration will be higher than that in the second digester. A higher
225 total VFA concentration in R1 than that on other reactor in this study is in accordance
226 with Baldi *et al.* (2019) who found that total VFA concentration of digested slurry in a
227 fermentative digester was significantly higher than that of digested slurry from
228 methanogenic digester.

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

235 Ammonia is one of the essential nutrients for the growth of microorganisms,
236 however it can inhibit the AD process if it is available at high concentrations (Yenigün
237 and Demirel, 2013). Under mesophilic conditions (35°C), the TAN inhibitory threshold
238 was in the concentrations of around 1700–1800 mg/L for unacclimated inoculum
239 (Yenigün and Demirel, 2013). TAN concentration of digested slurry in R1 was
240 significantly lower ($p < 0.05$) than that in R2 and R3. This fact can be attributed to a shorter
241 HRT in R1 than that in R2 and R3 therefore microorganisms in R2 and R3 can degrade
242 more protein in the substrate, subsequently producing more ammonia. However TAN
243 concentrations of digested slurry from all digester in this study were below the inhibitory
244 level as reported by Yenigün and Demirel (2013).

245 There was no significant effect ($p > 0.05$) of the application two stage compared to
246 single stage digesters on the VS reduction. No significant effect of phase separation on

247 volatile solid reduction in this study suggests that microorganisms in both reactor
248 configurations can work well.

249

250

CONCLUSION

251 It has been demonstrated that the application of a two-phase digester treating
252 DCM working at a tropical ambient temperature significantly increased methane
253 production by 29.98% compared to the single stage reactor in terms of digester volume.
254 However, no significant effect of this digester configuration on specific methane yield in
255 terms of VS. Both digester configurations can run properly with stable methane
256 production, low VFA, and TAN concentrations. Therefore the two-phase digester
257 configuration in tropical ambient temperature can be applied to increase methane
258 production in terms of digester volume. *However, to be implemented on an industrial
259 scale, further study is needed related to the addition of infrastructure to the two-phase
260 digester rather than the single reactor.*

261

262

CONFLICT OF INTEREST

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

266

267

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270

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338 **Figure caption**

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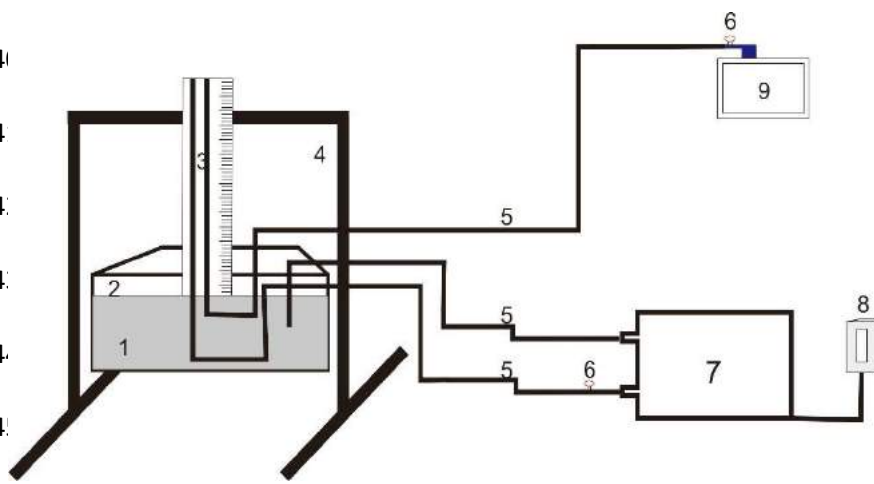
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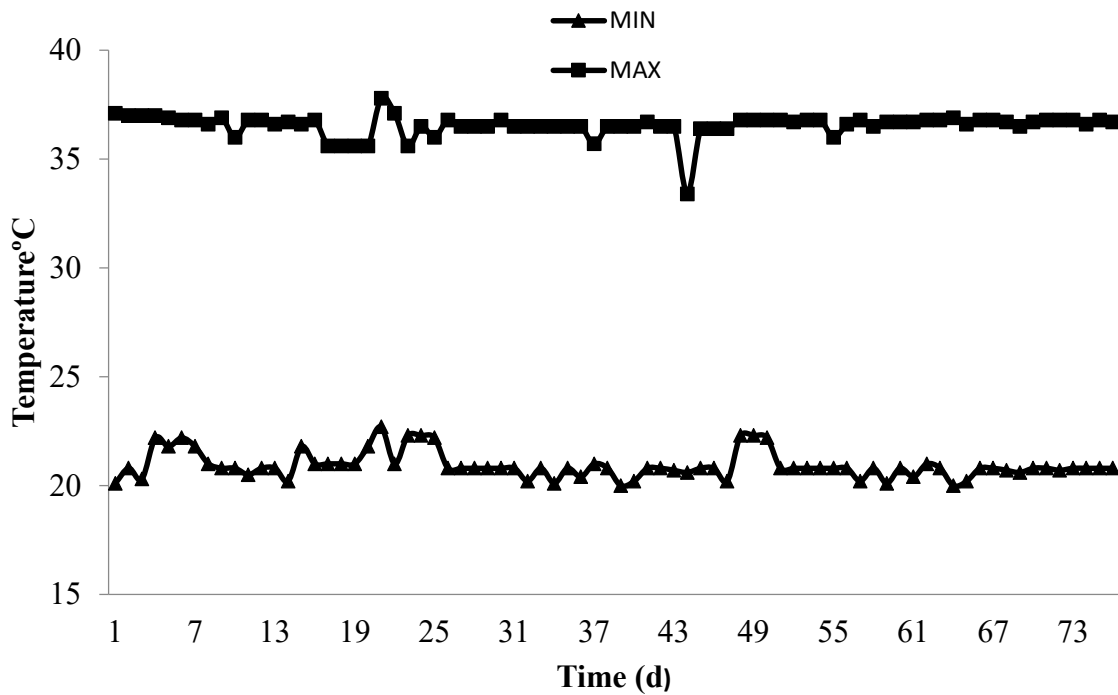
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- 1. Tap water
- 2. Water container
- 3. Measuring glass
- 4. Measuring glass holder
- 5. Teflon tube Ø 5 mm
- 6. Valve to pump and valve to tedlar gas bag
- 7. Water pump
- 8. Switch
- 9. Tedlar gas bag

347 Figure 1. Apparatus for measuring gas production

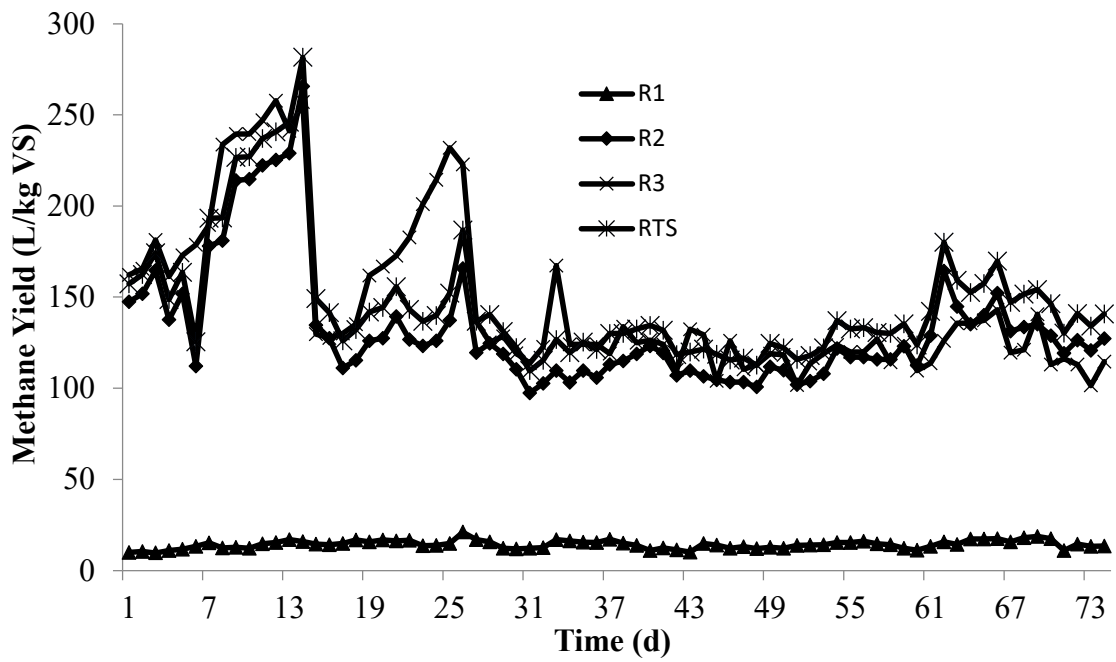
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350 Figure 2. Maximum-minimum ambient temperature during experiment

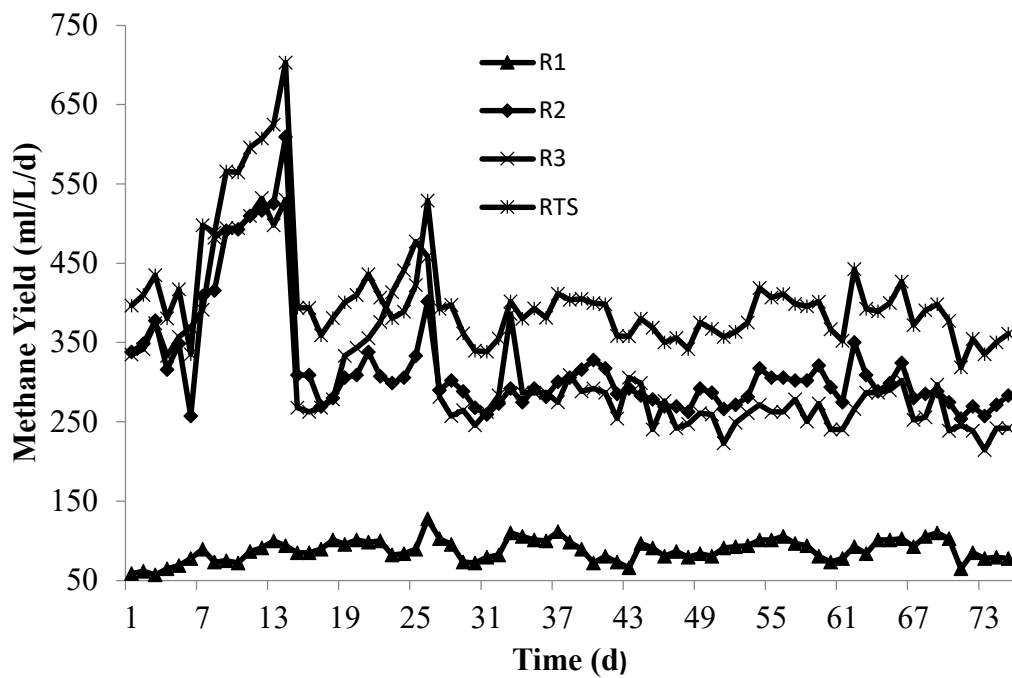
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353 Figure 3. A. Methane yield per kg VS added.

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

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358

359 **Table caption**

360 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				

361 ^{a,b}: Values in each column followed by the same letter are not significantly different ($p >$
362 0.05).

363 R1: First reactor of the two-phase digester

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

365 R3: Single-phase reactor

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



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4. Permintaan revisi dari editor terhadap hasil perbaikan pertama



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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




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. Perbaiki draft publikasi atas koreksi dari editor

Surat pengantar, form revisi, dan draft revisi kedua

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

7th July 2020

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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Tropical Animal Science Journal



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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:	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	<p>Thank you for the corrections. Why there is no superscripts in the methane yield of R1 and R2?</p> <p>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</p>
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1 **Performance Comparison of Single and Two-Phase Biogas Digester Treating Dairy**
2 **Cattle Manure at Tropical Ambient Temperature**
3
4

5 **ABSTRACT**

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

25
26 **INTRODUCTION**

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

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

43 Technically, the AD process can take places in three different temperature ranges:
44 (1) psychrophilic (cryophilic) temperature from 10 to 20°C; (2) mesophilic temperature
45 from 20 to 40°C; and (3) thermophilic temperature from 40 to 60°C (Burton and Turner,
46 2003). Based on those temperature range criteria, the AD process can be implemented at
47 tropical ambient temperatures. Moreover, operation of AD at tropical ambient
48 temperatures offers advantages compared to the operation of AD under mesophilic or
49 thermophilic temperatures since AD operation at higher temperatures **requires** significant
50 energy to maintain bioreactor temperature (Bandara *et al.*, 2012).

51 Among other biogas digester designs, the continuously stirred tank reactor
52 (CSTR) design is the most commonly applied bioreactor for treating agricultural waste
53 (Linke *et al.*, 2015). While in operation, the process of biodegradation of organic waste
54 can be in single or two-phases. The two-phase AD process has several advantages
55 compared to a single phase. These include the selection and enrichment of different
56 bacteria in each digester, increasing the stability of the process by controlling the
57 acidification stage therefore reducing the risk of overloading and the buildup of toxic
58 material. The first stage in the two-phase configuration can act as metabolic buffer
59 preventing pH shock to the methanogenic microorganisms and low pH in the first stage
60 due to a high organic loading rate favors the establishment of the acidogenic phase
61 (Sinbuathong *et al.*, 2012). On the other hand, single-phase bio-digester has also
62 advantageous as it is a simple and straightforward operation and for easier degradable
63 substrate such as fruit and vegetables waste single-phase process could be the preferred
64 rather than two-phase reactor (Ganesh *et al.*, 2014). Although previous studies have
65 evaluated the AD process at ambient temperature (Minale and Worku, 2014; Wei *et al.*,
66 2014; Murrugan and Appavu, 2018) and two-phase AD (Baldi *et al.*, 2019; Tsigkou *et*
67 *al.*, 2020), to the best of our knowledge there has been a lack of information regarding
68 direct comparison of single and two-phase AD of DCM at tropical ambient temperature.
69 Therefore the aim of this current study was to evaluate the process performance of single
70 and two-phase biodigester treating DCM and working in this specific area.

71

72

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

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

101 Substrate was taken from dairy cows in the lactation period and was collected
102 from the farm in the Faculty of Animal and Agricultural Sciences, Diponegoro
103 University. Manure was diluted with tap water in the ratio 1:1.5. Manure was collected
104 once per week and diluted with tap water directly and kept refrigerated. pH value, volatile
105 solids (VS) and total ammonia nitrogen (TAN) concentration in the inoculum were 7.11,
106 7.33% and 265.18 mg/L respectively, while pH value, VS, TAN and volatile fatty acids
107 (VFA) (C2-C5) concentration of DCM were 6.77, 7.40%, 97.98 mg/L and 142.93 mg/L
108 respectively.

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Analytical Methods

111 Biogas from the laboratory scale bio-digesters was passed up through 0.5 L
112 infusion bottles that contained 4% NaOH solution in order to absorb CO₂ using 5 ml
113 diameter Teflon tubing. Methane production was measured on a daily basis by collecting
114 the gas using 5 L Tedlar gas bags using a water displacement method (Figure 1). The
115 procedures to quantify gas production were: 1) valve to pump was in open position. 2)
116 water pump was switched on. Therefore air in the measuring glass head space was
117 removed, and the headspace was filled up with tap water. 3) valve to pump was closed.
118 4) water pump was switched off. 5) valve to Tedlar gas bag was opened therefore the
119 methane in the Tedlar gas bag will move to the head space of the measuring glass. 6) gas
120 volume was read in the measuring glass scale. When the gas volume in the Tedlar gas bag

121 exceeded the measuring glass volume, steps 1-6 were repeated. The net gas production
122 was corrected to STP conditions.

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

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RESULTS

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Ambient Temperature Variation

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Methane production

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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,

144 and 146 L/kg VS for R1, R2 and R3 respectively. Total methane yield of R1 and R2 (R_{TS})
145 was 147.13 L/kg VS (Table 1).

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Variables in the Liquid Phase

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DISCUSSION

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Ambient Temperature Variation

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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

167 There was no significant effect of the application of a two-phase bio-digester on
168 specific methane yield in terms of kg VS of substrate added when compared to that from
169 the single digester configuration (Table 1). However, methane production of R_{TS} was
170 significantly higher ($p < 0.05$) than that in R3 in term of L/L digester volume (methane
171 production/volume active). No significant effect of the application of the two-stage
172 digester than single-digester on specific methane yield in this study can be due to
173 anaerobic microorganisms activities in both reactors configuration operation efficiently.
174 This study used digested slurry from an active digester that operated at a tropical ambient
175 temperature, the same condition used in this study. This fact, along with the three weeks
176 adaptation period, contributed to the efficient microorganism's activity in both reactor
177 configurations in this study even though there was a large temperature difference between
178 day and night time. A study by Chae *et al.* (2008) found that using batch digesters and
179 treating swine manure, the methane production at 30 and 35°C were quite similar, but it
180 was higher by more than 13%-17% than that at 25°C. Temperature shocks caused a
181 reduction in the methane production rate compared to that of the control, but it recovered
182 rapidly. Once adapted, no significant effect on the methane production was observed
183 between the control and the temperature shock bio-digester. This fact therefore indicates
184 that, even though methanogenic archaea are quite sensitive to temperature shock they
185 have considerable ability to adapt to temperature changes (Chae *et al.*, 2008).

186 A study from Beneragama *et al.* (2013) using batch digesters with 16 d incubation
187 period at 55°C showed that methane production of DCM was 145.03 L/kg VS while the
188 study from Sutaryo *et al.* (2014) using continuous digesters with 20 d HRT at 35°C found
189 that methane production of DCM was 177 L/kg VS. Both those studies were performed
190 at constant mesophilic temperature while the study presented here was performed at

191 ambient variable mesophilic temperatures. However the result of this study is similar to
192 those previous results.

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

208 **Variables in the Liquid Phase**

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

215 fermentative digester was significantly higher than that of digested slurry from
216 methanogenic digester.

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

223 Ammonia is one of the essential nutrients for the growth of microorganisms,
224 however it can inhibit the AD process if it is available at high concentrations (Yenigün
225 and Demirel, 2013). Under mesophilic conditions (35°C), the TAN inhibitory threshold
226 was in the concentrations of around 1700–1800 mg/L for unacclimated inoculum
227 (Yenigün and Demirel, 2013). TAN concentration of digested slurry in R1 was
228 significantly lower ($p < 0.05$) than that in R2 and R3. This fact can be attributed to a shorter
229 HRT in R1 than that in R2 and R3 therefore microorganisms in R2 and R3 can degrade
230 more protein in the substrate, subsequently producing more ammonia. However TAN
231 concentrations of digested slurry from all digester in this study were below the inhibitory
232 level as reported by Yenigün and Demirel (2013).

233 There was no significant effect of the application two stage compared to single
234 stage digesters on the VS reduction. No significant effect of phase separation on volatile
235 solid reduction in this study suggests that microorganisms in both reactor configurations
236 can work well. Brown and Li (2013) found a VS reduction of 27% and 33% for batch of
237 AD, treating yard waste and combination of 90% yard waste and 10% food waste
238 respectively, and maintained at 36°C for 30 d. Meanwhile, a study from Sutaryo *et al.*

239 (2012) found a VS reduction in range of 27-35% for a reactor treating DCM with different
240 TS concentrations. Therefore the result of this study is in accordance with the result of
241 the previous study.

242

243

CONCLUSION

244 It has been demonstrated that the application of a two-phase digester treating
245 DCM working at a tropical ambient temperature significantly increased methane
246 production by 29.98% compared to the single stage reactor in terms of digester volume.
247 However, [there was no positive](#) effect of this digester configuration on specific methane
248 yield in terms of VS. Both digester configurations can run properly with stable methane
249 production, low VFA, and TAN concentrations. Therefore the two-phase digester
250 configuration in tropical ambient temperature can be applied to increase methane
251 production in terms of digester volume. [However, to be implemented on an industrial](#)
252 [scale, further study is needed related to the addition of infrastructure to the two-phase](#)
253 [digester rather than the single reactor.](#)

254

255

CONFLICT OF INTEREST

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

259

260

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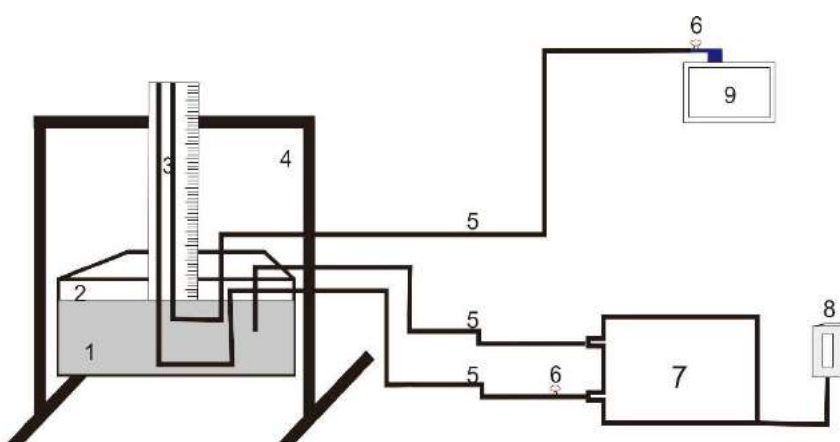
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1. Tap water
2. Water container
3. Measuring glass
4. Measuring glass holder
5. Teflon tube Ø 5 mm
6. Valve to pump and valve to tedlar gas bag
7. Water pump
8. Switch
9. Tedlar gas bag

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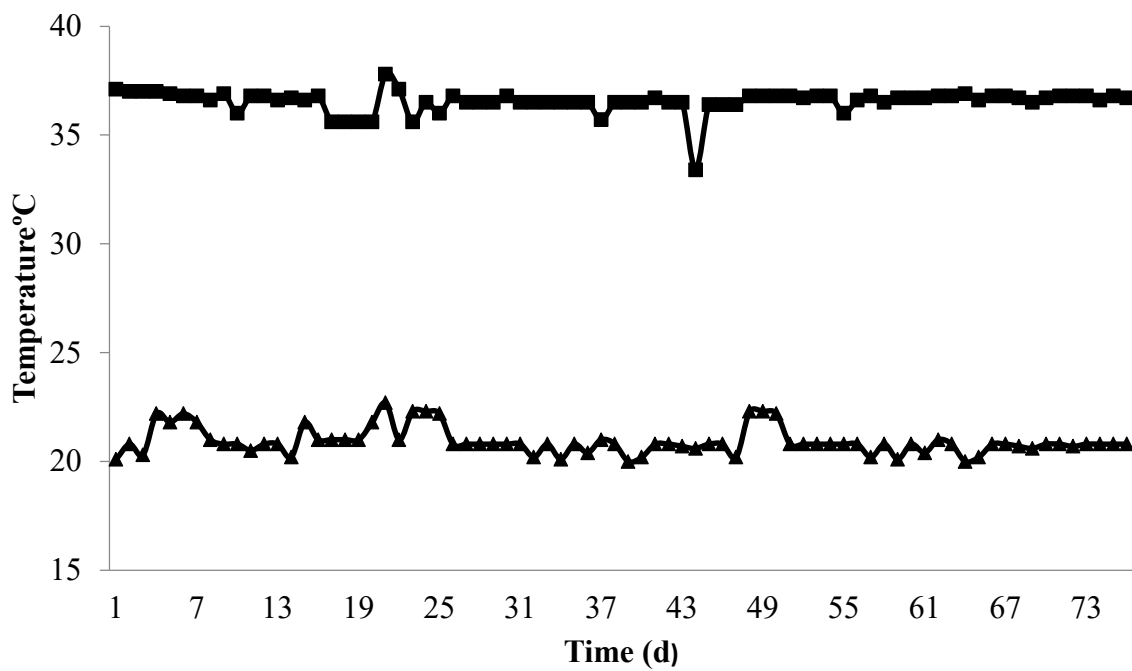
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338 Figure 1. Apparatus for measuring gas production

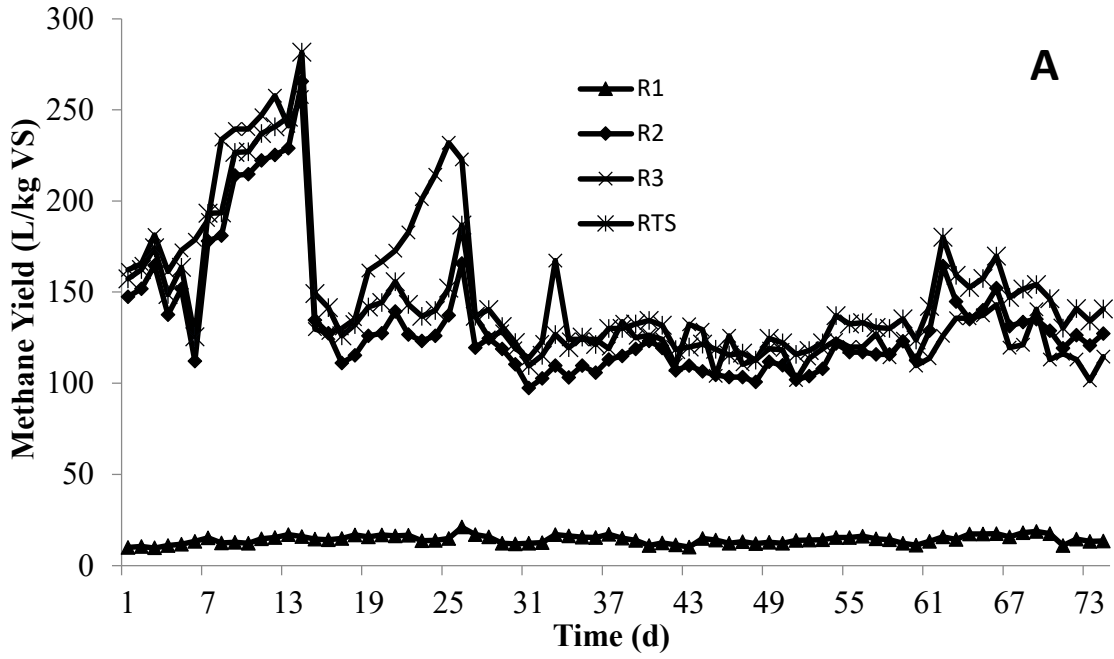


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340 Figure 2. Maximum-minimum ambient temperature during experiment. ■: maximum,

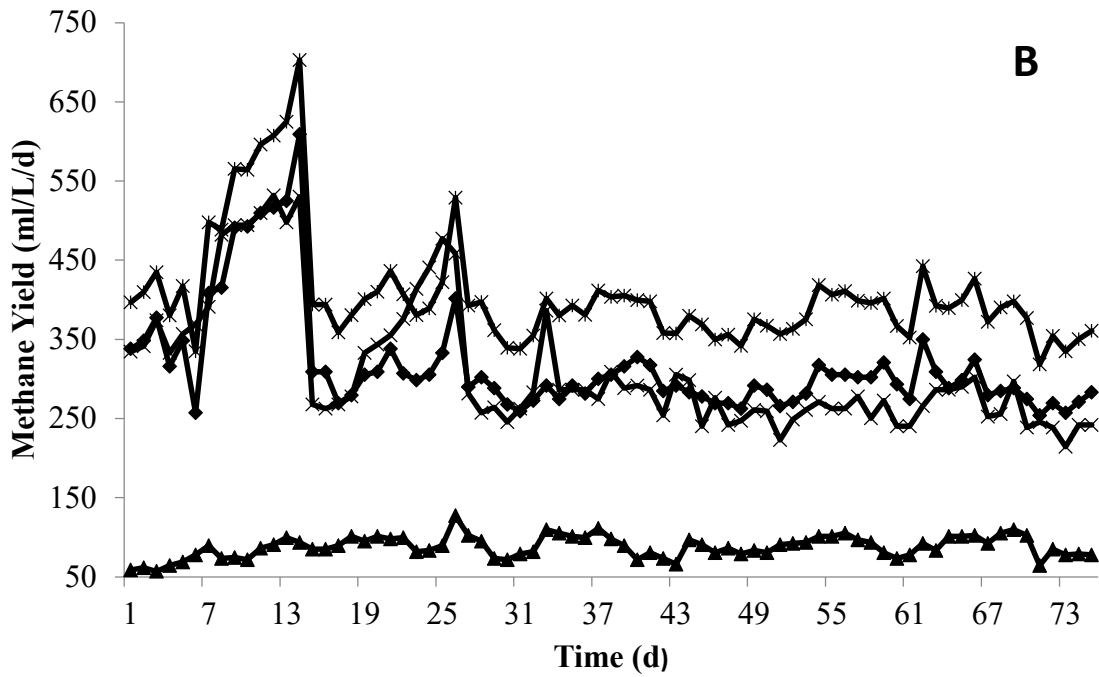
341 ▲: minimum.

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346 Figure 3. A. Methane yield per kg VS added. B. Methane yield per digester volume per

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day. ▲: R1, ◆: R2, ×: R3, *:R_{TS}.

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351 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 ±58.95	137.84 ^a ±45.32		6.46±0.17 ^a
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±0.17 ^b
R3	146.65±42.47	0.31±0.08 ^a	39.19 ^b ±23.23	185.86 ^b ±23.68	28.03 ^a ±3.19	6.90±0.28 ^b
R _{TS}	147.13±34.29	0.41±0.07 ^b				

352 Note: ^{a,b}: Values in the same column followed by the different superscript are significantly
353 different ($p < 0.05$). VFA: Volatile fatty acid, TAN: Total ammonia nitrogen, VS:
354 Volatile solid, R1: First reactor of the two-phase digester, R2: Second reactor of
355 the two-phase bio-degester, R3: Single-phase reactor, R_{TS}: Total sum methane
356 yield of R1 and R2.

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6. Draft publikasi dinyatakan diterima untuk dipublikasikan

 Prof. Dr. Komang G Wiryawan <jurnal@apps.ipb.ac.id>  Wed 7/15/2020 10:35 AM

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


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64 KB

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. Please find the attached invoice of the publication charge of your manuscript.
Thank you for your article submission, and we are looking forward to receive your incoming articles.

Prof. Dr. Komang G Wiryawan
Chief Editor
Tropical Animal Science Journal
kgwiryawan@yahoo.com

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You have a new notification from **Tropical** Animal Science Journal:

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To: Prof. Dr. Ir. Komang G. Wiryawan <kgwiryawan@yahoo.com>

Fri 10/30/2020 12:38 PM

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 Digester 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,

Sutarvo Sutarvo

28 production and low volatile fatty acids and total ammonia concentrations. The two-
29 phase bio-digester configuration can significantly increase methane production in terms
30 of digester volume.

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

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INTRODUCTION

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

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

50 Technically, the AD process can take place in three different temperature ranges:

51 (1) psychrophilic (cryophilic) temperature from 10°C to 20°C; (2) mesophilic

52 temperature from 20°C to 40°C; and (3) thermophilic temperature from 40°C to 60°C
53 (Burton & Turner, 2003). Based on those temperature-range criteria, the AD process
54 can be implemented at tropical ambient temperatures. Moreover, the operation of AD at
55 tropical ambient temperatures offers advantages compared to the operation of AD under
56 mesophilic or thermophilic temperatures since AD operation at higher temperatures
57 requires a significant amount of energy to maintain bioreactor temperature (Bandara *et*
58 *al.*, 2012).

59 Among the other biogas-digester designs, the continuously stirred-tank reactor
60 (CSTR) design is the most commonly applied bioreactor for treating agricultural waste
61 (Linke *et al.*, 2015). While in operation, the process of biodegradation of organic waste
62 can be in single or two-phases. The two-phase AD process has several advantages
63 compared to a single phase. These include the selection and enrichment of different
64 bacteria in each digester, increasing the stability of the process by controlling the
65 acidification stage, therefore reducing the risk of overloading and the build up of toxic
66 material. The first stage in the two-phase configuration can act as a metabolic buffer
67 preventing pH shock to the methanogenic microorganisms and low pH in the first stage
68 since a high organic loading rate favors the establishment of the acidogenic phase
69 (Sinbuathong *et al.*, 2012). On the other hand, single-phase biodigester has also
70 advantageous as it is a simple and straightforward operation and for an easier
71 degradable substrate such as fruit and vegetable waste, single-phase process could be
72 the preferred choice rather than two-phase reactor (Ganesh *et al.*, 2014). Although
73 previous studies have evaluated the AD process at ambient temperature (Minale &
74 Worku, 2014; Wei *et al.*, 2014; Murrugan and Appavu, 2018) and two-phase AD (Baldi
75 *et al.*, 2019; Tsigkou *et al.*, 2020), to the best of our knowledge there has been a lack of

76 information regarding to a direct comparison of single and two-phase AD of DCM in
77 tropical ambient temperature. Therefore, the aim of this current study was to evaluate
78 the process performance of single and two-phase biodigesters treating DCM and
79 working in this specific area.

80

81

MATERIALS AND METHODS

Experimental Set-Up

82 Evaluation of single and two-phase processes was conducted using three
83 identical digesters, namely R1, R2, and R3. The reactors were made from stainless steel,
84 and in order to minimize temperature fluctuations between day and night times, all
85 digesters were made with double layers. The two-phase digesters consisted of reactors
86 R1 and R2. Reactor 1 had a 2.1 L working volume (the minimum volume that can be
87 applied in the reactor) and 3 d hydraulic retention (HRT), while R2 had 5.25 L working
88 volume and 22 d HRT. Therefore in total, R1 and R2 had a 25 d HRT. R3 served as the
89 single-phase bioreactor and had 5.25 L working volume and 25 d HRT. Mao *et al.*
90 (2015) report that under mesophilic conditions, an average HRT in the range of 15-30 d
91 is required to treat waste.

92 The experiment was started by filling R1 with 1.4 kg inoculum and 0.7 kg DCM,
93 R2 with 5.011 kg inoculum and 0.239 kg DCM, and R3 with 5.040 kg inoculum and
94 0.210 kg DCM. From the second day, all digesters were fed as follows: 0.7 kg, 0.239kg,
95 and 0.210 kg DCM for R1, R2, and R3, respectively (after the first removing of the
96 same amount of digestate from a port at the base of the digesters) which continued for
97 the following 21 d adaptation period. The digesters were fed through a tube, the outlet
98 of which was submerged under the substrate level to avoid air ingress during the
99 feeding process. Data were collected after this 21 d startup period. During the data
100 collection period, R1 was fed 0.7 kg DCM. Effluent from this digester (0.239 kg) was
101 used to feed R2, while R3 was fed 0.210 kg DCM. Digesters were kept at ambient
102 temperature, and the experiment was run for a period of three HRT corresponding to 75
103 d in total.

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Inoculum and Substrate

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

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

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Analytical Methods

122 Biogas from the laboratory scale bio-digesters was passed up through 0.5 L
123 infusion bottles that contained 4% NaOH solution in order to absorb CO₂ using 5 mL
124 diameter Teflon tubing. Methane production was measured on a daily basis by
125 collecting the gas using 5 L Tedlar gas bags using a water displacement method (Figure
126 1). The procedures to quantify gas production consisted of 6 steps. 1) The valve to
127 pump was in an open position. 2) The water pump was switched on, therefore, air in the
128 measuring glass headspace was removed, and the headspace was filled up with tap
129 water. 3) The valve to pump was closed. 4) The water pump was switched off. 5) The

130 valve to Tedlar gas bag was opened therefore the methane in the Tedlar gas bag will
131 move to the headspace of the measuring glass. 6) The gas volume was read in the
132 measuring glass scale. When the gas volume in the Tedlar gas bag exceeded the
133 measuring glass volume, then the steps 1-6 were repeated. The net gas production was
134 corrected to STP conditions.

135 Daily maximum and minimum ambient temperatures were recorded using a
136 digital hygrometer thermometer HTC-2 (Taiwan). The sample pH value was measured
137 using a pH meter (Hanna® pH meter). Dry matter (DM) contents of samples were
138 analyzed by drying at 105°C for 7 h. Ash was determined by combusting the dried
139 samples at 550°C for 6 h, and VS was calculated by subtracting the ash weight from the
140 DM (APHA,1995). TAN concentration was measured using photometric kits (HACH®
141 USA: DOC316.53.01077) at 655 nm. VFA were determined using gas chromatography
142 (Shimadzu GC-8). The collected data were statistically analyzed manually using
143 ANOVA with 95% confidence level. Duncan's multiple range tests were used in post
144 ANOVA analysis when differences were found to be significant (Gomez & Gomez,
145 2007).

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RESULTS

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Ambient Temperature Variation

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

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Methane Production

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Variables in the Liquid Phase

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DISCUSSION

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Ambient Temperature Variation

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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).

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.

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

177 ambient temperature in this study (Figure 2) falls into the mesophilic category (Burton
178 and Turner, 2003).

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Methane Production

181 There was no significant effect of the application of a two-phase bio-digester on
182 specific methane yield in terms of kg VS of substrate added when compared to that
183 from the single digester configuration (Table 1). However, methane production of R_{TS}
184 was significantly higher ($p < 0.05$) than that in R3 in terms of L/L digester volume
185 (methane production/volume active). The non-significant effect of the application of the
186 two-stage digester than single digester on specific methane yield in this study can be
187 due to the activities of anaerobic microorganisms in both reactor configurations operate
188 efficiently. This study used digested slurry from an active digester that operated at a
189 tropical ambient temperature, the same condition used in this study. This fact, along
190 with the three weeks adaptation period, contributed to the efficient microorganism's
191 activity in both reactor configurations in this study even though there was a large
192 temperature difference between day and night time. A study by Chae *et al.* (2008) found
193 that using batch digesters and treating swine manure, the methane production at 30 and
194 35°C were quite similar, but it was higher by more than 13%-17% than that at 25°C.
195 Temperature shocks caused a reduction in the methane production rate compared to that
196 of the control, but it recovered rapidly. Once adapted, no significant effect on methane
197 production was observed between the control and the temperature shock bio-digester.
198 This fact therefore indicates that, even though methanogenic archaea are quite sensitive
199 to temperature shock, they have considerable abilities to adapt to temperature changes
200 (Chae *et al.*, 2008).

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

208 Methane production in terms of digester volume of R_{TS} was 29.98% higher than
209 that in R3. The positive effect ($p < 0.05$) of the application of two-phase digestion on the
210 methane production compared to that in the single-phase reactor can be attributed to a
211 shorter HRT period in R1 and R2 than that in R3, therefore the amounts of substrate
212 added to R1 and R2 were higher than that in R3. Since the amount of substrate added to
213 R2 (0.239 kg) was higher than that in R3 (0.210 kg) and in the same time, the active
214 volumes in both digester configurations were equal (5.25 L) therefore methane
215 production in term of digester volume R2 was higher than that in R3. In fact, methane
216 production in R_{TS} was the summation of methane yields in R1 and in R2. In this present
217 study, HRT in R1, R2, and R3 were 3 d, 22 d, and 25 d, respectively. Sinbuathong *et al.*
218 (2012) reported that one of the advantages of phase separation is the ability to handle a
219 higher organic loading rate than that in a single reactor. A similar study from Tsigkou *et al.*
220 (2020) found the same phenomenon, in that the application of a two-stage digester
221 treating co-digestion of used disposable nappies and expired food product at 60:40 (v/v)
222 ratio, working at mesophilic condition ($37 \pm 0.5^\circ\text{C}$) and 15 d HRT, the energy production
223 was 18.5% higher than that in the single reactor.

224

Variables in the Liquid Phase

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

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

239 Ammonia is one of the essential nutrients for the growth of microorganisms,
240 however, it can inhibit the AD process if it is available at high concentrations (Yenigün
241 and Demirel, 2013). Under mesophilic conditions (35°C), the TAN inhibitory threshold
242 was in the concentrations of around 1700–1800 mg/L for unacclimated inoculum
243 (Yenigün & Demirel, 2013). The TAN concentration of digested slurry in R1 was
244 significantly lower ($p < 0.05$) than those in R2 and R3. This fact can be attributed to a
245 shorter HRT in R1 than those in R2 and R3 therefore, microorganisms in R2 and R3 can
246 degrade more protein in the substrate, subsequently producing more ammonia.
247 However, TAN concentrations of digested slurry from all digester in this study were
248 below the inhibitory level reported by Yenigün & Demirel (2013).

249 There was no significant effect of the application of two stages compared to
250 single stage digester on the VS reduction. No significant effect of phase separation on
251 volatile solid reduction in this study suggests that microorganisms in both reactor
252 configurations can work well. Brown and Li (2013) found VS reductions of 27% and
253 33% for the batch of AD, treating yard waste and combination of 90% yard waste and
254 10% food waste, respectively, and maintained at 36°C for 30 d. Meanwhile, a study
255 from Sutaryo *et al.* (2012) found a VS reduction in the range of 27-35% for a reactor
256 treating DCM with different TS concentrations. Therefore, the result of this study is in
257 accordance with the result of the previous study.

258

259

CONCLUSION

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

268

269

CONFLICT OF INTEREST

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

273

274

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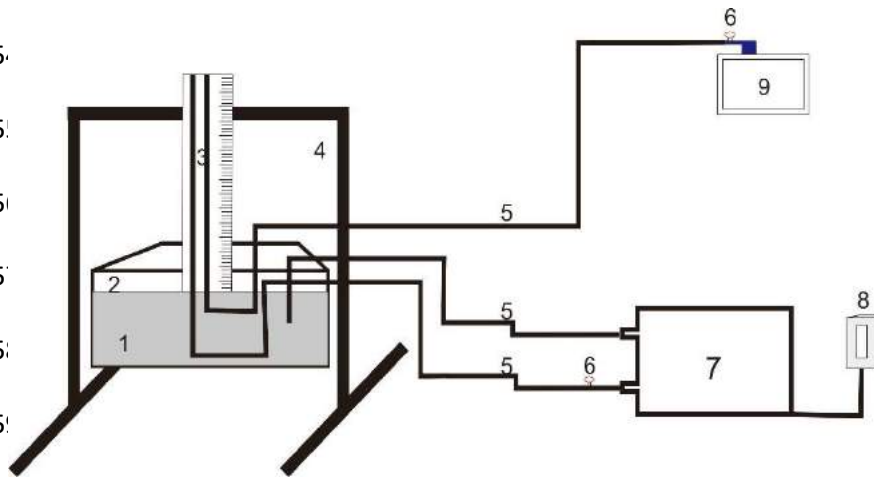
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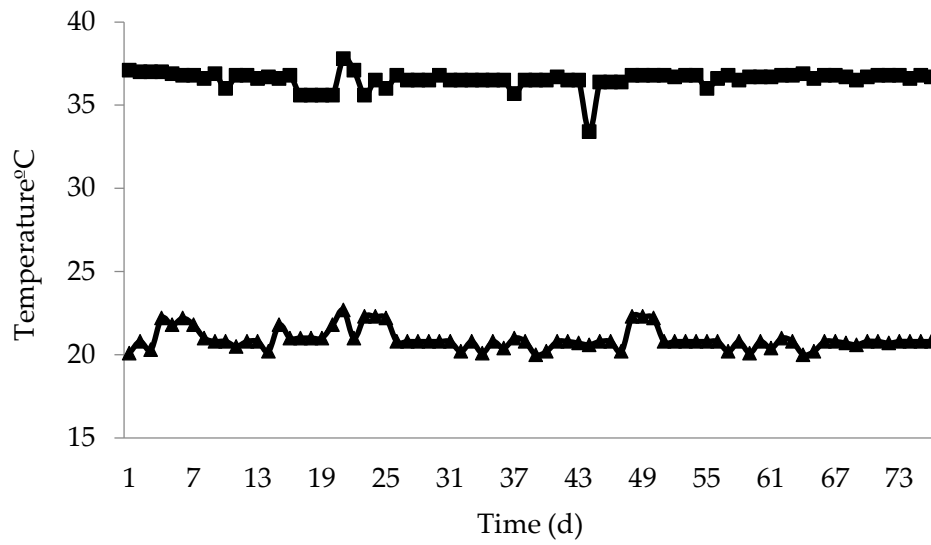


1. Tap water
2. Water container
3. Measuring glass
4. Measuring glass holder
5. Teflon tube Ø 5 mm
6. Valve to pump and valve to tedlar gas bag
7. Water pump
8. Switch
9. Tedlar gas bag

361 Figure 1. Apparatus for measuring gas production

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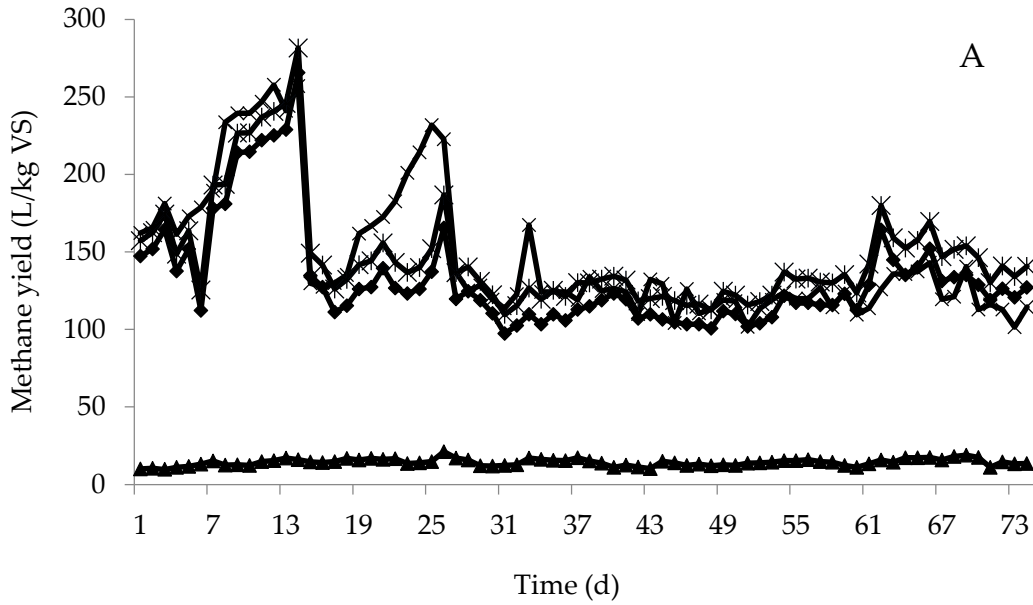
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365 Figure 2. Maximum-minimum ambient temperatures during experiment. ■: maximum,

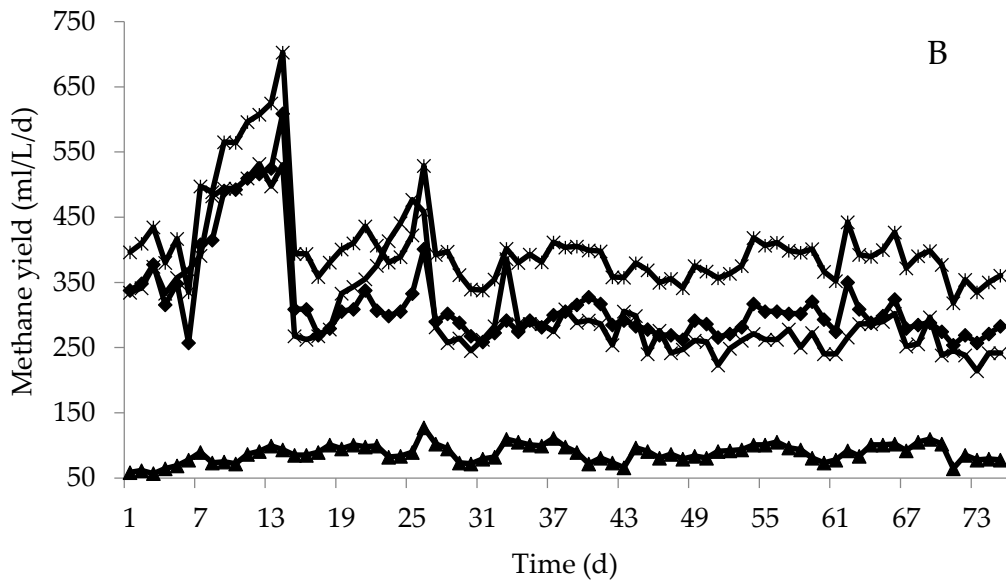
366 ▲: minimum.

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371 Figure 3. A. Methane yield per kg VS added. B. Methane yield per digester volume per

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day. ▲: R1, ◆: R2, ×: R3, *:R_{TS}.

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377 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 ^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				

378 Note: VFA= Volatile fatty acid; TAN= Total ammonia nitrogen; VS= Volatile solid;

379 R1= First reactor of the two-phase digester; R2= Second reactor of the two-
380 phase bio-digester; R3= Single-phase reactor, R_{TS}: Total sum methane yield of
381 R1 and R2. Means in the same **column** with different superscripts differ
382 significantly (p<0.05).



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10. Permintaan proofreading draft sebelum naskah terbit

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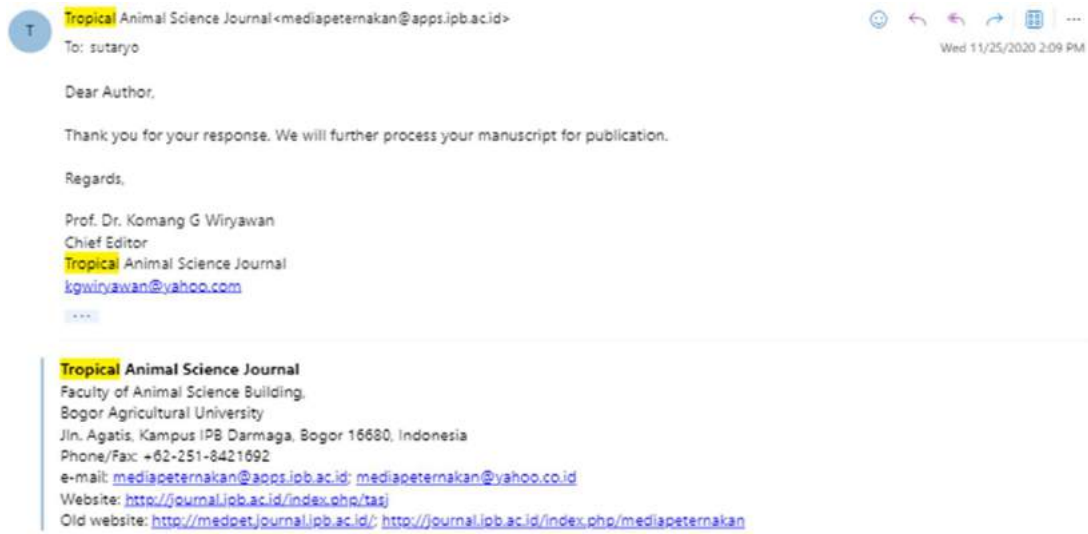
Thank you and best regards,

On behalf of all the authors,

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12. Draft memasuki proses produksi untuk penerbitan



13. Naskah publikasi terbit

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 bio-reactor temperature (Bandara *et al.*, 2012).

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

ling 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 advantages 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 CO₂ using 5 mL diameter Teflon tubing. Methane production was measured on a daily basis by collecting the gas using 5 L Tedlar gas bags using a water displacement method (Figure 1). The procedures to quantify gas production consisted of 6 steps. 1) The valve to pump was in an open position. 2) The water pump was switched on, therefore, air in the measuring glass headspace was re-

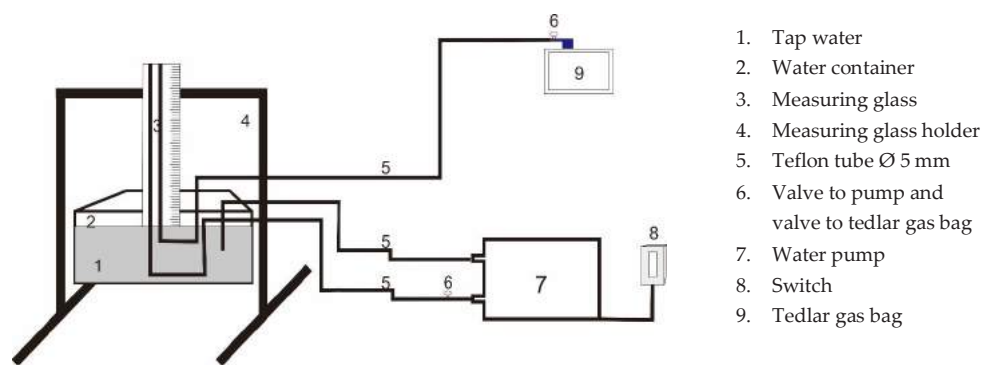


Figure 1. Apparatus for measuring gas production

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

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

RESULTS

Ambient Temperature Variation

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

Methane Production

The methane productions of the three bio-digesters throughout the experiment are presented in Figure 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).

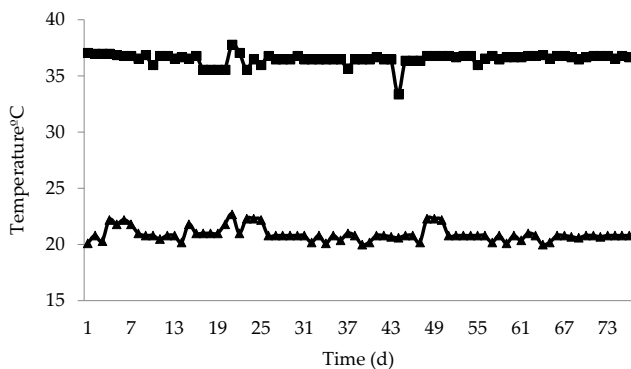


Figure 2. Maximum-minimum ambient temperatures during experiment. ■: maximum, ▲: minimum.

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).

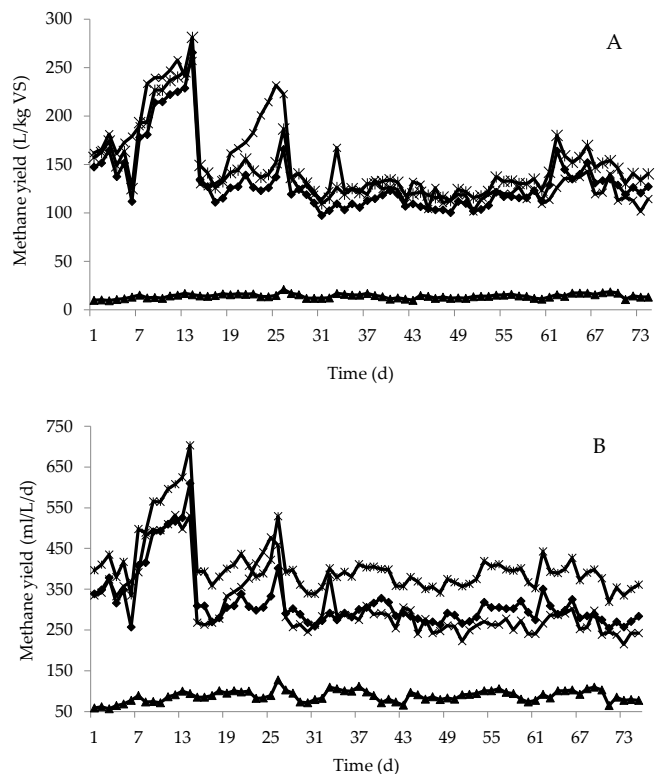


Figure 3. A. Methane yield per kg VS added. B. Methane yield per digester volume per day. ▲: R1, ◆: R2, ×: R3, *: RTS.

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

Reactors	Variables					pH
	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±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-digester; 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.

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