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Submission date: 18-Jun-2024 09:20AM (UTC+0700)
Submission ID: 2404515076
File name: APST_2.pdf (903.34K)
Word count: 4930
Character count: 24168

Asia-Pacific Journal of Science and Technology: Volume: 28. Issue: 03. Article ID.: APST-28-03-07. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research and Graduate Studies,
Khon Kaen University, Thailand

Increased methane yield from dairy cow manure by co-substrate with *Salvinia molesta*

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Received 24 April 2022
Revised 9 June 2022
Accepted 18 June 2022

Abstract

The objectives of this recent study were to examine the partial substitution of dairy cow manure (DCM) with aquatic weed *Salvinia molesta* (SM). The treatment consisted of replacing DCM with SM at four different levels of SM proportion in the combined substrate: 0%, 9.87%, 18.08%, and 25.02% in terms of volatile solid (VS), respectively. In addition, methane production of different parts of SM and biogas production of digested slurries were also evaluated. The result showed that methane production was increased significantly (p<0.05) by 21.89% and 22.73% in terms of substrate weight and active bio-digester volume with the highest proportion of SM (25.02%) in the combined substrate, with values of 11.86 L/kg and 0.54 L/L, respectively. The ultimate methane yield of the whole plant, shoot system (*i.e.,* the above ground part), and roots of SM were 118.49, 141.14, and 108.76 L/kg VS, respectively. The shoot system of SM had the highest methane yield and crude protein and the lowest ash and lignin concentrations, when compared to the whole plant and root system. As the proportion of SM in the combined substrate increased, so did the biogas production of the digested slurry. Therefore, when SM is used as co-substrate with DCM, the application of post digestion will be beneficial to capture the remaining biogas production of the digested slurry.

Keywords: Biogas, Co-digestion, Manure, Post-digestion, *Salvinia molesta*

1. Introduction

Salvinia molesta (SM) is known as the world's most invasive and dominant aquatic weed. It is a widespread and abundant aquatic weed that grows rapidly in both tropical and subtropical environments. When SM grows in a body of water, it quickly covers it, destroying all free-floating aquatic plants, cutting off sunlight for aquatic vegetation, limiting the supply of oxygen for photosynthesis beneath the SM bed, and resulting in poor physico-chemical quality of water in that area. This will eventually reduce biodiversity to the point where only a few species will survive. Other consequences, such as the loss of fisheries, stagnant water that facilitates mosquito breeding, and disruption of water-based transportation, will soon follow [1]. Along with its biomass production potential, SM also has a suitable chemical composition which allows for further processing into valuable products.

Previously, studies were conducted to investigate the handling of SM through anaerobic digestion (AD) to produce biogas, both as a single substrate [2] and as a co-substrate of SM and rice straw [3]. However, to the best of our knowledge, no studies have been conducted on the process performance of continuous stirred biogas reactors treating SM and dairy cow manure (DCM) in different volatile solids (VS) ratios, as well as batch digestion tests to evaluate methane production of SM whole plant, shoot system, and roots individually. Handling SM through AD in conjunction with DCM can have some positive effects by producing renewable energy in the form of biogas as a method of utilizing SM, while also improving the digested slurry quality as organic fertilizer.So far, AD of livestock manures alone to produce biogas is constrained by a low methane production per ton of substrate. This condition is caused by a low VS concentration, a high proportion of lignocellulosic

component (equal to or greater than 50%) in DCM [4], and a high concentration of ammonium and crude protein in pig and chicken manure [5]. In addition, the VS reduction of DCM in continuous stirred biogas digesters is only around 27-33% due to the high fibre content [6]. Anaerobic co-digestion of cattle manure (CM) with other organic materials with higher VS and nutrient concentrations than CM can be used to improve methane production. Study of [7] showed that anaerobic co-digestion of CM and pre-treated rape straw (PRS) with a VS ratio of 60:40 can increase methane production by 59.0% and 16.8%, respectively, over CM and PRS alone.

Co-digestion of DCM with SM is expected to improve the combined substrate's VS concentration, nutrient content, and methane production. However, because the substrate's hydraulic retention time (HRT) in the continuous feeding digester is limited, increasing the VS concentration may not allow all of the organic material to be degraded properly in the digester. This will result in lower digestion efficiency in terms of VS degradation in digesters with a high SM ratio. Therefore, a post-digestion test is required to assess the residual biogas yield of digested slurry, particularly in digesters with a high proportion of SM in the combined substrate. The objectives of this recent experiment were to: 1) determine the productivity of a continuous stirred tank reactor (CSTR) co-digesting DCM and SM in various VS ratios, 2) investigate biogas production of digested slurry following each treatment, and 3) evaluate methane production of SM whole plant, shoot system, and roots individually.

2. Materials and methods

2.1 SM collection

SM was collected from Pening Swamp located at District of Salatiga, Central Java. It was washed using tap water. For batch digestion tests, it was kept in the freezer until needed, whereas for use as a co-substrate with DCM, it was dried outdoors under shade and ground using a hammer mill with a 1 mm screen. Because this study used a laboratory-scale biogas digester, it was ground to obtain homogeneous samples for chemical analysis and to facilitate easy handling during co-digestion with DCM. The ground samples were kept at room temperature in sealed plastic containers. (Table 1). shows the chemical composition of the whole plant, shoot system, and roots of SM.

Table 1 Chemical composition of *Salvinia molesta*.

Component (% dry matter)	Root	Whole
Mass Balance (% of fresh)	54.41	100.00
Volatile solids	77.15	81.74
Ash	22.85	18.26
Crude protein	6.61	8.48
Extract ether	0.82	1.09
Crude fibre	20.21	24.37
Carbohydrate	44.37	41.6
Acid detergent fibre	54.01	57.44
Neutral detergent fibre	70.08	79.12
Lignin	43.03	41.67
Hemicellulose	16.07	21.68
Cellulose	10.98	13.83
C/N ratio	40.53	33.47

2.2 The inoculum and substrate

The inoculum for the continuous feeding experiment was the digested slurry from a previous experiment [8]. The pH, total solid (TS), and VS of the inoculum was 7.05, 4.05%, and 3.29%, respectively. The digested slurry from the CSTR digester experiment described in this section served as the inoculum for the batch digestion test. To degas the inoculum, the digested slurry was kept under anaerobic conditions for 1 week in an incubator (37 ºC), then it was filtered through a cloth and only the liquid fraction used as inoculum. The batch digestion test had a pH of 7.39, and its TS and VS were 1.14% and 0.56%, respectively. The basal substrate was made by diluting DCM with fresh water in a ratio of 1:1.75 (w/w), so its TS was around 7%, which is comparable to the 6-9% TS of DCM reported by [9]. The DCM was from cows in the lactation period and it was collected from the teaching farm at the Department of Animal Science, Diponegoro University, Semarang.

2.3 Experimental design

Three experiments were conducted in this study: batch digestion tests of SM fractions, process performance of a continuous biogas digester co-digesting SM and DCM in different VS ratio, and a post digestion test to assess

biogas production of digested slurry from all digesters. The first and the third experiment were conducted with 500 ml batch digestion tests using a method developed by [10], while the second experiment using a 7 L CSTR.

2.4 Batch digestion test

The batch digestion test was conducted to evaluate methane production of the whole plant, shoot system, and roots of SM. The test was performed using 500 ml infusion bottles with 200 g of inoculum and the necessary amount of sample (± 4 g) to achieve starter to sample proportion of 1:1 in terms of organic material concentration. Therefore, the batch digestion test had a working volume of approximately 204 ml. Control bio-reactors containing only starter were set up, and the control gas production was subtracted from the methane yield of bio-reactors containing sample to determine the sample's net gas production [10]. The digesters were then sealed with a rubber stopper and an aluminium crimp before being flushed with nitrogen for two minutes. The digesters were kept at 37ºC using an incubator. The batch digestion test was run for 90 d and was done with four replications. The configuration of batch digestion test is presented in (Figure 1).

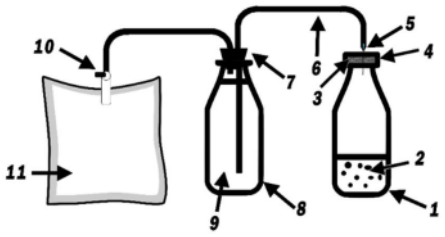

Figure 1 Batch bio-digester (1. Batch digester, 2. Inoculum and substrate, 3. Rubber stoper, 4. Aluminium crimp, 5. Hypodermic syringe, 6. Teflon tube, 7. Black rubber stopper, 8. Infusion bottle, 9. NaOH solution, 10. Valve, 11. Tedlar gas bag). Batch digester was connected to the series during gas collection period, the rest it was kept in the incubator.

2.5 Continuous feeding digesters test

Process performance of CSTRs co-digesting DCM and whole plant of SM in different VS ratio was conducted using four identical reactors (T1, T2, T3, and T4) with 7 L total capacity, 5.25 L working capacity, and 22 d HRT. Mao et al. [11] reported that at mesophilic temperatures, an average HRT in the range of 15-30 d is required to treat waste, so this study used 22 d per HRT cycle.

Therefore, there were four treatments with T1 digesting DCM alone and increasing proportions of SM from T2-T4 (Table 2). A propeller mixer with 36 revolutions per minute was used to mix the substrate in the bio-digester. The CSTRs were maintained at 37°C by keeping them in an incubator. The configuration of CSTR used in this study is illustrated in (Figure 2).

Table 2 Continuous digestion substrate properties.

Digester	TS (%)	VS (%)	Crude protein (%)	Proportion VS of SM (%)	DCM/SM VS ratio	pH	C/N ratio
T1	6.51	5.64	0.59	-	-	7.21	33.19
T2	7.82	6.51	0.75	9.87	9.13	7.16	30.14
T3	8.79	7.15	0.87	18.08	4.53	7.16	28.54
T4	9.89	7.75	0.92	25.02	3.00	7.12	29.25

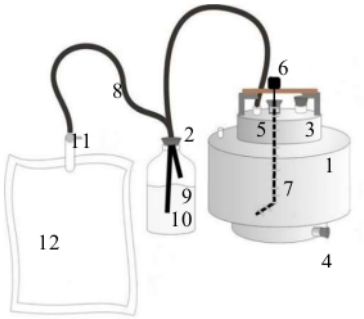

Figure 2 CSTR bio-digester [12] (1. Bio-digester, 2. Black rubber stoper, 3. Substrate inlet, 4. Digested slurry outlet, 5. Biogas outlet, 6. Dynamo, 7. Stirrer, 8. Teflon tube, 9. Infusion bottle, 10. NaOH solution, 11. Valve, 12. Tedlar gas bag). During the experiment, the digesters were kept in the incubator, while the infusion bottle and Tedlar gas bag were kept at room temperature outside of the incubator.

The study began by filling T1, T2, T3, and T4 with 5250 g of inoculum on the first day. From the second day on, 238.6 g of the basal substrate DCM was added after removing the same amount of digested slurry through the slurry outlet for each of T1-T4. During the adaptation period of two weeks, all digesters were fed the basal substrate. Following the two-week adaptation period, the treatment was initiated. The continuous feeding study was conducted three times during HRT, for a total of 66 d.

2.6 Post digestion test

On day 40-45 (after two times HRT), digested slurries from all CSTR digesters were collected and used for the post digestion test. The test was similar to the batch digestion test, but no inoculum was used in this study. Instead, 200 g of digested slurry was simply added to each 500 mL bottle, which was then sealed with rubber stoppers and aluminium crimps and kept at 37ºC in an incubator. The post digestion test was run for 30 d and was done in four replications for each CSTR digestate.

2.7 Analytical methods

Biogas was measured periodically using an acidified water displacement method as described by Møller et al. [13]. Methane productions were analysed by directing the produced biogas through a 0.5 L infusion bottle containing a 4% NaOH solution [14] using a 0.5 cm Teflon tube. The NaOH solution was replaced with a new one regularly. Methane was collected using a 1 L Tedlar gas bag for the batch digestion test and 5 L Tedlar gas bag for the continuous study (Hedetech-Dupont, China), and volume was measured periodically for the batch test and on a daily basis for the continuous study by liquid displacement method according [15].

The pH value of the sample was measured using a digital pH meter (Ohaus® ST300 pH meter). TS of the sample was determined by drying at 105°C for 7 hours, while ash concentration was analysed by combusting the dried samples at 550°C for 7 hours [16]. VS was calculated by subtracting the ash weight from the TS. Total Kjeldahl nitrogen (TKN) concentration was measured using the Kjeldahl standard method [16]. Crude protein was determined as TKN x 6.25. Total ammonia nitrogen (TAN) was evaluated by the distillation method [16]. Volatile fatty acid (VFA) concentration was analysed by titration method according to [16]. Total organic carbon (TOC) was estimated by dividing the VS value by 1.8 [17] and the C/N ratio was calculated from TOC/TKN. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin concentration were determined according to [18]. The concentration of hemicellulose in SM was calculated as NDF minus ADF, while the concentration of cellulose in SM was calculated as ADF minus acid detergent lignin (ADL), and lignin content was assumed to be equal to ADL [13].

The collected data were tabulated and were statically analysed using analysis of variance at the 5% confidence level [19]. Duncan multiple range tests were employed when there was a significant effect of the treatment on the observed variables.

3. Result and discussion

3.1 Batch digestion test

Methane productions of SM are presented in Table 3 and Figure 3. The results show that the ultimate methane yield (B_0 90d) of SM was 118 ± 8 ml/g VS. Results from previous studies showed that biogas yield of SM after 30 d of incubation was 330 ml/g VS [20] and 220 ml/g VS after 60 d fermentation [21]. In those two previous studies, the authors measured biogas production and methane concentration periodically and expressed the result as biogas rather than methane since the methane concentration varied across measurements. However, with the assumption of the methane concentration 50-55% in this study, therefore, the result presented here is comparable with Mathew *et al.* [21]. experiment result.

SM consists of stems, leaves, and roots. The branched stems grow flat, and the leaves can reach 30 cm in length [22]. This study evaluates the chemical composition and methane production of the different parts of SM, namely the whole of SM, the shoot system, and the roots (Figure 3, Table 1, and Table 3). Methane production of the different parts of SM was evaluated using a batch digestion test. Methane yield was statistically analysed on days 30, 60, and 90 of the incubation periods. Methane production of the shoot system of SM was the highest throughout the entire incubation period, whereas roots were the lowest. This is consistent with the order of crude protein content of the samples which showed the same pattern (Table 1). The ash and lignin content, in order of the highest value to the lowest, was found in roots, whole plant, and shoot system, respectively (Table 1). Li et al. [4] reported that a low methane production of DCM is caused by a high concentration of moisture, ash, and bio-fibre in DCM, while lignin is non-biodegradable in anaerobic environments [23].

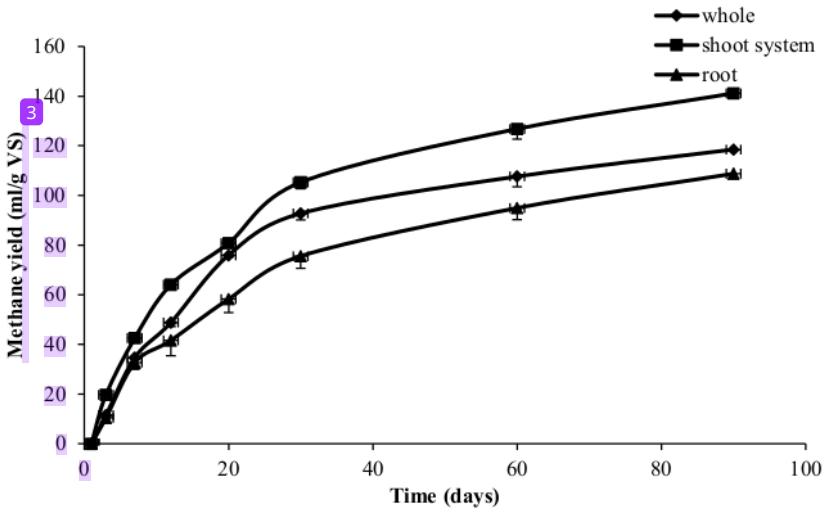

Figure 3 Cumulative methane production.

Table 3 Methane production of SM during batch digestion.

	Cumulative methane yield at 30 d (B_0: 30 d)	Cumulative methane yield at 60 d (B_0: 60 d)	Cumulative methane yield at 90 d (B_0 : 90 d)
	[ml/g VS]		
Whole plant	92.79 ± 9.36^{a}	107.67 ± 6.89^{a}	118.49 ± 8.10^{ab}
Shoot system	105.28 ± 1.03^{a}	126.80 ± 3.67^{b}	141.14 ± 7.23^{b}
Roots	75.55 ± 7.18^{b}	94.92 ± 1.95^{c}	108.76 ± 4.14^{a}

The values in each column that are followed by a different superscript letter differ significantly (p<0.05).

3.2 Methane production in continuous experiment

Methane productions of the continuous experiment are given in (Figure 4). and (Table 4). The average methane production of DCM in the control digester (T1) in this study was 172 ± 11 L/kg VS. The study from Sutaryo et al. [6] reported that methane yield of DCM in a CSTR at 35°C with 20 d HRT was 172 ± 10 L/kg VS. Another study by [24] found that methane yield of DCM in a plug flow digester with 25 d HRT, at 37-40°C was about

0.15-0.23 m^3/kg VS (150-230 L/kg VS). Thus, the result of this study was in line with the results of previous studies.

Methane production in terms of L/kg VS added was significantly ($p<0.05$) decreased by 6.37%, 7.39%, and 11.25% in T2, T3, and T4 respectively than that in T1. Methane yield in terms of L/kg VS added were 172.49 ± 11.38, 161.50 ± 15.43, 159.74 ± 15.74, and 153.08 ± 17.57 L/kg VS for T1, T2, T3, and T4, respectively. However, in terms of L/kg substrate, it increased by 8.02%, 17.37%, and 21.89% in T2, T3, and T4, respectively, compared to that in T1, while in terms of L/L digester volume it also increased by 9.09%, 18.18%, and 22.73% in T2, T3, and T4, respectively. Methane yield in terms of substrate VS weight decreased significantly ($p<0.05$) (Table 2) as the SM ratio in the sample increased. This was attributed to lower degradability of SM which resulted in a lower proportion of easily decomposed material per kg VS in the sample. The same phenomenon was discovered in [25], where methane yield in terms of substrate VS weight from a bio-reactor treating only swine manure was higher than that from a biogas digester handling a high concentration of solid fraction of swine manure, implying the latter was less degradable than the former. Moreover, [26] suggested that the potential methane production of a material relies both on the VS concentration of the sample and on the degree of digestibility of VS sample.

Co-digestion of DCM with SM gave a positive effect ($p<0.05$) to increase methane yield in terms of substrate weight and in terms of active bio-reactor volume. This phenomenon is explained by the fact that the increased proportion of SM in the substrate will increase the concentration of organic material. The increased methane production of biomass in terms of the L/kg sample is important since the economic calculation of methane production of the digester is based on L/kg biomass [27].

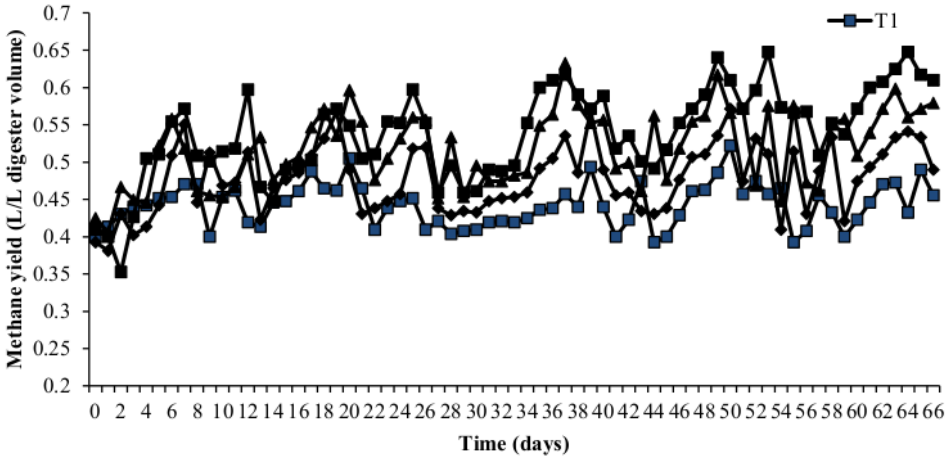

Figure 4 Methane yield in terms of L/L digester volume.

Table 4 Methane yield, TAN concentration, total VFA, VS digestibility and pH of digested bio-slurry.

	Methane yield				TAN		Total VFA	VS reduction	pH
	(L/kg substrate)	(L/L digester volume)	(L/kg VS added)		(mg/L)		(mM)	(%)	
T1	9.73 ± 0.64[a]	0.44 ± 0.03[a]	172.49 ± 11.38[a]		72.60 ± 14.42		154 ± 18.38	35.11 ± 2.89	6.94 ± 0.09
T2	10.51 ± 1.00[b]	0.48 ± 0.05[b]	161.50 ± 15.43[b]		75.40 ± 17.02		159 ± 31.07	37.03 ± 4.35	6.93 ± 0.07
T3	11.42 ± 1.13[c]	0.52 ± 0.05[c]	159.74 ± 15.74[b]		75.40 ± 15.81		166 ± 28.75	35.88 ± 2.55	6.95 ± 0.06
T4	11.86 ± 1.36[d]	0.54 ± 0.06[d]	153.08 ± 17.57[c]		79.60 ± 12.32		168 ± 13.98	35.28 ± 3.67	6.99 ± 0.08

Different superscripts in the same column are significantly different ($p<0.05$).

3.3 Variables in the liquid phase

Co-digestion of DCM and SM gave no significant effect ($p>0.05$) on the pH value of digested slurries, which were in the range of 6.93-6.99 (Table 4). This value is in the ideal stable range, as reported by [11] that the normal

pH for an AD process is about of 6.8 to 7.4. The VS reduction was in the range of 35-37%. There was no positive effect on VS digestibility with the utilization SM (p>0.05) as a co-substrate of DCM. Bruni et al. [28] reported that the biodegradation rate of livestock manure is about 40-50% of TS. The low biodegradation rate of manure is caused by the large fraction of lignocellulosic biofibres in animal manure.

Similarly, application of SM as a co-substrate with DCM (Table 4) gave no effect (p>0.05) on TAN concentration and total VFA. TAN concentrations were in the range 72-79 mg/L, while the total VFA concentration was 154-168 mM. The low concentration of TAN is in line with the low protein content in SM and in the combined substrate of SM and DCM. Ammonia is a decomposition product of protein in the substrate that will be used for growth and reproduction by anaerobic microorganisms. However, it can be toxic to anaerobic mesophilic microorganisms when the TAN concentration is over 1700-1800 mg/L [29]. There was a tendency that the total VFA concentration was slightly increased with the enhanced proportion of SM in the substrate.

3.4 Post digestion test

Biogas productions of digested slurries are presented in Table 5. The result showed that in terms of L/kg VS, the gas production was significantly different (p<0.05) with higher content of SM in the sample leading to lower biogas production of the digested slurry. This result was in line with the methane yield in the continuous study where higher SM concentration in the substrate led to lower methane production in term L/kg VS. However, biogas production of digested slurry in terms of L/kg digested slurry was significantly higher (p<0.05) with increased SM content in the substrate. This can be explained in the same way as the previous study, where SM addition was found to reduce degradability but increased the concentration of VS. These results indicated that when SM is used as a co-substrate with DCM in AD, post digestion is needed in order to capture the residual methane production in the digested slurry, or an HRT longer than 22 days is required.

Table 5 Biogas production of digested slurry.

	Biogas production in post digestion test	
	(L/kg VS)	(L/kg digested slurry)
T1	96.07 ± 5.06^{a}	3.58 ± 0.19^{a}
T2	92.02 ± 4.24^{ab}	3.97 ± 0.18^{b}
T3	88.23 ± 3.86^{b}	4.00 ± 0.22^{b}
T4	80.61 ± 4.15^{c}	4.60 ± 0.24^{c}

Values in each column followed by the different superscript letter are significantly different (p<0.05).

4. Conclusion

It has been demonstrated that using SM as a co-substrate of DCM at a level of 25.02% in terms of VS of combined substrate can significantly increase methane yield by 21.89% and 22.73% in terms of L/kg substrate and L/L digester volume when compared to the control digester that only uses DCM. The digester can operate smoothly, producing stable methane and having a low TAN concentration. Therefore, SM can be used as a co-substrate with DCM to boost DCM methane production. Residual biogas production of digested slurries in terms of L/kg digested slurry increased as the proportion of SM in that substrate increased. Biogas production from digested slurry at that level of co-substrate (25.02%) increased by 28.49% when compared to digested slurry from the control digester. Therefore, post digestion should be used to capture any remaining biogas in the material.

5. Acknowledgements

Our gratitude to the Directorate of Higher Education, Ministry of Education, Culture, Research and Technology of Republic Indonesia for financing this study (Grant Number: 187-20/UN7.6.1/PP/2021).

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8