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Increasing Biogas Production Rate from Cattle Manure Using Rumen Fluid as Inoculums

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Abstract— In this study, rumen fluid of animal ruminant was used as inoculums to increase biogas production rate from cattle manure at mesophilic condition. A series of laboratory experiments using 400 ml biodigester were performed in batch operation mode. Given 100 grams of fresh cattle manure (M) was fed to each biodigester and mixed with rumen fluid (R) and tap water (W) in several ratio resulting six different M:W:R ratio contents i.e. 1:1:0; 1:0.75:0.25; 1:0.5:0.5; 1:0.25:0.75; and 1:0:1 (correspond to 0; 12.5; 25, 37.5; 50, and 100 % rumen, respectively) and six different total solid (TS) contents i.e. 2.6, 4.6, 6.2, 7.4, 9.2, 12.3, and 18.4 %. The operating temperatures were at room temperature. The results showed that the rumen fluid inoculated to biodigester significantly effected the biogas production. Rumen fluid inoculums caused biogas production rate and efficiency increase more than two times in compare to manure substrate without rumen fluid inoculums. The best performance for biogas production was the digester with rumen fluid and TS content in the range of 25-50 % and 7.4 and 9.2 %, respectively. These results suggest that, based on TS content effects to biogas yield, rumen fluid inoculums exhibit the similar effect with other inoculums. Increasing rumen content will also increase biogas production. Due to the optimum total solid (TS) content for biogas production between 7-9 % (or correspond to more and less manure and total liquid 1:1), the rumen fluid content of 50 % will give the best performance for biogas production. The future work will be carried out to study the dynamics of biogas production if both the rumen fluid inoculums and manure are fed in the continuous system.

Keywords — rumen fluid, inoculums, anaerobic digestion, biogas production

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I. INTRODUCTION

Energy is one of the most important factors to global prosperity. The dependence on fossil fuels as primary energy source has lead to global climate change, environmental degradation, and human health problems. In the year 2040, the world predicted will have 9–10 billion people and must be provided with energy and materials [1]. Moreover, the recent rise in oil and natural gas prices may drive the current economy toward alternative energy sources such as biogas.

Anaerobic digestion (AD) is a technology widely used for treatment of organic waste for biogas production. AD that utilizes manure for biogas production is one of the most promising uses of biomass wastes because it provides a source of energy while simultaneously resolving ecological and agrochemical issues. The anaerobic fermentation of manure for biogas production does not reduce its value as a fertilizer supplement, as available nitrogen and other substances remain in the treated sludge [2].

Numerous studies had been conducted by several researchers in order to increase biogas yield in AD. An effort to improve biomass conversion efficiency and biogas yield conducted by several researchers i.e by using two continuously stirred tank reactors (CSTR) in series [3]-[4]; selectively retaining the solids within the reactor by holding mixing prior to effluent removal [5]; pretreatment of manure by separating solids from digested material in order to improve biodegradability and accessibility [5]-[7]; and improving bacterial nutritional requirement [8]-[9]. In addition, an effort to increase biogas yield also has been done by improving contact between bacteria and substrate using stirring [10]-[12]; immobilizing microbe using fixed film reactor [13]-[14] as well as Anaerobic Sequencing Batch Reactor (ASBR) [15]; improving substrate composition by co-digesting with others substrate [11], [16]-[17]; and controlling ammonia inhibition [18].

Different with other researchers mentioned before, an effort to improve methane yield was carried out by

increasing the inoculums content in biodigester [19]-[23]. Several results from these study i.e inoculums are substantially relevant in process kinetics of biogas production [19]; amount of methane produced seemed proportional to the initial cattle manure as inoculums [20]; a strong influence of the bovine rumen fluid inoculums on anaerobic biostabilization of fermentable organic fraction of municipal solid waste [22]; and the higher percentage of inoculums gave the higher production of biogas [23]. However, almost all of AD studied before, inoculums used were dominated by digested sludge from anaerobic digester. In addition, until right now, data concerning the study of the effect of inoculums content to biogas production rate are very limited.

Due to the highly anaerobic bacteria content in the rumen of the ruminant animals [24] and the abundance of rumen waste disposal from slaughterhouse, this study focuses on the use of rumen fluid as inoculums in anaerobic digestion of cattle manure. Biogas production with cattle manure as substrate on slaughterhouse has special condition that rumen as inoculums is supplied continuously from rumen waste disposal. To our best knowledge, so far there is very limited academic literature available on using rumen fluid as inoculums in anaerobic digestion of cattle manure. The aim of the current work was to obtain more data on the digestion characteristics of cattle manure under different total solid (TS) and rumen fluid content to biogas production

II. Materials And Methods

A. *Sample preparation.*

The cattle manures and rumen fluids used in this research were taken randomly from slaughterhouse located on Semarang city. The fresh raw manure was collected from animal holding pen unit while rumen was collected from evisceration unit. Rumen fluid was prepared as follows: rumen content is poured to 100 L tank and added 25 liter tap water. Solid content then be separated from slurry by filter cloth. To assure that solid content in solution are dominated by bacteria, solution obtained then be filtered by 10 micron cartridge filter. Before using, all of raw manure collected is homogenized by mixing with propeller mixer. Raw manure and rumen fluid sample was analyzed its dry matter (DM) and volatile solid (VS) content by mean heating at 105 and 600 ^{o}C, respectively. DM and VS content of fresh cattle manure and rumen fluid are presented in Table 1.

B. *Experimental apparatus set up.*

A series laboratory test of 400 ml biodigester was operated in batch system. The main experiment apparatus consists of biodigester and biogas measurement. Biodigester were made from polyethylene bottle plugged with tightly rubber plug and was equipped with valve for biogas measurement. The temperature of biodigester was maintained at certain value thermostatically controlled electrically heated water bath. Biogas formed was measured by 'liquid displacement method' as also has been used by Yetilmezsoy and Sakar [25]. The schematic diagram of experimental laboratory set up as shown in Figure 1.

TABLE 1.
DM AND VS CHARACTERISTICS OF FRESH CATTLE MANURE AND RUMEN FLUID

Parameter	Unit	Fresh manure	Rumen fluid
DM	%	22.75	1.3
VS	%	19.49	1.04
VS/DM	%	85.57	80

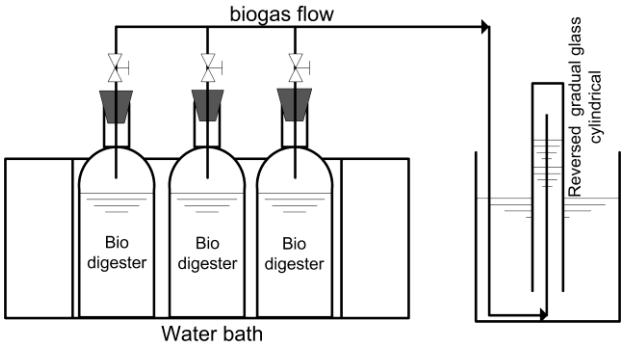


Figure 1. Schematic diagram of series laboratory batch assessment of anaerobic digestion

C. *Experimental design.*

The influence of rumen fluid inoculums to biogas production rate was studied by varying rumen fluid and TS contents in biodigester. A series of laboratory experiments using 400 ml biodigester were performed in batch operation mode. Manure used fixed on 100 gram. Given 100 grams of fresh cattle manure (M) was fed to each biodigester and mixed with rumen fluid (R) and tap water (W) in several ratio resulting six different M:W:R ratio contents i.e. 1:1:0; 1:0.75:0.25; 1:0.5:0.5; 1:0.25:0.75; and 1:0:1 (correspond to 0; 12.5; 25, 37.5; 50, and 100 % rumen, respectively). Composition of six manure samples used in the study as presented in Table 2. In order to study the influence of total solid (TS) content to biogas production, a series laboratory biodigester in several TS level in feed was investigated. Given 100 grams of fresh cattle manure was fed to each biodigester and mixed with fixed 50 ml of rumen fluid and different volumes of tap water resulting six different TS contents i.e. 2.64, 4.61, 6.15, 7.38, 9.23, 12.30, AND 18.40 % (Equivalent to Volatile Solid VS of 2.31, 4.4, 5.38, 6.46, 8.07, 10.76, and 16.74 %, respectively). Composition of six manure samples used in the study as presented in Table 3. Operating temperature was at room temperature. The biodigester performance was measured with respect to cumulative volume of biogas produced after corrected to standard pressure (760 mm Hg) and temperature 0 ^{o}C. All of treatment was carried out by triplication.

D. *The experimental procedures.*

The manure sample with certain TS and rumen fluid content as research variables was fed to biodigester and homogenized with mixer propeller. CO_2 gas was bubbled to biodigester to assure that biodigester in anaerobic condition. Biogas formed was measured every two days and stopped after biogas was insignificantly produced. The similar procedure was performed in three replications. Significance difference between treatments

was determined statistically by Duncan Multiple Range Test (DMRT).

TABLE 2.
COMPOSITION OF SIX MANURE SAMPLES IN DIFFERENT RUMEN FLUID CONTENT

M:W:R ratio (% R)	Manure, g	Water, ml	Rumen, ml
1:1:0 (0% R)	100	100	0
1:0.75:0.25 (12.5% R)	100	75	25
1:0.5:0.5 (25% R)	100	50	50
1:0.25:0.75 (37.5% R)	100	25	75
1:0:1 (50% R)	100	0	100
0:0:1 (100% R)	0	0	100

Remarks: M=manure;W=water; R=rumen fluid

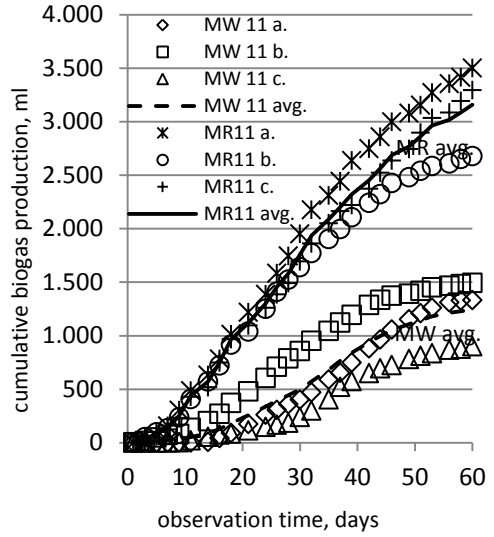
III. Results And Discussions

A. *The influence of rumen fluid to cumulative biogas production*

This research step was directed to study either the effect of liquid rumen to cumulative biogas production is significant or not. The substrate consists of 100 gram manure and 100 ml rumen (MR 11) was fed to the digester and compared to substrate of manure and water in equal weight ratio (MW 11). The research was carried out in triplication. The cumulative volume of biogas production was observed during 60 days as depicted in Figure 2(a). In other term, the cumulative biogas production per total VS added (specific biogas production) is presented in Figure 2(b).

Fig. 2 shows that, in general, biogas production rate tend to obey sigmoid function (S curve) as generally occurred in batch growth curve (this is especially more clearly for MW 11 sample). Biogas production is very slow at the beginning and the end period of observation. This is predicted due to the biogas production rate in batch condition is directly corresponds to specific growth rate of methanogenic bacteria in the biodigester [26]. In the around of the first 12 days observation, biogas production is very low or indeed do not formed yet due to the lag phase of microbial growth. In the range of 12 to 50 days observation, biogas production is sigificantly increase due to exponential growth of microorganisms. After 50 days observation, especially for manure without rumen fluid (MW 11), biogas production tend to decrease and this is predicted tend due to stationary phase of microbial growth.

From Fig. 2 (a) and (b) also can be seen that after 60 days observation still there is the tendency to increase biogas production and don't stop yet especially for manure mixed with rumen fluid (MR 11). This is predicted that the carbons contained by all waste constituents are not equally degraded or converted to biogas through anaerobic digestion. According to Richard [27] and Wilkie [28], anaerobic bacteria do not or very slow degrade lignin and some other hydrocarbons. In other word, the higher lignin content will lower biodegradability of waste. Animal manure such as waste used in this study include lignocellulosic rich materials, so anaerobically degradation also rather unoptimum [18].



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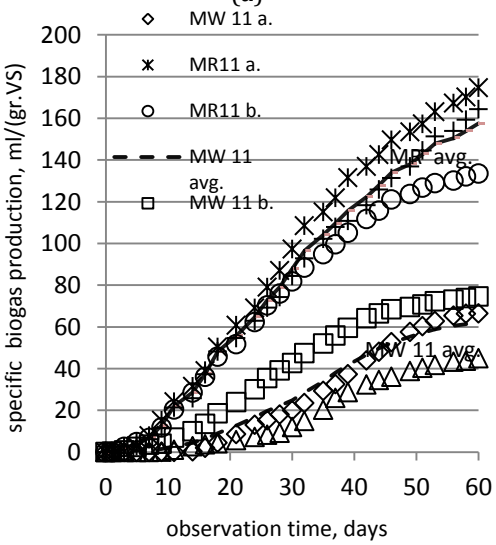


(b)

Figure 2. The influence of rumen fluid to biogas production; average from three bloc research with triplication; room temperature

Figure 2(a) and (b) also shows that, generally, substrates consist of manure and rumen (MR11) exhibit higher biogas production than substrates contain manure and water (MW11). In other terms, specific biogas production per gram VS added (Fig. 2.b) of MR11 is higher than MW11. The same behaviour is also shown in average biogas production curve. In the 60 days observation, average biogas production observed from MW11 and MR11 substrates were around 60 and 160 ml/(grVS). This result shows that the presence of liquid rumen in feed cause cumulative biogas production more than twice fold in compare to feed without liquid rumen. In other term, the substrates contain manure are statistically gave the significant effect to biogas production ($P<0.05$). This is suggest that high concentration of anaerobic bacteria content in liquid rumen works effectively to degrade organic substrate from manure. According to Aurora [24], rumen of the ruminant animals contains the highly anaerobic bacteria dominated by cellulolytic bacteria able to biodegrade cellulose material from manure. This is agree with other results of researcher before that amount

of biogas produced seemed proportional to the initial inoculums [20] and the bovine rumen fluid inoculums had a strong effect on anaerobic biostabilization of fermentable organic fraction of municipal solid waste [22]; as well as the higher percentage of inoculums gave the higher production of biogas [23].

From Fig. 2 also can be seen that the line slope of MR11 curve is sharper than MW11 line. The implication is that, biogas production rate (ml/grVS.day) of MR11 is higher than MW11. This indicated that the addition of liquid rumen to feed will increase biogas production rate in compare to feed without liquid rumen. Similar with this results, inoculums are substantially relevant in process kinetics of biogas production [19]. Finally, the most important finding from this research can be drawn the conclusion that the liquid rumen seeded to biodigester has significant effect to cummulative biogas production and biogas production rate. Mathematically, the discussion concerning the effect of inoculums to kinetics constant of biogas production rate will be presented in the further section.

From Fig. 2 also can be seen that the line slope of MR11 curve is sharper than MW11 line. The implication is biogas production rate (ml/grVS.day) of MR11 is higher than MW11. This is indicated that the addition of rumen fluid to feed will increase biogas production rate in compare to feed without rumen fluid. Similar with this results, inoculums are substantially relevant in process kinetics of biogas production [19]. Finally, the most important finding from this research can be drawn the conclusion that the rumen fluid seeded to biodigester has significant effect to cummulative biogas production and biogas production rate. Hence, the research to study the effec of rumen fluid concentration to biogas production will be carried out in the further step research.

B. *The effect of rumen fluid content to biogas production*

The effect of rumen fluid content to biogas production was studied by varying MWR ratio giving percent rumen fluid in mixed samples from 0 to 100 % rumen with fixed 100 gram manure. The TS content was presented in term of dry matter (DM). The research was carried out in triplication. The data obtained from the study then is averaged and the cumulative volume of biogas production was observed during 90 days as depicted in Figure 3(a). In other term, the cumulative biogas production per total VS added (specific biogas production) is presented in Figure 3(b). Numerical values of biogas yield in several days observation time is presented in Table 2.

Fig. 3 shows that, in general, substrates consist of manure and rumen (12.5 to 50 %R) exhibit higher cumulative biogas production than substrates contain manure and water only (0 %R). In other terms, specific biogas production per gram VS added (Fig. 2.b) of sampel contain rumen fluid is higher than sample no contain rumen fluid. The same behaviour is also shown in average biogas production curve. In the 80 days observation, cumulative biogas production of 12.5; 25; 37.5 and 50 %R are 112.5; 144,48; 162.18; and 191.38 ml/gVS, respectively. While sample with 0 %R give cumulative biogas production of 68.61 ml/gVS. In the fisrt 50 days observation, there is no significant differences between 25, 37.5 and 50 %R (P>0.05). While sample of 12.5 %R give the significant differences in biogas production with samples of 25, 37.5 and 50 %R as well as 0 %R (P<0.05).

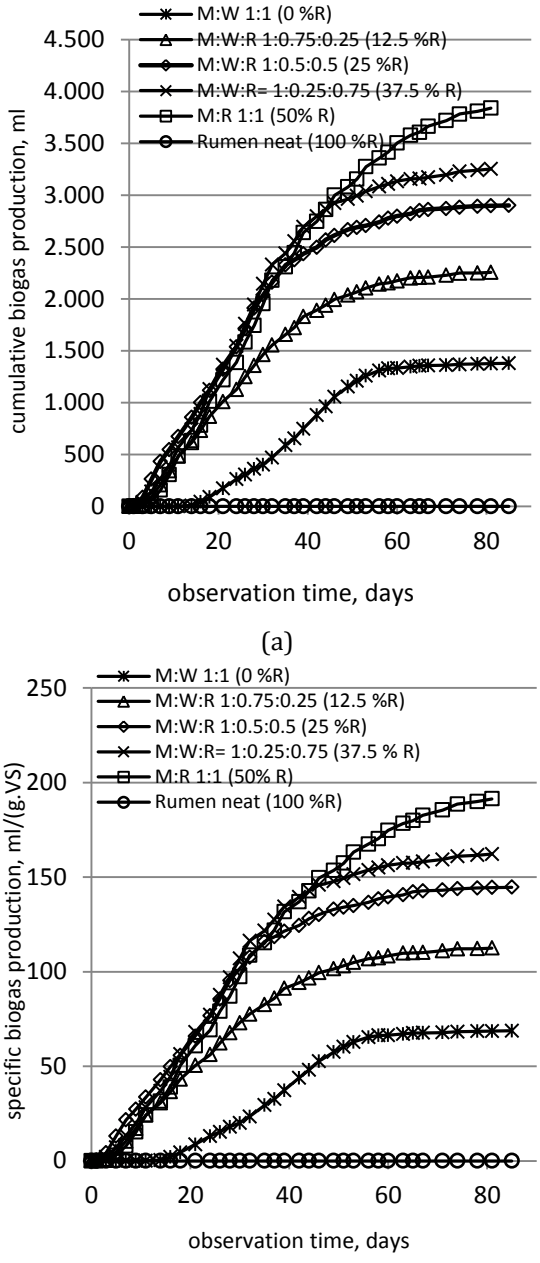


Figure 3. The effect of rumen fluid content to biogas production (room temperature)

These above result suggest that the optimum rumen fluid content for giving the best performance of biogas production is in the range of 25-50 %. Similar to this results, Lopes et al. (2004) reported that (a). no substantial difference was in evidence when 5% and 10% of the inoculum were used in preparation of the substrate; (b). in the range of 0 to 15 % rumen tested, the sample with the highest rumen content (15 %) gave the highest biogas production. Unfortunately, Lopes el at. [22] is not extensively investigate yet in using inoculums content more than 15 %. Hence, of course this study doesn't give data concerning optimum content of inoculums for biogas production. On the other hand, according to Foster-

Carneiro et al. [23], when treated food waste restaurant with 20 – 30 % inoculums, the best performance for food waste biodegradation and methane generation was the reactor with 30% of inoculums. However, we can not call this 30 % inoculums is the optimum condition because the research is not extensively investigate yet in using inoculums content more than 30 %.

Relatively different with other samples, samples with 50 %R exhibit still there is the tendency to increase biogas production after 90 days observation. This is suggest that, in case of very abundance of rumen fluid such as occur in slaughtrehouse, the rumen fluid content of 50 % (Manure : Rumen fluid ratio 1:1) will give the best performance for biogas production.

TABLE 2.
BIOGAS YIELD IN SEVERAL DAYS OBSERVATION TIME

Observation time, days	M:W:R ratio (%R)					
	1:1:0 (0 %R)	1:0.75:0.25 (12.5 %R)	1:0.5:0.5 (25 %R)	1:0.25:0.75 (37.5 % R)	1:0:1 (50% R)	Rumen neat (100 %R)
0	0.00	0.00	0.00	0.00	0.00	0.00
10	0.07	24.19	33.62	28.33	24.15	0.00
20	8.66	50.38	65.92	68.01	60.79	0.00
30	20.02	73.00	101.58	106.91	97.38	0.00
40	37.29	91.21	121.39	134.31	131.58	0.00
50	60.29	103.11	134.06	149.35	157.33	0.00
60	66.42	108.36	139.46	156.23	174.65	0.00
70	67.85	111.05	143.14	159.32	185.39	0.00
80	68.61	112.50	144.48	162.18	191.38	0.00

From Fig. 3 (a) and (b) also can be seen that after 90 days observation still there is the tendency to increase biogas production and don't stop yet. This is predicted that the carbons contained by all of waste constituents are not equally degraded or converted to biogas through anaerobic digestion. According to Richard [27] and Wilkie [28], anaerobic bacteria do not or very slow degrade lignin and some other hydrocarbons. In other word, the higher lignin content will lower biodegradability of waste. Animal manure such as waste used in this study include lignocellulosic rich materials, so anaerobically degradation also rather unoptimum [18]. Even, in other case, AD of organic matter such as municipal solid waste will not stop completely after 360 days observation [22].

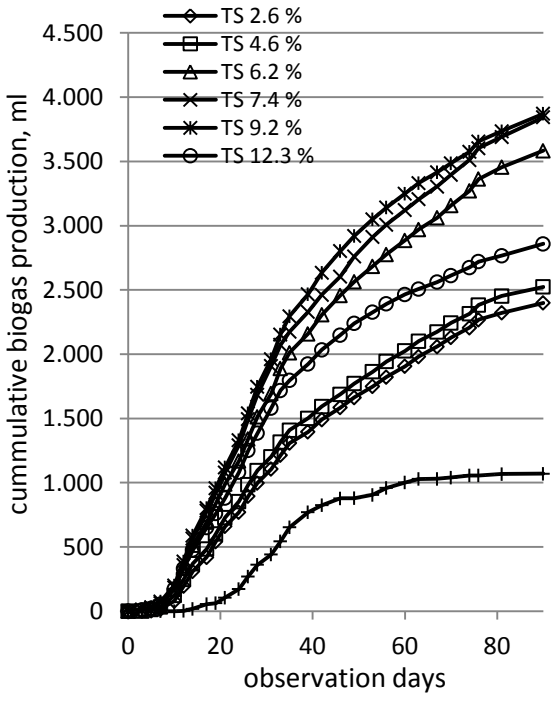
From Fig. 3 also can be seen that rumen neat (100 %R) do not contribute the biogas production. Hence, all of biogases produced during all of treatment are originated only from substrate contained by manure. The substrate content by rumen fluid estimated has been degraded to biogas durung storage. This is because rumen fluid used in this research has been stored in several months. However, although rumen fluid has been stored in several months, is predicted there is no deterioration in activities of microorganism contained. This is suitable with the information of Rajeswari [29] and Speece [30] that decay rate of anaerobic bacteria is very low below 45 ^{o}C. Even, anaerobic biomass can be preserved for months or years without serious deterioration in activity.

Finally, the conclusion can be drawn from this research that the best performance of biogas production will be obtained if rumen fluid is in the range of 25-50 %. Increasing rumen content will also increase biogas production. Due to the optimum TS content for biogas production between 7-9 % (or correspond to more and less manure and total liquid 1:1) [31]-[33], the rumen fluid content until 50 % will give the best performance for biogas production. However, intensively research need to be carried in further research to study interaction effect of TS and rumen content to biogas production. The further research is directed to verify the effect of rumen fluid content to biogas production at higher temperature.

C. *The effect of total solid (TS) content to biogas production*

The effect of TS content to biogas production was studied by varying TS from 2.64 to 18.40 %. The TS content was presented in term of dry matter (DM). The research was carried out in triplication. The data obtained from the study then is averaged and the cumulative volume of biogas production was observed during 90 days as depicted in Figure 4(a). In other term, the cumulative biogas production per total VS added (specific biogas production) is presented in Figure 4(b). Numerical values of biogas yield in several days observation time is presented in Table 3.

Fig. 4 shows that, in general, biogas production rate tend to obey sigmoid function (S curve) as generally occurred in batch growth curve and as also has be resulted by Budiyono et al. [34]. Biogas production is very slow at the beginning and the end period of observation. This is predicted due to the biogas production rate in batch condition is directly corresponds to specific growth rate of methanogenic bacteria in the biodigester [26]. In the around of the first 10 days observation, biogas production is very low or indeed do not formed yet due to the lag phase of microbial growth. In the range of 10 to 50 days observation, biogas production is sigificantly increase due to exponential growth of microorganisms. After 50 days observation, biogas production tend to decrease and this is predicted tend due to stationary phase of microbial growth [34].



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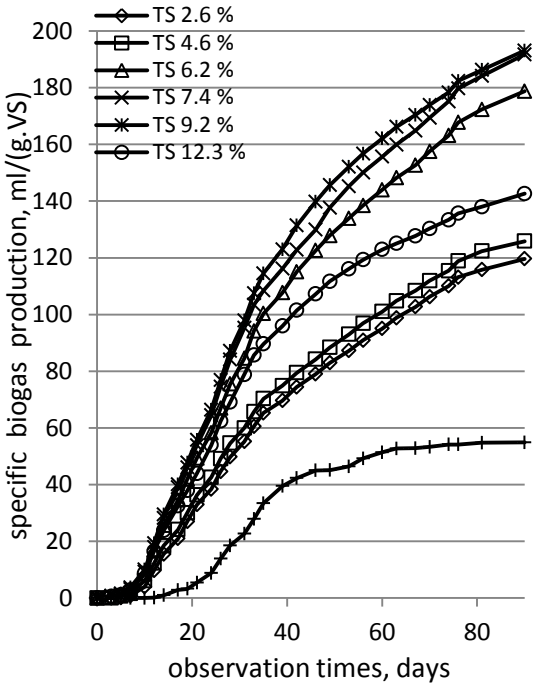

(b)

Figure 4. The effect of TS content to biogas production

From Fig. 4 (a) and (b) also can be seen that after 90 days observation still there is the tendency to increase biogas production and don't stop yet. This is predicted that the carbons contained by all of waste constituents are not equally degraded or converted to biogas through anaerobic digestion. According to Richard [27] and Wilkie [28], anaerobic bacteria do not or very slow degrade lignin and some other hydrocarbons. In other word, the higher lignin content will lower biodegradability of waste. Animal manure such as waste used in this study include lignocellulosic rich materials, so anaerobically degradation also rather unoptimum [18]. Even, AD of cattle manure will cease completely after 360 days observation.

TABLE 3
COMPOSITION OF SIX MANURE SAMPLES IN DIFFERENT TS CONTENT

TS, %	VS, %	Cattle manure, g	Water, ml	Rumen Fluid, ml
2.64	2.31	100	550	50
4.61	4.04	100	250	50
6.15	5.38	100	150	50
7.38	6.46	100	100	50
9.23	8.07	100	50	50
12.30	10.76	100	0	50
18.40	16.74	100	0	0

Furthermore, as shown in Fig. 4, the best performance for biogas production was the digester with 7.4 and 9.2 % of TS i.e. give biogas yield 184.09 and 186.28 ml/gVS, respectively after 90 days observation. While the other TSs content of 2.6, 4.6, 6.2, 12.3, and 18.4 % give the biogas yield 115.78, 122.33, 172.34, 137.99, 54.87 ml/gVS, respectively. In addition, in the range of all of the observation time, TS contents of 7.4 and 9.2 % are also exhibit the best performance in biogas yield as presented in Table 3. These results suggest that, based on TS content effects to biogas yield, rumen fluid inoculum exhibit the similar behaviour with other inoculums, respectively. This is similar with the information from Balsam [31] and Zennaki et al. [33] that the optimum solid content obtained for biogas production is in the range 7-9 %. Furthermore, Baserja [32] reported that the process was unstable below a total solids level of 7% (of manure) while a level of 10% caused an overloading of the fermenter. These results suggest that, based on TS content effects to biogas yield, rumen fluid inoculums exhibit the similar effect with other inoculums.

These results is predicted due the function of water in biodigester since the TS content will be directly correspond to water content. According to Sadaka and Engler [35], water content is one of very important parameter affecting AD of solid wastes. There are two main reason i.e (a). Water make possible the movement and growth of bacteria facilitating the dissolution and transport of nutrient; and (b) water reduces the limitation of mass transfer of non homogenous or particulate substrate. Mathematically, the function of water in AD processes organic wastes consists of elements of carbon (C), Hydrogen (H), and Oxyigen (O) reflected by reaction as follows [30]:

$$C_c H_h O_o + \left\lceil \frac{4c-h-2o}{4} \right\rceil H_2O \Longrightarrow \left\lceil \frac{4c+h-2o}{8} \right\rceil CH_4 + \left\lfloor \frac{4c-h+2o}{8} \right\rfloor CO_2 \quad \text{..... (1)}$$

Furthermore, the water needed for biogas production from organic wastes consists of elements of carbon (C), Hydrogen (H), and Oxyigen (O) and Nitrogen (N) is reflected by reaction as follows [16].

$$C_cH_hO_oN_n + \left\lceil\frac{4c-h-2o+3n}{4}\right\rceil H_2O ======> \left\lceil\frac{4c+h-2o-3n}{8}\right\rceil CH_4 + \left\lfloor\frac{4c-h+2o+3n}{8}\right\rfloor CO_2 + nNH_3 \quad \text{..... (2)}$$

The both two above equation depicted how important the water need in AD process for biogas production. Finally, the most important finding of this research that that the best performance for biogas production was the digester with 7- 9 % of TS similar with conventional processes used other inoculums. However, although the same optimum concentration of TS, rumen fluid inoculums caused biogas production rate and efficiency increase two to three times in compare to manure substrate without rumen fluid, as has been stated by Budiyono et al. [34].

IV. Conclusions

Biogas production rate was studied by performing a series laboratory experiment using rumen fluid of animal ruminant as inoculums. The most important finding from this research is that the rumen fluid seeded to biodigester has significant effect to cummulative biogas production and biogas production rate. Rumen fluid inoculums caused biogas production rate and efficiency increase two to three times in compare to manure substrate without rumen fluid. The best performance for biogas generation will be obtained if rumen fluid is in the range of 25-50 %. Increasing rumen content will also increase biogas production. In addition, the best performance for biogas generation was the digester with 7.4 and 9.2 % of total solid i.e. give biogas yield 184.09 and 186.28 ml/gVS, respectively after 80 days observation. These results suggest that, based on TS content effects to biogas yield, rumen fluid inoculum exhibit the similar effect with other inoculums. Due to the optimum TS content for biogas production between 7-9 % (or correspond to more and less manure and total liquid 1:1), the rumen fluid content until 50 % will give the best performance for biogas production. However, intensively research need to be carried in further research to study interaction effect of TS and rumen content to biogas production. The further research is directed to verify the effect of rumen fluid content to biogas production at higher temperature.

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