# Influence of Inoculum Content on Performance of Anaerobic Reactors for Treating Cattle Manure using Rumen Fluid Inoculum

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***Abstract*** **- Biogas productions of cattle manure using rumen fluid inoculums were determined using batch anaerobic digesters at mesophilic temperatures (room and 38.5 $^{o}$C). The aim of this paper was to analyze the influence of rumen fluid contents on biogas yield from cattle manure using fluid rumen inoculums. 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). The research showed that, either in room temperature as well as in 38.5 C, the best performance of biogas production was obtained with rumen fluid in the range of 25-50 %. Increasing rumen content will also increase biogas production. This is suggest that, 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. However, intensively research need to be carried in further research to study interaction effect of TS and rumen content to biogas production.**

**Keywords:** rumen fluid; inoculum; biogas yield

## I. INTRODUCTION

One of the most important challenges that our world will face in the twenty-first century will be continuing to meet the ever increasing energy needs of its citizen. Along with the need to find a renewable long term energy source is the need to find a more environmental friendly one. One of the promising candidates as a power source solution for the future world energy problem is biomass such as manure from animal agricultural waste (Nallathambi, 1997). Common terminology to describe the biological origin of a product includes terms such as biofuel, bioenergy and biogas. Anaerobic digestion (AD) is a biological method used to convert organic wastes into biogas and a stable product for land application without adverse environmental effects. The biogas produced can be used as an alternative renewable energy source. 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 (Alvarez and Lide´n, 2008).

Naturally, the fermentation of organic substrate during AD processes is similar to that occurs in digestive tract of ruminant animals (Hungate, 1966). The rumen is an exclusive organ of ruminant animals in which digestion of cellulose and other polysaccharide molecules occur through the activity of specific microbial populations. The capacity of cellulose digestion that these animals possess is related to the presence of anaerobic microorganisms in its rumen, which decompose glucose polymer chains up to acetate. According to Aurora (1983), rumen contains the highly anaerobic bacteria dominated by cellulolytic bacteria able to biodegrade cellulosic material. Methanogenic microorganisms also exist in rumen that converts acetate part in methane and carbon dioxide (Hungate, 1966; Bryant, 1979).

In fact, the research concerning the effect of inoculums to biogas production was conducted by several researchers with the results i.e. inoculums are substantially relevant in process kinetics of biogas production (Luengo and Alvarez, 1988); amount of methane produced seemed proportional to the initial inoculums (Castillo et al., 1995); the higher percentage of inoculums gave the higher production of biogas (Forster-Carnero et al., 2008); and the food to inoculums ration significantly affected the biogas production rate (Liu et al., 2009). However, almost all of AD studied before, inoculums used were dominated by digested sludge from anaerobic digester as well as animal manure. In case of the use of rumen fluid inoculums for biogas production, Lopes et al. (2004) reported that a strong influence of the bovine rumen fluid inoculums on anaerobic biostabilization of fermentable organic fraction of municipal solid waste. However, Lopes at al. (2004) don't be able to determine the optimum inoculums content yet due to the experiment were not extensively investigated yet in



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using inoculums content more than 15 %. In addition, Budiyono et al. (2009) has also reported that rumen fluid inoculated to biodigester gave significant effect to biogas production. Rumen fluid inoculums caused biogas production rate and efficiency increase two to three times in compare to manure substrate without rumen fluid. In addition, to our best knowledge, in case of using rumen fluid as inoculums; data concerning the study of the effect of inoculums content to biogas production rate from cattle manure are very limited. Hence, as also recommended by Budiyono et al. (2009), the aim of this research is to study the influence of rumen fluid content for biogas production from cattle manure.

## 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 L of 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.

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

| Parameter | Unit | Fresh manure | Rumen fluid |
|---|---|---|---|
| DM | % | 18.40 $\pm$ 0.65 | 1.71 $\pm$ 0.03 |
| VM | % | 16.74 $\pm$ 0.15 | 1.50 $\pm$ 0.01 |

### 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. Biogas formed was measured by 'liquid displacement method' as also has been used by Yetilmezsoy and Sakar (2008). The schematic diagram of experimental laboratory set up as depicted in Figure 1.

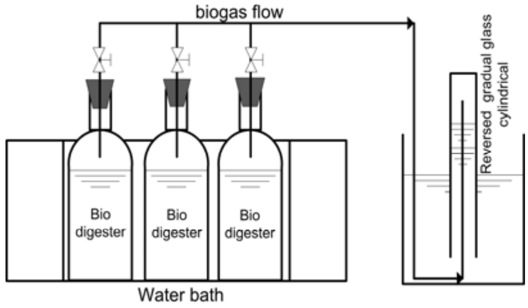


Figure 1. Schematic diagram of series laboratory batch assessment of anaerobic digestion

### C. *Experimental design.*

The influence of rumen fluid content to biogas production was studied by performing a series laboratory biodigester in several rumen fluids in feed. 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). Composition of six manure samples used in the study as presented in Table 2. Operating temperature was at room temperature and 38.5 $^{o}$C. 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.

TABLE 2. COMPOSITION OF SIX MANURE SAMPLES USED IN THE STUDY

| 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

### D. *The experimental procedures.*

The manure sample with certain MWR 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).



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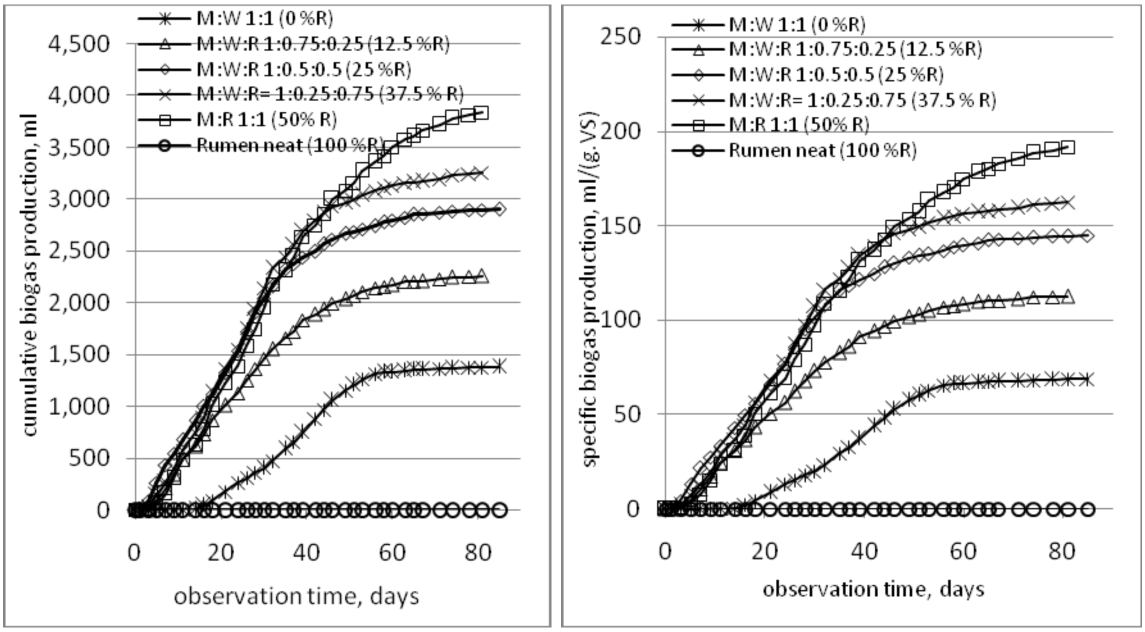
## III. RESULTS AND DISCUSSIONS

### A. *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 2(a). In other term, the cumulative biogas production per total VS added (specific biogas production) is presented in Figure 2(b). Numerical values of biogas yield in several days observation time is presented in Table 3.




(a) (b)

Figure 2. The effect of rumen fluid content to biogas production (room temperature)

TABLE 3. 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 |

Fig. 2 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 ($P<0.05$). These result suggest that the optimum rumen fluid content for



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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. (2004) 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 Foster-Carneiro et al. (2008), 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.

From Fig. 2 (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 (1996) and Wilkie (2005), 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 (Nielsen, et al., 2004). Even, in other case, AD of organic matter such as municipal solid waste will not stop completely after 360 days observation (Lopes, 2004).

From Fig. 2 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 (2000) and Speece (1996) 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) (Balsam, 2006; Baserja, 1984; and Zennaki et al. 1996), 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.

### *B. The effect of rumen fluid content to biogas production at 38.5 C*

One of the most important factors affecting anaerobic digestion of organic solid waste is temperature (Ahring, 1992). The temperature of 38.5 $^{o}$C was selected due to the fact that the rumen condition on animal ruminants is $\pm$ 38.5 $^{o}$C. The effect of rumen fluid content to biogas production at 38.5 C was conducted in the same variable and number of repication as before. 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 4.



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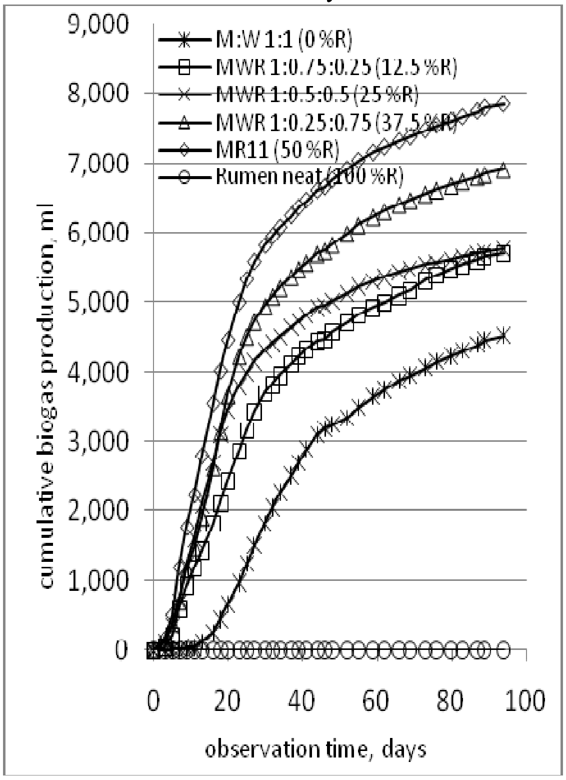


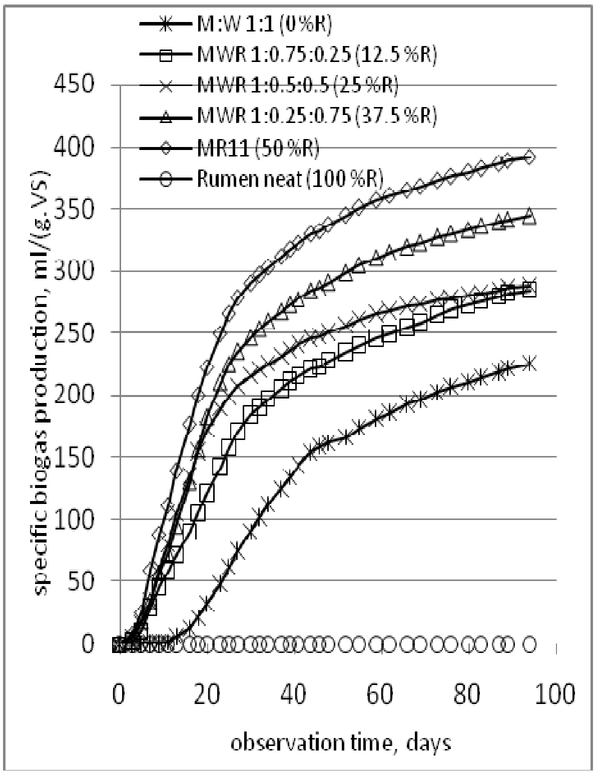


(a) (b)

Figure 3. The effect of rumen fluid content to biogas production at 38.5 C

TABLE 4. 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) | MR1:0:1 (50 %R) | Rumen neat (100 %R) |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 2.34 | 59.02 | 80.61 | 73.98 | 111.71 | 0.00 |
| 20 | 32.98 | 122.08 | 172.46 | 182.90 | 222.55 | 0.00 |
| 30 | 90.65 | 184.25 | 215.45 | 246.71 | 290.10 | 0.00 |
| 40 | 144.09 | 215.77 | 240.38 | 277.87 | 322.55 | 0.00 |
| 50 | 166.45 | 234.78 | 256.11 | 298.65 | 344.95 | 0.00 |
| 60 | 186.34 | 249.27 | 267.47 | 314.61 | 360.58 | 0.00 |
| 70 | 196.66 | 258.04 | 273.35 | 322.53 | 367.70 | 0.00 |
| 80 | 210.77 | 273.10 | 280.03 | 333.25 | 379.46 | 0.00 |
| 90 | 221.88 | 282.04 | 285.87 | 341.18 | 388.67 | 0.00 |

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. 3.b) of sampel contain rumen fluid is higher than sample no contain rumen fluid. These results are similar to the effect of rumen fluid content at room temperature. The same behaviour is also shown in average biogas production curve. In the 90 days observation, cumulative biogas production of 12.5; 25; 37.5 and 50 %R are 282.04; 285.87; 341.18 and 388.67 ml/gVS, respectively. While sample with 0 %R give cumulative biogas production of 221.88 ml/gVS. In the fisrt 25 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). These results also suggest that the optimum rumen fluid content for giving the best performance of biogas production is in the range of 25-50 %. From these results, the conclusion can be drawn that the effect of rumen fluid content to biogas production relatively similar either at room temperature or at 38.5 $^{o}$C. However, after 90 days observation, there is no significant differences between 12.5 and 25 % rumen fluid content (P>0.05). The further research was directed to study the effect of temperature on biogas production using rumen fluid as inoculum.



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*C. The comparation of biogas production at room temperature and 38.5 °C*

The study on comparation of biogas production at room temperature and 38.5 C is directed to know the effect of temperature on biogas production using rumen fluid as inoculum. The study resulted before were selected at manure to rumen ratio 1:1 (MR11) and manure to water ratio 1:1 (MW11). The results was depicted completely in Figure 4.

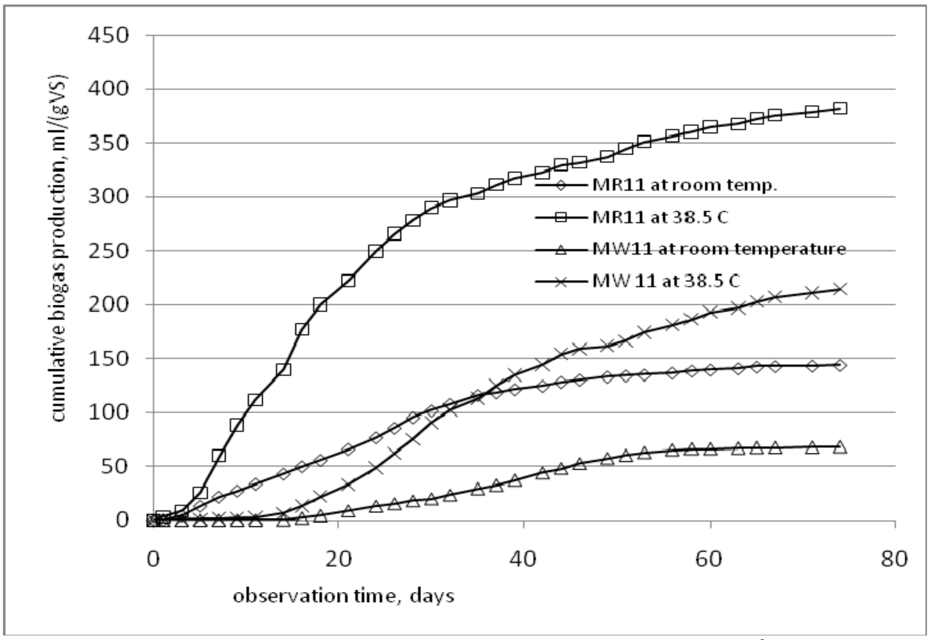


Figure 4. The comparation of biogas production at room temperature and 38.5 °C

Figure 4 shows that biogas production was significantly influenced by temperature either manure with rumen fluid inoculum (MR11) or manure without inoculum (MW11). Increasing temperature from room temperature to 38.5 °C caused maximum biogas production MR11 and MW11 from 144 to 382 and 68.21 to 214.25 ml/(gVS), respectively. This is because due to general rule that temperature is a very important operational parameter in an AD processes. The optimum temperature for all of bacteria groups is the range of 35-40 °C (Wenxiu and Mengjie, 1989; http://zorg-biogas.com/library/biogas-production-process). In addition, the 38.5 C is predicted as optimum temperature of bacteria due to the fact that the rumen condition on animal ruminants is 38.5 °C. The temperature difference of ± 2,5 °C can cause the decrease of the rumen microbial activity. This is agree with the overview conducted by Balsam (2002), that temperature variation around 2,5 °C can inhibit bacterial growth rate of methane former. However, the further intensive study is needed to access the optimum temperature of anaerobic digestion especially for AD using rumen fluid as inoculum.

*D. The effect of inoculums content to performance of biodigester*

Table 4 shows a summary of performance data at the end of the process for rumen fluids content studied. Correspond to biogas yield, based on TS content, the TSs content of 7.4 and 9.2 % exhibit the best performance for digestibility i.e. give digestibility 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.

Table 4 also shows that, in all cases, there is an increase of pH before and after digestion. Before digestion, pH varies from 6.37-6.62 while after digestion pH varies from 6.70-6.97. The increase of pH is predicted due to degradation of protein to give ammonia (equation 2). The final pH 6.70-6.97 indicate that the high crude protein in cattle manure (in this case 9.03 %) give the benefit effect for bringing pH toward the neutral pH i.e. optimum pH for methanogenic bacteria is 6.8-7.2 (Rajeshwari et al., 2000).

In addition, no significant variation of sludge protein content in all of variation of TS content. Crude protein varies between 1.67 to 2.53%. This result indicates that anaerobic sludge from all of TS variation still have benefit for being used as liquid fertilizer. Over all, there is no variation of fat, protein, and ash content in sludge after digestion. These results suggest that in point of view of fat, protein, and ash content, all of substrate has been degraded to biogas in the relatively same rate. Finally, the conclusion can be drawn that variation of TS content will give the significant effect to digestibility of TS content to be produced as biogas.



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TABLE 4. RESULTS OF 90-DAY BATCH ANAEROBIC DIGESTION OF CATTLE MANURE IN SEVERAL TS CONTENT

| No. | Rumen content, % | TS content, % | Sludge composition | | | | | Digest., %DM | pH | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Water, % | TS (DM), % | Fat, %DM | Protein, %DM | Ash, %DM | | Initial | Final |
| 1 | 0 | 9.2 | 91.11 | 8.89 | 1.08 | 2.53 | 5.95 | 19.47 | 6.17 | 6.72 |
| 2 | 12.5 | 9.2 | 91.66 | 8.34 | 0.93 | 1.83 | 6.32 | 24.45 | 6.34 | 7.03 |
| 3 | 25 | 9.2 | 92.54 | 7.46 | 0.78 | 1.72 | 4.23 | 32.39 | 6.41 | 7.09 |
| 4 | 37.5 | 9.2 | 93.42 | 6.58 | 1.02 | 1.67 | 7.88 | 40.40 | 6.53 | 7.21 |
| 5 | 50 | 9.2 | 94.97 | 5.03 | 1.09 | 1.78 | 6.53 | 54.46 | 6.65 | 7.23 |

Note: Initial raw manure composition : DM 18.4 %; Ash 19.34 %DM; Raw protein 9.03%DM; Lipid 1.28%DM; raw fiber 42.57%DM

## IV. CONCLUSIONS

The effect inoculum content to biogas production 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 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. 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.

The effect of rumen fluid content to biogas production relatively similar either at room temperature or at 38.5 $^oC$. Biogas production was significantly influenced by temperature either manure with rumen fluid inoculum (MR11) or manure without inoculum (MW11). The 38.5 C is predicted as optimum temperature of bacteria due to the fact that the rumen condition on animal ruminants is 38.5 $^oC$. However, the further intensive study is needed to access the optimum temperature of anaerobic digestion especially for AD using rumen fluid as inoculum.

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