The Effect of Feed to Inoculums Ratio on 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 was fed to each biodigester and mixed with rumen fluid and tap water resulting five different feed to inoculum (F/I) ratios (i.e. 17.64, 23.51, 35.27, and 70.54). The operating temperatures were varied 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. At four F/Is tested, after 80 days digestion, the biogas yield were 191, 162, 144 and 112 mL/g VS, respectively. About 80% of the biogas production was obtained during the first 40 days of digestion. The best performance of biogas production will be obtained if F/I ratio is in the range of 17.64 to 35.27 (correspond to 25 – 50 % of rumen fluid). 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, F/I ratio, anaerobic digestion, biogas production]

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 (Okkerse and Bekkum, 1999). 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 (Alvarez and Lide´n, 2008).

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 (Boe, 2006; Kaparaju et al. (2009); selectively retaining the solids within the reactor by holding mixing prior to effluent removal (Kaparaju et al., 2008); pretreatment of manure by separating solids from digested material in order to improve biodegradability and accessibility (Liao et al. (1984; Kaparaju and Angelidaki, 2008; Moller, 2008); and improving bacterial nutritional requirement (Kayhanian and Rich, 1995; Demirci and Demirer. 2004). In addition, an effort to increase biogas yield also has been done by improving contact between bacteria and substrate using stirring (Krylova et al., 1997; Callaghan et al., 1999; Karim, 2005); immobilizing microbe using fixed film reactor (Lo, et al., 1984; Vartak et al., 1997) as well as Anaerobic Sequencing Batch Reactor (ASBR) (Ndegwa et al., 2008); improving substrate composition by co-digesting with others substrate (Callaghan et al., 1999; Gelegenis et al., 2007; Lehtomaki et al., 2007); and controlling ammonia inhibition (Nielsen and Angelidaki, 2008).

Different with other researchers mentioned before, an effort to improve methane yield was carried out by increasing the inoculums content in biodigester (Luengo and Alvarez, 1988; Castillo et al., 1995; (Sans, C. et al. 1995; Lopes, W. S. et al. 2004; Forster-Carneiro, T. et al. 2008). Several results from these study 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 cattle manure as inoculums (Castillo et al., 1995); a strong influence of the bovine rumen fluid inoculums on anaerobic biostabilization of fermentable organic fraction of municipal solid waste (Lopes et al., 2004); and the higher percentage of inoculums gave the higher production of biogas (Forster-Carneiro et al., 2008). 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 (Aurora, 1983) 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 the cattle manure under different temperatures and different feed to inoculums (F/I) ratios to biogas production.

II. MATERIALS AND METHODS

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.

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

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 (2008). The schematic diagram of experimental laboratory set up as shown in Figure 1.

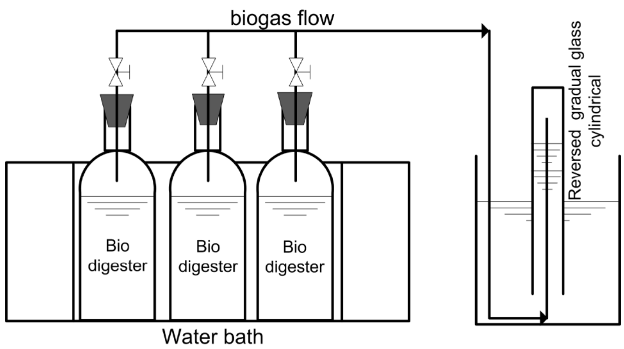

Figure 1. Schematic diagram of series laboratory batch assessment of anaerobic digestion

Experimental design. The influence of F/I ratio to biogas production rate was studied by varying manure, water, and rumen fluid ratio (MWR ratio) giving F/I from 0 to 70.54. Given 100 grams of fresh cattle manure was fed to each biodigester and mixed with rumen fluid and tap water resulting five different feed to inoculum (F/I) ratios (i.e. 17.64, 23.51, 35.27, and 70.54). Operating temperature was varied 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 by triplication. Composition of six manure samples used in the study as presented in Table 2.

TABLE 2.
THE CALCULATION OF F/M RATIO

Variables	Manure, gram	Rumen Fluid, ml	Water, ml	% Rumen fluid	F/M ratio
Neat Rumen fluid	0	100	0	100	0.00
MR11	100	100	0	50	17.60
MWR12575	100	75	75	37.5	23.46
MWR 155	100	50	50	25	35.19
MWR 17525	100	25	75	12.5	70.39
MW11	100	0	100	0	-

Remarks: TS and VS of neat rumen fluid was 1.3 and 1.14 %, respectively; TS and VS of cattle manure was 22.7 and 20.06 %.

The experimental procedures. The certain F/I ratio 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 produced insignificantly. The similar procedure was performed in three replications

Statistical data analysis. Significance difference between treatments was determined by Duncan Multiple Range Test (DMRT).

III. RESULTS AND DISCUSSIONS

The effect of F/I ratio to biogas production was studied by varying TS from 17.64 to 70.54. The F/I ratio was presented as ration between VS of feed and VS of inoculum used. The research was carried out in triplication. The data obtained from the study then is averaged and the cumulative volume of biogas production per total VS added (specific biogas production) was observed during 90 days as depicted in Figure 2. Numerical values of biogas yield in several days observation time is presented in Table 3.

Fig. 2 shows that, in general, substrates consist of manure and rumen fluid (F/I of 17.6 to 70.4) exhibit higher cumulative biogas production than substrates just contain manure and water (no inoculum). In the 80 days observation, biogas production of 17.64, 23.51, 35.27, and 70.54 F/I are 112.5; 144,48; 162.18; and 191.38 ml/gVS, respectively. While sample with 0 % inoculum give biogas production of 68.61 ml/gVS. In the fisrt 50 days observation, there is no significant differences between 17.64, 23.51, 35.27 of F/I ($P>0.05$). While sample of 70.54 F/I the significant differences in biogas production with samples of 17.64, 23.51, 35.27 of F/I as well as 0 % of inoculum ($P<0.05$). These result suggest that the optimum F/I for giving the best performance of biogas production is in the range of 17.64 to 35.27 (correspond to 25 – 50 % of rumen fluid).

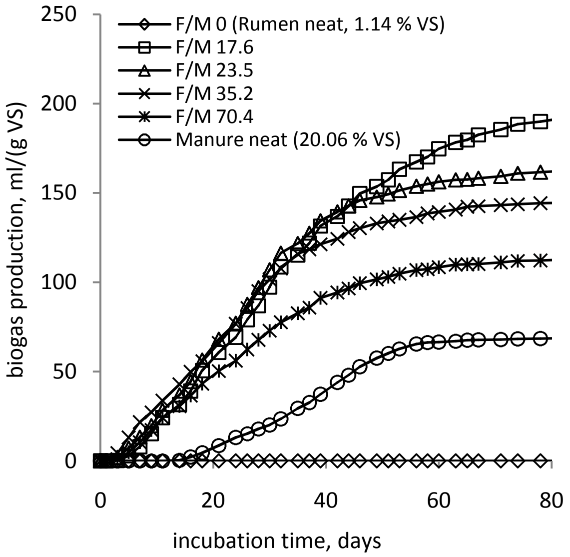

Figure 2. The effect of F/M ratio to biogas production; room temperature; TS and VS of rumen fluid was 1.3 and 1.14 %, respectively; TS and VS of cattle manure was 22.7 and 20.06 %

TABLE 3.
BIOGAS YIELD IN SEVERAL DAYS INCUBATION TIME

Inc. time days	F/I ratio					
	Manure neat	70.4	35.2	23.5	17.6	0
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

Similar to these 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 fluid 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 conclude 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 17.6 of F/I (50 % of rumen fluid) 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 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 et al., 2004).

From Fig. 2 also can be seen that rumen neat (100 % of rumen fluid) 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 F/I ratio is in the range of 17.64 to 35.27 (correspond to 25 – 50 % of rumen fluid). Decreasing of F/Is ratio 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 was directed to verify the effect of TS content to biogas production.

IV. CONCLUSIONS

The effect of F/I ratio 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 of biogas production will be obtained if F/I ratio is in the range of 17.64 to 35.27 (correspond to 25 – 50 % of rumen fluid). The effec of rumen fluid concentration to biogas production will need to be studied in the further step research. In addition, 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.

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