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Research Article

Potential Test on Utilization of Cow's Rumen Fluid to Increase Biogas Production Rate and Methane Concentration in Biogas

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Abstract

Objective: The study aims to determine the effects of rumen fluid addition on biogas formation rate, methane concentration in biogas and coliform content in the sludge from the biogas formation process. **Methodology:** In this experiment, biogas is produced from the slurry of cow's manure with the addition of a certain amount of rumen fluid. The amount of additional rumen fluid is varied in four treatments, which is 0, 20, 30 and 40% of the total volume of slurry. The ratio between livestock manure and water is 1:2 with total volume of 450 mL of slurry. The bio-digester are operated in the batch system under anaerobic condition. The observation is conducted for 30 days. Daily measurements of gas productions are recorded, whereas the methane concentration in biogas is measured on day 14, 20, 25 and 30 using the Gas Chromatography (GC). **Results:** The results showed that rumen fluid addition significantly shortens the time required for the initial phase of decomposition (hydrolysis and acidogenesis) prior to methane formation phase. With rumen fluid addition, the peak in biogas production is achieved on 20 days, while on the control sample (without rumen fluid addition) the peak is achieved later, on 30 days. Taking into account the value of methane concentration, rumen fluid addition is highly recommended since it can give maximum methane concentration (38.20% of the volume) on 20 days, which is 10 days earlier compared to the control sample (22.86% of the volume). **Conclusion:** It can be concluded that the addition of cow's rumen fluid as a starter can accelerate the biogas formation process in a shorter time which is 10 days and gives a positive effect on methane formation and methane concentration, compared to the treatment without rumen fluid addition.

Key words: Cattle manure, rumen fluid, biogas production, methane concentration, coliform

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Livestock farms and industries are part of national research agenda to address the problems of food security and energy. However, the waste can cause environmental problems such as pollution of water, soil and air. Various cases of environmental pollution and the deterioration of public health today are the effects of the wastes from many kinds of industrial activities, farms, hospitals, markets, restaurants and households. It is because the handling and processing of the waste have not been given serious consideration.

It can solve those problems by processing the waste into something useful. The organic material of the livestock and industrial waste can be used as a source of energy in the form of biogas to overcome the environmental problems and increase the energy security. Biogas is made up of 50-70% CH₄, 30-50% CO₂ and some notable impurities such as NH₃, H₂S, siloxane and halides¹. The concentration of each of these compounds depends on the composition of the raw materials and the process conditions used during the digestion². Transformation of organic waste (organic material of the livestock and industrial) to energy is the product of anaerobic digestion. Anaerobic digestion is a biological process of converting separated biodegradable solid wastes into methane gas. Methane is sourced from the decomposition of organic material, such as cattle slurry, food scraps and sewage³. Several renewable residues have been used for biogas production which is classified into organic wastes (municipal waste, wastewater sludge, swine manure, cow manure and other related residues), energy crops (sunflower, rape, jatropha, cardoon, etc.), agricultural residues (banana stem, barley straw, rice straw, softwood spruce, etc.), agricultural crops (maize, wheat, barley, sweet sorghum, etc.) and non-conventional feedstocks (glycerol, microalgae, etc.)⁴⁻⁷. Methane has similar thermal characteristics to natural gas and once the quality is upgraded it can be injected into the gas grid. Methane gas can be utilized as a renewable energy source to supply heat and electricity. In addition, waste to energy transformation is been recognized as an important and vital element in any Integrated Solid Waste Management (ISWM) plan⁸. However, long-term national energy problems are to the security of supply and sustainability of supply that can support the development and needs of the people of Indonesia for a long time. The population continues to increase resulted in higher consumption of fossil fuel.

One attempt to accelerate the production of biogas is the addition of starter into the biodigester. A starter (inoculum) is a group of microbes in a certain number and certain

physiological conditions that are ready to be inoculated in the fermentation media. Microbes are one of the key factors that will determine the success of the biological process of organic fluid waste. Their existence is important for various stages of the organic material recast. One of the starters that can be used is cow's rumen fluid which contains various microorganisms, including hydrolysis bacteria, acidogenesis and methanogenesis bacteria, protozoa and fungi. Different microbial systems participate in transforming organic residues in a number of reactions into biogas under anaerobic conditions, these include, hydrolysis-acidogenesis, acetogenesis and methanogenesis^{9,10}. Kalia and Singh¹¹ have reported about the adequate mixing can enhance biogas production and biodegradability due to the distribution of substrates, enzymes and microorganisms throughout the digester. Mixing also promotes heat transfer and particle size reduction, discharges gas bubbles trapped in the medium and avoids the sedimentation of denser particulate matter¹². These processes proceed under different conditions including low temperature <25 °C dominated by psychrophilic organisms, moderate temperatures between 25-45 °C for mesophilic organisms and high temperatures from 50 °C and above for thermophilic and other extremophilic species¹³.

After observing the above experience, it is necessary to conduct study that aims to determine the effects of rumen fluid addition on biogas formation rate, on methane concentration in biogas and on coliform content in the sludge from the biogas formation process.

MATERIALS AND METHODS

The tools needed in this study are a series of batch anaerobic reactor, which consists of a mini-biodigester (with the volume of 500 mL for mixing capacity of 450 mL), a hose with the length of 80 cm and diameter of 5 mm connecting the mini-biodigester to the volumetric burette, a hose with the length of 90 cm and diameter of 6.3 mm connecting the volumetric burette to the water container, a volumetric burette (volume of 50 mL) and a water container (volume of 250 mL) as shown in Fig. 1.

In this experiment, biogas is produced from the slurry of cow's manure with the addition of a certain amount of rumen fluid. The amount of additional rumen fluid is varied in four treatments, which is 0, 20, 30 and 40% of the total volume of slurry. The ratio between livestock manure and water is 1:2 with total volume of 450 mL of slurry. The biodigester are operated in the batch system under anaerobic condition.

The observation is conducted for 30 days and observes the formed gas volume by day-by-day recording the alteration

of the water surface in the scale tube in the same time. If the gas in that tube was close to 50 in the preparation time, that gas was release through a syringe. It aimed to normalize the water surface due to being 0. The hole formed by syringe then was closed by the fast-burning method. The value of CH₄ concentration was obtained with taking a gas sample by syringe from the hose that closes to mini-biodigester cover. After that, this gas then moved to the venoject tube. The methane concentration in biogas is measured on 14, 20, 25 and 30 days using the Gas Chromatography (GC).

RESULTS AND DISCUSSION

The data of the biogas production rate and biogas production accumulation of four kinds of treatments is presented in Fig. 2. Figure 2 shows that for device II with the addition of 10% of cow's rumen fluid, the result is almost the same as 0% of starter so that the percentage is changed to 20%. For device IV, the amount of gas generated turns out to

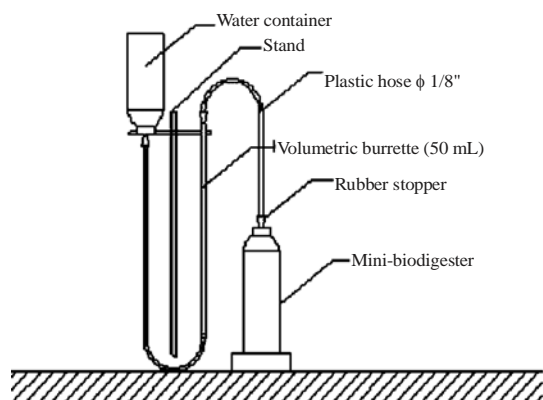


Fig. 1: Series of batch anaerobic reactor

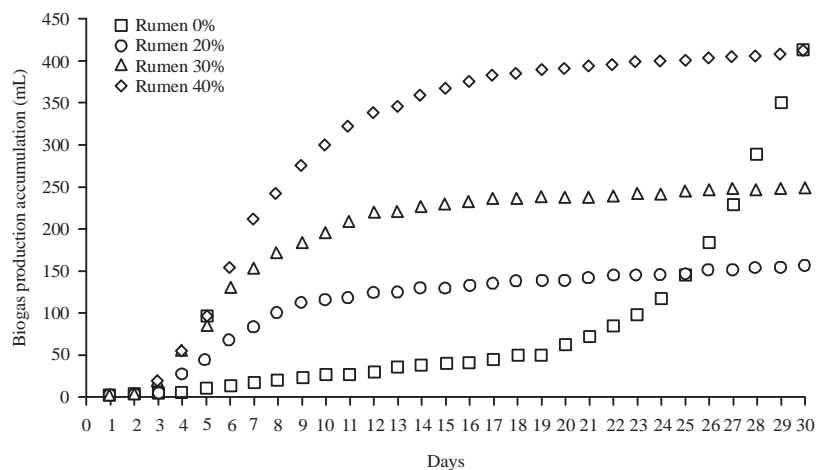


Fig. 2: Relation of biogas production accumulation and time

be very much but around the 12 days, the biogas production gets lower to 40%. The composition of the experimental material is listed in Table 1.

Table 2 and Fig. 3 show that on 20 days, methane concentration for treatment with a variation of additional cow's rumen fluid (20, 30 and 40%) is higher when compared to the control (without the addition of rumen fluid). This proves that the methane formation process can be accelerated by the addition of 20% of cow's rumen fluid on 20 days with the concentration of 38.20%, with biogas volume of 138.9 mL and the highest volume of biogas (391.3 mL) is obtained when 40% of cow's rumen is added. According to Weiland⁹ and Qasaimeh *et al.*¹⁰ that a starter (inoculum) is a group of microbes in a certain number and certain physiological conditions that are ready to be inoculated in the fermentation media. Microbes are one of the key factors that will determine the success of the biological process of organic fluid waste. Their existence is important for various stages of the organic material recast, one of the starters that can be used is cow's rumen fluid which contains various microorganisms, including hydrolysis bacteria, acidogenesis and methanogenesis bacteria, protozoa and fungi. Different microbial systems participate in transforming organic residues in a number of reactions into biogas under anaerobic conditions. In this study shows that the process of methanogenesis occurs between 10 and 20 days with the addition of rumen fluid. On 25 and 30 days, the concentration of methane is relatively constant. This may due to the diminishing substrate in the treatment with cow's rumen fluid addition and the diminishing activity of the methane-forming microbes, while the control still has much of the substrate that on day 25-30 days, the methanogenesis process still occurs and consequently the methane

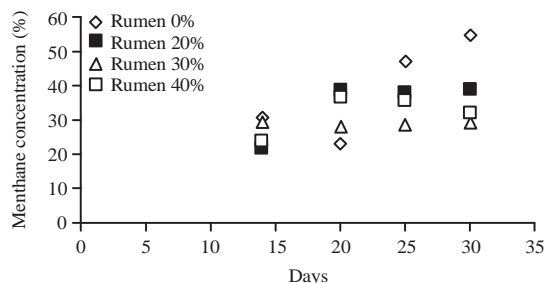


Fig. 3: Graph of methane concentration and time

Table 1: Calculation of the experimental material in volume

Treatment* (%)	Required amount (mL)			Total volume (mL)
	Starter	Cow's manure	Water	
0	0	150	300	450
20	90	150	210	450
30	135	150	165	450
40	180	150	120	450

*Treatment with variations in starter addition in the form of cow's rumen fluid to the volume of slurry

Table 2: Calculated amounts of microorganisms (Plate count of bacteria)

Sample type	Total plate count (amount g ⁻¹)
Cow's manure	9.55 × 10 ⁸
Cow's rumen fluid	1.32 × 10 ⁸

Table 3: Methane concentration (%) with variation of additional starter (cow's rumen fluid) to the volume of slurry during the observation period

Days	Methane concentration (%) with the variation of additional starter (Cow's rumen fluid)			
	0%	20%	30%	40%
14	30.84	21.95	29.87	24.02
20	22.86	38.20	28.76	37.00
25	47.60	37.71	29.63	35.65
30	54.88	39.15	30.16	31.38

concentration is higher when compared to the one formed in the treatment with rumen fluid addition. The biogas generation was started after seeding, kept increasing until reaching the peak and then began to decline. The biogas production and methane yield patterns were found similar to study of Jha *et al.*¹⁴ and Li *et al.*¹⁵. It means the biogas and methane generation showed an increased day after day until reached the maximum value and then decreased slowly day after day but biogas production was detected low in the reactor with mixing system during the 1st week of the fermentation process. Jha *et al.*¹⁶ adding that it happened due to high VFAs production, a decrease in pH value and lack of methanogens. However, the control takes a longer time, so if calculated economically, the treatment with the addition of rumen fluid is more likely to encourage selectivity towards the methane formation with higher concentration in a shorter time. The methane concentration (%) related to biogas production on day 14, 20, 25 and 30 is shown in Table 3.

Figure 4 shows that at the beginning of the experiment, the addition of rumen fluid (20, 30 and 40%) can produce a higher volume of biogas compared to the treatment without the addition of rumen fluid. The data graph in Fig. 4 shows that the highest concentration of methane occurs in the variation without the addition of rumen fluid (0% of rumen fluid) on 30 days. However, on 20 days, the variation of additional rumen fluid (20, 30 and 40%) produces a higher concentration of methane compared to the treatment without rumen fluid addition. And when compared with the volume of formed biogas, the addition of rumen fluid shows a very significant difference in the volume of methane than the treatment without rumen fluid addition (0% of rumen fluid). Montero *et al.*¹⁷ reported that during the process of fermentation in the production of biogas occurs the principal volatile fatty acids formed (acetic, butyric and propionic acids). Acetic acid was the dominant volatile fatty acid. The share of propionic and butyric acids was observed low because of the sufficient propionate and butyric-degrading syntrophs which could rapidly convert propionic acid and butyric acid to acetic acid. During this period, the acetic acid production rate was apparently higher than the acetic acid consumption rate. However, since from the beginning of the experiment until 13 days the methane test has not yet been conducted, the concentration of methane which is formed in those days is not known. Based on the previous study by Jha *et al.*¹⁶, the methane formation (methanogenesis process) will occur on 14 days.

The degradation of propionate and butyrate by syntrophic acetogenic bacteria produced acetic acid that was subsequently degraded into methane and CO₂ by acetoclastic methanogens¹⁷. Jha *et al.*¹⁶ added that during the methanogenic stage, acetic acid was started to convert into biogas such as methane and carbon dioxide. Thus as methanogenesis and methane gas yield have increased, the VFAs concentrations were decreased. No high VFAs accumulation was detected due to perhaps acetoclastic methanogens could consume acetate quickly in the digester to yield methane and carbon dioxide. Thus the results of study, it cannot be concluded that the methane concentration is high or low, so a verification is needed for further study by shortening the sampling interval at the beginning of the experiment in testing methane. Thus the addition of rumen fluid is needed to accelerate the degradation process which will lead to the acceleration of the methanogenesis process. The addition of rumen fluid is only necessary until 20 days and is not needed for the rest of the days because the biogas will be formed normally.

At this stage of the production of acid in the formation of biogas, bacteria acetogenic will remodel organic materials

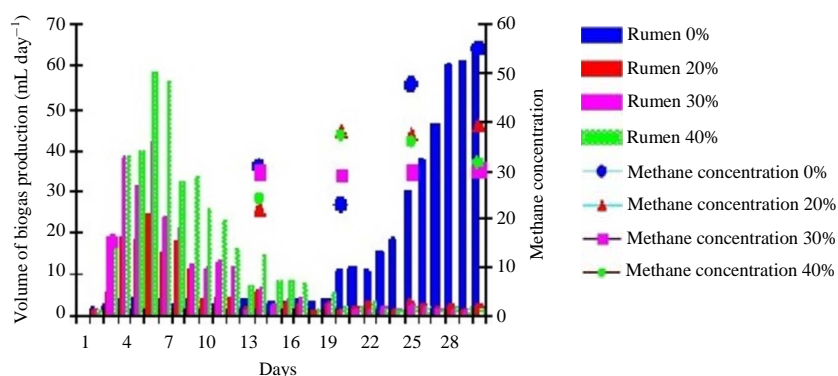


Fig. 4: Comparison of methane concentration to the biogas volume

dissolved in water into organic acids. Lactic acid can lower the pH of the substrate becomes 4-5, so that it will inhibit the growth of coliform requiring optimum¹⁸ pH 6-7. Based on the amount of coliform in the beginning of the study (which are 540×10^{14} , 240×10^{20} , 920×10^{19} and 220×10^{19} bacteria g^{-1}) compared to the result in the end of the study (which are 540×10^7 , 1600×10^6 , 540×10^5 and 540×10^6) in each treatment with the starter addition of 0, 20, 30 and 40%, a decrease in total coliform end of the study. Surono¹⁹ and Yuli *et al.*¹⁸ reported that in the hydrolysis stage reduced the number of coliforms. This is because the necessary nutrient is reduced and the accumulation result of excretion of the bacteria, thereby disrupting growth. In *Escherichia coli* metabolism colicins produce compounds which are compounds inhibiting bacterial growth.

CONCLUSION

The conclusion is that the addition of cow's rumen fluid as a starter can accelerate the biogas formation process in a shorter time (shortening the start-up time needed) which is 10 days, compared to the treatment without rumen fluid addition. Beside that, the addition of cow's rumen fluid on variations of 20-40% gives a positive effect on methane formation and methane concentration, thus it has potential to be used as a starter. In addition, the process of biogas formation is able to decrease the amount of coliform until nearly 100% compared to the time before the process begins. This makes the sludge safe to be used as organic fertilizer and for the environment.

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