The viability of Anaerobic Bacteria from Beef Cattle Feces in Liquid Media

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Abstract: The purpose of this paper is to know the viability of anaerobic bacteria from beef cattle feces in liquid medium incubated at temperature 25°C and 39°C aims to apply as biogas starter. The research was done by the explorative method and the obtained data was analyzed descriptively. The anaerobic bacteria were grown in 98-5 medium and incubated at temperature 25°C and 39°C, observed for 2 months and the analysis was done weekly. The parameters observed were a number of anaerobic bacteria, biogas volume, and percentage of biogas. The results showed that at the beginning of incubation at temperature 25°C, the number of anaerobic bacteria $1,250 \times 10^{10}$ cfu/mL, after incubating for 2 months, the number of anaerobic bacteria tend to decrease accumulatively reached 230×10^{10} cfu/mL, the volume of biogas production was 6 mL, the percentages of biogas production were $\text{CH}_4 = 0.0993\%$, $\text{CO}_2 = 1.1287\%$, N_2 and $\text{O}_2 = 86.163\%$. At temperature 39°C, the number of anaerobic bacteria, in the beginning, accumulatively reached 585×10^{10} cfu/mL, after incubating for 2 months, the number of anaerobic bacteria decreased accumulatively reached 180×10^{10} cfu/mL, the volume of biogas production was 4 mL, the percentages of biogas production were $\text{CH}_4 = 0.134\%$, $\text{CO}_2 = 2.4714\%$, N_2 and $\text{O}_2 = 89.4961\%$. It was concluded that an anaerobic bacterial of beef cattle feces incubated at temperature 39°C in a liquid medium generated a high survival and turn to be highly potential as a starter of biogas.

Keywords: beef cattle feces; anaerobic bacteria; biogas

1. Introduction

Biogas is a gas derived from the decomposition of organic materials by a consortium of bacteria in anaerobic conditions. Biogas production is influenced by organic material and anaerobic bacteria consortium activities [1]. Biogas formation utilizes indigenous bacteria from organic material so that the process is uncontrolled, and production of biogas is not maximal. However, biogas formation can utilize anaerobic bacterial consortium as a starter. Anaerobic bacteria consortium can be obtained from ruminant such as beef cattle that have various types of anaerobic bacteria in the rumen and will be carried in the feces during the process of defecation.

The consortium of anaerobic bacteria in the feces of beef cattle can be used as a starter in the formation of biogas but needs to be cultured first in a liquid medium and then tested its activity to their ability in the decomposing organic material into biogas.

The biogas formation process consists of three phases. Hydrolysis phase involve the anaerobic facultative bacteria degraded organic material into monosaccharide, peptide, and glycerol by *Clostridium acteinum*, *Bacteriodes ruminicola*, *Bifidobacterium* sp., *Eschericia* sp., *Enterobacter* sp., and *Desulfobio* sp. Acetogenesis phase involves acetogenic bacteria *Lactobacillus* sp. and *Streptococcus* sp. that oxidize long-chain carbon acids, such as acetate and alcohol. The last phase of methanogenesis involves methanogenic bacteria *Methanobacterium melianskii*, *Methanococcus* sp., and *Methanosarcina* sp. that utilize H_2 , CO_2 , and acetate for its growth to produce CH_4 and CO_2 [2-4]. However, the consortium of biogas-forming bacteria has to be isolated and incubated in a liquid medium before utilizing as a starter in producing biogas. Usually, the percentage of biogas production includes methane (CH_4) of 54-70%, carbon dioxide (CO_2) = 27-45%, nitrogen (N_2) = 0.5-3%, oxygen (O_2) = 0.1% and hydrogen sulfide (H_2S) <0.1% [4]. The highest biogas component is methane (CH_4) which is the source of energy, then followed by carbon dioxide (CO_2) together with H_2 and acetate converted by methanogenic bacteria into CH_4 . Thus, high biogas production does not necessarily contain high methane consequently analyzing the proportion of the produced gas is needed.

Bacterial activity except for the influence of nutrition availability, significantly influenced by environmental factors such as temperature and pH. The consortium of anaerobic bacteria in the rumen of beef cattle normally lives at 37°C according to the temperature present in the rumen [5]. The consortium of anaerobic bacteria in beef cattle feces lives at room temperature $\pm$ 25°C, naturally the activity is different compared with rumen. Therefore, investigation of the viability of anaerobic bacteria consortium of beef cattle feces in a growth media is very important. By growing the rumen bacteria into the growth medium is intended for the bacteria to adapt and allowed the bacteria easily to grow when applying as a starter biogas [6-8]. This paper aims to determine the viability of an anaerobic bacterial consortium of beef cattle feces in a liquid medium incubated at temperatures of 25°C and 39°C to evaluate the potency of this anaerobic bacterial consortium as a starter in the biogas formation.

2. Materials and Methods

The beef cattle feces was diluted with NaCl into 10^{-2} dilution and the solution was placed in Hungate tubes that contained melted pre-reduced media 98-5. The 98-5 media consisted of mineral solution I: dilution of K_2HPO_4 ; mineral solution II: dilution of NaCl, $(NH_4)_2SO_4$, KH_2PO_4 , $CaCl_2$, $MgSO_4 \cdot 7H_2O$; bacto-agar, resazurin solution, distilled water, rumen fluid, glucose, cellobiose, soluble starch, Cysteine-HCl. H_2O - $Na_2S \cdot 9H_2O$ solution and Na_2CO_3 solution.

The bacteria in a medium was maintained at 50°C to solidify the melted medium. The application of the roll-tube technique was applied to cultivate microorganism and obtaining the microorganism and 98-5 media spread evenly on Hungate tube wall. As the media solidified, the tube was incubated for 1 to 8 weeks at room temperature 25°C and 39°C. Bacteria were counted based on bacterial colonies growing on the walls of the Hungate tube. The produced gases were investigated by the Gas Chromatography (GC) analysis and determined their quality based on the production of methane (CH_4) and carbon dioxide (CO_2).

3. Results and Discussion

3.1. The Number of Anaerobic Bacteria Consortium

Figure 1 showed the result of the number of an anaerobic bacterial consortium of beef cattle feces in Hungate tube incubated at room temperature 25°C and incubator temperature 39°C. The results showed that the number of a consortium of anaerobic bacteria grown in each treatment, which was observed weekly to 8th week of the incubation period. An isolated consortium of anaerobic bacteria, incubated at room temperature 25°C, showed a high quantity at the beginning of growth at 1st week i.e. 1250×10^{10} cfu/mL then decreased rapidly until 8th weeks i.e. 230×10^{10} cfu/mL. In contrast, the anaerobic bacteria consortium isolate incubated at room temperature of 39°C, at 1st week showed the bacteria quantity of 585×10^{10} cfu/mL, then decreased relatively slow until the 8th week of 180×10^{10} cfu/mL. This result related to the phases of the biogas formation process, which was in the initial phase (hydrolysis) of the growing bacteria where anaerobic facultative bacteria and thrives at room temperature. This result was in agreement with the reported result in ref. [9] where the bacteria that play an important role in the hydrolysis phase at temperature 22°C were *Clostridium acteinum*,

Bacteriodes ruminicola, *Bifidobacterium* sp., *Eschericia* sp., *Enterobacter* sp., and *Desulfobio* sp. The observations of 2nd and 3rd weeks considered for the phase of acetogenesis involves the bacteria of *Lactobacillus* sp., *Streptococcus* sp. and commonly grows at temperature 37°C. At 4th week considered a methanogenesis phase involved the bacteria of *Methanogenetic melianskii*, *Methanococcus* sp., and *Methanosarcina* sp., and grow at a temperature of 30 – 40°C [10]. In the isolate of anaerobic bacteria consortium incubated at 39°C showed relatively less number of bacteria production. This might due to the growing bacteria was a methane-specific bacterium that normally grows at temperature of 55°C [11]. Therefore, the observation at 1st week showed that the number of growing bacteria was less than the isolate consortium of anaerobic bacteria incubated at room temperature 25°C. In contrast, the number of growing bacteria that grew relatively similar at 8th week.

Figure 1. The number of an anaerobic bacterial consortium of beef cattle feces in a Hungate tube incubated at room temperature 25°C and 39°C

3.2. Biogas Production

Figure 2 showed the production of biogas in Hungate tubes incubated at room temperature 25°C and 39°C which was observed weekly to 8th week of the incubation period. The isolate of anaerobic bacteria consortium incubated at room temperature 25°C produced biogas at 1st week was 1 to 10 mL and tended to decrease until the 8th week has produced a biogas around 6 mL. While the isolate anaerobic bacteria consortium incubated at 39°C produced biogas at 1st week was 7 mL and at 8th week was 4 mL. The number of biogas production was directly proportional to the growing number of anaerobic bacteria in which their activities and availability of organic materials determined the number of produced biogas [12]. In addition, several factors affect the potential of feedstock for biomethane production includes nutrient content, total and volatile solids (VS) content, chemical and biological oxygen demand, carbon/nitrogen ratio, and presence of inhibitory substances.

Figure 2. The biogas production in Hungate tubes incubated at room temperature 25°C and 39°C

3.3. Proportion of Biogas

The results of the percentage of biogas proportion in Hungate tubes incubated at room temperature 25°C and 39°C were listed in Table 1. In addition, the trend of the percentage of the biogas production in Hungate tubes incubated at room temperature 25°C and 39°C were represented in Figs. 3 to 5.

Table 1. Biogas proportions generated in Hungate tubes incubated at room temperature 25°C and incubator temperature of 39°C.

Weeks	Room Temperature (25°C)			Incubator Temperature (39°C)		
	CH ₄	CO ₂	O ₂ and H ₂	CH ₄	CO ₂	O ₂ and H ₂
	[%]			[%]		
1	0.0827	4.8113	94.935	0.1829	6.6686	91.9202
2	0.0943	4.4214	94.2942	0.323	5.783	91.1358
3	0.1069	3.1237	93.1558	0.3942	4.4303	90.5122
4	0.1007	2.3248	92.1371	0.2888	3.0271	90.0451
8	0.0993	1.1287	86.163	0.134	2.4714	89.4961

Figure 3. showed the percentage of methane (CH₄) production at incubated temperature 25°C and 39°C. This results indicated that the percentage of CH₄ production in Hungate tubes incubated at room temperature 25°C was similar for the 1st week up to 8th week [8]. In contrast, the percentage of CH₄ produced in Hungate tubes incubated at room temperature of 39°C was increased from a 1st week up to a 3rd week, then decreased until the 8th week. This result indicated that the activity of an anaerobic bacteria-forming methane gas consortium was more active at a treated incubated temperature of 39°C. This was in agreement to reported research [10] where the methanogenic bacteria were active at the temperature 30–40°C. However, the production of methane was still very low, it was assumed due to the availability of the organic material in the powder medium was limited and suspected that the biogas formation phase was still in the hydrolysis phase. This argument was supported by the evidence that a very high percentage of H₂ and O₂ were observed while for the methanogenic phase absolute anaerobic state was needed. Also reported in ref. [3] that the process of biogas formation mainly the microbial

growth affected by the availability of organic materials, pH, and temperature, and in turn, the microbial growth determined the number of biogas production.

Figure 3. The percentage of methane (CH₄) production at incubated temperature 25°C and 39°C.

Figure 4. The percentage of carbon dioxide (CO₂) production at incubated temperature 25°C and 39°C.

Figure 4. showed that the percentage of carbon dioxide (CO_2) production at incubated temperature 25°C and 39°C . The results showed that the percentage of CO_2 gas produced at two different temperature were a similar trend of decreasing for the observation of 1st to 8th week. This was due to produced CO_2 was utilized by methanogenic bacteria for growth and converted to CH_4 . Similarly reported in ref. [2-4] that CO_2 along with H_2 and acetate were exploited by methanogenic bacteria for its growth and producing CH_4 .

Figure 5 showed the percentage of H_2 and O_2 production at incubated temperature 25°C and 39°C . The percentage of H_2 and O_2 production was decreased for both incubating temperatures in line with the increased of the produced methane and CO_2 gas [13].

Figure 5. The percentage of H_2 and O_2 production at incubated temperature 25°C and 39°C .

4. Conclusions

In summary, the performance of an anaerobic bacterial of beef cattle feces isolated at incubating temperature 39°C was higher compared to incubated at room temperature in term of their number of a growing anaerobic bacteria consortium, the production of biogas, and the percentage of CH_4 , CO_2 , H_2 , and O_2 gas. Thus the obtained consortium of anaerobic bacteria from beef cattle feces incubated at incubator temperature (39°C) in a liquid medium potential to be applied as a starter for methane production.

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