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The Production of Anaerobic Bacteria and Biogas from Dairy Cattle Waste in Various Growth Mediums

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Abstract. The growth of anaerobic bacteria except the ruminal fluid quailty is strongly influenced by the media formulations. Previous researchers have set a standard media formulation for anaerobic bacteria from rumen, however the use of standard media formulations require chemicals with high cost. Moreover, other constraint of using standard media formulations is requires large quantities of media for anaerobic bacteria to grow. Therefore, it is necessary to find media with a new culture media formulation. Media used in this research were minimalist media consist of Nutrient Agar (NA), Lactose broth and rumen fluid; enriched media Rumen Fluid-Glucose-Agar (RGCA); and enriched media 98-5. The dairy cattle waste is utilized as source of anaerobic bacteria. The obtained data was analyzed by descriptive approach. The results showed that minimalist media produced anaerobic bacteria 2148 x 10^4 cfu/ml and biogas production: 1.06% CH_4, 9.893% CO_2; enriched media Rumen Fluid-Glucose-Agar (RGCA) produced anaerobic bacteria 1848 x 10^4 cfu/ml and biogas production 4.644% CH_4, 9.5356% CO_2; enriched media 98-5 produced anaerobic bacteria growth 15400 x 10^4 cfu/ml and biogas production 0.83% of CH_4, 42.2% of CO_2. It is conclude that the minimalist media was showed the best performance for the dairy cattle waste as source of anaerobic bacteria.

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INTRODUCTION

Many coal fields in Kalimantan Indonesia have low-calorific value thus not economical for industrial purposes. Coal Bed Methane (CBM) which is methane (CH_4) trapped and accumulated in the pores/cleats during coal formation found in coalfield. It has the potential to produce methane that can be utilized as an alternative energy source. Methane in CBM can be maximized by adding source of methanogenic bacteria and organic matter.

Methanogenic bacteria grow in dairy cattle waste which can be employed as a source of methanogenic bacteria and source of organic material. The growth of methanogenic bacteria outside the rumen is strongly influenced by the formulation of the media, therefore this study is trying to establish a media formulation for the growth of methanogenic bacteria in producing methane in form of biogas in Hungate tubes. The media used in this study is minimalist media consisting of Nutrient Agar (NA), Lactose broth and rumen fluid; Enriched media Rumen Fluid-Glucose- Agar (RGCA); and media 98-5. Feces of dairy cattle are used as a source of anaerobic bacteria. The parameters observed were the amount of anaerobic bacteria and the proportion of methane gas (CH_4) and carbon dioxide gas (CO_2) in biogas.

Biogas is a gas produced from the decomposition of organic matter by anaerobic bacteria activity. Biogas production is influenced by the amount of organic material and the number of anaerobic bacteria that degrade the organic material. The amount of biogas consists of several gases with high concentrations of methane gas (CH_4) and carbon dioxide (CO_2) gas, in addition there are nitrogen gas (N_2) and H_2S in small amounts [1-4]. The process of

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biogas formation includes three stages: Hydrolysis, Acetogenesis and Methanogenesis. Hydrolysis is decomposition of complex organic materials into simple organic materials by bacteria *Eschericia* sp., *Enterobacter* sp., *Pseudomonas* sp., *Acetobacter, Bifidobacter* sp. Acetogenesis is the decomposition of simple organic matter into organic acids (acetic acid, propionic acid, butyric acid, lactic acid by bacteria *Acetobacteriumwoodii, Sytrophobacterwolinii*, Methanogenesis stage is the decomposition of organic acids into biogas (CH_4, CO_2) by *Methanobacterium, Methanobacillus, Methanosarcina* and *Methanococcus* bacteria [5-7].

MATERIALS AND METHODS

Procedure

The first rumenal fluid is separated from dissolved solids using a centrifuge at 100 rpm for 20 min at 4^{o}C, then dairy cattle feces were diluted with NaCl up to 10^{-4}. The dilluted rumenal fluid immersed into the hungate tube no 1, 2 and 3, correspondingly using different media Nutrient Agar (NA), enriched media Rumen Fluid-Glucose-Agar (RGCA) and enriched media 98-5. The hungate tube 1 used a minimalist media contains a Nutrient Agar (NA), lactose broth, distilled water, resazurin, rumen liquid. The hungate tube 2 used a Rumen Fluid-Glucosa-Agar (RGCA) media consist of mineral solution I (K_2HPO_4 0.6 g in 100 ml distilled water), mineral solution II (1.2 g NaCl; 1.2 g $(NH_4)_2SO_4$; 0.6 g KH_2PO_4; 0.12 g $CaCl_2$;0.25 g $MgSO_4.7H_2O$ in 100 ml distilled water), 0.1 ml resazurin 0.1% in 500 ml distilled water), 2 g bacto-agar powder, 0.05 g glucose, 0.05 g cellobiose, 0.05 g Cystein-HCl-H_2O and 10 ml Na_2CO_3(8%). The hungate tube 3 used enriched media 98-5 containts mineral liquid I and II (15.0 ml), resazurin (0.1% 0.1 ml), distilled water (50 ml), ruminal fluig (40 ml), glucose (0.05 g), cellobiose (0.05 g), soluble starch (0.05 g), cysteine-HCl.H_2O-$Na_2S.9H_2O$ (5.0 ml), Na_2CO_3 (8%).

The performance was observed based on the produced anaerob bacteria and gas (ratio of methane and carbon dioxide). Before observing the number of anareob bacteria colonies growth, the culture tubes samples were incubated for 2, 5, 10, 14 and 30 days at 37^{o}C. While the produced gas samples were taken from culture tube using syringe and measured using Gas Chromatography (GC).

RESULT AND DISCUSSION

Number of Anaerobic Bacteria

The number of anaerobic bacteria (in 10^2 cfu/ml) from dairy cattle feces in Hungate tubes with minimalist media, enriched media (RGCA) and media (98-5) can be seen in Table 1. The growth of anaerobic bacteria tends to decrease on day 2 in NA, RGCA and 98-5 media (338, 247 and 165 x 10^2cfu/ml, respectively). The decrease of the number of anaerob bacteria considered due to two most important factors, the availability of nutrients and the environment of the media, i.e., availability of nutrients (10 macro elements C, H, O, N, S, P, K, Ca, Mg, Fe) and environmental conditions (pH, temperature) [1-4].

TABLE 1. Average Number of anaerobic bacteria from dairy cattle feces in Minimalist Media, Enriched Media (RGCA) and Media 98-5 (in 10^2 cfu/ml)

Day	Minimalist Media (NA)	Enriched Media (RGCA)	Media (98-5)
2	338	247	165
5	245	232	175
10	178	148	195
14	143	113	125
30	170	184	110

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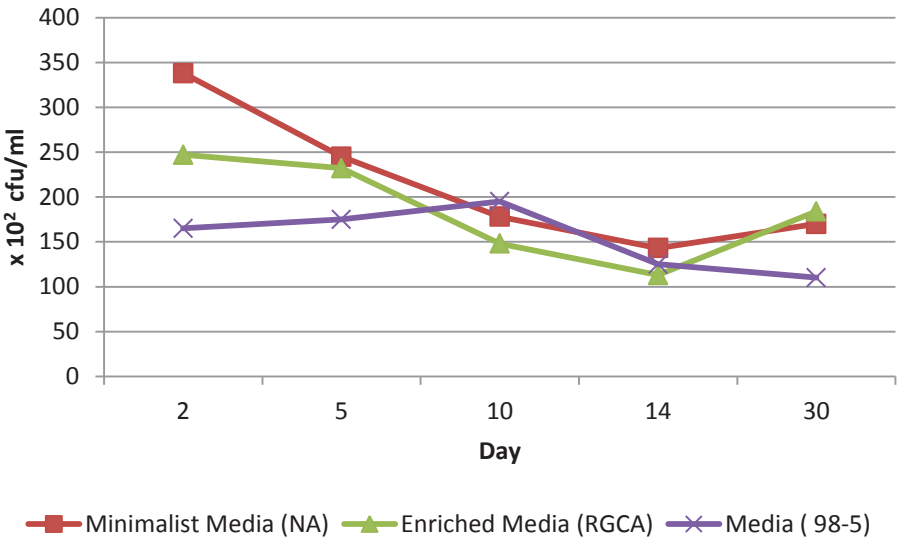

FIGURE 1. The number of anaerobic bacteria on different media

Figure 1 shows the growth pattern of anaerobic bacteria on three different media. The growth pattern on the enriched media RGCA and minimalist media was similar which is tend to decreased until day 14 and increased on day 30. This phenomena may due to the anaerobic bacteria on enriched media RGCA and minimalist media experienced adaptation process up to 14 days since the available organic nutrient did not ready to be comsummed by anaerob bacteria. It is wellknown that the anaerobic bacteria grow during biogas process consist of three groups of bacteria (hydrolysis bacteria, acetogenic bacteria and methanogenic) related to the decomposition of various organic nutrient and minerals as well [5-7]. Thus, inturn the growth of anaerobic bacteria would affect the production of biogas since the formation of biogas associated with fermentation time. In contrast, by using media 98-5, the number of anaerobic bacteria increased up to day 10, but decreased toward days 30.

Proportion of Methane (CH_4) and Carbon Dioxide (CO_2) in Biogas

The proportion of methane (CH_4) and carbon dioxide (CO_2) obtained from GC analysis is shown in Table 2. This result indicated that the percentage of the average values of methane (CH_4) and carbon dioxide (CO_2) concentration produced as a biogas in Hungate tubes from dairy cattle feces with NA, RGCA and 98-5 media. Generally, it was observed that the production of carbon dioxide (CO_2) was inversely proportional to the production of methane (CH_4). The production of methane (CH_4) on NA media increased until the tenth day and subsequently decreased until day 30, whereas in RGCA media, methane production increased until the 14th day and subsequently decreased until the 13th day. In contrast, methane production in media 98-5 increased up to thirty days.

TABLE 2. The average proportion of methane (CH_4) and carbon dioxide (CO_2) in biogas from dairy cattle feces in Minimalist Media, Enriched Media (RGCA) and Media 98-5

Day	Minimalist Media (%)		Enriched Media (RGCA) (%)		Media (98-5) (%)	
	CH_4	CO_2	CH_4	CO_2	CH_4	CO_2
2	0.93	98.63	0.90	95.45	0.05	53.03
5	2.39	97.61	2.39	97.61	0.04	63.70
10	2.39	97.58	7.91	92.09	0.14	42.11
14	1.06	70.56	10.54	89.46	1.69	29.86
30	0.88	99.02	1.48	98.52	2.23	22.3

Note: This table was based on GC results

For all samples, it were observed that the CO_2 composition was higher that other gas. The obtained biogas composed of methane (CH_4), carbon dioxide (CO_2), N_2, O_2, H_2. Usualy relatively small amount N_2, O_2, H_2 gas were

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available at the beginning of the biogas formation process, then gradually decreased according to the stages of the of biogas formation process. In the end of the stage, theoretically the highest gas proportion was methane (CH_4) followed by carbon dioxide (CO_2) and 54-70% methane can be used as energy resources. However, in this study, the proportion of carbon dioxide (CO_2) still higher than the methane indicated that the growth of methanogenic bacteria was still low, loonger time is reguired to completely convert the CO_2 into methane [11]. Similar result was reported when methane production of dried dairy cow 1 kg yield 0.0208% methane production [12, 13]

It concluded that omong three types of media, the production of biogas on NA media was best media for dairy ruminal fluid. In case of the RGCA and 98-5 media, it is also suggested that addition of Na_2CO_3 (buffer) to maintain pH at desired range for enhanching the growth of methanogenic bacteria; thus, the biogas formation is accelerated. However, other determinig factors also need to be considered such as organic loading rate, temperature, time, pH, C/N ratio [8-10].

CONCLUSION

The number of anaerobic bacteria from dairy cattle feces growing on the minimalist media (NA), enriched media (RGCA) and the 98-5 media during the biogas process in the hungate tube show similar pattern. The proportion of methane (CH_4) and carbon dioxide (CO_2) in biogas from dairy cattle feces in minimalist media (NA), enriched media (RGCA) and 98-5 media in hungate tube showed similar results which were low CH_4 and high CO_2. Suggestion for further research: addition of Na_2CO_3 (buffer) to maintain neutral pH (pH 6.8-8.5) which will be a suitable environment desired for the methanogenic bacteria.

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