The activation of microorganism inoculum from rumen of beef cattle

Y A Hidayati*, T B A Kurnani, E T Marlina, K N Rahmah and E Harlia
Faculty of Animal Husbandry, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang km 21 Jatinangor, Sumedang, West Java, Indonesia 45363

*yuli.astuti@unpad.ac.id

Abstract. The aim of this research was to investigate the activation of microorganism inoculum from rumen of beef cattle on lignite coal against number of anaerobic bacteria, the amount of volatile fatty acid (VFA) and biogas production. The research utilized completely randomized design with five treatments of various dosages of microbial inoculum from beef cattle rumen on lignite coal: P1 = 2%; P2 = 4%; P3 = 6%; P4 = 8%; P5 = 10%, and four repetitions. Parameters observed were the amount of VFA, the number of anaerobic bacteria and biogas production on day 2, 5, 10 and 15. Initial activation of beef cattle rumen microorganism inoculum utilized in-vitro technique with MC Dougall’s artificial saliva methods, by using combination of 70% concentrate + 30% forage as beef cattle feed. The rate of biogas production was observed on hour 2, 4, 6, 8, 24, 48 and 72. The highest biogas produced was selected as the optimal hour of inoculum source. The treatment used 250 mL bottle serum, as biogas digester, filled with 100 mL 98-5 media and 12 g lignite coal. Microbial inoculum inoculated in digester using 10 mL syringe and incubated at 39 °C. The result showed that the highest rate of biogas production on initial activation was 140 mL at hour 8. The highest average number of anaerobic bacteria was reached in P1, approximately 1068 x 10^10 cfu/mL; the highest amount of VFA on day 2 and 5 in P3, accounted by 181 mM (acetate acid 29.76 mM/L; propionate acid 6.93 mM/L; butyrate acid 2.48 mM/L); the highest biogas production was reached on P1, accounted by 37.17 mL (CH4 = 0.68%; CO2 = 99.32%).

1. Introduction

Microorganisms in the rumen of beef cattle have an important role in degrading organic materials from feed. Rumen microbes live under strictly anaerobic environment. Number and type of rumen microbes depend on type of diet. Organic material in rumen is degraded into a simpler organic compound, is break into organic acids and followed by gas formation, such as methane (CH4). Thus, rumen microbes, in particular beef cattle rumen microbes, can be utilized as starter in biogas formation.

Coal Bed Methane (CBM) is a natural gas formed through absorption processed of methane in liquid condition which trapped into coal pores. Similar with biogas, CBM contain carbon-dioxide (CO2), N2, H2, hydrogen sulfide (H2S), and O2. CBM is formed along with coalification. It can be formed through chemical changes from heat, namely thermogenesis, and microorganism activity under anaerobic condition, namely biogenesis. The quality of CBM relate to coal’s calorific rank which depends on coalification process: peat, lignite, bituminous and anthracite. In anthracite the quantity of CBM decreases.
This research study the activation of beef cattle rumen microbial inoculum in lignite coal against number of anaerobic bacteria, VFA and biogas production. This refers to CBM in lignite coal can be form biogenesis by microorganism activity under anaerobic condition. Result of this research is expected that microbial inoculum from rumen beef cattle can be utilized and activated in assisting methane gas formation process in CBM by observing the amount of anaerobic bacteria growing and the amount of VFA produced and the amount of biogas that is formed, so that in the end it can produce alternative energy in the form of methane gas ($\text{CH}_4$).

2. Materials and Methods

2.1 Tools and materials

The tools used in this research were Erlenmeyer, 250 ml serum bottle, 10 ml syringe, roll tube, and GC to analyze gas proportion on biogas produced. The materials used were rumen liquid of beef cattle, lignite coal, artificial saliva (Mac Dougall), concentrate, forage, 98-5 culture media consist of: 15.0 ml mineral liquid I and II, 0.1 ml Resazurin 0.1%, 50.0 ml distilled water, 2 g Bacto-agar Difco powder, 40.0 ml rumen fluid, 0.05 g glucose, 0.05 g Cellobiose, 0.05 g soluble starch, 5.0 ml Cysteine-HCl.H$_2$O-Na$_2$S.9H$_2$O liquid, 5.0 ml Na$_2$CO$_3$, 8%) [1].

2.2 Methods

In vitro fermentation: Initial rumen microbe activation utilized in vitro technique by using artificial saliva (Mac Dougall) and diet consists of 70% concentrate + 30% forage. Rate of biogas formation was observed at hour 2; 4; 6; 8; 24; 48; 72. The highest biogas production was selected as inoculum source.

Research design: Completely Randomized Design was utilized with five treatments: $P_1 = 2\%$ inoculum of beef cattle rumen microbes in lignite, $P_2 = 4\%$ inoculum of beef cattle rumen microbes in lignite, $P_3 = 6\%$ inoculum of beef cattle rumen microbes in lignite, $P_4 = 8\%$ inoculum of beef cattle rumen microbes in lignite, and $P_5 = 10\%$ inoculum of beef cattle rumen microbes in lignite, with 4 replications.

Parameter observed: Parameter observed were number of anaerobic bacteria, volatile fatty acid (VFA), biogas production Observation period was at day 2, 5, 10 and 15 after inoculation. The data were analyzed statistically with ANOVA test and followed with Duncan test using SPSS 23.

Research procedure: All treatments were utilized 250 ml bottle serum as biogas digester. Each bottle was filled by 100 ml 98-5 media and 12 g lignite. Microbial inoculum was inoculated by using syringe and incubated at 39°C for 7 days.

3. Result and Discussion

3.1 In vitro fermentation

The initial activation of rumen microbes using in vitro technique with artificial saliva (Mac Dougall) and diet combination of 70% concentrate + 30% forage can be seen in table 1.

| Hour | Biogas Production (ml) |
|------|------------------------|
| 2    | 40                     |
| 4    | 110                    |
| 6    | 140                    |
| 8    | 140                    |
| 24   | 100                    |
| 48   | 132                    |
| 72   | 36                     |

Biogas production was an indicator to show the rumen microbe activity. The highest biogas production was reached on hour 8 accounted by 140 ml. It assumed that anaerobic microorganism
from beef cattle rumen actively degraded organic material in diet consist of 70% concentrate and 30% forage with artificial saliva as buffer. It has a good agreement with other researcher who stated that growth curve of bacteria consists of adaptation, logarithmic, stationer and death phase [2].

3.2 Number of Anaerobic Bacteria
Average number of anaerobic bacteria growing on various treatments of inoculum dosages can be seen in figure 1.

![Figure 1. Number of anaerobic bacteria on various treatments at 15 days of incubation period.](image)

Note: treatments were not significantly different (P>0.05).

The result showed that the highest number of anaerobic bacteria were found in P1, however based on analysis of variance the average number of anaerobic bacteria growing not significantly different among the treatments (P>0.05). This condition occurred because the growth of anaerobic bacteria was affected by the ratio of available medium and number of inoculum as starter. Even though inoculum dosage that inoculated was different, the average of anaerobic bacteria grow was almost similar. It was allegedly because the growth of anaerobic bacteria greatly influenced by the nutrient availability. Other researcher stated that the growth of bacteria was affected by ratio of bacteria population-available nutrient and environment condition (temperature, pH) [3, 4]. Jing Yi et al. suggested that the content of total solid materials affected the anaerobic digestion performance and changes on total solid content resulted on changes of microbial morphology in rumen [5].

3.3 VFA Production
The average production of VFA on various treatments can be seen in Figure 4. Based on the result, addition of inoculum dosage until 6% tended to increase VFA production. However, the addition of more than 6% decreased VFA production. The highest VFA production at day 2 and 5 was reached on P3, accounted by 181 mM (acetate acid 29.76 mM/l; propionate acid 6.93 mM/l; butyrate acid 2.48 mM/l). This condition occurred because VFA production was influenced by the ratio of nutrient source (medium and lignite) and inoculum dosage. The optimum ratio of nutrient and inoculum was reached at 6% (P3) which showed the highest VFA production. Too much microbial population resulted competition in obtaining substrate, otherwise small population of microbes resulted substrate degradation was not maximal. This has good agreement with other researcher who argued that methane and VFA concentration had bimodal pattern which related to nutrient cycle [6, 7]. On other reference, it is suggested that although VFA production and organic polymeric hydrolysis increased, community of primary bacteria with different treatments had no substantial difference [8].
Figure 2. VFA production on various treatments at 5 days of incubation period.

Notes: treatments followed with same letter are not significantly different according to Duncan Multiple Test at 5% significance level.

**Biogas, CH$_4$ and CO$_2$ Production**

The average number of biogas, CH$_4$ and CO$_2$ production on various dosages of inoculum can be seen in figure 3 and 4. The highest biogas production was reached in P1. Based on analysis of variance, the biogas production was not significantly different ($P>0.05$). This related to number of anaerobic bacteria that grow. Biogas production was determined by organic material which was degraded and the activity of anaerobic bacteria. On other reference, it is suggested that ratio of carbon and nitrogen on degraded organic material affected biogas production. In addition, other researcher state that the addition of organic materials will affect the population of bacteria that activity so that will increase the biogas [9, 10].

Biogas production consists of CH$_4$ and CO$_2$ with different proportion. The highest CH$_4$ production obtained on P5 = 10% accounted by 13101.13 ppm, and the lowest CO$_2$ accounted by 271617.4 ppm. This condition illustrated that biogas formation was still in phase 3 where the proportion of CH$_4$ was low and CO$_2$ was high, in the next phase CO$_2$ could be reduced to CH$_4$ and H$_2$O [11].
4. Conclusion

The addition of inoculum of beef cattle rumen on lignite at 15 days’ incubation period was not affected number of anaerobic bacteria and biogas production, as well as CH$_4$ and CO$_2$ produced. However, the treatment of inoculum dosages significantly affected VFA production. Inoculum dose of microorganism from rumen of beef cattle up to 10% added to lignite coal with incubation period for 15 days, anaerobic bacteria growing $2 \times 10^{15}$ cfu / ml and the amount of biogas produced as much as 130 ml and CH$_4$ and CO$_2$ produced 50000 ppm and 250000 ppm, but the dose of inoculum affects the amount of Flying Fatty Acids produced on the 5th day $P \neq 0.05$ (Acetic Acid 29.76 mM / l; Propionic Acid 6.93 mM / l; Butyric Acid 2.48 mM / l).

Acknowledgement

We are profoundly indebted to Rector of Universitas Padjadjaran through the Academic Leadership Grant (ALG) and DPRM Universitas Padjadjaran. We thank Prof. Dr. Ellin Harlia, MS who led the Program at the Faculty of Animal Husbandry and provided insight and expertise that greatly assisted the research.

References

[1] Bryant M P and Robinson I M 1961 *J. Dairy Sci.* **44** 1446
[2] Rolfe M D, Rice C J, Lucchini S, Pin C, Thompson A, Cameron A D S, Alston M, Stringer M F, Betts R P, Baranyi J, Peck M W and Hinton J C D 2012 *J Bacteriol*. 2012 Feb **194** 686
[3] Leung D Y C and Wang J 2016 *International Journal of Green Energy* Volume **13** 119
[4] Shah F A, Mahmood Q, Shah M M, Perves A and Asad S A 2014 *Scientific World Journal* **2014** 183752
[5] Jing Yi, Bin Dong, Jingwei Jin and Xiaohu Dai 2014 *PLoS ONE* **9** e102548
[6] Robinson D L, Goopy J and Hegarty R S 2010 *Animal Production Science* **50** 630
[7] Dong R and Zhao G 2014 *PLoS ONE* **9** e116290
[8] Yang X, Liu X, Chen S, Liu G, Wu S and Wan C 2016 *Archaea* **2016** 1698163
[9] Dioha J, Ikeme C H, Nafi’u T, Soba N I and Yusuf M B S 2013 International Research Journal of Natural Sciences 1 1
[10] Cioabăa A E, Djuricb A, Dumitrelc G A, Ėd D C and Pode V 2017 Studia Ubb Chemia LXII 51
[11] Chen J, Falivene L, Caporaso L, Cavallo L and Chen E Y X 2016 J. Am. Chem. Soc. 138 5321