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Production of volatile fatty acid in biogas digester with coal media

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Abstract

The need for environmentally friendly energy can be sought by using a consortium of microorganisms originating from dairy cow feces in a biogas digester with sub-bituminous coal media. This study aims to determine the effect of adding consortium from dairy cow feces on coal media in biogas digester on pH of digester and volatile fatty acid production. This study used an experimental method using a randomized block design consisting of four treatments based on the consortium microorganism inoculum given (P1 = powder inoculum (mixture of inoculum from coal and dairy cow feces), P2 = inoculum from coal, P3 = inoculum from dairy cow feces, and P4 = without using inoculum) and six groups based on fermentation time (I = 10th day, II = 20th day, III = 30th day, IV = 40th day, V = 50th day, and VI = 60th day). The pH conditions of the digester in all treatments ranged from 6.438 -6.597, and the production of volatile fatty acids ranged from 153.083-162.333 mM. The results of the analysis in this study indicated that the addition of various types of consortium microorganism inoculums in the biogas digester medium produced similar pH conditions and volatile fatty acids. The production of increased concentrations of volatile fatty acids was directly proportional to the decrease in pH conditions.

Keywords: pH, volatile fatty acid, biogas, dairy cow feces, digester

Introduction

Biogas contains compounds such as methane (CH_4), carbon dioxide (CO_2), hydrogen (H), oxygen (O_2), and hydrogen sulfide (H_2S). Compounds that play a major role in the formation of biogas are methane. Methane is formed naturally from organic materials such as livestock manure, human waste, household waste, vegetable waste, and fruit waste. Methane is also found in coal which is referred to as coal bed methane (CBM). In the process of methane formation, volatile fatty acid (VFA) is an important indicator. This is because in the Acetogenesis stage, acetogenic bacteria such as *Syntrophomonas* and *Syntrophobacter* converts VFA to acetic acid and hydrogen which will later be used by methanogens to form methane (Schink, 1997). Anaerobic decomposition depends on pH, because each group of microbes involved has a specific pH range to grow optimally. Based on pH, microbes in anaerobic digestion are included in microbial neutrophils, this is because the optimal acidity conditions in anaerobic digestion are around pH 6.8-8, the rate of digestion will decrease at higher or lower pH conditions (Tuti, 2006). Decreasing pH accompanied by accumulation of volatile fatty acids is a major cause of reactor toxicity and failure in the anaerobic digestion process (Ahring et al. 1995). The process of digesting livestock waste in the digesting tank can last for 60-90 days (Sweeten 1979, in Fontenot et al. 1983), while according to Sahidu (1983) only lasts 60 days. Biogas is formed on the 10th day and increases by 50% on the 30th day of fermentation (Hadi, 1980). The VFA formation process occurs through the degradation of complex organic material by acidogenic bacteria into organic acids such as acetate, butyrate, propionate, or valerate.

Generally the accumulation of VFA in the process of biogas formation can reduce the pH value, but this depends on the content of the buffer formed, and the type of biomass used. For example, livestock manure contains high alkaline compounds so that high VFA concentrations are needed to reduce the pH value of the digester, but too high a concentration of VFA can actually inhibit the process of methane formation (Seadi et al. 2008).

Methane is formed through the conversion of acetate, formic, and hydrogen into methane and CO_2 with the help of microbial methanogens (Krzysztof et al. 2012). VFAs with long carbon chains such as propionate and butyrate are utilized by acetogenic bacteria such as syntrophomonas or syntrophobacter to form acetate and hydrogen (Schink, 1997). VFA with short carbon chains namely acetate and formic is utilized by methanogens to form methane from CO_2 reduction using H_2 (Krzysztof et al. 2012). The acetate formation process describes the level of efficiency of biogas production, because about 70% of methane is formed from the conversion of acetate to methane. In the acetogenetic process around 25% acetate and 11% hydrogen are formed (Krzysztof et al. 2012).

The aim of this research is to utilize microbial consortium from dairy cow feces and coals for environmental friendly biogas formation. If abandoned and stacked in the environment, dairy cow waste can easily run off into river and contribute in increasing BOD and COD in the water bodies. As a biogas fermentation indicator, there are factors in biogas formation including pH and VFA that will observe during fermentation.

Materials and methods

The research materials used were microbial consortium inoculums from dairy cow feces; 985 liquid medium for anaerobic microbes consisted of rumen fluids, distilled water, various mineral solution (K_2HPO_4, NaCl, $(NH_4)_2SO_4$, KH_2PO_4, $CaCl_2$, and $MgSO_4$), glucose, cellubiose, soluble starch, cysteine, and Na_2CO_3 8% solution (Ogimoto & Imai, 1980); medium for digester; rumen fluid; and sub-bituminous coal.

Research methods

Preparation of inoculum: Microbial consortium from dairy cow feces and coals were prepared through in vitro techniques and adaptation process for 14 days. The 985 medium liquid was put into a bottle of 1 liter each. The microbial consortium inoculum was inserted as much as 5% using a syringe. The planting of the inoculum was adjusted according to the treatment of powder inoculum (P1), coal inoculum (P2), dairy cow feces inoculum (P3), and without inoculum (P4). After inoculation, each bottle was incubated for 7 days at 37°C.

Preparation of medium for digester: The rumen liquid used was taken from the Cibinong Slaughterhouse. The rumen contents were filtered using a total of 12 layers of gauze to produce 12 liters of rumen liquid for each treatment. Each liquid was put into a biogas digester and added 18 liters of distilled water. Each digester was added mineral mix 0.5%, dextrose 0.05%, and starch 0.05% was carried out as a substitute for minerals and sugar so that the digester medium resembled a medium 985. The medium was heated in the digester at 100°C for 15 minutes until it was ready for use after the temperature drops to 35-37°C.

Biogas fermentation in 30 liter digester: After incubation, each bottle was poured into a digester containing 30 liters of medium with a temperature of 35-37°C. Addition of coal was carried out before. The digester was tightly closed and CO_2 gas was inserted to create an anaerobic atmosphere in the biogas digester.

This research utilized randomized group design with 4 treatments (P1, P2, P3, and P4) and 6 replication of observation time during 60 days. Parameters observed were pH conditions, volatile fatty acids production, and methane production. Data were collected every ten days for 60 days, which is in day 10 (I), day 20 (II), day 30 (III), day 40 (IV), day 50 (V), and day 60 (VI).

Results and discussion

pH in digester

The observation of pH Digester conditions in various types of consortium of microorganisms from dairy cow feces and coal in the medium in the digester can be seen in **Table 1** as follows:

Table 1 pH Digester on various addition of microorganism consortium from dairy cow feces and coal.

Group/Replication	pH Digester			
	P_1	P_2	P_3	P_4
I	6.82	6.92	6.83	6.85
II	6.60	6.79	6.51	6.43
III	6.35	6.41	6.39	5.95
IV	6.02	5.45	5.95	5.69
V	6.80	6.82	6.81	6.89
VI	6.99	6.91	6.99	6.82
Average	6.59	6.55	6.58	6.43

Note: P1 = powder inoculum, P2 = coal inoculum, P3 = dairy cow feces inoculum, P4 = without inoculum;
I = 10^{th} day, II = 20^{th} day, III = 30^{th} day, IV = 40^{th} day, V = 50^{th} day, and VI = 60^{th} day

The results of analysis of variance showed that the treatment of the difference in consortium microorganism inoculums given to the media did not have a significant effect ($P > 0.05$) on the pH conditions of the digester. This indicated that the addition of various types of inoculums from the microorganism originating from dairy cow feces and coals had similar pH condition of the digester.

The pH condition of the digester on all observation days of this study ranged from 6.43 to 6.59, this was slightly lower than the statement of Hasyimi (2010) which stated that the optimum pH for bacterial growth ranged from 6.50 to 7.50 and the opinion of Tuti (2006) which stated that optimal acidity conditions in anaerobic digestion were around pH 6.80 to 8.00. The lower pH value indicated that there was methanogens acted like *Methanobacterium ruminantium*, according to the opinion of Ogimoto and Imai (1981) which stated that methanogens such as *Methanobacterium ruminantium* grow well at temperatures of 38 to 42°C, pH 5.00 to7.50, and under anaerobic conditions.

The results showed that in each treatment during observation group of day 10 (I) to day 40 (IV) there was a decrease in pH. This indicated that microbial activity occurred. This was in line with the statement of Tillman et al. (1991) which stated that the atmosphere of acidic rumen pH (low pH) can cause increased rumen microbial activity. In addition to microbial activity, the increased production of volatile fatty acids resulted in a decrease in the pH condition of the digester. In the process of biogas formation, pH affected the growth of microorganisms in term of the diversity and population of microorganisms in biogas reactors (Baily & Ollis, 1986).

Production of volatile fatty acids

The results of volatile fatty acid production in various additions to the consortium of microorganisms from dairy cow feces and coal in the medium in the digester can be seen in **Table 2**.

Table 2 VFA Production in addition of various microorganism consortium from dairy cow feces and coal.

Group/Replication	VFA Production			
	P_1	P_2	P_3	P_4
	mM			
I	132.00	136.50	148.50	127.00
II	152.00	156.50	158.00	166.00
III	183.00	183.50	184.50	186.00
IV	194.00	213.00	195.00	188.50
V	137.50	149.50	144.50	143.00
VI	120.00	135.00	110.00	133.00
Total	918.50	974.00	940.50	943.50
Average	153.08	162.333	156.75	157.25

Note: P1 = powder inoculum, P2 = coal inoculum, P3 = dairy cow feces inoculum, P4 = without inoculum;
I = 10th day, II = 20th day, III = 30th day, IV = 40th day, V = 50th day, and VI = 60th day

Based on **Table 2**, it can be seen that the average production of volatile fatty acids in this study from observation of the 10th day to the 60th day ranged from 153,083 mM to 162,333 mM, this was higher when compared to the opinion of Sutardi et al. (1983) which stated that the optimum levels of volatile fatty acids ranged from 80 to 160 mM.

The results of variance analysis showed that the treatment of different microorganism inoculums given to the media had no significant effect ($P > 0.05$) on the amount of volatile fatty acid production. This showed that the addition of various types of consortium inoculum of microorganisms from dairy cow feces and coals produced similar production of volatile fatty acids.

Volatile fatty acid production in this study increased from the 10th day observation group (I) to the 40th day (IV) observation. Sub-bituminous coal had an organic carbon content of 25% (ASTM, 2004). The increase that occurs was indicated by the organic content contained in coal in the form of long chain alkanes which were converted into fatty acids with long chains and acetic acid through the process of bacterial respiration, while single ring aromatics from coal were fermented into phenol and benzoate compounds by fermentative bacteria. The long chain fatty acids and phenol and benzoate compounds were then converted to propionate and butyrate through syntrophic bacteria activities (Elizabeth et al. 2010).

The decrease in all treatments in the 50 (V) and 60 (VI) day observation groups indicated that the volatile fatty acids formed had been reused to be utilized by microbes as the methane forming substrate (**Figure 1**). Volatile fatty acids with long carbon chains such as propionate and butyrate were utilized by acetogenic bacteria such as *Syntrophomonas* or *Syntrophobacter* to form acetate and hydrogen (Schink, 1997). Volatile fatty acids with short carbon chains namely acetate and formatting were utilized by methanogens to form methane from CO_2 reduction using H_2 (Krzysztof et al., 2012). The acetate formation process described the level of efficiency of biogas production, because about 70% of methane was formed from the conversion of acetate to methane. In the acetogenetic process it formed around 25% acetate and 11% hydrogen (Krzysztof et al. 2012).

In the process of methane formation, VFA was one of the important indicators, this was because in the stage Acetogenesis, acetogenic bacteria such as *Syntrophomonas* and *Syntrophobacter* converted VFA to acetic acid and hydrogen which would later be used by methanogens to form methane (Schink, 1997).

Methane production

The figure below showed the methane production during observation period.

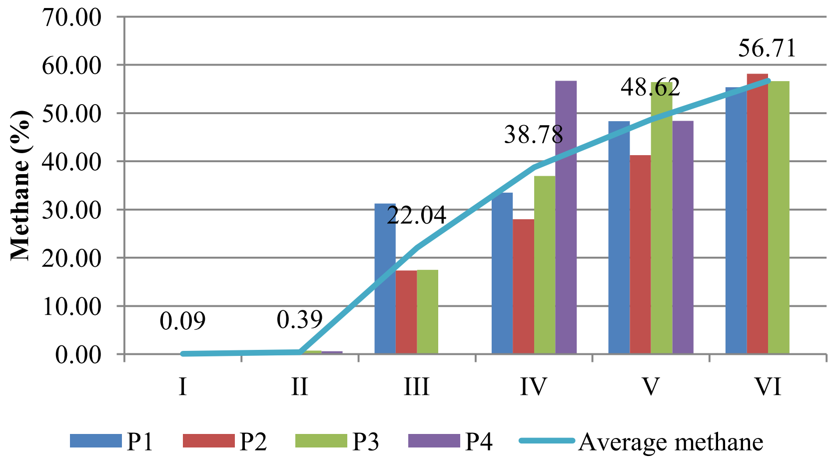

Figure 1 Percentage of methane production during 60 days of observation period;
I = 10th day, II = 20th day, III = 30th day, IV = 40th day, V = 50th day, and VI = 60th day

Methane production was generally increased following the observation period. At day 10, average methane production only reached 0.09%, and increased tremendously into 56.71% at day 60. Based on **Figure 1**, rapid increased occurred in day 20 to 30 and increased continuously until day 60. There were several stages of methane formation: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Zieminski & Fac, 2012). Methanogenesis had begun in day 14-21 depend on its substrate. This was in accordance with the result of methane production in **Figure 1** which increased promptly in day 30. Methane production was highly depended on VFA production, specifically acetate acids which converted by acetogenic bacteria namely *Syntrophomonas* and *Syntrophobacter* into methane (Schink, 1997). Methane production in digester at day 60 has reached more than 50% which means it can be utilized as biogas (Ilaboya et al. 2010).

Relation between pH and VFA production

Based on observations it was found that there was a pattern of increase and decrease in pH conditions and the production of volatile fatty acids resulting from the addition of inoculums to the biogas digester media. This can be seen in **Figure 2**.

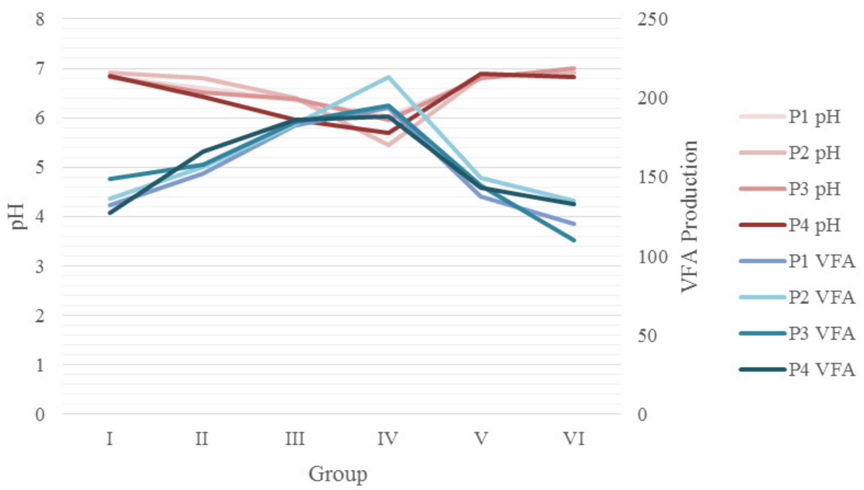

Figure 2 Relation between pH and VFA production.

Based on these illustrations, it could be seen that in each treatment there were the lowest pH conditions and the peak point of volatile fatty acid production at the 40th day of fermentation (IV). The illustration also showed that high volatile fatty acid production would be accompanied by low pH conditions. This was in line with the statement of Hasyimi (2010) which stated that the presence of VFA would reduce the pH value. Generally, the accumulation of VFA in the biogas formation process could reduce the pH value, but this depends on the content of the buffer formed, and the type of biomass used (Seadi et al. 2008). Based on these observations, it showed that there was a relationship between pH conditions and the production of volatile fatty acids, where the increase in VFA concentration was directly proportional to the decrease in pH.

Conclusions

It can be concluded that the addition of various type of microorganisms (mix powder of coal and dairy cow feces, coals, dairy cow feces, and without inoculum) in anaerobic digester containing coal medium produced similar pH condition and VFA production. In general, VFA production and pH value reached its peak in day 40.

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References

American Society for Testing And Materials. (2004). *Annual Book of ASTM Standard section five Petroleum, Lubricants, and Fossil Fuels, Vol. 05.06.* Philadelphia: ASTM International.

Baily, JE., & Ollis D.F. (1986). *Biocemical Engineering Fundamental*. 2nd eds. New York: McGraw-Hill.

Guo, H., Yu, Z., Liu, R., Zhang, H., Zhong, Q., & Xiong, Z. (2012). Methylutropic methanogenesis governs the biogenic coal bed methane formation in Eastern ordos basin, China. *Applied Microbiology and Biotechnology 96*(6), 1587-1579.

Ilaboya, I.R., Asekhame, F.F., Ezugwu, M.O., Erameh, A.A., & Omofuma, F.E. (2010). Studies on biogas generation from agricultural waste; analysis of the effects of alkaline on gas generation. *World Applied Sciences Journal 9*(5), 537-545.

Jones, E.J.P., Voytek, M.A., Corum, M.D., & Orem, W.H. (2010). Stimulation of methane generation from nonproductive coal by addition of nutrients or a microbial consortium. *Applied and Environmental Microbiology 76*(21), 7013-7022.

Kementerian Energi dan Sumber Daya Mineral. (2015). *Rencana strategis kementerian energi dan sumber daya mineral 2015-2019*. Kementerian Energi dan Sumber Daya Mineral, Jakarta.

Ogimoto, K., & Imai, S. (1980). *Atlas of rumen microbiology*. Tokyo: Japan Scientific Societies Press.

Schink, B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews 61*(2), 262-280.

Seadi, T.A., Rutz, D., Prassl, H., Köttner, M., Finsterwalder, T., Volk, S., & Janssen, R. (2008). *Biogas Handbook* (pp. 21-28). Denmark: University of Southern Denmark Esbjerg.

Sutardi, T., Sigit, N.A., & Toharmat, T. (1983). *Standarisasi mutu protein bahan makanan ruminansia berdasarkan parameter metabolismenya oleh mikroba rumen*. Bogor: Fakultas Peternakan Institut Pertanian Bogor.

Tillman, A.D., Hari, H., Soedomo, R., Soeharto, P., & Soekanto, L.D. (1991). *Ilmu makanan ternak dasar*. Fakultas Peternakan UGM: Gadjah Mada University Press.

Tuti, H. (2006). *Biogas: Limbah peternakan yang menjadi sumber energi alternatif.* Bogor: Balai Penelitian Ternak.

Zieminski, K., & Frac, M. (2012). Methane fermentation process as anaerobic digestion of biomass: Transformations, stages, and microorganism*. African Journal of Biotechnology 11*(18), 4127-4139.