ARTIKEL 1

Judul : The Effect of Rumen and Mixed Microorganisms (Rumen and Effective Microorganisms) on Biogas Production From Rice Straw Waste

SEKOLAH TINGGI KEGURUAN DAN ILMU PENDIDIKAN
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[21]THE EFFECT OF RUMEN AND MIXED MICROORGANISM (RUMEN AND EFFECTIVE MICROORGANISM) ON BIOGAS PRODUCTION FROM RICE STRAW WASTE

INTRODUCTION

Biomass is very potential to be biogas energy resource. It can be converted directly into biogas with low production cost. The output-input energy ratio of biogas is 28 MJ/MJ [1]. Biomass is the best choice to keep the world's energy fulfilled in the future [2]. Anaerobic process is used to convert biomass into biogas. Anaerobic process is a degradation process of organic waste without oxygen and involve anaerobic microorganism [3] which produce methane (biogas), carbon dioxide and organic fertilizer to improve agricultural land [4].

[25]There are various kinds of biomass that can be used in anaerobic process to produce biogas. Biomass from food (oil and simple carbohydrate) such as corn, cane, and non-food such as leaves, tree branch, coffee pulp and husk can be used in anaerobic process and involve special microbes for pretreatment of waste to increase the yield of methane and the stability of the end product. The usage of food waste in bioprocess can reduce the environment pollution [5, 6]. Energy production from biomass is an important technology to continue the generation of renewable energy [7].

There are some problems to convert biomass to biogas because cellulose biomass has three similar polymers: [3]cellulose, hemicellulose and lignin [8]. Lignin derivate with aldehyde group or its polar substituent is very toxic in methanogen process [9]. Due to the strong bond in every polymer molecules, it form physical barrier

to prevent the absorption by hydrolysis enzyme [10, 11]. The problem of methane production can be solved by pretreatment to remove physical barrier from biomass material [7] and with the addition of microorganism, because anaerobic process needs high microorganism concentration. First of all, toxic components (such as tannin and phenol) were degraded by microorganisms to produce biogas [2]. [3]The aim of this study is to compare the effect between rumen fluid microorganism and combination of rumen and effective microorganism from rice straw waste in anaerobic process. [19]The optimum condition and high production rate in the production of good quality of methane were also investigated.

METHODOLOGY

The batch anaerobic process was chosen in this research. Batch system is very good process in methane production from lignoselulosic material, because metanogens bacteria is strictly anaerob, the presence of litte air can hinder the methane production. In another research, semi batch process was used, but in this research we compared some variable of micoorganisms to obtain the best operating conditions from all variables that used, therefore the batch system was necessary.

Preparation

Rice straw waste was collected from rice field in Sumenep, Madura Island, Indonesia. Before delivered to laboratory, it was spread out to the sun for 3-4 days and grinded to become powder. The feed substrate was made by mixing of rice straw powder and water (1 : 2), then the mixing was filled to every digester with dosage 60 % volume of digester and additional nutrition was filled to every digester that instead of 2 g/l CH_3COONa, 4 g/l NH_4Cl, 0.06 g/l KH_2PO_4, 0.025 g/l $CaCl_2$, 0.005 g/l $NiCl_2$,
0.005 g/l $MnCl_2$, 0.005 g/l $CoCl_2$, 0.1 g/l yeast extract,
0.025 g/l $MgCl_2$, and 0.03 g/l Fe-EDTA (7).

Rumen fluids were taken from Surabaya slaughters house freshly from cut cattle. [10]Rumens was filtered by 1 mm x 1 mm sieve to remove coarse solid until collected 10 liters, then delivered to the Biochemichal Technology Laboratory at Chemical Engineering Department of ITS. [10]Then stored in fluid storage and filled by Nitrogen at 30oC - 35oC for incubation

Effective Microorganisms was purchased from PT. Songgolangit Persada. A liter of EM-4 (Effective Micoorganisms-4) contains 1.5 x 106 cfu/ml Lactobasillus casei, 1.5 x 106 cfu/ml Saccaromyces cerevisiae and 1.[3]0 x

106 cfu/ml Rhadopseudomonas palustris which it product was registered from Agriculture Ministry of Republic of Indonesia,No.D.11064101 FTC and label certification No. IDM000073421.
[10]Cow dung microorganisms were isolated from 5000 liters biogas plant at LTD Laboratory at Chemical Engineering Department of ITS Surabaya. The digestion was done in 7 month earlier with cow dung and molasses as feed or substrate. Then 550 ml cow dung microorganisms were incubated in incubator shaker for 12 hours before inoculated into rice straw digester [2, 7].

Starter was made by injecting every volume variables of microorganisms into Erlenmeyer. Then it was added by nutrition. 0.35 g rice straw powder and 0.35 g L-Cyscteine were added to 5 % (v/v) of starter, 0.7 g rice straw powder and 0.7 g L-Cyscteine were added to 10 % (v/v) of starter and 1.05 g rice straw powder and 1.05 g L-Cyscteine were added to 15 % (v/v) of starter. After the addition of the nutrition (rice straw powder and L-Cysteine), the erlenmeyer must be closed tightly. Then the starter was placed in an incubator shaker for 12 hours with 137 rpm and 37 oC [7]. After 12 hours, the starter was ready to be filled into every digester.

Anaerobic Digestion

Before batch digestions were started, dosage of rumen fluid microorganism, combination of rumen fluid and effective microorganisms were prepared by 5%, 10%, and 15 % volume variables in 21 days, with pH 6-7, temperature 30-40 oC and 1 atm pressure [13]. Digester volume that used was 6 liter with working volume 3.7 liter [7].[3]The response variables are COD value, VFAs production, yield of CH_4, composition, and heating value of biogas

GC Analysis

Methane and VFAs (Volatile Fatty Acid) were analyzed directly by Hewlett Packard gas chromatography provided with flame ionization detector (FID).[3]The chromatograph that used was Agilent 19095P-Q04 HP Plot Q column which allow to determine methane (CH_4) in the mixture as a function of digestion time.[3]The temperature of FID, oven and injector port was 280 oC, 150 oC and 275 oC respectively.[3]Helium was used as carrier gas at flow rate of 30 mLmin-1.[3]Biogas samples were analyzed by collecting the gas in venojeck, and injecting to column by syringe.[3]The VFAs concentrations were analyzed by using gas chromatography (Hewlett Packard) provided with flame ionization detector (FID), equipped with poraplot-Q04 1 µl direct, working at 275oC and flow rate 45 mL min-1. H_2 and CO_2 gas were analyzed by gas chromatography (Shimadzu GC-2010 plus) provided with a thermo-conductivity detector (TCD).

Cellulose, Hemicellulose and Lignocellulose Analysis

After conducted pretreatment of rice straw, cellulose, hemicellulose and lignocellulose were analyzed by gravimetric methods [14].

COD Analysis

COD was analyzed by APHA methods [15].

RESULT AND DISCUSSIONS

In this study, lignocellulosic biomass that used was rice straw. Rice straw consists of 37.71% cellulose; 21.99% hemicellulose; and 16.62% lignin [16]. In 2010, Agriculture Ministry of Republic Indonesia estimated that rice straw amount reached 84 million tons across Indonesia. The microorganism used was rumen microorganism. In former study, this microorganism produced the highest methane conversion as high as 73.4% without mechanical, thermal and chemical pretreatment The amount of rumen fluid waste was 1,560,000 liters/month at RPH (slaughter house) Pegirian, Surabaya, Indonesia. In Japan rumen fluid waste that must be processed was 116,000 tons each year [17]. Rumen fluid waste is a source of methane. Methane comeas from enteric fermentation process / livestock and it is one of the sources of greenhouse gas (GHG) [17]. The greenhouse effect from methane is 23 times stronger than carbon dioxide. Therefore, rumen fluid is very potential to produce biogas from rice straw because it is low cost production and it is very abundant in Indonesia.

Effective microorganism was rarely used in former study, especially in methane production from lignocellulosic biomass. [3] Effective microorganism can reduce the growth of pathogen bacteria which produce H_2S in anaerobic process [18, 19].

Combination between rumen and effective microorganism was used as a hypothesis in this study. Cow dung microorganism was used as control microorganism. Based on the previous study, cow dung microorganism was used to convert lignocellulosic biomass which is coffee pulp waste and gave very low conversion rate and in the first 1.5 months, the yield still less than 10% [2].

From the present research, there were many experimental data. The data and discussion were shown below in details.

[3] Chemical Composition of Rice Straw

Chemical composition of rice straw from Madura Island that used in this research has special composition as listed in Table-1

VFAs Production During Anaerobic Process

At acidogenic phase, acetate, propionate, butyrate, isovalerate and valerate were formed in methane production [7]. But in this research,only acetate, propionate and butyrate were analyzed as representative of volatile fatty acid during anaerobic process in 21 days.

Methane formation (4th reaction) was covered by several (5th reaction) path ways of reaction. There were hydrogenotropic and acetropic methanogens [23]. VFAs involves at acetropic methanogens. Acetropic methanogens is reaction of acetate to form methane. Propionate and butyrate also can be degraded to acetate to form methane, beside direct reaction to form methane as shown at 2nd and 3rd reaction [23], as shown in Figure-2 below

The effect of digestion time on VFAs concentration as product of anaerobic digestion was shown in Figure-3. Generally the acetate, propionate and butyrate level increased faster in 5-10 days. Then it decreased after 15-21 days,actually in acidogenic phase. It showed that rumen digestions was an effective catalyst to convert lignoselulosic biomass to VFAs [7], as shown by 15 % rumen, 10 % mixture, 5 % mixture, 5 % rumen and 10 % rumen volume. The trends showed acetate, propionate and butyrate effectively were converted to methane as acetropic methanogens. The phenomena affected methane yield as shown in figure.5. The different phenomena was occured at 15 % mixture, and cow dung, which the trends were very low in 5-10 days and increased after 15-21 days. It showed that VFAs were not converted or less converted to methane. Therefore methane yield were very low. Rumen microorganisms and mixture microrganisms had higher acetate, propionate and butyrate value than cow dung microorganisms. Cow dung has not or less microorganism that can be contributed to lignocellulose degradation. So that the VFAs were converted slowly to methane [2, 24, 25]. These also caused by toxicity of lignocellulose material to cow dung microorganisms [23].

High yield of VFAs production was affected by the substrate as reported in research that aquatic plants and agricultural residues like corn stover, cabbage, soya and wheat straw when used as substrate of rumen digestions resulted high yield [13, 26]. VFAs represent the ability of biomass to convert economically to more desirable compounds [27]. VFAs was converted to CH_4 and CO_2 and other products [28

COD (Chemical Oxygen Demand)

Chemical oxygen demand can be explained as the demand of oxygen chemically that affected by increasing organics materials in solutions. Oxygen was needed by microorganisms in the reactor to digest organics complex materials to other simpler compounds like VFAs.

curve at all variables of microorganisms showed the digestive ability of rumen microorganisms to lignoselulosic biomass was very effective than cow dung microorganisms. VFAs and COD degradation affected volume and methane yield resulted by every microorganisms in this research. The highest methane

Degradation of organics material formed CH4,

volume was 15 % rumen vol. 0.52611 Nm

, after that 10
3

CO2 and other gas and water. The biogas formation was indicated by decreasing COD level. Higher COD removal would impact to amount of biogas. Digestion time affected to COD removal, because microorganisms need time to degrade organic compounds to be biogas. Higher COD removal gives higher biogas volume [29].
The change of COD level indicated methane formation; the decreasing of COD level showed concentration decreasing of organic material that converted to methane digestion. While for cow dung's digestion COD level was very high at the beginning until acidogenic phase, but at methanogenic phase the COD level was smooth or less decreasing. The trend of COD

% and 5 % mixture vol. were 0.32153 Nm , 0.28322
Nm3,then 5 % rumen, 10 % rumen vol. were 0.27212 Nm3, 0.05611 Nm3, and 15 % mixture volume was 0.02191 Nm3 and the lowest was cow dung with 0.01245
Nm3 in 21 days digestion. This result showed that rumen microorganisms, both as itself or in mixing with effective microorganisms were very dominant in cellulose, hemicellulose and lignocellulose digestion than cow dung.
Rumen microorganisms were powerful microorganisms in naerobic digestion of rice straw. Figure-4 shows that the highest COD removal was obtained by using rumen and mixture microorganisms
Methane Yield
Calculation of methane yield was based on methane volume in Nm3 per kgCOD removal. Actually 350 ml of methane was produced from 1 g of COD [11].
Figure-5 shows that the highest methane yield in 21 days were 0.611
Nm3/kgCODremoval for 15 % rumen

vol., after that 10 % mixture vol., 5 % mixture vol. were
0.365 Nm3/kgCODremoval and 0.296,5 Nm3/kgCODremoval respectively, then 5 % rumen vol. and 10 % rumen vol. were 0.274 and 0.199 Nm3/kgCODremoval respectively, and the lowest yield is cow dung with 0,033
Nm3/kgCODremoval.
This result indicated that lignocelluloses biomass was very difficult to digest by cow dung microorganisms. The methane yield of anaerobic digestion by cow dung microorganisms was very low before 60 days [2]. The microorganism composition of cow dung also affected the result of methane yield. Microorganisms population of cow dung were dominated by Bacteroides SP, Colistridium SP, dan Bifidobacterium, then anaerob facultative and patogen like Enterobacteriaceae; e.g. E. Coli, Salmonella Spp, Shigella Spp, etc. [24]. There were no microorganisms for lignocelulose digestion. The highest production rate of methane also showed by 15 %

rumen vol. with 0.02505 Nm3/day. And the lowest was cow dung with 0.00059 Nm3/day.
The highest result of this research was 0.6111 Nm3/kgCODremoval. This result is equal to 61.11 % (in percentage). It was slightly lower than the research of Baba et al. [7]. He examines the process of anaerobic waste paper using rumen microorganisms with the yield of 73.4% for 21 days. However, this result was higher and faster than the research of Corro et al. [2], which it used cow dung microorganisms and coffee pulp and the yield was 60% after the anaerobic process for 8 months. More comparative data with the results of other studies are presented in the Table-2 below.
Lower result from this study was caused by different substrates that used in previous studies. Baba et al. [7] used waste paper, while the paper has less lignin through the process of delignification in paper production.[3]

Meanwhile the lignin content of rice straw used in this research was still quite high. When we compared with the research of Corro et al. [2], the process of methane formation in this study was faster because the rumen microorganisms have the better ability than cow dung microorganisms in producing methane.

Biogas Composition

Biogas composition (CH_4, CO_2, H_2) were analyzed after 21 days and 30 days digestion. The digestion were continued until 30 - 50 days to get stationary phase. The longer digestion time, the methane concentration will increase until stationer phase [30]. The lower biogas volume from 10 % rumen, 15 % mixture and cow dung were not continued because the rate production of biogas was slow and time in stationer phase was long. Biogas composition impacted the heating value of biogas. Table-3 showed the comparation of composition from every microorganisms variables

Qualitative Test (Combustion Test)

The one of the combustion test was shown by Figure-7 below. Generaly the combustion test showed blue fire as in Figure-7 from 15 % rumen vol. with heating value 670.37BTU/SCF. The other biogas from 5 % rumen vol, 5 % mixture vol. and 10 % mixture vol. also showed the blue fire at qualitatife test.

Figure-7. One of combustion test of biogas from 15 % rumen vol. showed bluefire with heating value 670.37 BTU/SCF, combustion after 30 days digestion time of rice straw.

CONCLUSIONS

The highest yield and production rate of methane in 21 days was 0.611 Nm3/kgCODremoval and 0.02505 Nm3/day in 15% (v/v) of rumen. In 10% (v/v) mixture microorganisms, the yield of methane was 0.365 Nm3/kgCODremoval and the yield of methane from cow dung microorganism was 0.033 Nm3/kgCODremoval with production rate of methane 0.01530 Nm3/day and 0.00059 Nm3/day for 10% mixture microorganism and cow dung respectively. Rumen microorganism was very dominant in rice straw digestion, by rumen only or mixture with effective microorganisms. After stationary phase, digestion of rice straw/rumen fluid microorganisms and rice straw/mixture microorganisms with rumen 5% vol., 15 % rumen vol., 5 % mix vol., and 10 % mixture vol. generates final biogas composition and the highest heating value was 744.72 Btu/Scf in 5 % rumen vol. At combustion test the fire colors of biogas from digestion of rice straw/rumen fluid microorganisms and rice straw/mixture microorganisms were blue. These results indicate that rumen microorganisms and mixture microorganisms were effective in digestion with rice straw, the biogas quality was good and production rate was high than using cow dung microorganisms.

ACKNOWLEDGEMENT

This research was funded by a research grant, "Penelitian Unggulan Perguruan Tinggi-Batch I", in 2015 no. 003246.146/IT2.11/PN.08/2015, through the Ministry of Research, Technology and Higher Education of Republic Indonesia (Kementrian RISTEK-DIKTI RI) and all of members of biochemistry laboratory, Hasrul Anwar, Farah Amirotus Salma, Veny Ulil Amrinah, Violita Anggraeni, Yumarta Tansil, for the support to this research.

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