

Anaerobic Co-Digestion of Human Excreta and Corn Stalk for Biogas Production

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Abstract

The anaerobic decomposition process of human feces substrate with a C/N ratio of 14.6 has failed to produce biogas optimally. In order to produce biogas maximally, the C/N ratio in the substrate should be in the range of 20-30. In this study, a combination of human feces substrate (C / N = 14.6) with corn stalk waste (C / N = 66.5) was carried out. Corn stalks were soaked first in a NaOH solution to separate lignin before being mixed with human feces. In this study the effect of the C/N ratio from the combination of feces and corn stalks as well as the effect of the type of activated sludge on the rate of biogas production were evaluated. The C/N ratios were varied at 20, 25, and 30 with F/M of 0.5. As the source of microbes is the activated sludge of human feces. A further experiment was carried out by varying the types of microbes where sludge from cow's rumen, activated sludge from feces, and activated sludge from rotten corn stalk was employed at a C/N ratio of 30 and F/M of 0.5. The results of this study showed that the optimal biogas production was obtained at a C/N ratio of 30 with a cumulative gas volume of 13.185 ml for 60 days. The type of microbes that produce maximum biogas production was the activated sludge from the rumen. The optimum biogas yield was obtained at 4.184 liters/kg COD, which was achieved in the stationary phase with a C/N ratio of 30.

Keywords: anaerobic co-digestion; biogas; corn stalk; human feces

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INTRODUCTION

Biogas is a gas mixture of CH₄ and CO₂ as well as other constituents (H₂, NH₃, and H₂S) in low concentration. The gas is produced from the anaerobic decomposition of organic substrates (Jogersen, 2009). To produce maximum biogas yield, there are several factors that necessary to be considered, such as substrate composition (C/N ratio), temperature, pH, retention time, and alkalinity (Noraini, 2017).

Human feces have a C/N ratio of 14.6 (Sun et al., 2017). Biogas can be produced in large quantities

at a substrate ratio (C/N) of around 25-30 (Maishanu and Hussani, 1991). Therefore, feces require to be combined with other substrates in order to achieve the proper C/N ratio. One of the substrates that can be used as an additional substrate is corn stalk, which has a C/N ratio of 66.5 (Tompkins, 2005).

Sambang (2015) has reported the potential of human feces sludge as a biogas substrate and found the low biogas yield was obtained at the level of 25.13-45.72 L/kg.

Corn stalks still contain lignin, which is very difficult to be decomposed; therefore, delignification is required by soaking the corn stalk into a NaOH solution before being mixed with human feces. The combination of human feces and corn stalk waste is conducted to obtain the optimal C/N ratio. It is expected to be able to produce biogas maximally.

A series of processes that occur during biogas generation are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Al Saedi *et al.*, 2008). To produce maximum biogas, there are several parameters that require an optimization such as anaerobic environment, temperature, pH, type of substrate, substrate size, C/N ratio, ratio (F/M), and a substrate with microbes (Jogersen, 2009). Microbes are microscopic organisms with 4 growth phases consists of adaptation phase, growth phase, stationary phase, and death phase.

Feces has the same potential with livestock manure as a substrate for biogas production since both of them are from anaerobic degradation in the digestive tract; thus, it already contains high content anaerobic bacteria. Feces can be converted into biogas with the produced volume equivalent or higher than livestock manure as substrate. Moreover, the methane content in biogas can reach up to 70% (Febrianto and Priyono, 2012). The utilization of feces as a substrate is advantageous since it has a pH of 7.3 (Andriani *et al.*, 2015), which is the optimum pH in biogas production. However, it is limited by the low C/N ratio that is only 14.6 (Sun *et al.*, 2017).

Corn stalks have a high C/N ratio between 63.5 - 66.5 (Tompkins, 2005; Zhou, 2014). Lignin contained in corn stalks causes it difficult to be decomposed; thus, it inhibits the hydrolysis process. Pre-treatment of the corn stalk to remove lignin is required prior to the hydrolysis process. The pre-treatment process decomposes the complexes substance into simpler substances that are easier to be hydrolyzed (Dinuccio *et al.*, 2010; Zheng *et al.*, 2014). There are several methods to pre-treat corn stalk, such as physical, chemical, biological, and combination treatments (Yao *et al.*, 2018).

Anaerobic decomposition process requires anaerobic microbes. In this study, the anaerobic microbes were supplied from activated sludge of human feces, activated sludge of rotten corn stalks, and activated rumen cattle. Rumen is a mixture of microbes and grass that has not been fermented and fully digested (Ramli and Hartanto, 2015). The rumen is an inoculum that contains bacteria, fungi, archaea, and protozoa (Yue *et al.*, 2013). One of the bacteria contained in the rumen is *Methanosarcina sp.*, where these bacteria play an important role in the process of biogas production (Fithry, 2010). Rumen microorganisms consume $N-NH_3$ to synthesize proteins. The high concentration of nitrogen ammonia is useful for bacterial growth. However, it inhibits the growth of methanogenesis bacteria (Dai *et al.*, 2017; Tian *et al.*, 2018). Microorganisms in the rumen can

increase methane production (Ozybram *et al.*, 2017) using hydrogen and carbon as precursors for the process of methanogenesis (Elghandour *et al.*, 2017).

MATERIALS AND METHOD

Human feces and chopped corn stalk waste, microbes (cow's rumen active sludge, fecal anaerobic sludge, and decomposed anaerobic sludge) were used as substrates in this study. NaOH and p.a chemicals for C, N, COD analysis were obtained from the Undip Environmental Engineering Laboratory. The analysis technique of each parameter refers to the SNI (Indonesian National Standard).

The equipment sets used in this study are biodigester, universal pH indicator, and water displacement apparatus.

The dependent variables are the cumulative volume of biogas produced, the daily production rate, and the biogas yield. The independent variable in this study is the decomposition time, and the controlling variables are the C/N ratio (20, 25, 30) and the type of microbes (rumen activated sludge, feces anaerobic sludge active, and anaerobic sludge activated sludge).

The experiment was performed at a laboratory scale consisting of the preparation of the substrate, production of biogas with variations in the C/N ratio and variations in the type of activated sludge. The flame test was carried out to evaluate biogas quality instead of chemical composition analysis of biogas. The schematic of process equipment based on this study is shown in Figure 1.

The substrate preparation stage is carried out by chopping the corn stalks into small pieces and then grinding until smooth size. The prepared corn stalk was pre-treated using the alkaline method.

The corn stalk was mixed with 0.1 M NaOH solution (the ratio of corn stems to the solution is 1:4), the slurry was soaked for 2 h. the solid was then separated from the solution, subsequently washed the remaining solids using deionized water. The solid size

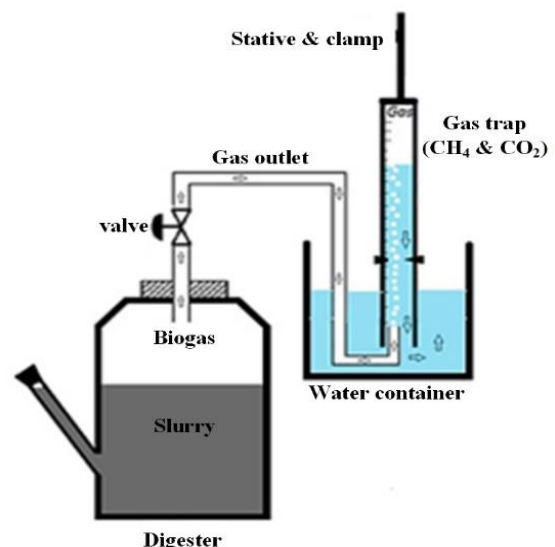


Figure 1. Equipment setup for biogas production

was reduced using the size reduction device and sieved using 200 mesh sifters. Human feces, anaerobic activated sludge from feces, rumen and rotten corn stalks are filtered. Both the substrate and the activated sludge were filtered using 200 mesh filter to obtain uniform size for further process.

The anaerobic digestion process for the first objective was conducted by mixing the human feces substrate with corn stalks at a C/N ratio of 20, 25, and 30. The slurry was then mixed with the active rumen in an F/M ratio of 0.5, and pH was maintained at 7. The total volume of the substrate mixture was 2 l. The mixture of substrate and microbes was filled into 5 l biodigester.

The anaerobic digestion process for the second objective was performed by mixing human feces with corn stalks at the C/N ratio of 30, F/M ratio of 0.5 and pH at 7. The microbe was varied using 3 types of microbes from different sources. This experiment was carried out to evaluate the effect of microbe type on biogas production.

Initial COD analysis was carried out when the substrate was mixed at a certain C/N and periodically analyzed every day. COD analysis procedure of the substrate followed SNI 6989.73: 2009 method. The biogas flame test was performed to examine the presence of methane gas in produced biogas. The decomposition process was carried out for 60 d.

The collected research data, such as biogas volume and biogas production rate with various C/N ratio and the type of microbes, were displayed in graphical form. The COD measurement data was displayed in the form of a CODC graph as the function of time for each variable.

RESULTS AND DISCUSSIONS

The effect of biogas production rate as a function of time at various C/N ratios

In this study, the C/N ratio was varied at 20, 25 and 30. F/M ratio was 0.5 and pH was maintained at 7. The decomposition temperature was carried out at ambient temperature. The time function biogas production rate at various C/N ratio shows different results. An overview of trends in biogas production rates is shown in Figure 2 and Table 1.

From Figure 2, the rate of biogas production profile is similar to the profile of the microbial growth rate that consists of the adaptation phase, the increase/growth phase, the stationary phase, and the decrease/death phase. Rumen microbes require up to 6-13 days to adapt to this substrate combination.

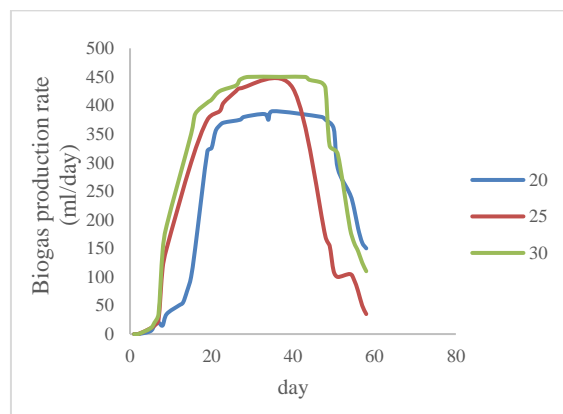


Figure 2. The correlation of biogas production rate with digestion time at various C/N ratio, F/M ratio of 0.5, pH at 7, and rumen as microbe source

The production rate increased sharply on day 7-19th and started stationary on the 19th day until the 54th day. After that, the rate declined, starting on the 40th day. The largest biogas production rate (465 ml / day) was obtained when the C/N was 20.

Rolfe et al. (2012) revealed that the duration of the adaptation phase was influenced by several factors such as inoculum size, microbial age, and environmental compatibility for the microbial growth.

After the adaptation phase, the rate of biogas production sharply increases until it reached the peak of production. At this phase, microbes were in a growth phase. The growth phase occurs when bacterial cells continue to divide over time and depend on the type of bacteria and the available nutrients. The bacterial growth continues until it reaches the condition of limited nutrients availability. In this study, there was no addition of micronutrients such as Co, Ni, Mg, etc. to enhance biogas production (Mancinni et al., 2016). Therefore, the process of bacterial growth becomes slower and reaches a state where the number of bacteria does not increase and tends to stagnate, or it can be said there is no increase in the rate of biogas production. This phase is called the stationary phase (stationary phase) which causes biogas production does not show a significant increase as in the growth phase; the biogas production tends to be stagnant at this phase (Todar, 2004). The end of the stationary phase is indicated by a sharp decline in the biogas production rate. This phase is called the death phase where in this phase microbes tend to die due to the limited substrate availability.

Table 1. Biogas production rate with rumen microbe at various C/N ratio (20, 25, and 30)

Phase	dV biogas / dt, (ml/day)					
	day	C/N 20	day	C/N 25	day	C/N 30
Adaptation	0-13	48	0-7	8	0-6	6
Increase/growth	14-18	270	8-18	137	7-19	257
Stationer	19-54	465	19-40	275	20-47	435
Decrease/death	55-58	102	41-58	68	48-58	239

Table 2. Cumulative volume of biogas at various C/N ratio (20, 25, and 30) with rumen as microbes' source

The cumulative volume of biogas (ml)					
day	C/N 20	day	C/N 25	day	C/N 30
0	0	0	0	0	0
13	195	7	50	6	30
16	520	16	1.415	19	2.340
54	9.810	40	8.020	47	11.035
58	10.525	58	10.065	58	13.185

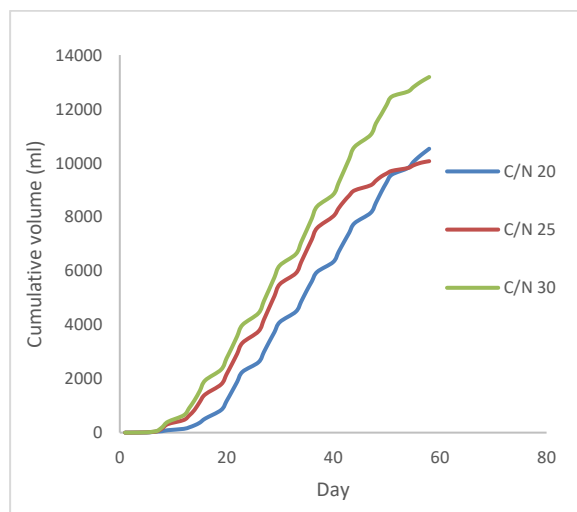


Figure 3. Cumulative volume of biogas as a function of time at various C/N ratio, F/M of 0.5, and rumen as microbe source

From the results of the experiment that has been conducted, the obtained cumulative biogas volume as a function of time is shown in Table 2. For a more detailed description of the C/N ratio effects on the cumulative biogas volume, it can be observed in Figure 3.

In Table 2 and Figure 3 shows the cumulative volume of biogas produced from a combination of human feces substrate and corn stalks with C/N ratios of 20, 25, and 30. At a C/N ratio of 20, 25, and 30, the total volumes of biogas produced during 60 days are 10.525 ml, 10.065 ml, and 13.185 ml, respectively. The C/N ratio of 30 was considered as the best C/N ratio due it stabilize the pH of the substrate, and the activity of methanogenic bacteria increased (Tanimus, 2014).

The effect of biogas production rate as a function of time at various type of microbes in activated sludge

The objective of this study is to investigate the correlation between the rate of biogas production as a function of time in various types of activated sludge as a microbial source for anaerobic decomposition. The microbial activated sludge is derived from cow rumen activated sludge, decomposed corn stalk activated sludge and human feces anaerobic activated sludge. The combination of the substrate was conditioned at a C/N ratio of 30, an F/M ratio of 0.5 with an incubation time of 60 days. The effect of activated sludge types

on the volume of generated biogas is displayed in Figure 4.

From Figure 4, it can be seen that there are four phases of microbial growth: the lag phase, exponential growth phase, stationary phase, and death phase. Biogas productions on days 0 to 6th are not significant because it is still in the lag phase. Biogas production has increased significantly on days 7-19th for the rumen as the microbial source, days 5-19th for corn stalks as the microbial source, and days 7-21th for human feces as the microbial source. This is caused by the exponential growth phase. While biogas production tends to be constant on days 20-47th for the rumen, days 20-46th for corn stalks, and days 22-53th for human feces. This is called the stationary phase. Then biogas production tends to decrease starting from day 48th for the rumen, day 47th for microbial from corn stalks, and day 54th for human fecal microbes. From Table 3, it can be seen that the most optimum activated sludge in producing biogas at the stationary phase is rumen activated sludge, compared to the other two activated sludges. This is because the rumen at least has nine bacterial species: six isolates are hydrolytic bacteria (*Bacteroides nordii*, *Clostridium perfringens*, *Prevotella bivia*, *Porphyromonas asaccharolytica*, *Ruminococcus gnavus*, *Lactobacillus acidophilus*) that grow in an obligate anaerobic habitat; two isolates are methanogenic archaea (*Methanobacterium formicicum* and *Methanosarcina siciliae*) which grow in anaerobic, facultative habitat and another isolate is acetogenic bacteria (*Acetobacter syzygii*) (Christy et al., 2014).

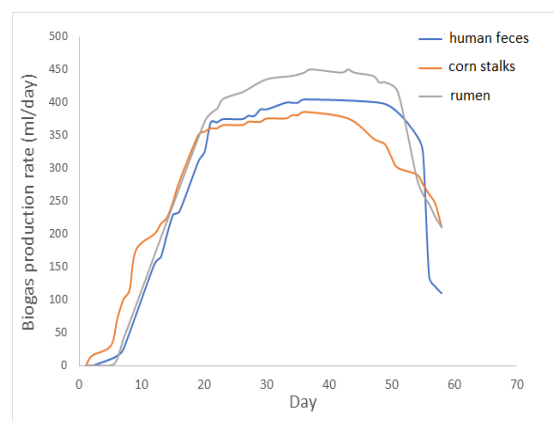


Figure 4. The correlation of biogas production rate as a function of time with various activated sludge as a microbial source

Table 3. Biogas production rate as function of digestion time at C/N ratio of 30 with various activated sludge types

dV Biogas /dT (ml/ day)					
day	Rumen	day	Corn stalks	Hari	Human feces
0-6	6	0-4	5	0 - 6	5
7-19	257	5-19	183	7-21	152
20-47	435	20-46	375	22-53	406
48-58	239	47-58	202	54-58	208

Table 4. The effect of Activated Sludge Variations on the Cumulative Volume of Biogas

The cumulative volume of Biogas (ml)			
Day	Rumen	Corn stalks	Human feces
0	0	0	0
10	400	505	195
20	2.750	2.380	1.690
30	6.200	5.315	4.720
40	8.825	7.615	7.190
50	12.145	10.180	10.610
58	13.185	11.160	12.035

The increase of produced biogas volume is related to the availability of easily digested organic matter and the conditions of bacteria that have adapted to their environment. Hydrolytic bacteria can hydrolyze carbohydrate and protein compounds into organic compounds with a lower molecular weight in order to be easily consumed by bacteria. Methane gas in biogas is produced from organic acids (acetogenesis). The addition of rumen can accelerate the process of acetogenesis. This was also stated by Susilowati (2009) that the addition of the rumen might shorten the decomposition time, i.e., hydrolysis and acetogenesis as initial processes in methane generation or methanogenesis.

From the results of this experiment, it was also obtained the cumulative volume of biogas as a function of time that is shown in Table 4. The produced biogas volume within 60 days by utilizing microbes from cattle rumen activated sludge was up to 13.185 mL, while using activated sludge from corn stalk waste was obtained as much as 11.160 mL, and human feces as activated sludge was 12.035 mL.

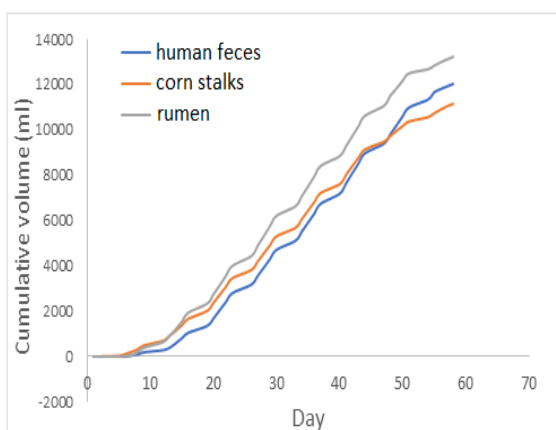


Figure 5. Relationship of biogas cumulative volume to time in a variety of active sludge

The experimental results show that the best microbe in producing biogas is from rumen activated sludge.

The collected biogas volume from the digestion process by using microbes from corn stalks activated sludge, and human feces produced lower volumes of biogas when compared to the digestion with microbes from rumen activated sludge. This is due to differences in microbes present in each activated sludge. Corn stalks contain lignin, hemicellulose, and cellulose, which can be converted into other compounds biologically. *Trichoderma harzianum* can accelerate the decomposition of organic material since it contains the enzyme cellobiohydrolase (CBH), which is active in hydrolyzing cellulose, the active endoglucanase enzyme for hydrolyzing dissolved cellulose, and the glucosidase enzyme that actively hydrolyzes the cellobiose unit; however, it contains fewer methanogenic bacteria.

Biogas Yield

In this study, COD levels from the substrate were analyzed as a function of time. The results obtained from the COD measurements periodically during the digestion process are shown in Figure 6.

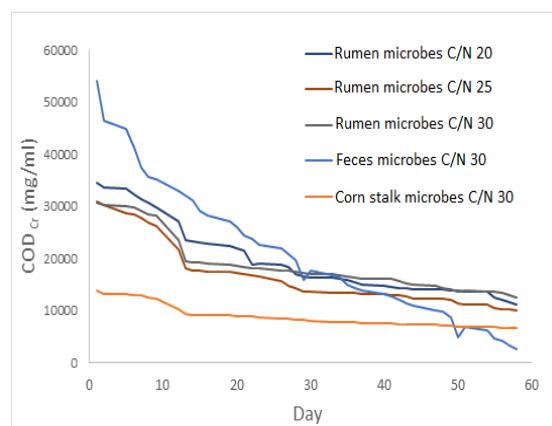


Figure 6. The correlation of COD declines on the biogas production time

Table 5. Rate of Biogas production on COD levels declines in each phase

Phase	Biogas yield ($\Sigma v/dCOD$) (liter/kg)				
	Rumen C/N 20	Rumen C/N 25	Rumen C/N 30	Corn stalks C/N 30	Human feces C/N 30
Adaptation	18	16	30	31	2
Growth	901	144	226	501	130
Stationer	1.073	1.540	2.234	4.184	533
Death	493	676	1.316	3.457	290

In Figure 6, it can be seen that the COD level decreases with increasing decomposition time. This is because COD is a measure of the amount of organic matter in the substrate which is food from microbes (P.J. Jorgensen, 2008). As decomposition time increases, more and more organic matter is consumed by microbes, which results in decreasing organic compounds in the substrate represented as COD. This is consistent with research conducted by Nining *et al.* (2015), which discusses the degradation of COD using palm oil liquid waste in the biogas formation. The decrease in COD is due to the high organic matter in the substrate as a nutrient for microorganisms in producing biogas.

In Figure 6 it can be seen that at a C / N ratio = 20, for 60 days the COD value decreased from 34.424 mg / l to 11.035 mg / l (68%). C / N ratio = 25 decreased the value of COD from 30.852 mg / l to 9.998 mg / l (68%). For C / N ratio = 30, the COD value decreased from 30,779 mg / l to 12.571 mg / l (59%).

In the variation of types of activated sludge with a C / N ratio = 30, the type of activated sludge from rotten corn stems decreased COD value from 13.724 mg / L to 6,657 mg / L (51%), while the activated sludge of human feces decreased the COD value from 53.929 mg / L to 2.688 mg / L (95%)

Anaerobic digestion process can reduce the COD substrate; in other words, the anaerobic digestion process can reduce the burden of contamination from a combination of human feces and corn stalk with the rumen. A decrease in COD levels in anaerobic digestion indicates that material other than acids can be degraded. It can be said that the process of degradation of complex organic matter into methane gas and biogas is effective. Trisno Saputra *et al.* (2010) stated that the more biogas volume is formed, the greater the decrease in COD content. The increase in the volume of biogas to reduce the level of COD decomposes for more details can be observed in Table 5.

From Table 5, it can be seen that the highest yield of biogas products in this study was 4184 liters of biogas/kg COD achieved on day 20th to 44th in the stationary phase. The highest yield of biogas products was achieved at the C/N ratio of 30 with corn stalks as microbial source. This result shows that the combination of human feces and corn stalks produces higher yield of biogas products; however, the flame test of produced biogas from it shows shorter flame

duration than biogas obtained using rumen as the microbial source. Wu (2007) stated that co-digestion at a C/N ratio of around 30 had better nutrient balance as well as the digester performance; thus, the production of biogas was higher.

CONCLUSIONS

Based on the research that has been conducted, the biogas production from a combination of human feces substrate and corn stalk waste exhibited an optimal biogas production at a C/N ratio of 30 with a cumulative gas volume up to 13.185 ml. The optimal biogas production by varying the activated sludge types was obtained when using activated sludge from rumen with a collected cumulative gas volume up to 13.185 ml. The fermentation process is carried out within 60 days. The optimum biogas yield was obtained at 4.184 liters/kg COD, which was achieved on day 20th to 44th in the stationary phase with the C/N ratio of 30 and rotten corn stalks as the microbial source.

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