

Potential of Methane (CH₄), Nitrogen (N₂), and Carbon Dioxide (CO₂) from Eco-Enzyme with the Addition of Cow Feces Starter

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Abstract

Eco-enzyme solutions are generally used in the manufacture of disinfectants, floor cleaners, liquid fertilizers, preservatives and others. In this study, eco-enzyme was used as the main ingredient in the manufacture of biogas fermentation with the addition of cow feces as an additional starter in the fermentation. The aim of this study was to determine the content of CH₄, CO₂, N₂, pH, temperature, and pressure in eco-enzyme fermentation with the addition of cow feces starter. This research is a quantitative research with the type of experimental research as well as direct observation data collection techniques and data analysis techniques using descriptive statistical analysis. The results showed that the treatment that produced the highest methane (CH₄) gas was the P5 treatment with a concentration of 2.889%. 6,1. The highest volume pressure value was produced in the P5 treatment which was 70 ml. Nitrogen gas (N₂) and carbon dioxide gas (CO₂), the concentration of nitrogen gas (N₂) from reactor P0 - P4 is in the range of 95.875-99.669 % and the concentration of carbon dioxide (CO₂) from reactor P0 - P4 is in the range of 0.237 - 4.125 %.

Keywords: CH₄; CO₂; Eco-Enzyme; N₂; pH; Pressure.

INTRODUCTION

During the pandemic, the consumption of fruit was in great demand by the community, especially on the household scale. This is done by the community as an effort to increase body immunity. Based on an analysis of the projections for 2018-2022 consumption of fruits and vegetables will increase with an average growth rate of 2.50% and 1.50% (Alfaena, et al, 2018). The increase in fruit consumption can cause a new problem, namely household organic waste in the form of fruit residue. The waste of this fruit, which can be in the form of fruit peels, seeds or other parts, is generally not reused and is just thrown away. This will have a serious impact on the environment if not handled properly. This waste problem will be very important to find a solution because it will have an impact on the balance of the environmental ecosystem (Prabekti and Ahmadun, 2011). Therefore, a solution is needed to process the waste (fruit residue) into useful by-products.

Environmentally friendly waste treatment can be a solution to overcome this problem. The principle of zero waste waste treatment can be used to solve household waste problems. The principle of zero waste is management by sorting, composting and collecting salable goods (Widiarti, 2012). Utilization of household organic waste in the form of other fruit residues can be

processed by fermentation to produce a multi-purpose liquid or known as eco-enzyme (Najaga Bumi Learning Class Module Compilation Team, 2020). There are many by-products that can be produced from fruit residues which are processed into eco-enzymes, including clearing clogged drains, used to water plants (as liquid fertilizer), repel nuisance insects, as a home cleaning agent, and others (Megah, et al. al 2018).

The type of bacteria used to produce optimum biogas is methanogenic bacteria (Mia et al., 2016). These methanogen bacteria will stimulate the fermentation substrate to produce methane gas (CH₄). This methane gas makes the biogas produced have an ignition power so that the biogas can be used as fuel. Meanwhile, the basic material used to make biogas in this study is eco enzyme derived from fruit waste.

Cow dung is considered capable of being a starter in eco enzyme fermentation to produce biogas. The optimum production of biogas and methane from a mixture of cow dung and elephant grass was 72.42 l/kgTS and 102.86 l/kgVS respectively (Afrian, C. et al 2017). Thus, based on the background of this problem, an analysis of the quality of biogas from eco-enzymes with the addition of cow dung starter and based on fruit composition and fermentation time needs to be carried out.

METHOD

The type of the research is quantitative with experimental methods. The research method was designed using 5 treatments with 1 repetition. The eco-enzyme used is made from fruit-based ingredients that have been fermented for 3 months which is the optimal time for eco-enzyme fermentation. After the eco-enzyme is produced, a fermentation process is carried out by adding cow dung starter. The addition of cow dung starter was carried out to maximize this fermentation process to produce methane gas (CH₄). The fermentation process was carried out for 37 days. 7 days the resulting gas is released into a bucket of water. Gas storage for analysis was carried out on day seven and so on until day 37. The following is the treatment plan in this study:

- P1 : 3000 ml eco enzyme + 600 g cow dung starter
- P2 : 3000 ml eco enzyme + 800 g cow dung starter
- P3 : 3000 ml eco enzyme + 1000 g cow dung starter
- P4 : 1000 ml eco enzyme + 1000 g cow dung starter
- P5 : 1500 ml of water + 1500 g of cow dung starter

Making Eco-enzyme Solutions

The fermentation solution used for biogas was an eco enzyme solution with the addition of cow dung starter. Eco enzyme was made with a ratio of 3: 1: 10. 3 for the waste used; 1 for molasses and 10 for the ratio of water used. In this study, the manufacture of eco-enzymes was carried out at the Al Fatah Natar Islamic Boarding School, South Lampung. Eco enzyme was made in large quantities in drums and fermented for 3 months. The waste material used for the manufacture of eco enzyme is fruit and vegetable waste. A total of 10,000 mL of eco enzyme solution was used in this study.

Making Storage Tanks

The fermentation tank was made of a 5 L gallon. The lid on the top of the gallon was perforated for a gas outlet pipe with a diameter of 1/2 inch. Two faucets are then placed above the fermentation tank before the gas collection point and one more valve is used for gas discharge. The collection point for the fermented gas (biogas) was made of plastic.



Figure 1. Digester tube circuit. Image description: 1. digester tube; 2. tube cover; 3. main faucet; 4. gas flow hose; 5. compressor faucet; 6. gas flow hose; 7. plastic gas holder.

Production of Biogas from Eco-Enzyme

The eco enzyme solution that has been fermented for 3 months was then ready to use. This solution was then mixed with cow dung to be fermented again for 37 days to produce biogas. During the fermentation process, specially designed vats are connected by a hose to the biogas storage vat (Chandra et al, 2020). Cow dung is added to the digester tube with varying weight according to the dosage. Cow dung is used as a starter in fermentation to produce methane gas. Then the molasses was also put into the digester tube according to the dosage determined in this study and labeled for each treatment. Biogas samples were taken on the 37th day of fermentation.

Biogas Sampling

Biogas samples were taken on the 37th day of fermentation using a previously prepared breathing bag. First sampling close the main faucet and compressor faucet, after the faucet is closed all the hoses of the digester tube and compressor faucet are separated. The end of the compressor faucet is directly connected to the breathing bag hose, after connecting the valve on the breathing bag is opened and the plastic container is pressed until the contained gas moves into the breathing bag. Next, close the breathing bag valve immediately after gas the one transferred to the breathing bag is sufficient and close the compressor faucet again and remove the breathing bag.

RESULTS AND DISCUSSION

Results

Biogas volume pressure

Measurements made to measure volume pressure using technical Archimedes' law with the following results:

Table 1. Results Biogas volume pressure.

Reactor	Increase (mL)
P1	26
P2	60
P3	56
P4	50
P5	70

The results of the pressure measurement of biogas volume in table 1 show that the highest value was found in reactor P5, which was 70 mL, and the lowest value was in reactor P1, which was 26 mL.

pH and Temperature

Data on the results of measuring the pH acidity level on the initial day of manufacture and the last day of sampling are as follows:

Table 2. Result of pH value.

Reactor	pH	
	Before	After
P1	3,4	3
P2	3,5	2,9
P3	3,5	3
P4	4	3
P5	6,3	6,1

Table 3. Results of temperature measurements at the start and end of the eco-enzyme fermentation.

Variation	Suhu (°C)	
	Before	After
P0	29,1	28,7
P1	28,8	28,1
P2	28,8	27,9
P3	28,9	28,1
P4	28,8	28,2

Based on the results of measuring the acidity level of the pH of the fermentation solution in table 4.2, it shows that the lowest level of acidity on the first day of making the fermentation was in treatment P1 and on the last day there were in treatments P1, P3, and P4. The highest acidity level value on the first and last day was in the P5 treatment.

In table 4.3 the results of initial and final temperature measurements show insignificant differences between each treatment. There was a decrease in temperature with a range of 0.40.9°C. The highest temperature decrease was in treatment 2 of 0.9°C.

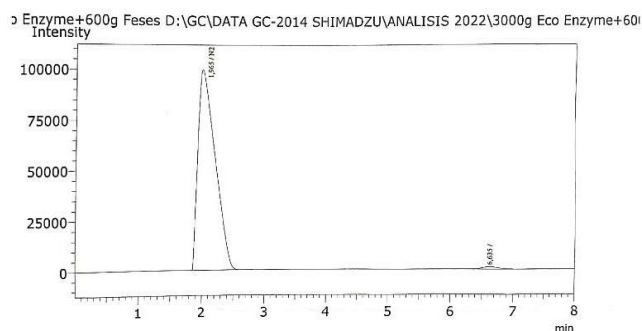
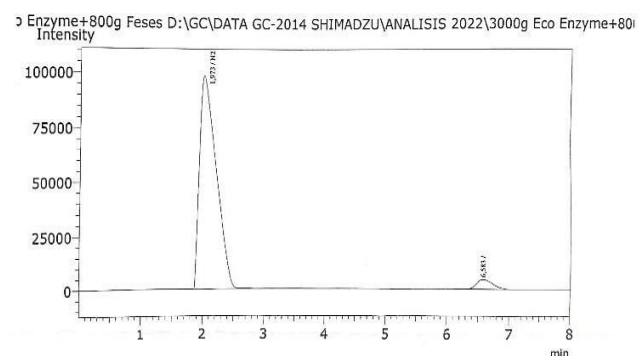
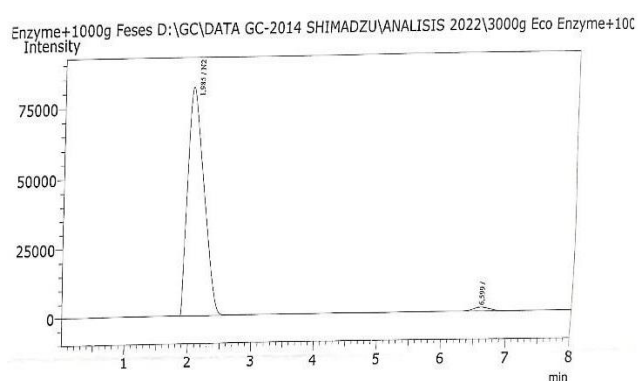
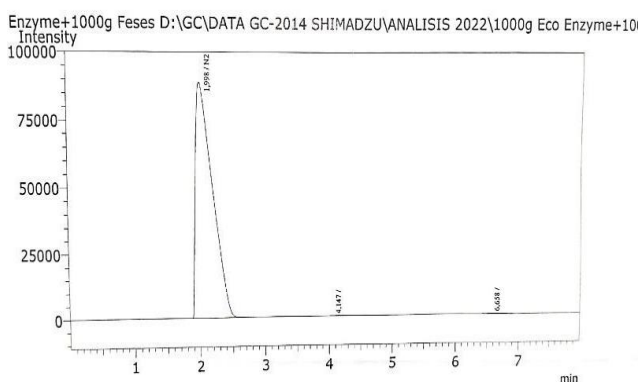
Metana (CH₄)

Data on methane concentration measurements that have been tested for biogas content are as follows:

Table 4. Rated Yield (CH₄).

Reactor	Concentration (%)	Height (%)	Area(%)
P1	0	0	0
P2	0	0	0
P3	0	0	0
P4	0,094	107	1486
P5	2,889	3435	50080

The results of measuring the concentration of methane (CH₄) in table 4 show that the highest concentration level was found in treatment P5, which was 2.889, followed by treatment P4, which was 0.094. Whereas in the P1 - P3 treatment it did not show any concentration of methane (CH₄) in it. The following is a graphic image of the results of the analysis of methane gas (CH₄).

**Figure 1.** Graph of P1 Analysis Results.**Figure 2.** Graph of the results of the P2 analysis.**Figure 3.** graph of the results of the P3 analysis.**Figure 4.** graph of the results of the P4 analysis.

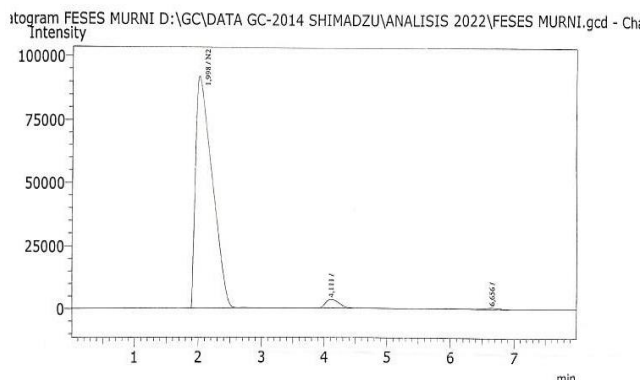


Figure 5. graph of the results of the P5 analysis.

Gas Content of N₂ and CO₂ in Fermentation

The results of the analysis of N₂ and CO₂ content in biogas fermentation are presented in table 5:

Table 5. Results of N₂ and CO₂ Values.

Sample Type	Gas Production (%)	
	N ₂	CO ₂
P0	96,565	0,546
P1	99,014	0,986
P2	95,875	4,125
P3	98,270	1,730
P4	99,669	0,237

Table 5 shows that the production of nitrogen and carbon dioxide gases in the 5 types of samples tested has different and fluctuating values. The highest nitrogen production was in sample P4 (99.669%) and the highest amount of carbon dioxide was produced in sample P2 (4.12%).

Discussion

This research was conducted to analyze several parameters in testing biogas from the main eco enzyme substrate with modifications of several materials. The addition of a starter is needed in this study with the aim of stimulating the growth of methanogenic bacteria which produce methane gas as the main gas in biogas. The initial stage of this research was to make eco enzyme from household organic waste with a ratio of 3:1. This waste is used based on the results of observations (pre-research). Methane gas is produced after going through eco enzyme fermentation with added starter which lasts for 1 month.

Eco-enzymes are made with the commonly used ratio of 3 (organic matter): 1 (molasses): 10 (water). All ingredients are put in a sterile container (large plastic bucket with a wide lid) and covered for 3 months of fermentation. In the first month of fermentation it will produce gas, if the place used is made of glass there will be a risk of breaking. Places with a metal base are not recommended. This is because it will trigger corrosive

(rusty) at the end of the fermented product which is acidic (pH below 4). Stirring needs to be done before the fermentation place is closed. This aims to flatten all the basic ingredients for making eco-enzymes.

Eco enzyme was chosen as the main substrate in forming the fermentation process, it needs to be added with molasses. Molasses contains 30% sucrose, 25% glucose and fructose. All of these ingredients are important elements as a source of energy for microbes. The carbon element (C) from glucose will be needed to regulate the carbon value in the substrate. Besides that, glucose adds value to carbon which is a source of nutrition for microbes that break down methane gas (Zulkarnaen at al., 2018).

The main element in biogas is the presence of methane gas. Methane gas is produced from methanogenic bacteria. This bacterium can be obtained from several sources, one of which is by using starter from cow dung. Cow manure is high in cellulose content (Asmiarti, 2019), contains matanogenic bacteria that produce methane gas (Karlina, 2017).

All of these treatments were fermented for 30 days. This is based on previous sources which state that 30 days of fermentation is the best time for biogas production. The most methane gas is produced with a presentation of 50.4%, a temperature of 350°C and a flame of 72 seconds (Mirwan and Nadia, 2021).

This fermentation process is carried out with the aim of producing methane gas. The gas produced from this process is accommodated in a gas holder in a tool that has been used assembled. The gas is then put in a sampling bag to be tested for biogas content using chromatography.

Based on the pressure research data, the data shows the results of different pressure values. It can be seen in table 4.1 that the highest pressure value is found in the P5 treatment reactor with a variation of 1500 g of water and 1500 g of cow feces producing a pressure value of 70 ml, in P4 with a variation of 1000 eco-enzyme and 1000 cow feces producing a pressure value of 50 ml, in treatment P3 with material variations 3000 g eco-enzyme and 1000 g cow feces produced a pressure value of 56 ml, in treatment P2 with material variations 3000 g eco-enzyme and 800 g cow feces produced a pressure value of 60 ml, in the reactor P1 treatment with 3000 g eco-enzyme and 600 g cow feces resulted in the lowest pressure value of 26 ml. The volume pressure value in the P2 treatment could be higher than the P3 treatment, this could be due to the unequal ratio of carbon (C) and nitrogen (N).

If the ratio (C/N) is too high (the C value is higher than the N value) then metabolism becomes inadequate which means that there is carbon in the substrate that is not fully converted, so it cannot achieve maximum methane yield which also results in unstable pressure values. On the other hand, if the N value is excessive, the amount of ammonia (NH₃) can be reduced, which even in low concentrations will inhibit bacterial growth

and can even cause the collapse of the entire population of microorganisms (Zulkarnain et al., 2018).

The main factor that can affect the difference in the volume pressure value of the biogas produced from each treatment is the physical properties of the filling material caused by the water content and acidity of the media (pH level) (Mara and Alit, 2011). Apart from that, it can also affect the difference in the amount of volume produced by each treatment, one of which is the reduced number of microorganisms (Novita et al., 2018), the number of organisms can be reduced due to the acidity of the pH which is too acidic which can cause the number of organisms to decrease.

Different pressure values can be influenced by the amount of methane gas produced from each reactor as in treatment P1 it does not produce methane gas, so the resulting pressure value is also small, namely 26 ml because the gas contained only contains nitrogen (N₂) and carbon dioxide (CO₂). Meanwhile, the P5 treatment had the greatest pressure value of 70 ml, because the gas contained in the P5 treatment reactor had a fairly large concentration of methane gas, namely 2.889.

The acidity level of pH is one of the determining factors for success in the formation of biogas. An acidity level that is too high or low will affect the amount of biogas produced. The degree of acidity of this pH is monitored every time a sample is taken to determine the condition of the substrate in the digester tube (Budiharjo, 2009).

Testing the acidity level of pH in eco-enzyme fermentation with the addition of cow feces starter was carried out using a pH meter. Testing the pH acidity level was carried out twice, namely on day 0 (beginning of fermentation) and on day 37 (last day when sampling).

Based on research data, the value of the pH acidity level in ecoenzyme fermentation on day 0 was the lowest pH acidity level in treatment P1, namely 3.4, in treatment P2, namely 3.5, in treatment P3, namely 3.5, in treatment P4, namely 4, and in treatment P5, which was equal to 6.1 is the highest pH acidity level value in the first test. Meanwhile, in the second test on the last day the acidity level of each treatment reactor decreased overall. In treatment P1 it fell to 3, in treatment P2 it fell to 2.9, in treatment P3 it fell to 3, in treatment P4 it fell to 3, and in treatment P5 it fell to 6.1. The optimum condition for the pH acidity level is between 6.8-7.2. If the substrate drops, it will cause the process of converting the substrate into biogas to be hampered, resulting in a decrease in the quantity of biogas. A pH value that is too high must also be avoided, because it will cause the final product to be CO₂ as the main product (Dwivannie et al., 2019), whereas in this study the treatment almost reached the optimum conditions for biogas so that it can produce methane gas, namely in treatment P4 (4 and 3) and P5 (6.3 and 6.1). The results of research by Rahim et al., (2017) biogas in this case the CH₄ content in general will produce high in pH conditions that are close to neutral. This is related to the development of methane bacteria

which will be encouraged to grow and reproduce optimally, so that it will have an impact on biogas production.

According to Ramdiana, in the pH range of 6-6.7, very few methanogenic bacteria are able to survive to produce gas because the acidity of the substrate can kill the bacteria, so that methane gas is still produced, even in small quantities. The microorganisms that work in the early stages are microorganisms in the hydrolysis-acidogenesis process which produce volatile acids so that the pH value drops (Ni'mah 2014).

In the initial and final temperature tests, it was seen that there was a decrease in each of the treatments tested. The temperature drop is not too big, still in the range of 0.4 – 0.9°C. This is influenced by the condition of the environment around the test site which is drizzling. This condition slightly affects the temperature in the digester. This statement is in line with research conducted by Widodo (2022), the digester temperature is similar to the ambient temperature in the morning or evening.

The measurement results in the P0 and P4 treatments had a larger value and produced methane gas (CH₄) in small amounts. As research conducted by Widodo (2022), the higher the temperature of the fermentation indicates that the decomposition of organic matter is occurring, which is the basic ingredient for producing methane, carbon dioxide and other gases. The best temperature for fermentation is the mesophilic temperature, which is around 20-40°C (Syaichurrozi, 2020). In this study, the initial and final temperatures were measured in the mesophilic temperature range (27-29°C). Methanogenic bacteria will work well at mesophilic fermentation temperatures and the best (optimum) temperature for mesophilic bacteria to produce biogas is 35°C (Adiani et al., 2019). The optimum temperature is the perfect temperature for bacterial metabolic processes. High or low temperatures will cause imperfect bacterial metabolism and can stop.

At low temperatures there are parts that turn into gel, one of which is the double layer of lipid membrane which generally forms a liquid into a gel. This can cause damage to protein function (denaturation) and leakage so that the protein cannot pump ions. Another impact is the late bacterial growth (Asiaah, 2020). At high temperatures it can damage the bacterial cell membrane. If this membrane is damaged, protein denaturation will occur and activity in the bacterial cell will decrease (Roma et al., 2021).

This decrease in bacterial activity will eventually lead to the death of the bacteria. Bacterial cell death has an impact on reducing the composition of methanogenic bacteria. This change in composition results in the accumulation of gas resulting from the hydrolysis and acidogenesis stages (fatty acids, glycerin, monosaccharides, amino acids, acetic acid, butyric acid and propionic acid) which are not converted into methane gas.

Testing the sample for methane (CH_4) content in the eco-enzyme fermentation solution which was added to the cow feces starter was carried out using a gas analyzer. Judging from the research results table 4.3, the P1-P3 treatment did not show methane (CH_4) content, while in Figure 4.4 it can be seen that the P4 treatment showed a (CH_4) content of 0.094 with a graphic height of 107 and an area of 107. In addition, in Figure 4.5 can be seen that the need for P5 also indicates the presence of content (CH_4), which is 2.889 with a graph height of 3435 and an area of 50080.

It can be seen from the research data table 4.3 that the greater the ratio of the eco-enzyme solution given, the smaller the possibility of the presence of (CH_4) content, whereas the higher the ratio of cow feces starter, the greater the possibility of (CH_4) content. This is because in treatment P1 the ratio used in the fermentation solution was 3000 g of eco-enzyme and 600 g of cow feces.

In the P2 treatment the comparison used was 3000 eco-enzyme and 800 g of cow feces, and in the P3 treatment the comparison used was 3000 eco-enzyme and 1000 cow feces. This much larger amount of eco-enzyme can affect the performance of the bacteria in the fermentation process, because the ingredients contained in the eco-enzyme solution are ingredients that contain antimicrobials or inhibit bacterial growth such as pineapple, orange peel, aloe vera, noni, cultivar. bananas, and others (Rochmayani et al., 2020).

Another factor that affects the amount of methane gas (CH_4) produced is only a small amount and is only produced in the P4 and P5 treatments because the number of comparisons used in P4 giving eco-enzyme is comparable to giving cow feces, namely 1000 g eco-enzyme and 1000 g cow feces, whereas in treatment P5, the ratio of water to cow feces was used, namely 1500 water and 1500 cow feces. Thus, methane gas (CH_4) can be produced in treatments P4 and P5. Another influencing factor can be seen from the acidity level of the pH of the fermentation solution, because the optimal pH conditions required for biogas production are in the range of 6.6 to 7.5 (Fitri and Trisna, 2018).

In treatment P1-P3 the pH acidity level is below optimal conditions, namely in treatment P1 the pH on day 0 is 3.4 and the pH of the 37th day is 3. So the bacteria that help the process of forming methane gas (CH_4) are hampered or can even die. The low population of methanogenic bacteria results in the accumulation of volatile acids that have not been converted into methane gas, so that little or no gas is produced (Roma et al., 2021).

The N_2 and CO_2 gas content that has been analyzed can be seen in table 4.5. The highest yields obtained were nitrogen (N_2) and carbon dioxide gas (CO_2). The concentration of nitrogen gas (N_2) from reactors P0-P4 was in the range of 95.875-99.669% and the concentration for carbon dioxide (CO_2) from reactors P0 - P4 was in the range of 0.2374.125%.

Stirring the reactors P0, P1, P2, P3 and P4 by shaking the digester tube. The digester tube is made using a batch type with an anaerobic system so that it cannot be opened when stirring. This is thought to cause non-optimal contact between the organism and the substrate, thereby affecting the methane gas concentration value. In line with research which states that stirring in the digester will allow direct contact between microorganisms and gas-forming substrates. The higher the stirring frequency, the higher the opportunity for microorganisms to degrade the substrate. Using a stirrer, methane gas trapped in solution can be immediately released and enters the gas reservoir. Stirring provides sufficient contact between the substrate and the microorganism to produce a homogeneous condition. A digester with a stirrer produces more optimal biogas compared to a biodigester without a stirrer (Suryani et al., 2018).

Reactor P0 is a reactor with control treatment. Reactor P0 and reactor P4 both produce methane gas. This is presumably because both of them use filling materials in a 1:1 ratio. In the P0 reactor using a ratio of 1.5 L of water + 1500g of cow dung. The addition of water to the P0 reactor was carried out because based on research it was stated that microorganisms in metabolism need water. The amount of water needed for biogas varies depending on the material used. In order to function normally, biogas-producing microbes require a substrate with a moisture content of 90% and a solids content of 8-10%. The raw material should be diluted 1:1 if the raw material is 100 kg of manure, then the water needed is 100 kg (Kamal, 2019). In the P4 reactor, a ratio of 1 L of water + 1000 g of cow dung is used. Based on research results, it is stated that biogas with a 1:1 ratio between substrate and starter produces the most optimal biogas (Widodo, 2022). Even though there is methane gas in the P0 reactor and P4 reactor, the nitrogen (N_2) content obtained is still quite high in all reactors. This is probably due to the gas reservoir and sample container not being vacuumed, resulting in air contamination from outside the digester (Amanda, 2020).

CONCLUSION

The conclusion was based on the results of research analysis of methane content (CH_4), pH, and pressure in eco-enzyme fermentation with the addition of cow feces starter. This research was conducted for 37 days from July to August, the results can be summarized as follows:

- The treatment that produced the highest methane gas (CH_4) was the P5 treatment with a concentration of 2.889%.
- The pH acidity level that reached optimal conditions was in the P5 treatment, namely on the 0th day of sampling it was 6.3 and on the 37th day it was 6.1.
- The highest volume pressure value was produced in treatment P5, namely 70 ml.

Competing Interest: The authors declare that there are no competing interests.

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