Effect of Organic Waste Addition into Animal Manure on Biogas Production Using Anaerobic Digestion Method

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ABSTRACT. One biomass form with a high potential to replace fossil fuels is biogas. Biogas yield production depends on the raw material or substrate used. This research was aimed to investigate a biogas production technique using an anaerobic digestion process based on a substrate mixture of a starter, cow dung, chicken manure, tofu liquid waste, and cabbage waste. The anaerobic digestion is a promised process to reduce waste while it is also producing renewable energy. Moreover, the process can digest high nutrients in the waste. The anaerobic digestion results showed that the combination producing the highest biogas amount was 200 mg starter mixed with a ratio of 70% cow dung, 15% chicken manure, and 15% tofu liquid waste. The larger the amount of cabbage waste, the lower the biogas production. The quadratic regression analysis was obtained for the variable with the highest yield and the estimated kinetic parameters based on the Gompertz equations revealed that the value of $P\infty = 2,795.142$ mL/gr.Ts, Rm = 113, 983.777 mL/gr.Ts, and t = 10.2 days. The results also concluded that the use of tofu liquid waste produced more biogas than cabbage waste. This study also successfully showed significant development in terms of the amount of biogas produced by adding organic waste to animal manure as the substrate used.

Keywords: biogas, anaerobic digestion, tofu liquid waste, cow dung, chicken manure, cabbage waste
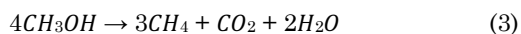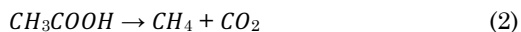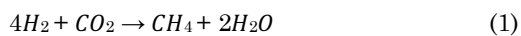
1. Introduction

Renewable energy sources are necessary to overcome the crisis in energy reserves such as oil and coal. Various alternative energy sources have been developed, including wind energy, hydro energy, and geothermal energy (Ikram *et al.*, 2019). However, limited funds for capital investment and complex technology development make implementing these energy sources difficult. Nonetheless, one renewable energy source that requires simple, easy-to-develop technology is biomass; thus, it has enormous potential to become an alternative energy source with affordable capital investment and easy technology development (Sakhmetova *et al.*, 2017).

One alternative energy worldwide is biomass, which can be transformed into various kinds of energy such as heat, steam, and electricity. Biomass can also be converted into biofuels like methanol, ethanol, and biodiesel. In Indonesia, one of the most developed forms of biomass is biogas which is derived from organic, living matter just as biomass is. Biogas should reduce gas emissions in Indonesia because it is carbon-neutral, so it doesn't produce gas emissions. Furthermore, any consumption of biogas that replaced the usage of fossil fuels will lower CO_2 gas emissions (Ariae *et al,* 2019). The biogas production results depend on the substrate and the method used.

Tofu waste is one substrate which is being discussed (Adisasmito *et al.,* 2018). During the production process, factory tofu waste is mostly in the form of liquid. Tofu enterprises are spread throughout Indonesia, with a total of almost 84,000 enterprises. Therefore, various biogas production technique using tofu waste have been performed. For example, Budiyono and Syaichurrozi (2020) investigated biogas production from tofu waste using a two-phase anaerobic digestion method where biogas becomes the top product while the effluent becomes the bottom product. A renewable energy technology promises to reduce waste while producing renewable energy known as anaerobic digestion. Anaerobic digestion is an interesting method because it can also digest high nutrients. The nutrients available after the digestate application are nitrogen, phosphor, sulphur, calcium, magnesium and micronutrients (Ichsan *et al,* 2014). Therefore, anaerobic digestion has many benefits for the environment, since it reduces the amount of waste material, lowers the emission of hazardous materials such as greenhouse gases, allows for the production of organic fertilizers, and reduces the odor of waste materials. The anaerobic digestion method is considered as the right approach for biogas production, especially from organic substrate (Igoni *et al.*, 2008). A decomposition reaction of organic matter occurred, and the organic matter was

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converted into biogas by anaerobic bacteria. Biogas contains methane and carbon dioxide as the main products and small amounts of hydrogen, ammonia, carbon monoxide, and H₂S as by-products. Biogas formation reaction can be described in reaction 1 to 3.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad (1)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad (2)$$

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \qquad (3)$$

Reaction 1 is the mechanism of hydrogenotrophic methanogenesis. It can be described as the reaction between carbon dioxide and hydrogen to produce methane and water. Reaction 2 is the acetoclastic methanogenesis mechanism, which is the reaction of acetic acid become

methane and carbon dioxide. Finally, reaction 3 is the mechanism of methylotrophic methanogenesis which is the reaction of methanol and become methane, carbon dioxide, and water. Figure 1 presents the complete mechanism of the biogas formation phase.

Rahmat *et al.* (2014) compared biogas production from tofu liquid waste mixed with cabbage vegetable waste and that from tofu liquid waste mixed with sheep dung by using a fixed dome biogas digester. The biogas amount produced by a mixture of tofu liquid waste and sheep dung was almost twice as much as the mixture of tofu and cabbage vegetable waste (Rahmat *et al.*, 2014). The result shows stone, sand, and gravel on the digester's bottom and detected as residual impurities. The waste remained semi solid and resembled fiber material which is difficult to digest.
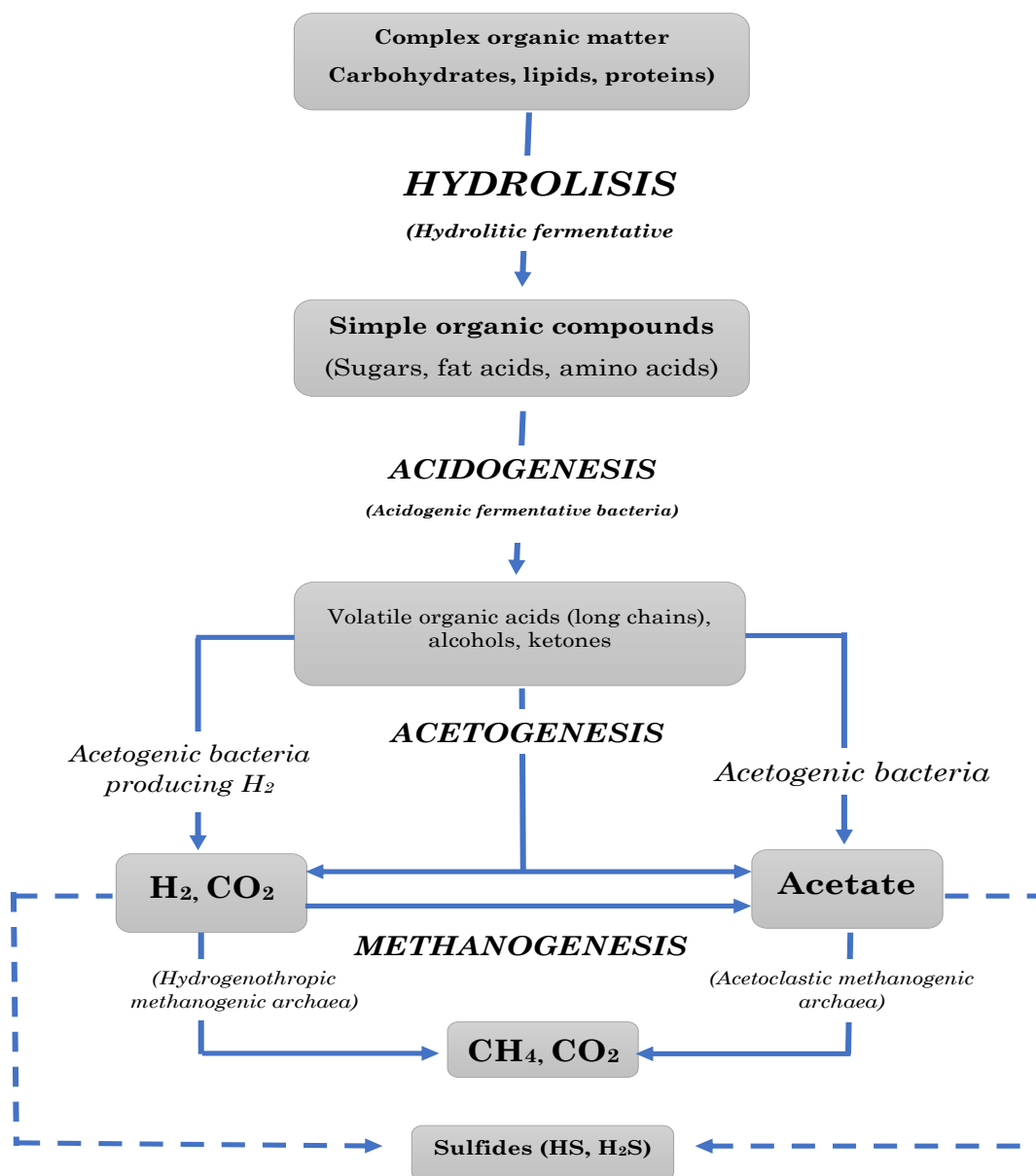

Fig. 1 Phases of anaerobic digestion (Moraes, *et al.*, 2015)

The fiber material will be on the top of the digester and above the supernatant. The flammable gas mixture will rise to the top of the tank. It shows that the use of fixed dome biogas digester is inefficient and not environmentally friendly.

Because sheep manure in Indonesia is rarely obtained owing to the small number of sheep farms, realizing industrialization scale will be difficult. Usually, chicken and cow manure represent the livestock manure often processed into biogas (Darja and Andreja, 2020). Many studies about biogas production from organic waste do not produce an adequate biogas yield. The biogas produced from tofu waste is only 77.01 mL/g.TS (Syaichurrozi *et al.*, 2016) and requires further research to increase the biogas yield produced. Also, it needs a better method to produced biogas in order to minimize the waste produced during the process.

This study focused on optimizing a new substrate mixture using an anaerobic digestion method that had never been analyzed before. We aimed to produce a high biogas yield using substrate from a mixture of tofu liquid waste and animal manure (cow and chicken dung), then compare it with substrate from a mixture of tofu liquid waste, cow and chicken dung, and cabbage vegetable waste. Moreover, various combinations with different component ratios were analyzed to determine the best variables, and analyses with quadratic regression and reaction kinetics analyses were conducted.

2. Materials and Methods

2.1 Materials

Tofu liquid waste was obtained from a tofu factory in the Bandungan area, Indonesia. Cabbage waste as raw materials were obtained from traditional markets in the Semarang area, Indonesia. Dung from cows and chickens was obtained from the Faculty of Animal Husbandry-Agriculture, Diponegoro University, Indonesia. In this study, the used NaOH material came from Merck Pro Analyst (Darmstadt, Germany). Furthermore, an incubator with temperature control was used, and a set of devices for measuring biogas volume, including a 1000 mL Pyrex measuring cup and a Tedlar bag from ShilpEnt Kaipa Enterprises, was used.

2.2 Raw material pretreatment and initial characterization

The raw material for the tofu liquid waste was prepared, and the cabbage waste was reduced in size. Then fresh cow and chicken manure as a substrate mixture was taken directly from chicken and cow farms. The starter came from fermented cow dung. Materials were pretreated to determine the C/N ratio (from the ratio between C Total and N Total), total solid (TS), and volatile solid (VS). The C total was determined based on ash content after the material burned at 550°C. The N total was determined by the Kjeldahl method. The number of N total showed by titration number with chloride acid after being distilled with sulphuric acid, NaOH, H_3BO_3, and BCG-MR. Chloric acid, sulphuric acid, and NaOH were obtained from Merck Pro Analyst (Darmstadt, Germany). H_3BO_3 and BCG-MR were purchased from CV Indrasari, Semarang, Indonesia. The TS and VS analysis according to the standard method (APHA, 1995).

2.3 Initial mixing and analysis of substrate

The raw materials such as tofu liquid waste, cabbage waste, and chicken and cow manure were mixed into a digester, which already contained a starter in the form of fermented cow dung. The starter is needed to ensure a maximum biogas yield volume. Dung from ruminants is a very good source of inoculums for biogas production because it already contains native microbial flora. Cow dung was chosen due to its balanced C/N ratio (25-30:1). The mixing was performed according to the variables presented in Figure 2.

Both variables A and B have four different percentages in terms of raw materials. Variable A uses cow dung, chicken manure, and tofu liquid waste as raw materials. Variable A1 consists of 100% cow dung; variable A2 consists of 80% cow dung, 10% chicken manure, and 10% tofu liquid waste; variable A3 consists of 70% cow dung, 15 % chicken manure, and 15% tofu liquid waste; variable A4 consists of 60% cow dung, 20 % chicken manure, and 20% tofu liquid waste. Variable B used cow dung, chicken manure, tofu liquid waste, and cabbage waste as raw materials.
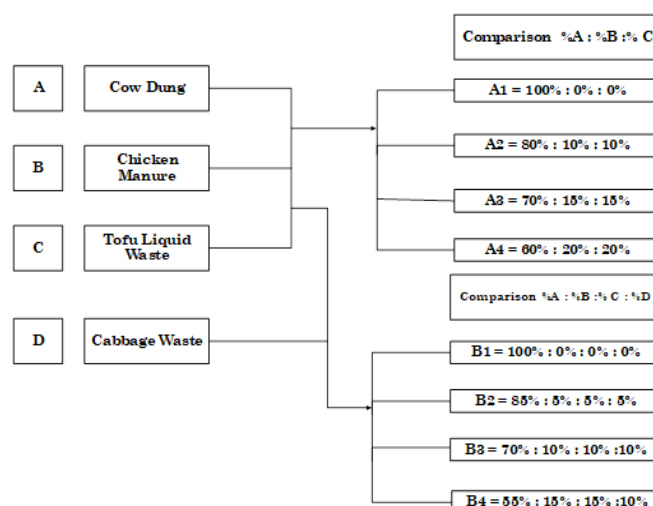

Fig. 2 Experimental variables used in the experiments

Variable B1 consist of 100% cow dung ; variable B2 consists of 85% cow dung, 5% chicken manure, 5% tofu liquid waste, and 5% cabbage waste ; variable B3 consists of 70% cow dung, 10% chicken manure, 10% tofu liquid waste, and 10% cabbage waste; variable B4 consists of 55% cow dung, 15% chicken manure, 15% tofu liquid waste, and 15% cabbage waste. The target of experimental variables is to clearly determine the effect of tofu liquid waste and cabbage waste in animal manure mixture. Therefore, all of 8 variables must be conducted properly to study the effect of organic waste addition. The weight of the starter in each digester was 200 g. An anaerobic digestion process was performed to produce biogas in each digester used. The volume of material that was placed into the digester was 70% × the total volume of the digester (Wang *et al.,* 2018). This experiment used a digester with total volume of 350 mL. The digester was shaken until a homogeneous material was obtained.

2.4 Process of biogas production via anaerobic digestion

Nitrogen gas was passed through each digester for two min. Furthermore, the digester was connected to a NaOH vessel and a Tedlar bag. Sodium hydroxide (NaOH) functions to absorb CO_2, so that the biogas produced is pure and does not contain CO_2 (Deublein & Steinhauser, 2008). The experimental was done in design is shown in the incubator (Figure 2). The incubator maintained the temperature so that the biogas formation process could run optimally, i.e., at 55 °C. Figure 3 shows the incubator design. The biogas production process was observed for 90 days, and the biogas volume data were collected every two days.

2.5 Analysis of pH and Chemical Oxygen Demand (COD)

The substrate mixture in each variable was tested for pH and COD before and after anaerobic digestion treatment. For pH analysis using pH meter (model 526, Germany). For COD analysis using the standard from SNI 6989.02:2019 by close reflux method. The COD value was defined by the amount of Cr_2O_7 oxidant that reacted with the sample and expressed as mg for every 1000 mL of sample (Wahyudi *et al.,* 2020) .
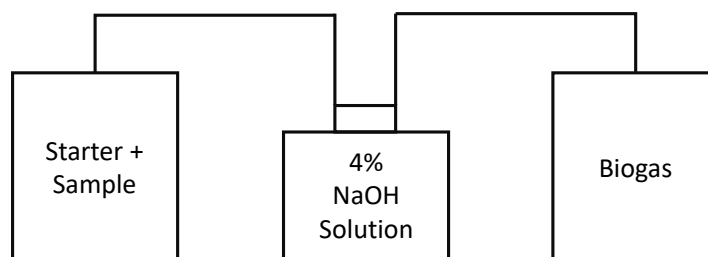

Fig. 2 Series of experiment tools

Fig. 3 Incubator of experiment tools

2.6 CO_2 removal analysis

The yield of biogas in each variable was analyzed for CO_2 content by using gas chromatography (GC) method. The GC method used GC-FID 2025 Shimadzu with Rtx-wax, and microliter syringe 10 μL (SGE) to find the concentration of CO_2 in each sample of biogas product. If the absorption of CO_2 by NaOH done well, there will be no CO_2 inside the product of biogas.

2.7 Quadratic regression analysis and reaction kinetics

From the biogas volume data obtained, a model can be constructed to analyze the relationship between time and the amount of biogas produced and the equation for the quadratic regression analysis is shown by Eq 4.

$$Y = \alpha X^2 + \beta X + C \qquad (4)$$

Where: Y is dependent variable (gas production), α is regression coefficient X^2 against Y, β regression coefficient X against Y, C constant and X independent variable (time).

Furthermore, the reaction kinetics was investigated using the Gompertz equation (eq 5).

$$P = P_\infty \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{A}\,(\lambda - t) + 1\right]\right\} \qquad (5)$$

Where: P is cumulative production of methane (mL/gr.Ts), $P\infty$ is methane production potential (mL/gr.Ts), Rm: maximum specific speed methane production (mL/grTs.day), then λ is lag phase period or minimum time to produce biogas (day), e is math constant (2.7182), and t is biogas production cumulative time (day).

3. Results and Discussion

3.1 Initial characterization

Table 1 presents the raw material initial characterization results such as C&N Total, the ratio of C/N, TS, and VS values, which are the most important in biogas yield production. The optimum C/N ratio to produce biogas is 25-30:1 (Faisal et al., 2016).

Table 1 shows that the largest C content was from cabbage waste (42% total C), while the largest N content was from chicken manure (1.27% total N). The biggest number of TS and VS contained on chicken manure, while the most optimum TS is showed by tofu liquid waste with 8.975%. The best percentage of TS content obtained for biogas production is in the range 7-10%. Total solid below 7% made the process unstable, while the total solid above 10% may cause the fermenter to become overload (Baserja, 1984). Table 1 shows that cow dung has a TS value of 22.725%, while chicken manure has a TS value of 32%. The number of TS in both materials is not in the optimum range (7-10%), and can cause an overloading of the fermenter. However, the fermenter used in this experiment has a larger capacity to prevent overloading in the fermenter/digester. Moreover, the starter's presence in this experiment is important because the starter also contains water to help the process of anaerobic digestion. Water will make bacteria become possible to move and grow. The presence of water also makes the process of dissolution and transport of nutrients easier. Water can minimize the mass transfer of non-homogenous or particulate substrate (Budiyono et al., 2010).

3.2 Biogas production

Biogas production results in variable A which consisted of a mixture of starter, animal manure (dung from cow and chicken) and tofu waste, as illustrated in Figure 5. The figure shows the results of biogas production within 90 days for variable A. Variable A3 produced the highest amount of biogas, which yielded a total of 2780 mL of gas in 90 days. Gas volume measurement started from the second day and was performed every two days. The biogas can be produced when it is also optimum in C/N ratio, percentage comparison in each material, and TS content. This means variable B3 has the optimum conditions required to produce the best/highest results pf biogas yield. Variable A in this research provided a higher yield than that obtained in the experiment by Latinwo & Agarry involving biogas with a mixture of chicken manure and cow dung (Latinwo & Agarry, 2015).

For variable A3, the phase corresponding to the highest gas production in the graph is the lag phase from day 2 to 8, followed by an exponential phase from day 10 to 20. Day 8th to 28th is a stationary phase. On day 28th, anaerobic bacteria began to die, so that the biogas production start to decline every day. From day 68th to day 90th, there was no more gas production. Furthermore, for comparison, biogas production under variable B consists of starter, a mixture of animal manure (cow dung & chicken manure) and cabbage vegetable waste as illustrated in Figure 6.

Table 1
Initial characterization on raw material

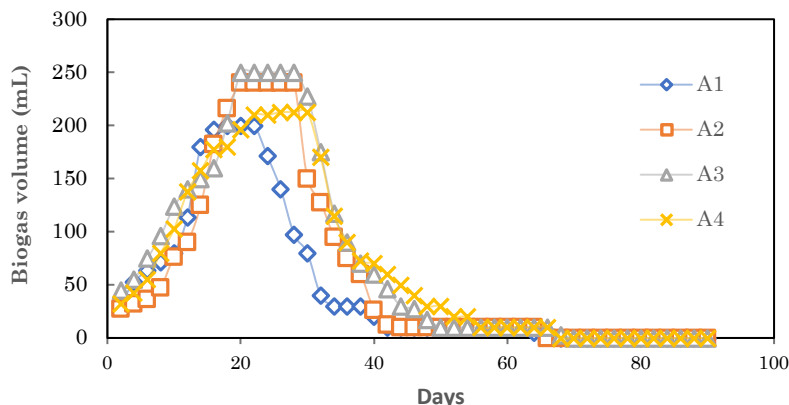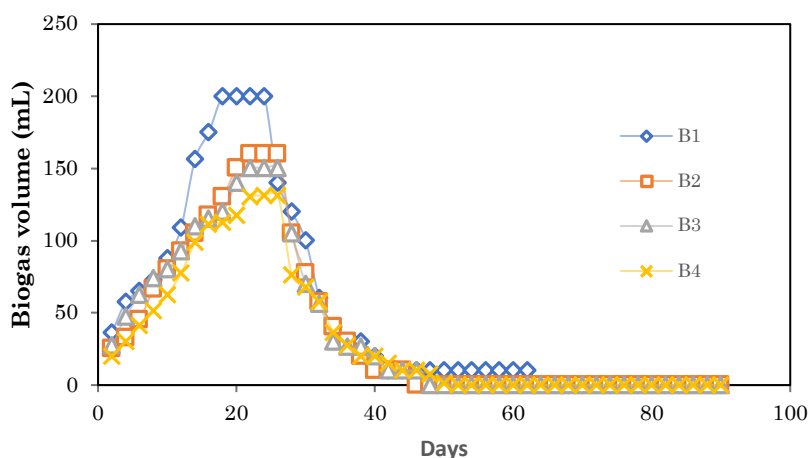| No	Raw Material	Total of C (%)	Total of N (%)	Ratio of C/N	TS (%)	VS (%)
1	Cow dung	27.31	0.95	28.74	22.725	18.1125
2	Chicken manure	19.51	1.27	15.36	32	18.375
3	Liquid tofu waste	10.28	0.21	48.95	8.975	8.550
4	Cabbage waste	42	0.7	60	5.925	5.375

Fig. 5 Biogas production for variable A

Fig. 6 Biogas production for variable B

The average biogas production yield under variable B was lower than that under variable A. It is caused by cabbage waste in variable B which is not an optimum C/N ratio. The variable that produced the highest amount of gas was variable B1, which consisted of 100 mg starter and 100% cow dung. As shown in the graph, the exponential curve of variable B1 reached a point higher than the other variables curves. This means that the anaerobic digestion process under this variable was the most effective for the substrate mixture of cow dung and starter. Anaerobic microorganisms decompose organic matters without dissolved oxygen (anaerobic conditions), and produced the highest biogas yield for variable B.

The higher the cabbage waste content, the lower the gas yield. Under variable B, less gas was produced because cabbage waste has a very high C content, which increases the C/N ratio. In contrast, the optimum C/N ratio for gas production is 20–30 (Faisal et al., 2016). On the one hand, a too high C/N ratio will result in inadequate metabolism, which means that the carbon in the substrate will not be completely converted, and thus, the maximum biogas yield will not be achieved.

Also, a low C/N ratio will result in a nitrogen surplus. Large amounts of nitrogen will produce excessive amounts of ammonia (NH_3). The presence of NH_3 will inhibit bacterial growth and in the worst case can lead to the collapse of the entire microorganism population, leading to inhibited and inadequate biogas production (Ridlo, 2017).

This research shows that the presence of cabbage waste in the substrate affected biogas production. The cabbage waste tended to reduce the amount of biogas produced. The production process became inhibited and below optimal because of the high value of the C/N ratio. It can be optimized using another raw material as a substrate to replace cabbage waste, and the optimum C/N ratio is between 20-30:1. However, the overall research shows upgrading quality based on the biogas amount produced by variable A3. When a substrate mixture of animal manure (cow dung and chicken manure) was mixed with tofu liquid waste, a higher biogas amount was produced than in the research by Rahmat *et al.* who used sheep dung and tofu liquid waste as the raw material (Rahmat *et al.*, 2014). The research by Budiyono *et al.* that used cattle manure and rumen fluid inoculum produced 186.28 mL (g.VS)$^{-1}$ of biogas yield (Budiyono *et al.*, 2010). It means, the use of raw material (animal manure from cow and chicken dung and tofu liquid waste) as a substrate in this research gives better results in the amount of biogas produced.

3.3 pH and COD analysis

The pH in each sample being tested before treatment and after anaerobic digestion treatment of 90 days. Figure 7 shows the results of the pH value in each sample, while Figure 8 shows the results of the COD value in each sample.
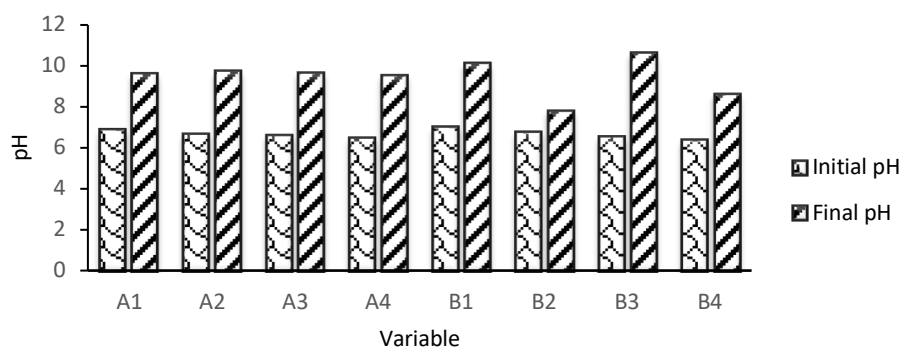
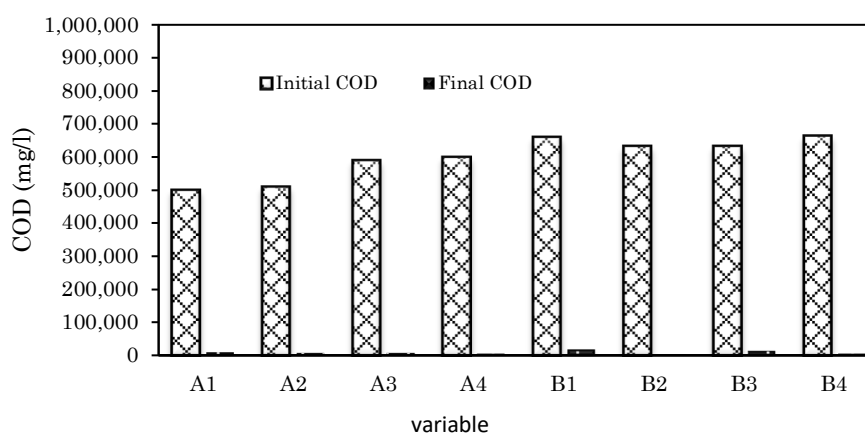**Fig. 7** The results of pH analysis in each sample

Fig. 8 The results of COD analysis in each sample

In Figure 7, the graph shows the comparison between the initial and the final pH, and it reveals that the final pH has increased at the end of fermentation. According to Ramdiana, in the pH range 6-6.7, methanogenic bacteria are able to survive to produce very little gas because the acidity of the substrate can deactivate bacteria, so that methane gas is still produced even in small amounts (Ramdiana, 2017). Microorganisms that work in the early stages are microorganisms in the hydrolysis-acidogenesis process which produce volatile acids so that the pH value decreases (Ni'mah *et al.,* 2014). On the following day the pH value increased closer to normal conditions. As long as the pH is still in an acidic state, biogas and CH_4 production will continue. After 12th days, all samples has reached normal pH, where methanogenic bacteria are gradually more active until the optimal pH is reached for biogas production (Ni'mah, 2014). On the 12th day of measurement, the methanogenesis process was going well because methanogenic bacteria could produce methane at a pH of 6.8-8.5 (Ramdiana, 2017). The results at the final study pH tended to rise for all variables compared to the baseline test. This can occur because methanogenic bacteria cannot work in acidic conditions and therefore the pH will increase. The optimum conditions for pH to produce biogas are at pH 6.8-7.2. However, until a pH of 8.5, it still produces biogas even though the amount is decreasing. Long operating time and high pH reduce the amount of biogas production so that the biogas production in this study begins to terminate in the 60th day.

In Figure 8, at the beginning of the experiment, the variable with the highest COD content was B4 with 660,000 mg/L and all variables initially have very high COD levels and are very dangerous if disposed directly into the environment. Therefore, the anaerobic digestion process can reduce COD levels in the mixture of ingredients. The COD value in each digester decreased during the fermentation process. The largest amount of COD removal showed by variable B2 which means that the number of organic compounds that can be chemically oxidized in variable B2 is higher than the other variables. According to Kresnawaty *et al* (2008) the hydrolysis process decreases the COD value. The hydrolysis process makes organic material from the substrate is used by microorganisms as nutrients and converts them into simpler compounds (Kresnawaty *et al.*, 2008).

3.4 CO₂ removal

The major composition of biogas is methane and carbon dioxide. There are also minor components such as nitrogen, hydrogen, hydrogen sulfide, oxygen, and water vapor. However, only methane is needed as an energy source while the presence of CO_2 is undesirable. Therefore, we used NaOH to remove the CO_2 component, and the methane gas can be captured in a Tedlar bag. Results of

CO_2 removal using NaOH via GC showed that all of the samples had a 0% CO_2 concentration and 0 value of retention time. The retention time (RT) is the time required for a solute to pass through a chromatography column, from injection until detection. The area and height detected in the GC screen are also zero which means there is no CO_2 detected in each sample.

The CO_2 absorption by NaOH in biogas production can be defined by reaction 6.

$$CO_{2(g)} + 2NaOH_{(aq)} \rightarrow Na_2CO_{3(s)} + H_2O_{(l)} \qquad (6)$$
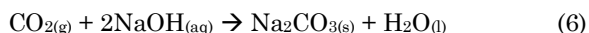
The absorption process of CO_2 by NaOH is a chemical absorption process. The absorption reaction is irreversible, where the CO_2 in the gas phase will be absorbed by NaOH liquid. If the gas approaches the liquid interface, the CO_2 in the gas phase will dissolve and react immediately with the NaOH solution. Under alkaline conditions, the formation of bicarbonate is negligible because it reacts with OH^- to form CO_3^{2-}.

3.5 Quadratic regression analysis

A quadratic regression analysis was performed on the variable that produced the gas with the most volume. Of the eight existing variables, variable A3 (70% cow dung, 20% chicken manure, and 20% tofu liquid waste) produced the most gas. The graph of the quadratic regression analysis of variable A3 is presented in Figure 9.

From the graph of the quadratic equation generated in Figure 9, an equation can be derived in Table 2. From the data in Table 2, a quadratic equation (Eq 7) can be obtained.

$$Y = 2E\text{-}13 - 14.743X^2 - 0.2457X \qquad (7)$$

To analyze the relation of the independent and dependent variables, analysis of variance showed in Table 3.

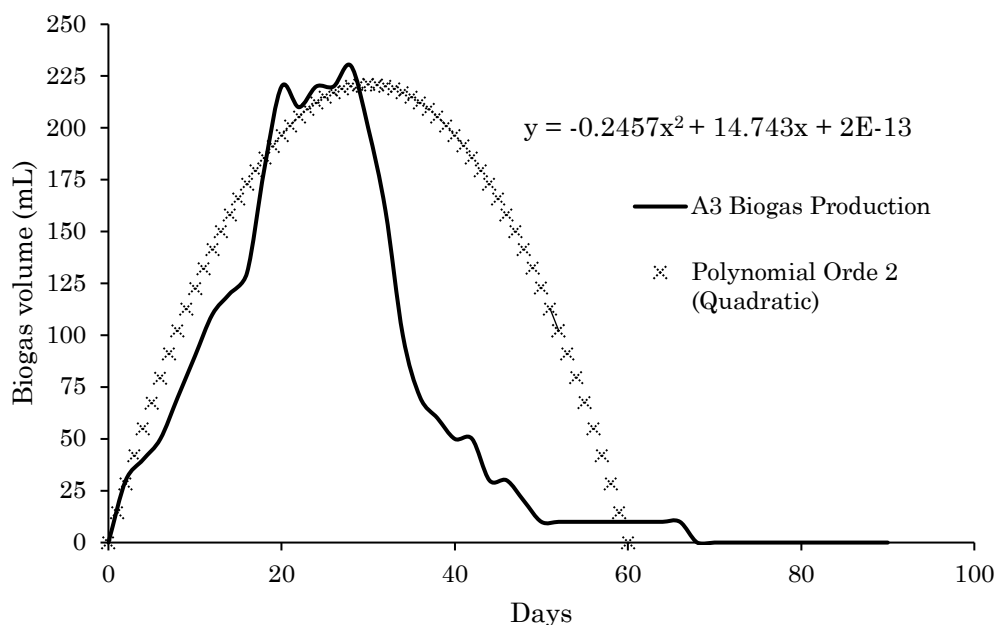**Fig. 9** Regression analysis results for variable A3

Table 2
Data analysis of quadratic regression equation for variable A3

	Unstandardized coefficients		Standardized coefficients	t	Significance
	β	Std. error	β		
Days	−0.2457	1.344	−132	−285	0.9984
Days**2	14.743	0.014	−.552	−1,193	0.777
(Constant)	2E-13	9.2429		4.722	0.000

Table 3
Analysis of variance results of the relationship between time and biogas production amount

|	Sum of squares	df	Mean square	F	Significance
Regression	118,628.522	2	59,314.261	18.075	0.000
Residual	137,829.255	42	3,281.649		
Total	256,457.778	44			
	The independent variable is time (day)				

From the output, the F-value is 18.075, and the significance level shows a value less than 0.001. The significance level below 0.05 ($p<0.05$) indicates that time (day) significantly influences the biogas production amount.

3.6 Reaction kinetics of biogas formation

The Gompertz kinetic model equation indicates the value of the methane production potential, i.e., $P\infty$. In the equation, R_m is the maximum specific speed of methane production, while t is biogas production cumulative time.

For variable A3, which produced the highest biogas amount, through the Gompertz equation, the values of $P\infty$, R_m, and t are presented in Table 4.

From the existing Gompertz equation, it was simplified through a nonlinear regression analysis method using Microsoft Excel with λ value as constant value = 2.718, and the results were obtained as $P\infty$ = 2,795.142 mL/gr.Ts, R_m = 113, 983.777 mL/gr.Ts, and t = 10.22 days. The experimental data from variable A3 then compared with a kinetic model of Gompertz as shown in Figure 10.
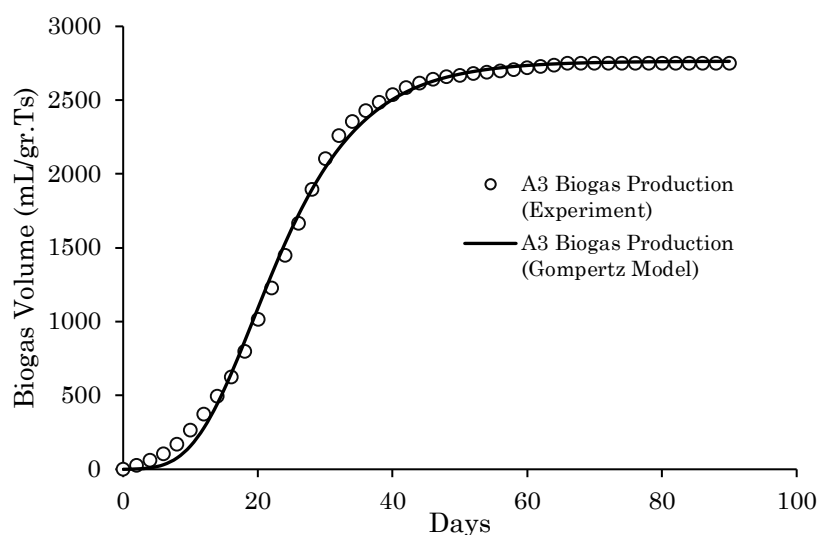
Fig. 10 Comparison of experimental data and kinetic model of Gompertz for variable A3

Table 4
Reaction kinetics for variable A3

P_∞ (mL/gr.Ts)	R_m (mL/gr.Ts.Day)	T (Days)	λ (Days)
2,795.14	113,983.77	10.22	2.72

3.7 Discussion

This research aimed to determine the best substrate variable for biogas production by using an anaerobic digestion method. The biogas from anaerobic digestion come from anaerobic decay (Kaparaju & Angelidaki, 2008). The production of methane rich biogas depends on the types of raw materials used. Cow dung, chicken manure, tofu liquid waste, and cabbage waste were the raw materials in this research. The results show that the presence of cabbage waste decreases the quantity of biogas produced. In the future, as a substitute for cabbage, microalgae can be utilized to produce biogas by an anaerobic digestion process (Ichsan *et al,* 2014). Microalgae is suitable for anaerobic digestion which can be mixed with liquid tofu waste for better results. However, the biogas obtained in this study can be

improved by removing the H_2S content. The liquid waste was introduced into anaerobic digesters, while anaerobic bacteria converted the liquid containing organic compounds into biogas. The produced biogas still contains impurities such as H_2S and CO_2. The H_2S in biogas may cause engine corrosion via emission of SO_2 from combustion, especially when the engine is not operated continuously (Wellinger & Linberg, 2000). Many attempts to remove H_2S content have been proposed, such as biological conversion with air/oxygen, commercial biological process, and adsorption on solids such as activated carbon, iron hydroxide or oxide. Most of them need efficiency improvement due to high capital, energy and media costs. In the future, to upgrade the results so that the biomethane can be applied efficiently. It can use biofiltration, membrane, or activated carbon to eliminate the H_2S (Budiyono and Syaichurrozi, 2020). Also, it is necessary to analyze the pretreatment of raw material. Pretreatments are needed to accelerate the hydrolysis process, so the number of methane yields produced can be increased. It is also important to analyze the effect of factors that influencing the enhancement of biogas such as organic loading rate (OLR), initial volatile solids concentration, pH, temperature, and hydraulic retention time (HRT) in the biogas produced. The rate of organic load same as the number of organic contents that are fed into the biogas digester and it was calculated in each day per unit size of biogas digester. It is also the same with the sum of BOD or COD demand used in the digester every day. The OLR is affected by the types of raw materials, temperature, and type of biogas digester that can affect the OLR results (Tchobanoglous *et al.,* 2003). The pH shows if any substance is acid, neutral, or alkaline. The optimum pH to produce biogas is between 5.5 and 6.5. The pH value is important to maintain the systems working in the equilibrium phase (Rittmann BE & McCarty,2001). Temperature is a crucial and important parameter that influences biogas formation. The temperature between 0-97°C can produce methane gas, even if the microorganism has different optimum temperature ranges to live. Another parameter that affected biogas production is hydraulic retention time (HRT) (Haryanto *et al*, 2018). HRT is the total time for the substrate to remain in the biogas digester before disposal. The process of deterioration of biomass needs 10-30 days for the biogas production (Taghinazhad *et al* 2017). HRT was affected by substrate, condition/ environment, and the type of digester (Fulford, 2001). Further analysis of H_2S content can be conducted to determine the best method for H_2S removal from the biogas produced. For anaerobic digestion can also be improved using two-phase anaerobic digestion systems. This research used a traditional digester with a single phase anaerobic digestion system. It can be improved by using a two-phase anaerobic digestion system. A two-phase anaerobic digestion system will use the first for the hydrolysis and acidogenesis phases, while the second reactor/digester utilize methanogenesis phase.

4. Conclusion

Biogas production by mixing a starter with animal manure (cow dung and animal manure), and tofu waste in a form of liquid can increase biogas production. The combination that produced the highest biogas amount was 200 mg starter with 70% percentage of cow dung, 15% percentage of chicken manure, and tofu liquid waste with a percentage of 15%. A larger amount of cabbage waste will reduce the biogas production amount. The quadratic regression analysis and kinetics model based on the Gompertz equation was obtained for A variable with the highest yield, which has a comparison of 70% cow dung, 15% chicken manure, and 15% tofu liquid waste. From the kinetics model based on Gompertz equations, the value of $P\infty$ was 2,795.142 mL/gr.Ts, Rm was 113, 983.77 mL/gr.Ts, and t was 10.22 days. The results concluded that tofu liquid waste was more effective for producing a larger amount of biogas rather than cabbage waste. Moreover, this study successfully showed a significant development in biogas production by adding organic waste to animal manure as the substrate used.

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