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Effects of Comparison of Feed Composition, pH, and Preliminary Treatment of Biogas Production from Cow Blood Waste and Molasses

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Abstract - *Energy problems in Indonesia are issues that are not easy to solve. If the energy needs dominated by BBM continue to increase without any changes in the pattern of energy use, then Indonesia's sustainability and energy security will be disrupted. Therefore, Indonesia really needs alternative energy. Biogas is an alternative energy produced from the anaerobic degradation of organic compounds and can be a substitute for natural gas and fossil fuels. Cow's blood which is a waste from slaughterhouses can be used as a substrate for anaerobic biogas production by rumen and molasses. The objectives of this study are (i) To examine the comparison of the concentration of blood and molasses added to the volume of biogas produced, (ii) To examine the effect of pasteurization on blood on the volume of biogas produced, (iii) To examine the effect of initial system pH regulation on the volume of biogas produced, (iv) Assessing the pH setting and without adjusting the pH after measuring the volume of biogas produced. This research was conducted by making variations in the composition of feeds, pH stabilization, and blood pasteurization. The process of biogas formation is carried out for 40 days at room temperature with the response of quantitative results in the form of biogas volume every 2 days. Biogas production in cow's blood gets the best results at C/N 30, using pH 8. Pretreatment of blood pasteurization and pH stabilization also shows the best biogas results.*

Keywords *– Biogas, cow's blood, pasteurization, pH*

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1. Introduction

People in the world need energy, which is used to drive various tools that support human life. In 2014, world energy consumption rose to around 13 billion tons of oil, increasing by 22% and 54% respectively compared to 2004 and 1994. High demand accelerates the exploitation of energy resources from the natural environment, and ultimately bringing Severe challenges are energy scarcity and climate change (G. Q. Chen & Wu, 2017).

Indonesia's energy needs from year to year increase along with economic growth and the population of Indonesia. The increasing average of energy demand per year is 36 million barrels of oil equivalent (BOE) from 2000 to 2014. While non-renewable energy sources, such as petroleum, natural gas, and coal are running low (Sa'adah et al., 2017). Energy use in Indonesia still uses fossil-based energy, especially petroleum and coal fuels. If in the near future no significant new energy sources are found in 2046, it is feared that Indonesia will get an energy deficit (Jaelani et al., 2017). Internationally, carbon dioxide emissions from fossil fuel sources are increasing, contributing to global warming and climate change. Countries that sign on the 'Paris Agreement' in 2015 in the United Nations Work Agreement on Climate Change are committed to reducing the level of emissions for global security to below 2 ° C (Ralph & Hancock, 2019).

The use of renewable energy must be the main concern of the Indonesian government not only to reduce the use of fossil energy but also to establish clean or environmentally friendly energy (Jaelani et al., 2017). At present, energy supply and environmental pollution are the two most important problems of human societies throughout the world (Nosratpour et al., 2018). Energy is considered as a key factor for economic growth, a key driver for society and civilization, and an important driver of social development. The energy needed for several industrial purposes, transportation, housing facilities, and services (Azzuni & Breyer, 2018).

Indonesia is an agricultural country with the potential for animal husbandry as the spearhead of finance. The potential thing is to fill up the needs of meat both locally and abroad through animal husbandry. One commodity that can be developed is a cow commodity. In the last five years (2013-2017) the number of dairy cows and buffalo population increased quite high by 6.95%. National beef/buffalo production in 2017 was 564.02 thousand tons, an increase of 2.48% compared to 2016 which was 550.39 thousand tons. The increase in meat production in 2017 compared to 2016 is in line with the increase in cattle/buffalo slaughter rate received by 0.78%. Cattle slaughter rate in 2016 was 2,278,033, and increased in 2017 by 2,295,722 (Ministry of Agriculture, 2017).

Livestock waste is waste from livestock business activities such as livestock business, slaughterhouses, and animal product processing. The waste contains solid and liquid waste such as feces, urine, food scraps, embryos, eggshells, fat, blood, feathers, nails, bones, horns, and rumen contents (Gamayanti et al., 2012). The rumen is one of four gastric sections in ruminants, and rumen fluid contains a complex anaerobic microbe consisting of 1010-11 cells/ml of bacteria, 103-5 cells/ml of fungi, and 104-6 cells/ml of protozoa. Its microorganisms produce various enzymes to digest lignocellulose, including glycoside hydrolase and reserve esterase (Takizawa et al., 2018). But on the other hand, rumen fluid contains high levels of ammonia and phosphorus, thereby reducing the slaughterhouse causing pollution of the environment. This rumen fluid causes eutrophication when transferred to soil and airways. Therefore, it is important to find alternative uses of rumen fluid from slaughterhouses to prevent environmental pollution (Sarteshnizi et al., 2018). In general, animal blood is liquid waste, obtained after slaughter. The blood is one of the main sources of organic waste (80 gL - 1 of Total Organic Carbon (TOC); 300 gL - 1 of Chemical Oxygen Demand (COD)) and protein waste (100 - 200 gL - 1 of protein). Organic carbon content contained in blood containing blood has a great potential to be used as a raw material in the process of making biogas (Langone et al., 2019).

In the cane sugar industry, besides producing cane sugar, molasses are also produced which are by-products during the sugar whitening process. In some sugar factories, molasses are exported abroad at relatively low prices, in many places, this waste is of little use and is often a problem of environmental pollution because molasses contain calcium oxide which can reduce soil oxygen content. Molasses contains nutrients high enough for the needs of bacteria, so it is used as an alternative material as a source of carbon in the fermentation media. Molasses contains a lot of sugars and organic acids (Fifendy et al., 2013).

Biogas is renewable energy in the form of gas produced from organic materials through the fermentation process in biodigesters. Materials that can be used for biogas production are organic matter in the form of vegetable waste, fruit waste, household waste, restaurant waste, and livestock manure (Schlüter et al., 2008). Currently, biogas is not only used as fuel for cooking but is also used to generate electricity (Kadam & Panwar, 2017). By-products from biogas can also be used as fertilizer (Koszel & Lorencowicz, 2015). The biogas component consists of 50-70% methane, 30-40% carbon dioxide, and a small number of other gases such as nitrogen, hydrogen, and oxygen (Schlüter et al., 2008). Biogas production from biomass raw materials has been carried out since several decades ago, such as in India which has been producing biogas since the 1950s (Divya et al., 2015).

One of the processes of making biogas is the anaerobic digestion process. Anaerobic digestion is recommended as a sustainable and environmentally friendly method for converting high organic waste into renewable energy. This process can be divided into four phases: the first three phases include hydrolysis, acidogenesis, acetylation, and methanogenesis (Jin et al., 2018). To produce good biogas requires a high-quality substrate that contains biodegradable organic material with a C/N ratio of 15-35 (Łochyńska & Frankowski, 2018). Ideally, the substrate should have a high carbon fraction that is biodegradable (Moset et al., 2018).

Seeing the potential and threats of the rumen and blood, the negative impacts must be prevented by utilizing rumen waste and cow blood by processing the waste into biogas. Therefore, making biogas with anaerobic digestion method from the rumen and blood as an effort to reduce waste and alternative energy sources can be developed for the benefit of the community.

2. Materials and Methods

The research method used to achieve the objectives of this study was by conducting laboratory-scale experiments conducted at the Chemical Engineering Waste Treatment Laboratory, Faculty of Engineering, Diponegoro University. The process and method of processing are carried out using the anaerobic digestion method in a mesophilic state which is the ambient temperature (25-35^{o}C) using feed intake with a batch system. The materials used are rumen and cow blood from the Semarang Penggaron Slaughterhouse, molasses, NaOH, HCl, and Water. The tools used are plastic bottles (biodigester), beaker glass, stirrers, plastic hoses, water reservoirs, pH indicators, valves, measuring cups, glue guns, filters, porcelain evaporating dish, measuring flask. The research design can be seen in Figure 2.

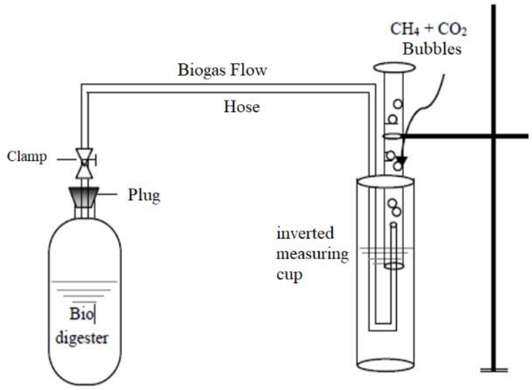

Figure 1. Design of Research Tools

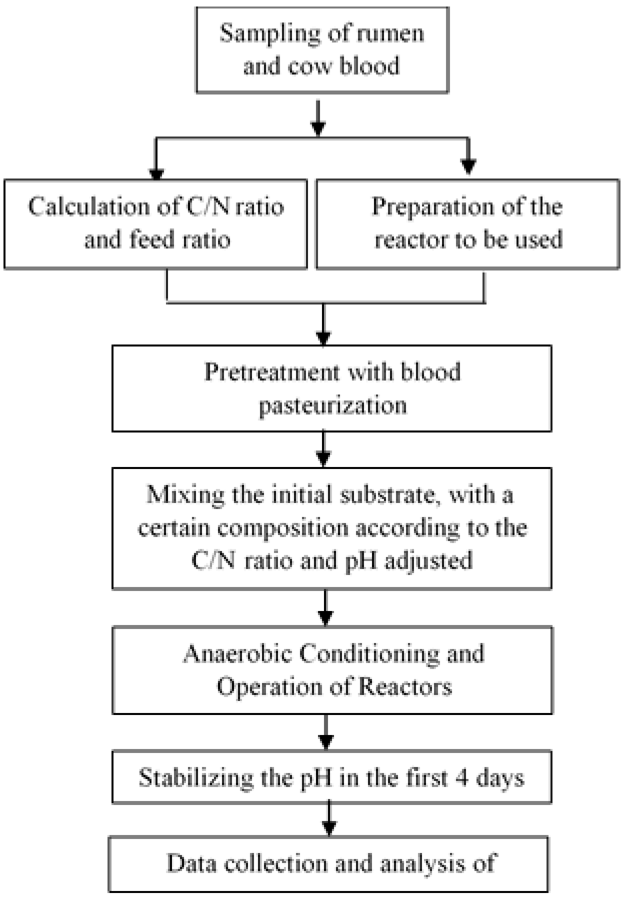

Figure 2. Research Design

Control Variable

1. Operating conditions (batch)
2. Time of observation (for 40 days)
3. Ambient temperature (25-35^{o}C) and atmospheric pressure (1 atm)
4. Reactor volume 600 ml
5. Comparison of blood with rumen 1: 1

Independent variable

1. Pretreatment by pasteurizing the blood by heating it at 70^{o}C for 60 minutes
2. Pretreatment by stabilizing the pH in the first 4 days
3. Comparison of initial pH of feed (6, 7, and 8)
4. C/N ratio (25, 28, and 30)

Dependent variable

The response observed from this study was the total biogas volume once every 2 days for 40 days

Preparation phase

The tool is prepared and designed according to the research variables. Take cow's rumen, cow's blood, and molasses in a proportion according to the variable then mixed and put in a container.

Data Collection and Analysis Phase

Biogas volume data is analyzed in the form of graphs of the relationship of biogas volume to time. Graphical data analysis and theoretical phenomena that occur on the influence of pretreatment, pH differences and CN feed comparison to the results obtained on the graph of research results

3. Result and Discussion

The Effect of Feed Composition on the Volume of Biogas Produced

In this study, the feed consisted of 120 ml of blood, 360 ml of molasses, and 120 ml of rumen had a C/N value of 25. The feed consisted of 85 ml of blood, 430 ml of molasses, and 85 ml of rumen had a value of C/N 28. While the feed consisted of 65 ml of blood, 470 ml of molasses and 65 ml of rumen had a C/N value of 30. Figure 3. and Figure 4. can be seen in the comparison of biogas produced between the C / ratio N 25, C/N 28, and C/N 30.

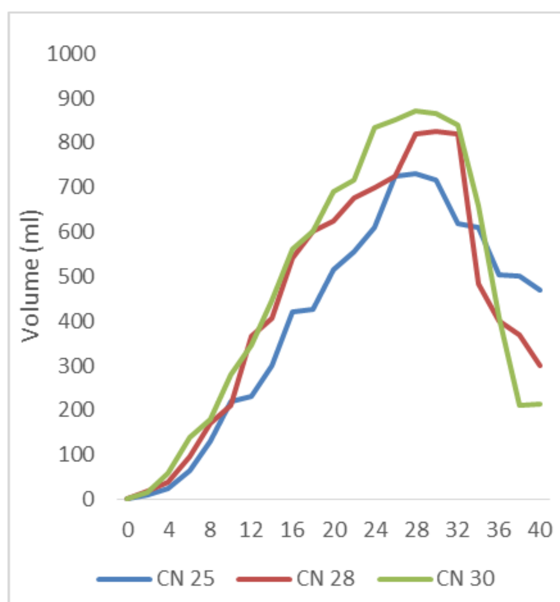

Figure 3. Daily biogas production with C/N values of 25, 28 and 30 feeds on the volume of biogas produced

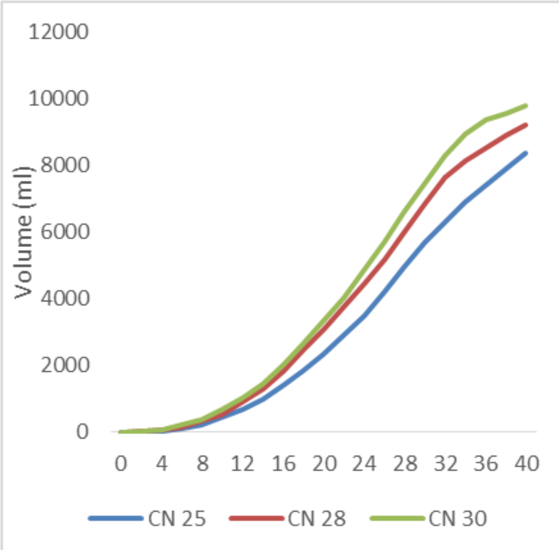

Figure 4. Accumulation of daily biogas production with C/N values of feed 25, 28, and 30 of the volume of biogas produced

In Figure 3. and Figure 4. it can be seen that the biogas yields produced with CN 25, 28, and 30 tend to increase until the 30th day. Feed with a C/N ratio of 25 produce biogas with a total biogas volume of 8380 ml. While feed with a C/N ratio of 28 produces biogas with a total volume of biogas of 9190 ml. A feed with a C/N 30 ratio produces biogas with a total biogas volume of 9785ml.

C-N ratio is the ratio of carbon content (C) and Nitrogen content (N) in material units. All living things are made of large amounts of Carbon (C) and Nitrogen (N) material in small amounts. For the anaerobic digestion process to run smoothly, the nutrient elements needed by microbes must be available in a balanced manner (Wiratmana et al., 2012). Therefor the C/N ratio of the substrate is very important. According to (Yan et al., 2015), the recommended CN ratio is 30. If the C/N ratio is too high (lots of C and not much N), metabolism becomes inadequate which means that there is carbon in the substrate not fully converted, so it doesn't maximum methane yield will be achieved. According to (Dioha et al., 2013), carbon content will increase carbon dioxide formation and lower pH values thereby reducing the quality of biogas produced and according to (Wiratmana et al., 2012), too high carbon content can also cause microbial development longer gas forming. In the opposite case, a nitrogen surplus can cause the formation of excessive amounts of ammonia (NH_3), which even in low concentrations will inhibit bacterial growth and the worst risk can cause the entire population of microorganisms to die (Zulkarnaen et al., 2018). And also according to (Yan et al., 2015), a lower C/N ratio causes ammonia accumulation and a pH value exceeding 8.5 which are both toxic to methanogenic bacteria.

The Effect of Pasteurization Pretreatment on the Volume of Biogas Produced

Research at this stage aims to study the effect of pretreatment by pasteurizing the volume of biogas produced. At this stage comparing feeds that have a C/N value of 30 which are carried out pasteurization process and without pasteurization in cow blood. In Figure 5. and Figure 6. can be seen the comparison of biogas produced between the ratio of feed that has a C/N value of 30 which is carried out the pasteurization process and without pasteurization.

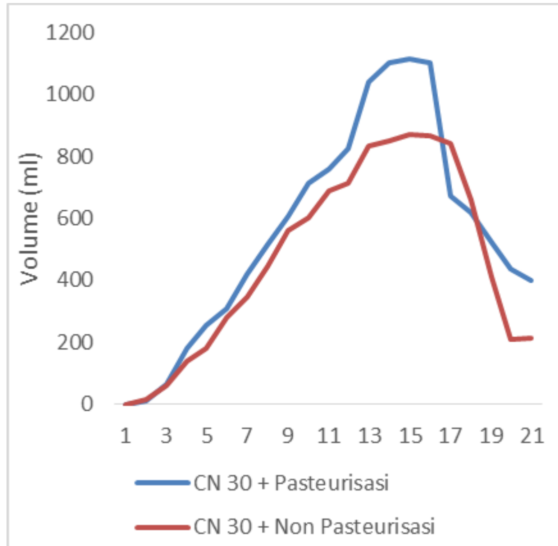

Figure 5. Daily biogas production with a C/N value of 30 feeds with pasteurization and without pasteurization on the volume of biogas produced

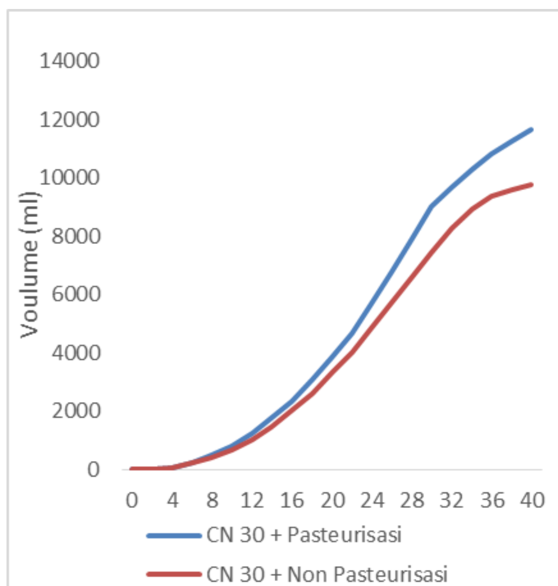

Figure 6. Accumulation of daily biogas production with C/N 30 feed through pasteurization and without pasteurization of the volume of biogas produced

Figure 5. shows the total biogas production with a C/N value of feed 30 through the pasteurization process and

without the pasteurization process. Feeds that go through the pasteurization process produce biogas with a total biogas volume of 11665 ml. While the feed without going through the pasteurization process produces biogas with a total volume of biogas of 9785 ml.

This experiment is done by comparing pasteurized raw materials and without the pasteurization process produces different biogas production yields. In the biogas raw material which is pasteurized, the yield gives a greater yield compared to the biogas raw material which is not carried out in the pasteurization process. Pasteurization is a heat treatment that is repeated or periodically given to a raw material by reaching temperatures below the boiling point with the aim of sterilizing a raw material (Rahardjo, 2010). Pasteurization can be carried out in various raw material processing processes for various products. In the research that has been done, the raw material for cow's blood is pasteurized at 70°C for 60 minutes. The pasteurization process in cattle blood aims to deactivate pathogens in the blood that have the potential to become inhibitors in the anaerobic digestion process (Langone et al., 2019).

The Effect of Initial pH on the Volume of Biogas Produced

Research at this stage aims to study the effect of the initial pH of the feed on the volume of biogas produced. At this stage comparing feeds that have a C/N value of 30 through pasteurization with pH 6, pH 7, and pH 8. In Figure 7. and Figure 8. it can be seen the comparison of biogas produced between the ratio of feeds that have a C/N value of 30 pasteurization with pH 6, pH 7, and pH 8.

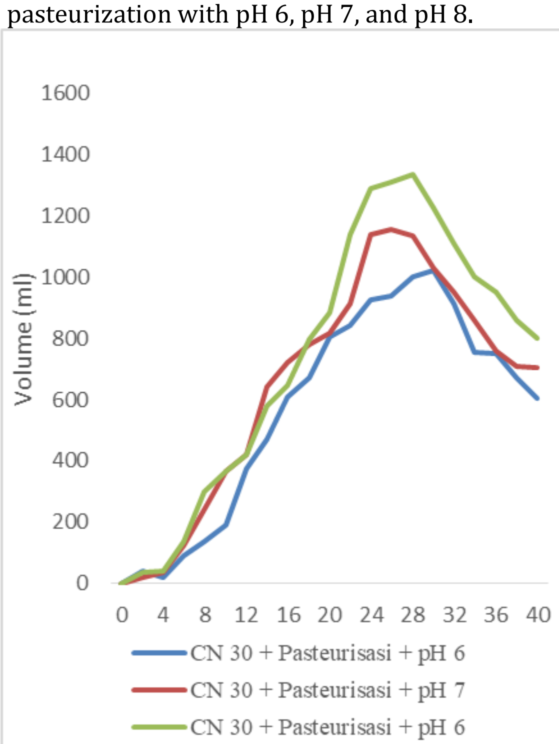

Figure 7. Daily biogas production with 30 pasteurized C/N feeds with pH 6, 7, and 8 on the volume of biogas produced

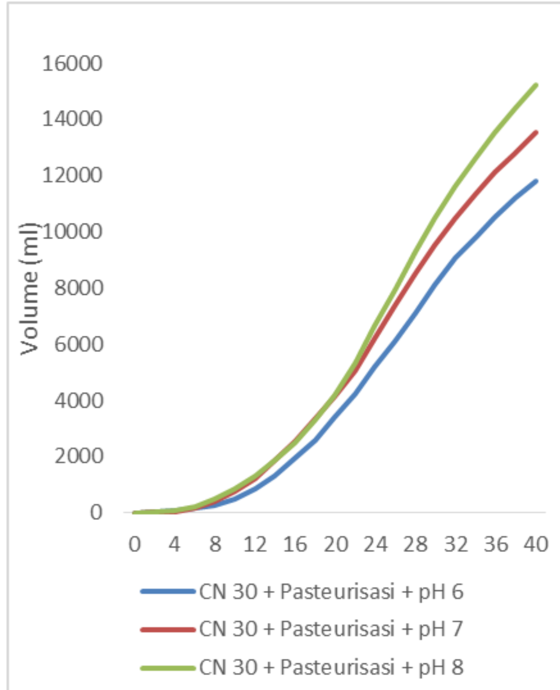

Figure 8. Accumulation of daily biogas production with pasteurized 30 C/N feeds with pH 6, 7, and 8 on the volume of biogas produced

Figures 7. and 8. show the results of biogas production with C/N 30 feed pasteurized with pH 6, 7, and 8. A feed with pH 6 produces biogas with a total biogas volume of 11825 ml. Whereas feed with pH 7 produces biogas with a total biogas volume of 13520 ml. A feed with a pH of 8 produces biogas with a total volume of biogas of 15220 ml.

The degree of acidity is an expression of the concentration of hydrogen ions, [H+] whose magnitude is expressed in minus the logarithm of the concentration of hydrogen ions, namely:

$$pH = -\log [H^+].$$

The degree of acidity (pH) has a strong influence on the work of the microbes present in the digester. The amount of biogas produced from the anaerobic digestion process is very sensitive to the pH value of the feeds. PH conditions that are too acidic or too basic greatly affect the work of these microbes.

At low pH, the decomposition of organic matter is carried out by bacteria that can live at low pH and the resulting decomposition is not optimal because the bacteria work well in neutral pH conditions. If the pH value is below 6.5, the activity of methanogenic bacteria will decrease and if the pH value is below 5.0, the fermentation will stop (Yani & Darwis, 1990). If the fermentation process takes place under normal and anaerobic conditions, the pH will automatically range between 7 - 8.5 (Lee et al., 2009; Liu et al., 2008). If the pH is higher than 8.5 it will have a negative effect on the population of methanogenic bacteria, so that it will affect the rate of biogas formation in the reactor.

In the biogas production process, anaerobic digestion goes through several stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In the acidogenesis stage, acid formation occurs from simple compounds. At this stage, small molecules produced from hydrolysis are converted by acid-forming bacteria (acidogenic) into a mixture of volatile fatty acids (VFA) such as propionic acid, butyric acid, acetic acid, hydrogen, and carbon dioxide. Acidogenesis is usually the fastest step in the anaerobic conversion of complex organic matter in the digestion of the liquid phase (L. Chen & Neibling, 2014).

$$C_6H_{12}O_6 \longleftrightarrow 2CH_3CH_2OH + 2CO_2$$
$$C_6H_{12}O_6 + 2H_2 \longleftrightarrow 2CH_3CH_2COOH + 2H_2O$$
$$C_6H_{12}O_6 \longleftrightarrow 3CH_3COOH$$

In the acetogenesis stage, acetogenic bacteria convert volatile fatty acids into acetate, CO2, and hydrogen. Organisms that convert substances such as propionate, butyrate, lactate and ethanol into acetate are acetogenic bacteria. Excess hydrogen often causes an increase in longer chain VFA and a decrease in pH so that it inhibits acetogenesis (David Broughton, 2009). This is the reactions that occur at the acidogenesis stage:

$$CH_3CH_2COO^- + 3H_2O \longleftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$$
$$C_6H_{12}O_6 + 2H_2O \longleftrightarrow 2CH_3COOH + 2CO_2 + 4H_2$$
$$CH_3CH_2OH + 2H_2O \longleftrightarrow CH_3COO^- + 3H_2 + H^+$$

In both stages it allows the pH change to be lower. Low pH changes can cause the condition of the methanogen microbes to not work properly. Thus, feed with a pH of 6 allows changes in pH to the lowest compared to feed 7 and 8. This causes a feed with a pH of 6 to produce the lowest biogas compared to variables 7 and 8.

The Effect of pH Stabilization on the Volume of Biogas Produced

The research at this stage aims to study the effect of stabilizing the feed pH during the fermentation process on the volume of biogas produced. At this stage comparing feeds that have a C/N value of 30 through pasteurization with pH 6, pH 7, and pH 8 are stabilized in the first 4 days. In Figure 4.7 and Figure 4.8 we can see the comparison of biogas produced between the ratio of the feed which has a pasteurization C/N value of 30 with pH 6, pH 7, and pH 8.

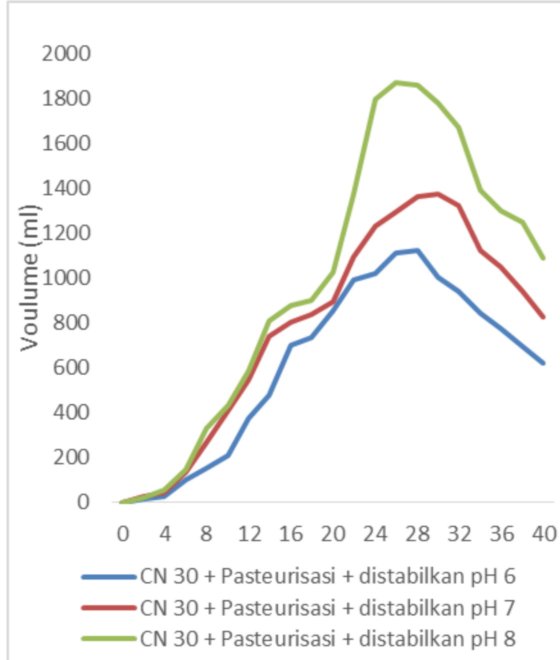

Figure 9. Daily biogas production with pasteurized 30 C/N feed with a stable pH of 6, 7, and 8 on the volume of biogas produced

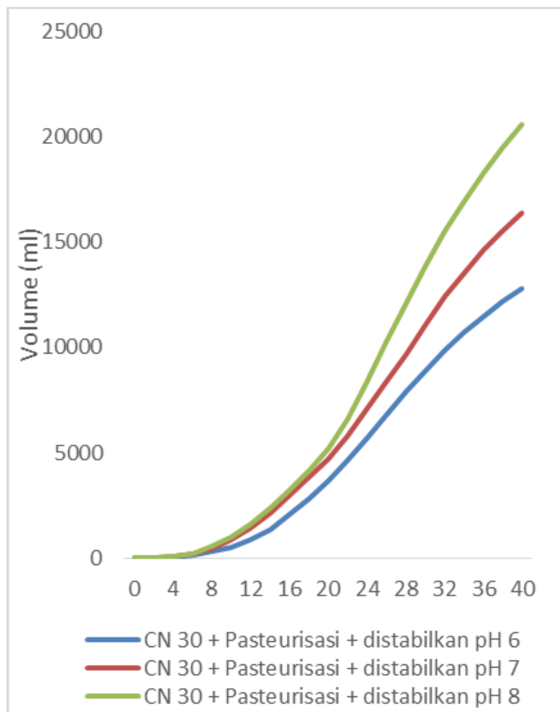

Figure 10. Accumulation of daily biogas production with pasteurized 30 C/N feed with a stable pH of 6, 7, and 8 on the volume of biogas produced

Figure 3.7 and 3.8 show the production of biogas with C/N 30 feed pasteurized with a stable pH of 6, 7, and 8. Feed with a pH of 6 produces biogas with a total biogas volume of 12785 ml. While the feed with a pH of 7 produces biogas with a total volume of biogas of 16350 ml. Feed with pH 8 produces biogas with a total volume of biogas of 20590 ml.

The degree of acidity is an expression of the concentration of hydrogen ions, [H+] whose magnitude is expressed in minus the logarithm of the concentration of hydrogen ions, namely:

$$pH = - \log [H^+].$$

The degree of acidity (pH) has a strong influence on the work of the microbes present in the digester. The amount of biogas produced from the anaerobic digestion process is very sensitive to the pH value of the feed. pH conditions that are too acidic or too basic greatly affect the work of these microbes.

At low pH, the decomposition of organic matter is carried out by bacteria that can live at low pH and the resulting decomposition is not optimal because the bacteria work well in neutral pH conditions. If the pH value is below 6.5, the activity of methanogenic bacteria will decrease and if the pH value is below 5.0, the fermentation will stop (Yani & Darwis, 1990). If the fermentation process takes place under normal and anaerobic conditions, the pH will automatically range between 7 - 8.5 (Lee et al., 2009; Liu et al., 2008). If the pH is higher than 8.5 it will have a negative effect on the population of methanogenic bacteria, so that it will affect the rate of biogas formation in the reactor.

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In both stages it allows the pH change to be lower. Low pH changes can cause the condition of the methanogen microbes to not work properly.

A reaction system with an uncontrolled pH produces less biogas. This may occur because the organic acids produced have a large amount and produce severe acidification. Montañés et al. (2014) illustrate that low pH will stimulate acidogenic activity and inhibit methanogenic activity. However, pH higher than 8 is also not suitable for methanogen growth, which will inhibit methanogenic activity and produce less biogas.

Yang et al. (2015) conducted a study that the pH of the reaction system in the process of anaerobic digestion to suit the conditions of methanogenic bacteria with a pH adjustment mode. Organic acids that are accumulated will be more utilized to produce biogas. In addition, pH adjustment in the anaerobic digestion process can increase the activity of enzymes of microorganisms, which are beneficial for the hydrolysis of nutrients from the substrate, and methanogenic processes can be increased.

Liu et al. (2008) and Lee et al. (2009) states that pH 7 and 8 are suitable conditions for producing biogas in the anaerobic digestion process. Whereas Westerholm et al. (2011) said that the anaerobic digestion process will remain stable and the methane yield will remain normal at a pH value of 7.9. In studies that have been conducted, feed with a stabilized pH 8 has a greater yield compared to feeds that have a pH of 6 and 7.

4. Conclusion

Biogas production in cow blood added with molasses gives optimum results when the C/N value is 30. Giving pretreatment pasteurization in cow blood gives optimum biogas results than when cow blood is not pasteurized. Biogas production in cow blood added with molasses gives optimum results when the pH value is 8. The provision of pH stabilization treatment in the first four days of the digester gives optimum biogas results than when the digester does not do pH stabilization. Continuous research needs to be done to determine the length of fermentation in biogas production, test the gas content produced to determine the methane gas content and other gases, test the fuel value of the biogas produced.

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