Dilution of Biogas Bioactivator to enhance microbial growth

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Abstract. The addition of bioactivator to biogas unit is a breakthrough to accelerate and increase the formation of gas. Bioactivator are made from fruit waste that is commonly found in local markets, including bananas, papaya and pineapples. This study aims to find the level of fruit waste dilution to obtain consistency that supports an ideal environment for microbial growth in biogas unit. Fifteen treatments on 15 plastic containers contain fruit waste with a dilution rate of 1, 2 and 3 times with a curing period of 25 days. The parameters were bioactivators colour and smell, pH, and microbial population of bacteria and fungi. The study result was that treatment with dilution rate of 3 times gave better result than other treatments. Bioactivator colour was yellow to orange with tapai/alcohol odour. The pH varies from 3.1 to 4.2. The largest microbial population were as follows bacteria 9.45x10^7 cfu/ml and fungi 12.32x10^5 cfu/ml.

1. Introduction
Biogas is an alternative energy that has multiple benefits, among others as an energy source for household cooking and for roasting coffee and organic fertilizer sources in rural areas [1], besides that it is also an energy source for cooking pork food such as that found on Samosir Island [2] Biogas is also used for heating on growing phase of chicks [3]. In China, biogas that utilizes chicken manure can contribute up to 20% of natural gas [4].

In rural Indonesia, generally, in the process of producing biogas used manure livestock, generally cow manure. There are supporting materials that can help microbial growth in biogas, such as molasses and urine. Both of these materials principally provide nutrients for microbes. However, there is other material that also could be used to support the growth of microbial in the process of biogas formation, for example bioactivator. Bioactivator is biologically active ingredient used to increase the activity of biological processes. Bioactivator is substance that contain effective microorganisms that can actively help decompose organic matter.

In this study, bioactivator is utilized, which is processed from fruit waste that is commonly found in rural areas. It is known that fruit waste contains simple sugar/sucrose, which microbial is needed in its metabolism as an energy source [5]. Bioactivator from fruit wastes also contain microbes, which will assist in the process of overhauling biogas inputs such as cow manure. [6] stated that in the bioactivator of fruit waste 4 types of bacteria were found, namely Clavibacter, Agrobacterium, Clostridium and Pseudomonas fluorescents. [7] stated that in research on bioactivator, several bacteria were found include Enterobacter sp and Bacillus sp.

Organic matter such as cow manure contains 15-60% cellulose, hemicellulose 10-30% and lignin 15-30% [8]. Therefore, it is assumed that bioactivators from fruit waste contain microbes such as...
bacteria could help to remodel organic matter and help speed up the decomposition process while accelerating biogas formation.

Fruit wastes contain different levels of water. [9] banana water content is 63% while papaya is 76% [10] and pineapple is 90% [11]. Therefore, in making bioactivator dilution is necessary in order to obtain ideal consistency. This is because there is a basic need for microorganisms, especially bacteria, for a normal life, depending on organic matter/consistency. To obtain ideal consistency, a dilution is carried out. Dilution of organic material which generally contains high water content, usually requires dilution of 1: 1, if the water content is less, more dilution is needed, for example 1: 1.25 or 1: 2 [12].

[13] found that bioactivators using tofu waste with 1: 2 dilution produced a consistency that supported larger bacteria's growth. This study aims to find the best dilution level that supports microbial growth in bioactivator from banana, papaya, and pineapple waste.

2. Materials and methods
This research was conducted in the Animal Production laboratory. Fifteen treatments in plastic containers were filled with banana, papaya and pineapple waste in the same percentage as much as 4 kg. Dilution rate were 1, 2 and 3 times. The parameters were the bioactivator solution's characteristics, including colour and odour, pH, and population of bacteria and fungi. The design used was a Completely Randomized Design because the research units used were the same. The research treatments were as follows:
T1 (fruit waste with 1x dilution) T2 (fruit waste with 2x dilution) T3 (fruit waste with 3x dilution)
Curing time was 25 days and stirring is every day. In this study, there were five replications. Microbial was identified and counted on the Soil Microbiology Laboratory of Agriculture Faculty, North Sumatra University and used Plate Count Method. The unit of microbial was colony forming unit / ml (CFU / ml).

3. Results and discussion
3.1. Characteristics of bioactivator solutions: Colour and odour
In the treatment of T1 to T3 the colour of the bioactivator was yellow and yellow to orange with a fragrant aroma like tapai / alcohol. There are white moulds on the surface of the bioactivator. The colours and odours of the bioactivator are detailed in Table 1.

| Treatments | Colour         | Odours          |
|------------|----------------|-----------------|
| T1         | Yellow to orange | Tapai/Alcohol   |
| T2         | Yellow to orange | Tapai/Alcohol   |
| T3         | Yellow          | Tapai/Alcohol   |

The colour of the bioactivator was influenced by the colour of the basic ingredients of the bioactivator namely banana, papaya and pineapple. At 1-time dilution, the colour of bioactivator was yellow to orange, so was 2 times dilution. However, a larger dilution of 3 times caused the colour concentration to change to yellow. Bioactivator aroma like tapai / alcohol. This was due to the microbial in the bioactivator such as the fungus breaks down sucrose, glucose and fructose becomes ethanol [14]. The colour of the bioactivator which was brown was found by [6] due to the basic ingredients of the bioactivator namely golden snails, banana weevil and fruit waste at a balanced percentage. [13] found a yellow colour in bioactivators derived from tofu pulp and cow urine.
3.2. pH of Bioactivator

Fruits have a variety of pH, such as papaya and pineapple are alkaline fruits because they have a pH of around 8.5, while bananas are acidic because they have a pH of around 5. In this study, the mixture of the three has a basic pH, but in line with the decomposition process, many acids were produced, such as lactate, so that the pH turns to acid, which was around 4.

The condition of the pH of the media is very influential in the types of microbes that grow. This is consistent with what was found [15] that pH is one factor that influences the rate of decomposition of organic matter. Microbes are very suitable for growing at neutral pH / 7 and will be excellent in environments containing many simple sugars/carbohydrates such as waste fruits rich in carbohydrates, especially in pineapple and bananas. Based on the pH of life, microbes are divided into 3 groups, namely asidofil microbes (microbes that can grow at pH ranges from 2.0 to 5.0); neurofil microbes (microbes that can grow at pH ranges from 5.5 to 8.0) and alkali fil microbes (microbes that can grow at pH ranges from 8.4 to 9.5) [8].

| Treatments | pH |
|------------|----|
| T1         | 3.1|
| T2         | 3.7|
| T3         | 4.2|

From Table 2 it is known that T3 had a higher pH than the others. This correlates with dilution 3 times so that the very acidic pH concentration was corrected.

3.3. Microbial population of Bioactivator from fruit waste: banana, papaya and pineapple

The highest total bacterial and fungal population found at T3 fell significantly different to T2 and significantly different from T1 due to differences in pH in the bioactivator solution due to different dilution rates. In this study, the amount of fruit waste prepared was the same at 4 kg with the percentage between the same type of fruit, banana, papaya and pineapple. As a result of the decomposition process lactic acid formation occurs which correlates to pH. However, the degree of dilution from the beginning has affected the pH due to the pH of the water and the volume of the dilution water. The pH of the mountain water used was 7.5.

| Parameters | Treatments | Average |
|------------|------------|---------|
| Total Bacterial (CFU/ml) | T1 | T2 | T3 | 6.06 x 10^3 |
| Total Fungal (CFU/ml) | 4.82 x 10^6a | 6.97 x 10^5a | 12.32 x 10^5b | 8.03 x 10^5 |

The degree of dilution also affects the viscosity of the bioactivator solution. The treatment of T1 with a one-time dilution caused the solution to become too thick and affect microbial metabolism. [16] stated many things that influence growth and ability to live from microbial. In the ecosystem, the competition takes place in addition to obtaining nutrients as well as competing in space, oxygen, and other essential substances.

Bacteria have optimum growth pH in the range of 6.5-7.5 while yeast or fungus in the range 4.5-5.5 [17]. The degree of acidity of the bioactivator is closely related to the production of organic acids by microbes especially lactic acid which can reduce the pH to 5.0 or less. Lactic acid is an acid which is classified as weak and can dissociate by releasing hydrogen ions. The release of hydrogen ions...
changes the balance of the solution so that the pH becomes low with low pH conditions lactic acid bacteria will dominate growth in the media. This is consistent with the results of the study that the bacterial population is greater than the fungal population.

4. Conclusions
Bioactivator from fruit waste needs to be diluted to meet microbial needs for growing. Dilution made biactivator has yellow to orange colour and tapi/alcohol odours. Three times dilution made bioactivator had better pH, which supports microbial growth as population of either bacteria or fungi higher than dilution of one and two times. Population of bacteria as much as 9.45 x 10^6 cfu/ml and fungi as much as 12.32x10^6 cfu/ml in bioactivator will help decomposition of organic matter on bio gas process.

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Acknowledgements
The author gratefully acknowledges that financial support from Universitas Sumatera Utara, Medan 2022 Indonesia under TALENTA Research Grant 2019 No. 1389/UN5.1.R/SK/PPM/2019: 29 March 2019.