Anaerobic digestion of solid and liquid organic waste with microorganism from manure

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Abstract. Biogas is a valuable byproduct of anaerobic digestion of organic compounds. Biogas production depends on various factors including pH, temperature, microbes, substrate, and C/N ratio. From anaerobic digestion product, we can obtain not only fertilizer but also Biogas that could be used for cooking, lighting, and electricity. This research aims to obtain biogas from solid waste (household waste) and liquid waste (sago processing waste) added microbe from cow rumen and municipal sediment. These research steps are (1) biogas production; (2) methane gas analysis. The result of this research indicates that methane gas is able to be generated from several variations of waste: bacteria mixture. The result showed that high biogas production from household wastes was obtaining at 7% (b/v) concentration of waste, 0.5% (b/v) urea, and ratio microbial of municipal sediment and rumen was 2:3 at 5% (b/v), the incubation period was 35 days. The total volume of biogas was resulted by 687 mL biogas/gram waste that consist(s) of 165.56 mL gas of CO2 and 521.44 mL gas of CH4 (75.89% (v/v)). Maximum biogas production is recorded from sago processing waste treatment (1 L) added with bacteria from cow rumen 18x1010 bacteria. Fermentation duration is 84 days. Total of biogas generated is 17,481 L consisting of 105.37 mL CO2 (0.60% v/v) and consisting of biogas without CO2 17,375.63 L (99% (v/v)).

1. Introduction

Due to declining energy inventories fossil fuel crisis, atmospheric pollution, and global warming have prompted researchers to conduct this research to find forms of renewable energy. Recently, studies on waste recovery and alternative energy sources have been popular in the scientific area. Many studies have argued that biogas production can be done using various types of organic waste and plants. Some alternative energy sources that can be used are various animal wastes such as cow dung, goat droppings or chicken manure. Remaining leftovers from restaurants and organic waste from various plants and fruits can also be used.

Various studies show that the biogas produced contains about 65 percent of methane at a temperature of 34 degrees [1]. Anaerobic biogas production depends on various factors, microbial diversity and abundance, which is influenced by the composition of the microbial community, which is involved in the fermentation process, is certainly very decisive, the influence of fermented ingredients, environmental conditions, pH and the design of the digester also cannot be ignored.

In various anaerobic digestion processes, organic compounds are broken down by the consortium of microorganisms in the absence of oxygen and digest these compounds to eliminate biogas which consists mainly of methane and carbon dioxide. Initially, anaerobic digestion is considered a two-stage process involving sequential action of acid-forming and methane-forming bacteria, but now known as...
the complex fermentation process carried by the symbiotic association of various types of bacteria. Products produced by one bacterial group function as substrates for the next group. Primary metabolic reactions can be sequentially classified into four major groups involving reactions: hydrolysis, acidogenesis, acetogenesis, methanogenesis [2-4]

In the initial steps of the anaerobic fermentation process, hydrolysis functions to reduce macromolecules (including proteins, complex fats, and polysaccharide) into their constituent, namely, amino acids, long chain fatty acids, and monosaccharide sugars. Other bacteria, including acidogenesis and acetogenesis, will further decompose them into smaller intermediate compounds (such as acetate, carbon dioxide, and hydrogen) [2-4].

In the next step, methanogenesis, a biological reaction where acetates are converted into methane and carbon dioxide, while hydrogen is consumed. Therefore, a thorough understanding of the composition, structure, and function of microbes in anaerobic reactors is very important to develop a fermentation strategy to increase the yield of methane from existing biogas reactors as well as new design ideas.

| Table 1. Biogas composition [1]. |
|-----------------|-----------------|-----------------|
| Substrate       | Chemical Formula| Percentage (%)  |
| Metane          | CH₄             | 50-70           |
| Carbondiokside  | CO₂             | 30-40           |
| Hydrogen        | H₂              | 5-10            |
| Nitrogen        | N₂              | 1-2             |
| Water (vapour)  | H₂O             | 0-3             |
| Hydrogen Sulfide| H₂S             | Sisa            |

Hydrolysis [2-4]

\[
\text{C}_6\text{H}_10\text{O}_4 + 2 \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_12\text{O}_6 + \text{H}_2
\]

Acidogenesis [2-4]

\[
\begin{align*}
\text{C}_6\text{H}_12\text{O}_6 & \rightarrow 2 \text{CH}_3\text{CH}_2\text{OH} + 2 \text{CO}_2 \\
\text{C}_6\text{H}_12\text{O}_6 & \rightarrow 2 \text{CH}_3\text{CH}_2\text{COOH} + 2 \text{H}_2\text{O} \\
\text{C}_6\text{H}_12\text{O}_6 & \rightarrow 3 \text{CH}_3\text{COOH}
\end{align*}
\]

Acetogenesis [2-4]

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{COOH} + 3 \text{H}_2\text{O} & \rightarrow \text{CH}_3\text{COO}^- + \text{H}_2 + \text{HCO}_3^- + 3 \text{H}_2 \\
\text{C}_6\text{H}_12\text{O}_6 + 2 \text{H}_2\text{O} & \rightarrow 2 \text{CH}_3\text{COOH} + 2 \text{CO}_2 + 4 \text{H}_2 \\
\text{CH}_3\text{CH}_2\text{OH} + 2 \text{H}_2\text{O} & \rightarrow \text{CH}_3\text{COO}^- + 3 \text{H}_2 + \text{H}_2\text{O}
\end{align*}
\]

Methanogenesis [2-4]

\[
\begin{align*}
\text{CH}_3\text{COOH} & \rightarrow \text{CH}_4 + \text{CO} \\
\text{CO}_3^- + 4 \text{H}_2 & \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O} \\
2 \text{CH}_3\text{CH}_2\text{OH} + \text{CO}_3^- & \rightarrow \text{CH}_4 + 2 \text{CH}_3\text{COOH}
\end{align*}
\]

2. Materials

2.1. Chemicals

Chemicals used in this study, using chemicals obtained by online purchases and based on the purity from Merck. Biogas fermentation substrate: sago processing waste (liquid waste) and household waste (solid waste). Microbes: cow rumen.
2.2. Methods
This study aims to obtain produced biogas from solid waste (household waste) and liquid waste (sago processing waste) by adding microbes from the cattle rumen and sewer water. The proximate analysis of the substrate was carried out to determine the carbon, nitrogen, pH, ash content and moisture content. Whereas contained bacteria were characterized by morphology and acting ability. The gas formed from the anaerobic digester is measured using a simple method at standard temperature and pressure. To obtain the fermented gas in a simple digester, it is done by varying the various concentrations of bacteria and substrate.

Table 2. Substrate composition biogas production.

| Experimental code | Waste                  | Volume (mL) |
|-------------------|------------------------|-------------|
|                   |                        | Cow Rumen   | Municipal sediment |
| Household waste   |                        |             |                    |
| A                 | 70 g waste + 880 mL aquadest | 25          | 25                 |
| B                 | 70 g waste + 880 mL aquadest | 10          | 40                 |
| C                 | 70 g waste + 880 mL aquadest | 30          | 20                 |
| Water Sago processing waste | 900 mL | 100          | 0                  |
| E                 | 800 mL                 | 200         | 0                  |
| F                 | 750 mL                 | 250         | 0                  |
| G                 | 700 mL                 | 300         | 0                  |

3. Results and Discussion

3.1. Composition waste
Household solid waste is a serious problem in big cities with high population density. Population increase is directly proportional to the increase in the amount of waste produced. Waste has become a problem because it requires land for landfills, management fees, and handling facilities. The types of household waste generally are (a) organic waste, or wet waste, which consists of leaves, wood, paper, cardboard, bones, livestock, vegetable, fruit, etc., and as (b) inorganic waste, or dry waste consisting of cans, plastics, iron and metals (Table 3).

Table 3. Composition of mixed household solid waste of Andunohu district (Kendari, Indonesia).

| No. | Constituents | Percentage (%) |
|-----|--------------|----------------|
| 1.  | Organic      | 45.8           |
| 2.  | Inorganic    | 24.42          |
| 3.  | Metal        | 6.10           |
| 4.  | Wood         | 9.16           |
| 5.  | Glass        | 6.10           |
| 6.  | Rubber       | 3.05           |
| 7.  | Stereiform   | 5.37           |

Organic waste can still be utilized, one of which is to be made into biogas, through an anaerobic fermentation process. This process involves anaerobic microorganisms. To be able to live microorganisms need nutrients, as an energy source and a source of nitrogen/protein. Energy sources for microorganisms come from chemical compounds (such as carbohydrates, fats) or sunlight. Protein/amino acid are a source of nitrogen. Minerals function to help the metabolic process. In addition, waste will be easily overgrown with microorganisms if it has high water content. Wastes are containing nutrients and high water is a good growth medium for microorganisms. Based on the
nutritional composition of household waste and sago processing liquid waste, fulfills the requirements as a growth medium for microorganisms (Table 4).

**Table 4.** Chemical composition of the Water Sago processing and Household solid waste of Kendari, Indonesia.

| Constituents    | Household solid waste | Water Sago Processing Waste |
|-----------------|------------------------|-----------------------------|
| Carbohydrate    | 21.50                  | 0.69                        |
| Protein         | 5.26                   | 3.69                        |
| Fatty           | 6.20                   | 0                           |
| Ash             | 5.26                   | 4.05                        |

The production process of sago flour begins with the feeling of sago trees which are considered worthy of harvest. After the tree has been raised, then the tree bark is peeled to take part in the soft stem. This part is shredded and extracted using water by squeezing or trampling it to get the starch out. The next water is stored in the tub for 1 day. After the starch settles, the extracting water is removed. In the extraction process, the waste is produced can be either solid or liquid. Solid waste in the extraction process in the form of squeezed residual fiber, while the waste liquid in the form of water used in the extraction process. According to [5], to produce 1 kg of sago flour will be produced around 20 L of wastewater. In general, liquid waste produced by the extraction process is only discarded. Sago extraction liquid waste still contains solids in the form of starch and fiber pulp from extraction so that if it is dumped into a river or land it will reduce the quality of the water and give negative impacts on the environment, such as foul odor. This condition is caused by waste is a good place for the growth of microorganisms.

### 3.2. Composition Microorganism

Microorganisms are able to decompose organic waste into simple organic compounds by converting it into the forms of carbon dioxide (CO$_2$), methane (CH$_4$), hydrogen (H$_2$) and hydrogen sulfide (H$_2$S), and water (H$_2$O) and energy for the growth and reproduction process [1]. In this study, to improve the characteristics of microorganism raw materials fermentation used (Table 5).

The fermentation process can be accelerated by adding a starter containing methane bacteria. Starters that can be used are known as three types, namely: a) Natural starter: if the source is from nature which is known to contain groups of methane bacteria such as activated sludge, old waste deposits, ruminants [6], b) Semi-artificial starter: if the source comes from a biogas tube that is expected to contain the methane bacteria in the active stage. c) Artificial starters: if the source is intentionally made, both with natural media and artificial media.

In ruminants such as cows have four abdominal compartments consisting of the rumen, reticulum, omasum, and abomasum. Among these four compartments, the rumen is the largest compartment and has a diverse microbial community consisting of bacteria (Table 5), archaean, protozoa, and fungi [7]. Rumen microbes have a very important role for livestock because they can utilize plant nutrients efficiently as a source of energy. These microbes are involved in initiating the conversion of plant feed polymers into monomers and leading to the formation of VFA (Volatile fatty acids). Then it will be converted into methane gas (Figure 1). Rumen microbes are anaerobic.
Table 5. Gram Staining and Observation of Bacterial in Rumen Cow and Municipal Sediment.

| Isolates code | Color of the colony | Form of cell | Gram coloring test |
|---------------|---------------------|--------------|--------------------|
| **From Rumen** |                     |              |                    |
| 1 0.1.R       | White round big     | Coccus       | Positive           |
| 2 0.2.R       | Red                 | Coccus       | Negative           |
| 3 0.3.R       | White               | Bacillus     | Negative           |
| 4 0.4.R       | White               | Spiral       | Negative           |
| 5 0.5.R       | Yellow dark         | Coccus       | Positive           |
| 6 0.6.R       | Bright red          | Bacillus     | Positive           |
| 7 0.7.R       | Yellow              | Bacillus     | Negative           |
| 8 0.8.R       | Yellow              | Coccus       | Negative           |
| 9 0.9.R       | White               | Coccus       | Positive           |
| 10 0.10.R     | Brown white         | Bacillus     | Positive           |
| 11 0.11.R     | White round bright  | Coccus       | Negative           |
| 12 0.12.R     | Small yellow round  | Coccus       | Negative           |
| 13 0.13.R     | Light brown         | Coccus       | Positive           |
| 14 0.14.R     | Small white round   | Coccus       | Positive           |
| **From Municipal sediment** |                     |              |                    |
| 1 0.1.S       | Big white round     | Coccus       | Positive           |
| 2 0.2.S       | Bright red          | Bacillus     | Positive           |
| 3 0.3.S       | Big white jagged    | Bacillus     | Negative           |
| 4 0.4.S       | White               | Coccus       | Negative           |
| 5 0.5.S       | Yellow              | Coccus       | Positive           |
| 6 0.6.S       | White               | Coccus       | Positive           |
| 7 0.7.S       | Dark yellow         | Coccus       | Positive           |
| 8 0.8.S       | White               | Coccus       | Positive           |
| 9 0.9.S       | Small round yellow  | Coccus       | Negative           |
| 10 0.10.S     | Small white round   | Bacillus     | Positive           |
| 11 0.11.S     | White light yellow  | Bacillus     | Negative           |
| 12 0.12.S     | Dark green          | Coccus       | Positive           |
| 13 0.13.S     | White               | Coccus       | Positive           |
| 14 0.14.S     | Brown round         | Coccus       | Positive           |
| 15 0.15.S     | Light pink          | Coccus       | Positive           |
Municipal sediments are materials found at the bottom of the water. There is a lot of organic material that becomes this sediment or dissolved material, such as the remnants of household waste, such as food scraps. In sewer deposits, there are microorganisms that have organic or bio-decomposer decomposition activities. These microorganisms generally have the ability to decompose fibers, lignin, starch, fat and protein from organic remains from dead tissue plants or animals, into simple molecules of glucose, amino acids, fatty acids and glycerol (Figure 1). Microorganisms found in Municipal sediments are not all good, there are some that may be pathogenic. The more nutrients available, the microorganisms will grow faster.

The liquid waste from sago processing, which is usually thrown into the river, causes a bad smell. Based on research by [8], indigenic bacteria found in sago processing wastewater is a group of lactic acid bacteria that have amylolytic activity. Therefore, this bacterium can hydrolyze starch into a simple molecule (glucose) (Figure 1).

### 3.3. Biogas production

The new paradigm views waste as a resource that has economic value and can be utilized, for example, energy, compost, fertilizer or for industrial raw materials. Organic waste is an important source for making biogas. From organic waste, combustible gas can be produced. Making biogas from organic or household waste can be a solution to reduce dependence on fossil fuels. Biogas can be produced through the fermentation process of organic waste by anaerobic bacteria that live without oxygen. Biogas composition is: methane by 60%, carbon dioxide 38%, and 2% are other gases such as \( \text{O}_2, \text{H}, \text{N}, \text{and H}_2\text{S} \). This biogas can burn like LPG, even on a large scale it can be used as a power plant.

The amount of energy produced by biogas is highly dependent on its main gas, namely methane gas. The higher the amount of methane gas, the higher the energy produced. By adding cattle rumen in organic waste, more methane gas produced in biogas. This is because in the cattle rumen there are methanogenic microorganisms [7].

Biogas is a gas produced by anaerobic activity or fermentation of organic materials including: household solid waste and biodegradable water sago processing wastes (Table 6).
Table 6 Biogas production.

| Experimental Code | Volume (mL) | Time incubation (days) | Total Gas Volume (L) | CH₄ (%) |
|-------------------|-------------|------------------------|----------------------|---------|
| Cow Rumen         | Municipal   |                        |                      |         |
| Household solid waste 7% + urea 5% |             |                        |                      |         |
| A                 | 25          | 25                     | 35                   | 0,146   | 63,00   |
| B                 | 10          | 40                     | 35                   | 0,611   | 65,00   |
| C                 | 30          | 20                     | 35                   | 0,916   | 75,89   |
| Water Sago Processing Waste |      |                        |                      |         |
| D                 | 100         | 0                      | 84                   | 6,197   | 98,86   |
| E                 | 200         | 0                      | 84                   | 5,901   | 98,60   |
| F                 | 250         | 0                      | 84                   | 7,881   | 98,96   |
| G                 | 300         | 0                      | 84                   | 17,481  | 99,39   |

3.4. Biogas dari Household solid waste

Dilution of feedstock will affect the normal activity of the methane microbes requiring about 90% water and 7-10% dry matter from the input material for fermentation. Thus the most abundant source of biogas is containing 7-9% dry matter. To get the dry content of this amount, the raw material is usually added to the water with a certain ratio.

Figure 2 shows that the concentration of organic waste 10% (w / v) decreases the amount of biogas produced. This happens because at this concentration there is an increase in carbon sources for...
microorganisms that play a role in the process of biogas formation. If there is too much element C in the material (high C/N), then the element N will be used up first, so that the element C is a lot left. This will cause bacteria to stop working [9].

The comparison of carbon-nitrogen (C/N) feedstock (substrate) is one of the factors that influence the formation of biogas. From a biological point of view, the digestive chamber (digester) is said to be a place of life for bacteria where they eat, multiply and convert organic matter into gases, fertilizers, and so on. Elements of carbon from organic matter (in the form of carbohydrates), Nitrogen (in the form of protein, nitric acid, ammonia, and others) is a portion of food for anaerobic bacteria. Carbon element (C) is used for energy and nitrogen elements to build cell structures from bacteria.

4. Conclusion
The fermentation time for solid waste (35 days) requires a little bit longer time than liquid waste (84 days). The amount of biogas produced from solid waste is more than liquid waste. The more bacteria added to the fermentation process, the more biogas is produced.

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