Utilization of waste as biogas substrate by dominant microbes identified

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Abstract. Indonesia as the tropics have a source of biomass feedstock which is very large, so the waste biomass can be used optimally as an energy source in the form of biogas. This study was conducted to obtain alternative energy from domestic waste materials, given the limited availability of petroleum and natural gas sourced from fossil fuels. This methodology is an experimental method, the process conditions at room temperature 25-27 °C, pH adjusted to the growth of microbes to produce biogas, retention time 20-60 days, the bioreactor is operated with a batch system, the volume of waste in the bioreactor is made permanent, so that the production of biogas in large scale will increase the pressure inside the bioreactor. Biogas is formed accommodated then distributed to the stove. Factors that determine the formation of biogas is a microbial species capable methanogens convert acetate into biogas. From the results of microbial identification of the isolates in the bioreactor, has identified three types of bacteria methanogens namely Methanospirillum hungatei, Methanobacterium polustre and Methanolacinapoynteri. The results of this study, domestic waste can be utilized as a substrate in biogas production, with the highest methane composition reaches 50.79%. This result is expected to increase public knowledge to utilize the waste into biogas as a renewable energy to sufficient the energy needs of household, so it does not depend on the energy derived from fossil fuels.

1. Introduction
Indonesia as a tropical region has an enormous source of biomass feedstock, so its biomass waste can be utilized as substrate in renewable energy production in the form of biogas [1]. Biogas is a product produced from the anaerobic biomass fermentation process [2]. Methane gas is the main gas with relatively more percentage compared to other gases [3]. The biogas component comprises ± 60% CH4 (methane), ± 38% CO2 (carbon dioxide), ± 2% N2, O2, H2, H2S [4] is produced by several stages of the process, i.e. hydrolysis acididation (acid fermentation), acetogenesis, methanogenesis [5]. Microbial methanogen species are important in the process of methanogenesis [5]. Microbial methanogens are able to convert organic material into acetate to be produced into biogas [5]. The benefits of this research, in addition to addressing waste issues, also utilize waste to be a useful thing, namely as a renewable alternative energy in the form of fuel [6], that is environmentally friendly.
2. Methods

Domestic waste to be used in this study, before it is fermented in an aerobic bioreactor, first waste is analyzed, the results presented in Table 1

| No. | Material                        | content | Parameters |
|-----|---------------------------------|---------|------------|
| 1   | Water content                   | 90.3%   | %          |
| 2   | Carbon Organic                  | 11.21%  | %          |
| 3   | Total Nitrogen (Kjeldahl)       | 0.17%   | %          |
| 4   | Phosphat                        | 0.004%  | %          |
| 5   | C:N                             | 66      |            |
| 6   | C:P                             | 2.803   |            |
| 7   | N:P                             | 43      |            |

Domestic waste in the form of kitchen garbage and garden waste has been fermented in bioreactor with batch system by using microbial starter sourced from cow rumen at research center of cattle in Cikole lembang. The aerobic batch bioreactors used can be seen in Figure 1 and 2.

**Figure 1.** Bioreactor Diagram for Hydrolysis of waste

**Figure 2.** Batch Bioreactor Diagram for methae production

The reactor consists of 3 unit:
Reactor A: Contains solid organic waste with Ratio of Waste and water 1:1
Reactor B: Contains solid organic waste with Ratio of Waste and water 1:1/2
Reactor C: Contains solid organic waste only without water
The reactor is filled to ¾ volume of the reactor.

Measurement method of biogas composition carried out qualitatively through gas chromatography, the results presented in Table 2 and 3. Condition of biogas production process, consisting of temperature and acidity presented in Table 4. Microbes to be identified were obtained from microbial sampling of gas bioreactor [7], microbe isolated, microbial growth, morphology test, cell staining and biochemical test. Result of morphology test and cell staining presented in Table 5.
### Table 2. Biogas Composition from the waste

| Ratio of Waste and water | 1 : 1 | 1 : ½ |
|--------------------------|-------|-------|
| Days | CO₂(%) | H₂(%) | N₂(%) | CH₄(%) | CO₂(%) | H₂(%) | N₂(%) | CH₄(%) |
| 12   | 0.0978 | 0     | 99.89 | 0.0137 | 0.1017 | 0     | 99.89 | 0.0268 |
| 19   | 6.1756 | 0.017 | 89.34 | 44.706 | 5.2948 | 0.052 | 91.69 | 3.0052 |
| 25   | 13.075 | 53.09 | 31.829| 5.5454 | 0.408  | 79.06 | 15.025|
| 33   | 8.2227 | 0     | 51.02 | 40.754 | 6.6082 | 0     | 60.69 | 32.624 |

### Table 3. Biogas Composition from the waste without water

| Days | CO₂(%) | H₂(%) | N₂(%) | CH₄(%) |
|------|--------|-------|-------|--------|
| 12   | 0.1003 | 0     | 99.8991| 0       |
| 19   | 1.1179 | 0.1135| 98.0457| 0.703  |
| 25   | 2.26039| 0     | 93.2561| 4.14   |
| 33   | 2.2935 | 0.877 | 91.7084| 5.121  |

### Table 4. Condition of biogas production process

| Days | Temperature Condition | The acidity (pH) at reactors |
|------|-----------------------|-----------------------------|
|      |                       | A  | B  | C  |
| 0    | 25                    | 7  | 7.5| 7.82|
| 3    | 25                    | 7  | 6.6| 6.59|
| 12   | 26                    | 6.37| 6.59| 6.55|
| 19   | 26                    | 6.3 | 7.05| 7.09|
| 26   | 27                    | 6.45| 7.5 | 7.06|
| 33   | 27                    | 6.5 | 7.09| 7.98|

### Table 5. Cell morphology and microbial Gram staining

| Biochemical Test | Microbial Anaerob I | Microbial Anaerob II | Microbial Anaerob 3 | Microbial Methanogen I | Microbial Methanogen I |
|------------------|---------------------|----------------------|---------------------|------------------------|------------------------|
| Cell Shape       | Toccoid             | Toccoid              | Toccoid             | Toccoid                | Toccoid                |
| Gram staining    | G                   | G                    | G                   | G                      | G                      |
| Form of cell colony | circular,    | circular,             | circular,            | circular,                | circular,              |
| Elevatiaon       | flat,              | filamentous,          | convex,             | convex,                  | convex,                |
| Margin           | Entire (smooth)    | Lobate               | Entire (smooth)     | Entire (smooth)          | Entire (smooth)        |
3. Results and Discussion

3.1. Biogas

Biogas composition, consisting of CH₄, CO, N₂, CO₂, H₂ [8]. The biogas composition of reactor A, reactor B and reactor C, which contains solid organic waste formed on the 10th, 19th to 25th day and the 33rd day after acclimation, is shown in Figure 2.

Figure 3. Biogas composition of reactor A

Figure 4. Biogas composition of reactor B

Figure 5. Biogas composition of reactor C

Figure 4 shows that on reactor B, which contains waste with ratio of volume of organic and water waste 1: ½, biogas production is relatively smaller compared to biogas production from bioreactor A, containing waste with ratio of volume of organic and water waste 1: 1. At reactor A, Methan biogas, formed better. On the 33rd day of methane produced in reactor A formed 40.754%, at the reactor B 32.6236%, while in reactor C, the reactor containing the substrate, without the addition of microbes, the biogas formed only 5.121. Because in cow dung there are methanogen microbes. The specific characteristics of the methanogen group are autotrophic or heterotrophic, the energy produced from oxidation of hydrogen / format / acetate with methane formation [7]. The amount of methane and biogas composition formed depends on the ratio of waste volume to the microbial starter. Observations made up to week 4 after acclimation, Water is very important. Water content is used to dissolve organic content and as a nutrient solvent for microorganisms [6]. The existence of an anaerobic biological waste treatment to become biogas, then the application of this system will produce CO₂ and methane gas, CH₄ [9] which is the greenhouse gas emissions that contribute to global warming. The resulting methane is actually a gas that can be combusted (flammable gas) so that if used the gas can be used as a source of renewable alternative energy (bioenergy).

3.2. Process conditions for biogas production

The process conditions for biogas formation need to be considered because in the process of fermentation to produce biogas products such as methane gas, Temperature, pH and alkalinity are very influential. The pH will affect the solubility of the compound and the occurrence of the reaction [10]. The hydrolysis of polymers usually occurs slowly in anaerobic conditions, and several factors may affect the degree and rate at which the substrate is hydrolysed [5]:

- Operational temperature of the reactor
- Residence time of the substrate in the reactor
- Substrate composition (e.g. lignin, carbohydrate, protein and fat contents)
- Size of particles
- pH of the medium
- Concentration of NH₄⁺–N
• concentration of products from hydrolysis (e.g. volatile fatty acids)

Temperature and acidity (pH) conditions in Bioreactors A, B and C to biogas production rate of biogas formation, presented in Figure 6 and Figure 7

![Figure 6. Temperature (°C) conditions in Bioreactors A, B and C](image1)

![Figure 7. Acidity (pH) conditions in Bioreactors A, B and C](image2)

The pH will affect the solubility of the compound and the occurrence of the reaction. The low pH value and excess production and acid accumulation can be an inhibitor for metanogenesis bacteria because it will damage the bicarbonate buffer and increase the concentration of carbon dioxide [11]. In general, the reaction rate of methanogenesis increases at temperatures up to 60°C. Two optimum temperature ranges are mesophilic bacteria, growth temperatures of about 35°C and thermophilic, growth temperatures range from 55 to 60°C. If growth is at temperatures above 70°C, the process of methane formation decreases, although the organic substrate is sufficiently available. Although the process of producing methane biogas products can take place rapidly in the thermophilic process, but by economic calculations, the expenditures and benefits gained are not comparable. Thus in this case the process is better designed to be conditioned on the mesophilic temperature range[12]

3.3. Identification of species of methanogenic bacteria

Methanogenic bacteria are bacteria that produce methane gas from hydrogen gas and CO2 or acetic acid [5]. Identification is done on various biochemical tests of microbes to be determined species, this biochemical test is a stage of activity after the microbe is known cell form and Gram staining. From the results of this biochemical test to know what kind of reaction possessed by microorganisms in growth metabolism [13], presented in Table 6.

Observations of metabolic activity are known from the ability of microbes to use and decompose complex molecules, such as starch substances, fats, proteins and nucleic acids. In addition, observations are also performed on simple molecules such as amino acids and saccharides [7]. The results of these tests were used for the identification and identification of microbes [8]. For example, in the test of citrate usage, if this test microbe grown on the media to Simon's citrate and if positive, then the growing media will change from green to blue in a positive sign of the increase in pH and that microorganisms are able to use citrate as a source of carbon. A method for knowing the production of H2S as a product of the reduction of cysteine, an amino acid protein constituent, H2S reacts with heavy metals (e.g. Pb) to produce colored compounds. The presence of H2S is seen from the increasing darkness of the media in the former inoculation [1]. Based on identification of microbial determination on species of isolates, three methane species have been identified, Methanospirillum hungatei, Methanobacterium Polustre and Methanolacinapoynteri [8]
Table 6. Biochemical test of microbial methanogen that has been isolated

| Biochemical Test | Microbial Anaerob I | Microbial Anaerob II | Microbial Anaerob 3 | Methanogen I | Methanogen II |
|------------------|---------------------|----------------------|---------------------|--------------|--------------|
| Starch           | −                   | −                    | +                   | +            | −            |
| Fat              | −                   | −                    | −                   | −            | −            |
| Casein           | +                   | +                    | +                   | +            | +            |
| Gelatin          | −                   | −                    | +                   | +            | −            |
| Glucose          | −                   | −                    | −                   | + , AG       | −            |
| Sucrose          | −                   | −                    | −                   | + , AG       | −            |
| Lactose          | −                   | −                    | −                   | −            | −            |
| Mr               | −                   | −                    | −                   | −            | −            |
| Vp               | −                   | −                    | −                   | −            | −            |
| Nitrate          | +                   | −                    | −                   | +            | +            |
| Tripton          | −                   | −                    | −                   | Ft−          | Ft+          |
| Sediment         | −                   | −                    | −                   | +            | +            |
| Urea             | −                   | −                    | −                   | +            | +            |
| Citric           | −                   | −                    | −                   | +            | +            |
| S - H2S          | −                   | −                    | −                   | −, G         | −            |
| I – indol        | −                   | −                    | −                   | −            | −            |
| Motilitas        | Motil               | Motil                | Motil               | Non          | Motil        |

RESULTS IDENTIFICATION

| Methano spirillum hungatei | Methano bacterium polustre | Methano lacinapo ynteri | Methanogen 4 | Methanogen 5 |
|---------------------------|---------------------------|--------------------------|--------------|--------------|

Sed= Sedimentn ; Ft = Finer turbidy

4. Conclusion
Identified methanogen bacteria were able to utilize domestic waste as a substrate in biogas production, with the highest methane composition reaching 50.79%. Composition ratio of solid organic waste with waste ratio and water 1:1 optimal for use as substrate in biogas production.

5. References
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