Treatment of *S. cerevisiae* and dairy cow manure on organic waste for biogas production

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Abstract. The organic waste should be used to minimize environmental problems. Organic waste can be mixed with *Saccharomyces cerevisiae* and cow manure as raw material for biogas production. The right concentration of *S. cerevisiae* and cow manure is needed to get the best biogas production as fertilizer that meets the standards. This study aims to determine whether the utilization of organic waste with the addition of *S. cerevisiae* and cow dung can provide good biogas production. This research was conducted experimentally based on a completely randomized 4 × 3 design with factorial patterns. Treatment consists of some factors including the addition of different concentrations of *S. cerevisiae* (0 gr, 2 gr, and 4 gr) and the combination of organic waste and cow manure with the following ratio (1:0, 2:1, 1:1, 1:2 v/v) with three replications. The results showed that 2 gr *S.cerevisiae* in kitchen waste and cow manure with a ratio of 1:1 (F2S1) can produce 0.0128 m³ biogas with a decrease in the C/N ratio of 60.07 % and a sludge final pH of 6.81, respectively.

Keywords: biogas; *S. cerevisiae*; organic waste

1. Introduction

Wastes are a complicated problem faced by big cities in Indonesia for many years. Bandung as one of the biggest waste contributor has 6,479 m³ waste volume per day, of which 3,926.9 m³ is a residential waste. From that waste volume, only 2,356.1 m³ (65% of total residential daily waste production) can be transported to the landfill site. Meanwhile, extensive landfills are now available, totalling only 17 hectares and 74 air transport vehicles also available [1]. Along with the increasing amount of disposed wastes, environmental pollution problems also increase. Piles of wastes will also reduce the aesthetics due to the unsightly. Moreover, household waste disposal activities are endless. During this time, some local governments experienced difficulties in collecting wastes and getting a place or land that was really safe to be used as a landfill [2]. Therefore, a better waste management system is needed so that various problems that arise can be better resolved. As is known that the problem of waste in Bandung is from organic waste, which reaches 63.5 % of the total waste produced [3].

As it is defined, biogas is a processed from organic material with the help of microorganism without involving oxygen or which is called anaerobic digestion. This production process produces gas, which is mostly (more than 50%) composed of methane gas. The methane gas produced from the degradation of organic materials carried by anaerobic bacteria in the digester, which is usually carried out in wet conditions or called wet anaerobic fermentation. The wet anaerobic fermentation requires
the use of high water, a larger reactor, the pretreatment stage is needed (such as melting the substrate),
handling the substrate in the digester (such as the continuous stirring process), and handling liquid
sludge waste that considered not easy [4]. This causes high costs and energy consumption to run large
enough production of around 10-30% [5]. Given these various constraints, biogas production should
be carried out through dry anaerobic fermentation.

Furthermore, the dry anaerobic fermentation with more organic waste raw material provides
benefits. Some of these advantages include the capacity of the digester needed is smaller, with a large
volume of waste, does not require the addition of water, and easier handling [6]. The fresh organic
waste is a good medium for microorganism growth, allowing biogas production through dry anaerobic
fermentation without the need to add water. These advantages enable biogas production through dry
anaerobic fermentation to be easier and more profitable.

Biogas production from organic waste naturally requires a relatively long time. Organic waste as
raw material for biogas production is lignocellulosic biomass with a high fibre content, consisting of
cellulose, hemicellulose, and lignin, which are compounds that are naturally difficult to degrade. This,
then, causes substrate degradation to be longer, retention time to be slower, and the methane gas
produced is not optimal. Therefore, it is necessary to add a starter in biogas production with organic
waste material, which is a deliberate renovation mechanism to accelerate degradation and improve the
quality of the degradation products [7].

The fact is that the addition of a starter is expected to short the retention time in biogas production,
produce high methane gas and resulting good quality sludge as fertilizer, so that it will provide greater
benefits both for the economy and the environment by reducing waste. One type of microorganism
that can degrade fiber and produce organic acids is S. cerevisiae. As it is known that S. cerevisiae is a
yeast that can be used as a starter in biogas. S. cerevisiae is an anaerobic microorganism that can
increase fibre degradation and stimulate cellulolytic bacterial and fungal growth and increase pH in
digesters through organic acid production [8].

The addition of S. cerevisiae to dairy cow dung is often used in biogas production. Dairy cow
dung is used because it contains many bacteria that produce methane (methanogens bacteria) [9]. If
this dairy cow manure is added as a starter, the methanogens bacteria found in dairy cow manure can
produce methane from organic acids through fermentation process carried out by S. cerevisiae.
Therefore, the proper addition of S. cerevisiae and dairy cow manure in biogas production with
organic waste raw materials needs to be known.

2. Materials and Methods

The materials used in this research include organic waste from the Sukamenak Indah settlement. The
composition of the waste taken consists of various types of vegetables, food leftovers, and fruit peels.
The Sengon sawdust is added as carbon source so that the C/N ratio is in accordance for the anaerobic
process. A starter of S. cerevisiae was obtained from the Bandung Institute of Technology (ITB) and
cow manure was taken from the dairy cage of the Dairy Farm Laboratory of the Faculty of Animal
Husbandry, Padjadjaran University.

This research has been conducted experimentally in a laboratory using a randomized design with a
factorial test. The study consisted of two factors that will be tested. The first factor is the
concentrations of S. cerevisiae which are 0, 2, and 4 mL/kg (v/w) organic waste. The second is the
organic waste and cow manure ratio which are 2:1, 1:1 and 1:2, respectively. In the study, there were
9 combinations of treatments with three repetitions, therefore there are 27 parameters as shown below:

| F0         | Comparison of garbage and dairy cow manure 1:0 |
| F1         | Comparison of garbage and dairy cow manure 2:1 |
| F2         | Comparison of garbage and dairy cow manure 1:1 |
| F3         | Comparison of garbage and dairy cow manure 1:2 |
| S0         | 0 mL/kg S. cerevisiae |
| S1         | 2 mL/kg S. cerevisiae and |
| S2         | 4 mL/kg S. cerevisiae |
The \textit{S. cerevisiae} with concentration of 0, 2, and 4 mL/Kg (v/w) cow manure is added as an inoculum. Meanwhile, the number of organic waste and dairy cow manure used are 2:1, 1:1 and 1:2 (w/w). Fermentation under anaerobic conditions at temperatures of 25-27 °C for up to 32 days until biogas is formed. The volume of biogas that supply the infuse bottle chamber will be proportional to the volume of 1.5 \% \textit{NaOH} solution, which drops into the container bottle. Biogas is collected then measurements are made on the volume of biogas produced as well as C/N ratio and final pH of sludge. Data will be analysed by Analysis of Variance, the significant results were followed with Tukey Test.

3. Results and Discussions

3.1. Effect of \textit{S. cerevisiae} and dairy cow manure treatments on biogas production

Based on Table 1, it was known that the highest average biogas production produced was 12.76 ± 0.097 L that resulted from a combination of 1:1 organic waste and dairy cow manure, and also the addition of 2 mL/kg \textit{S. cerevisiae}. The lowest average of biogas production was 5.23 ± 0.24 L resulted from the use of organic waste treatment without the addition of dairy cow manure without \textit{S. cerevisiae} addition.

Results of variance analysis have showed that each treatment and combined treatments contribute significant effect on the biogas production. The analysis results followed by the Tukey test to determine the differences among treatments.

\textbf{Table 1.} Average of biogas production (in litre).

| Treatment | F0   | F1   | F2   | F3   | Total  | Average |
|-----------|------|------|------|------|--------|---------|
| S0        | 5.23 | 7.06 | 6.77 | 6.24 | 25.29  | 6.32    |
| S1        | 7.55 | 12.31| 12.76| 9.79 | 42.41  | 10.60   |
| S2        | 7.45 | 11.37| 11.38| 8.38 | 38.57  | 9.64    |
| Total     | 20.22| 30.73| 30.91| 24.40| 85.57  | 26.57   |
| Average   | 6.74 | 10.24| 10.30| 8.13 |        |         |

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{The average of biogas production}
\end{figure}
Figure 1 shows that the use of dairy cow manure and addition of \textit{S. cerevisiae} produced higher biogas volume. The increased of biogas production occurred at the rate of dairy cows manure as much as 2:1 and 1:1, then decreased in 1: 2 of organic wastes and dairy cow manure ratio. Meanwhile an increase of biogas production also occurs along with the increase of \textit{S. cerevisiae} concentrations 2 ml/kg. However 4 mL/kg of \textit{S. cerevisiae} addition has caused a decrease in biogas production. The treatment marked with the same letter in Figure 1 shows no significant difference at the level of 5% according to the Tukey test.

In the treatment without dairy cow manure addition, biogas production were lower than other treatments. This was presumably due to the low number of microorganism that can degrade the substrate and produce gas. The percentage of anaerobic microorganism contained in the digester will be influenced by the amount of dairy cow manure added [11]. The results showed that there was an increase in the treatments of 2:1 (F1) and 1:1 (F2). This was presumably due to the number of microorganism that can degrade the substrate and produce biogas were increases with the increasing concentration of dairy cow manure. Microorganism from dairy cow manure such as rumen fluid microorganism are good starters for fibre digestion because they produced complex enzymes with the ability to digest the fibre, including α-amylase, galactosidase, hemicellulase, cellulase, and xylanase [12]. Fibre hydrolysed due to the synergistic and interacting influence of the complex microorganism available in dairy cow manure.

However, the ratio of organic waste and dairy cow manure 1:2 shown different results wherein this treatment, biogas production is lower than treatment F1 and F2. Dairy cow manure is known to have high lignin residues from leaves, stems, seeds, and branch, which can inhibit the microbial degradation to be occurred [13]. Therefore, the high amount of dairy cow manure could reduce the rate of substrate degradation. The addition of 2 mL/kg \textit{S. cerevisiae} seems to have an effect on increasing biogas production. The balance of the substrates number with microorganism has supported the optimum biogas production. \textit{S. cerevisiae} plays a role in stimulating anaerobic bacteria and fungi on cellulose degradation and lactic acid utilization [8,14]. In addition to digest cellulose and lactic acid, \textit{S. cerevisiae} also produces growth factors for faecal microorganism in the form of Vitamin B, amino acids and organic acids that can be utilized by anaerobic microorganism to sustain their lives [15]. Addition of 4 mL/kg \textit{S. cerevisiae} has produced lower biogas. \textit{S. cerevisiae} was known to have the ability to increase the concentration of acetic acid and Volatile Fatty Acid (VFA) by stimulating the growth of acetic acid-producing bacteria found in dairy cow manure [16]. Besides that, treatment of organic waste and dairy cow manure ratio up to 1:2 with the addition of \textit{S. cerevisiae} equal to 4 mL/kg produced lower biogas. \textit{S. cerevisiae} was known to have the ability to increase the concentration of acetic acid and Volatile Fatty Acid (VFA) by stimulating the growth of acetic acid-producing bacteria found in dairy cow manure [16]. Besides that, treatment of organic waste and dairy cow manure ratio up to 1:2 with the addition of \textit{S. cerevisiae} equal to 4 mL/kg produced lower biogas. This might be due to some hydrolytic microorganism such as \textit{Fibrobacter succinogenes}, \textit{Butyrivibrio fibrisolvens}, and several other microorganisms were found. In conclusion, biogas production is influenced by several factors, including conditions in the digester, pH, nutrient, temperature, C/N ratio, and starter concentration [17]. Fermentation conditions of anaerobic digester with acidity in the range of 6.9-7.0 and C/N ratio organic waste of 30 were support biogas production.

### 3.2. Effect of \textit{S. cerevisiae} and dairy cow manure addition towards C/N ratio of sludge

The average reduction in the C/N ratio was presented in Table 2. Table 2 shows that the highest average of C/N ratio decrease was 64.10, which is produced by the treatment of organic waste and dairy cows manure ratio as much as 2:1, and the addition of 2 mL/kg \textit{S. cerevisiae} (F1S1). The lowest average of C/N ratio was 51.00 which produced by the treatment of organic waste without the addition of dairy cow manure and without the addition of \textit{S. cerevisiae} (F0S0).

The results in Table 2 were then tested using analysis of variance. The results of the analysis of variance show that each treatment and combination of treatments has given a significant effect on the C/N ratio. Therefore, the results of the analysis are followed by the Tukey Test to find out the differences between treatments (Figure 2). The treatment marked with the same letter in Figure 2 shows no significant difference at the level of 5% according to the Tukey test.
Table 2. Average of C/N ratio decrease in the sludge.

| Treatment | F0  | F1  | F2  | F3  | Total | Average |
|-----------|-----|-----|-----|-----|-------|---------|
| S0        | 51.00 | 51.89 | 58.51 | 56.74 | 218.15 | 54.54   |
| S1        | 54.87 | 64.10 | 60.07 | 61.85 | 240.89 | 60.22   |
| S2        | 53.53 | 60.61 | 61.54 | 57.50 | 233.18 | 58.29   |
| Total     | 159.40 | 176.60 | 180.12 | 176.09 | 692.21 |         |
| Average   | 53.13 | 58.87 | 60.04 | 58.70 |        |         |

Figure 2. Average of bar chart the C/N ratio of sludge

Figure 3 shows that the decreasing C/N ratio has a correlation with the biogas production. The increase in biogas production will occur along with the high decrease in the C/N ratio. This happens because the microorganism contained in the substrate will use carbon and nitrogen available to degrade the substrate and finally produce biogas. Microorganism need a proper amount of carbon and nitrogen as energy sources to grow and produce biogas [18].

Figure 3. The average of C/N ratio reduction from sludge and biogas production
There are some circumstances in which increasing C/N used by microorganism does not always resulting in high biogas production. The F2S2 treatment has showed an increase in the use of C/N instead of produced biogas, which was lower than the previous treatment (F2S2). In addition, the high use of C/N in all treatments F3 (F3S0, F3S1 and F3S2) also has showed similar things. This might happen due to the rate of glycolysis in anaerobic fermentation is regulated to maintain the ratio of ATP/ADP in the cytoplasm. Anaerobic bacteria consumed carbon about 30 times faster than nitrogen. If C/N was too high, nitrogen will consumed rapidly by methanogenic bacteria to meet their growth requirements, and only a few will react to the carbon, which resulting a low biogas production. Conversely, if the C/N ratio was low, nitrogen will be released and accumulated in the form of ammonia (NH₃) which can cause pH increase. If the pH is higher than 8.5, the population of methanogenic bacteria will be disrupted and the production of methane will not be optimum. The C/N ratio was a matter that needs to be consider in microorganism growth, especially in biogas production. The C/N ratio of 30 is optimum for the microorganism growth and biogas formation [10].

3.3. Effect of S. cerevisiae and dairy cow manure addition towards final pH of sludge

The average final pH of organic waste presented in Table 3. The highest final pH was 6.82, which was produced by the combination of organic waste and dairy cows manure ratio as much as 2:1, and the addition of S. cerevisiae 2 ml/kg and 4 ml/kg. Whereas the lowest final pH was 6.76 produced by a combination of 1: 2 organic waste and dairy cow manure ratio, without S. cerevisiae addition.

| Table 3. The average of final pH. |        |        |        |        |        |        |
|----------------------------------|--------|--------|--------|--------|--------|--------|
| Treatment                        | F0     | F1     | F2     | F3     | Total  | Average|
| S0                               | 6.79   | 6.77   | 6.78   | 6.76   | 27.10  | 6.78   |
| S1                               | 6.81   | 6.82   | 6.81   | 6.80   | 27.24  | 6.81   |
| S2                               | 6.81   | 6.82   | 6.81   | 6.79   | 27.23  | 6.81   |
| Total                            | 20.41  | 20.41  | 20.40  | 20.35  | 81.57  |
| Average                          | 6.80   | 6.80   | 6.80   | 6.78   |

The average final pH results then tested using analysis of variance. The analysis of variance results show that the comparison of organic waste and dairy cow manure along with S. cerevisiae addition have given no significant effect on final pH. However, the single treatment of S. cerevisiae has given a significant effect on the final pH. Therefore, the results of the analysis are followed by the Tukey test to determine the differences among treatments.

The Tukey test results showed that the combination of organic waste and dairy cow manure accompanied by the addition of S. cerevisiae had no significant effect on pH sludge (Figure 4). The higher the ratio of dairy cow manure and organic waste accompanied by an increase in S. cerevisiae, the lower pH produced. This was presumably along with the high amount of dairy cow manure; microorganism are difficult to recast the substrate so that the pH rate becomes lower. In contrary, the single treatment of S. cerevisiae takes longer time to break down the substrate considering the fermentation takes place at a temperature of 25 to 27.5 °C, which was not the optimum temperature for the microorganism growth.

Figure 5 shows that a single treatment of S. cerevisiae addition has a significant effect on sludge pH. The production of organic acids largely influences the degree of acidity due to carbohydrate hydrolysis. Along with the increase of organic acids production at the acidogenesis stage, the pH will decrease then will increase again during methanogenesis as shown in Figure 6. These organic acids are converted into acetate and eventually transformed into methane gas [19]. The treatment marked with the same letter in Figure 5 shows no significant difference at the level of 5% according to the Tukey test.
The pH decreasing at the beginning of the fermentation process influenced by the production of lactic acid from the digestion of carbohydrates by anaerobic microorganism contained in manure. At this acidogenesis stage, one type of lactic acid bacteria, namely *Lactobacillus* sp., has experienced a rapid growth phase and produced a lot of acid [20]. In addition, adding *S. cerevisiae* will help to accelerate the degradation process; therefore it produces higher organic acids and decreases pH faster.

The addition of *S. cerevisiae* was accelerated the degradation process and produced higher organic acids that also could utilize carbon sources in organic acids. *S. cerevisiae* played a role in releasing pyruvate dehydrogenase enzyme to convert organic acids to pyruvate [21]. *S. cerevisiae* could stimulate the growth of the lactic acid-consumed bacteria such as propionic bacteria which can convert lactic acid to propionic acid and acetic acid [22]. *S. cerevisiae* could also utilize other organic acids such as citric acid, malic acid, succinic acid and tartic acid which are fermented substances as one of the nutrients for their growth [23].
4. Conclusion

From the discussion above, it can be concluded that the treatment of waste consist of organic waste and dairy cow manure 1: 1 with the addition of *S. cerevisiae* 2 mL/kg (F2S1) produce the best biogas yield of 0.0128 m$^3$ with a reduction 60.07 % in C/N ratio and final sludge pH 6.81, respectively.

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