The effect of cow manure and cow rumen fluid on biogas production using *Oscillatoria* sp. biomass

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Abstract. In order to meet the growing demand for energy and to reduce the dependency on non-renewable energy sources, a renewable alternative energy source is needed. *Oscillatoria* sp. biomass has the potential to be processed into a renewable energy source because it can be produced continuously in sufficient quantities, environmentally friendly, and does not disrupt agricultural land. This study was conducted to identify the ability of microbes contained in cow manure and rumen fluid to produce biogas from *Oscillatoria* sp. biomass and optimize the concentration of these microbial sources. Cow manure and rumen fluid were utilized as a source of inoculum and the concentration was further optimized through central composite design (CCD) using a response surface methodological (RSM) approach. The results showed that cow manure had a more significant effect on biogas production compared to cow rumen fluid. In addition, fluctuations in pH values appear to affect biogas production activity. According to the model developed, the amount of optimum biogas produced was estimated can reach around 324 mL with the content of cow manure and rumen fluid are 128.03 mL and 21.97 mL respectively. The results of the validation experiments show that the cumulative biogas production reaches 350.33 mL or 8.12% more than the predicted biogas production from the regression model produced.

Keywords: Biogas, Microalgae, Cow Manure, Cow Rumen Fluid, Central Composite Design (CCD), *Oscillatoria* sp.

1. Introduction
The world's population is increasing at an alarming rate. In the mid of 2017, the world's population is already close to 7.6 billion with an increase of 1.10 per cent per year. It is projected that the world's population will reach 8.6 billion in 2030, 9.8 billion in 2050, and 11.2 billion by 2100 [1]. Every human being needs a certain amount of energy to do their activities, so the demand for energy will increase as the human population grows [2]. The total world energy consumption will increase by 28% from 2015 – 2040 [3]. By 2015, 78.4% of the energy consumed globally comes from fossil fuels that are a source of non-renewable energy [4].

Dependency on fossil fuels is predicted to continue as fossil fuels are projected to contribute as much as 77% of total global energy consumption by 2040 [5]. Indonesia also experienced an increase in energy consumption from year to year. This condition will continue to deplete national energy reserves such as
crude oil, gas and coal reserves that will be run out within 23 years, 59 years and 82 years [6]. The dependence on non-renewable energy consumption is always associated with greenhouse gas emissions and is not related to the process of carbon fixation or reduction of gas emissions. The use of non-renewable energy have a close connection to environmental damage.

The increased demand for energy, the depletion of natural resources, and the emergence of environmental problems that caused by the use of non-renewable energy hence urging deep exploration to find a renewable alternative energy to meet current and future energy needs [7]. Biomass-based energy is the most popular form of renewable energy that used in recent years. By 2015, the use of biomass-based energy accounts for 14.1% of 19.3% total renewable energy consumption globally [4]. One type of renewable energy that can be generated from biomass is biogas.

Based on the problems that have been elaborated, it is necessary to use non-conventional biomass sources that are easier to produce, can be grown on non-arable land using saline or waste water, environmentally friendly, and can be produced continuously in sufficient quantities. The source of biomass that has characteristics according to these needs is microalgae biomass [8]. Microalgae are suitable feed materials to be used for biogas production because they also contain a negligible or low amount of lignin and tend to contain high amount of lipid, carbohydrate and protein [9].

In general, microalgae can multiply it's biomass within 24 hours [10], so in a short period of time we can obtain a large amounts of lipid, carbohydrate and protein from the microalgae biomass, which later can be processed into biogas and valuable co products. This research was conducted to produce biogas by utilizing Oscillatoria sp. biomass as a substrate for biogas production and using cow manure and cow rumen fluid as a source of microbial consortium that plays a major role in anaerobic digestion. Until now, the Oscillatoria sp. biomass has not been utilized optimally as a substrate for biogas production. The objective for this research is to study the effect of using Oscillatoria sp. biomass as a substrate on biogas production, to investigate the ability of cow manure and cow rumen fluid to produce biogas, and optimize the concentration of cow manure and rumen fluid to produce biogas via central composite design (CCD) using a response surface methodological approach.

2. Methods

2.1. Preparation of Oscillatoria sp.
The microalgal biomass was obtained from the Center for Environmental Technology (PTL) Laboratory, Indonesian Agency for Assessment and Application of Technology (BPPT), Tangerang Selatan, Indonesia. A 1-L microalgal culture was inoculated into 9 L Bold Basal Medium (BBM) which had been autoclaved and the culture was incubated for 30 days. The cultivation process was carried out in a 11-L plastic container with a working volume of 10 L, at room temperature and given aeration via the aerator as well as lighting for 24 hours using 100 W white neon lamps.

2.2. Preparation of cow manure and rumen fluid
Cow manure and cow rumen are obtained from a slaughterhouse (RPH) in Tangerang City, Jalan Bayur, Kampung Baru, Subdistrict Karawaci. Rumen fluid was collected right after the cow was slaughtered and the manure was collected from the cage. Both materials were stored for 12 hours before being put into the reactors. Cow manure and rumen fluid were separately diluted using aquadest (distilled water) with a ratio of 1 : 1 then homogenized by manually stirring.

2.3. Experimental setup
The experiments were conducted in HDPE plastic bottles with 1 L working volume as a simple batch reactors and operated at room temperature. The amount of biogas produced was measured daily by using the liquid displacement method (LDM) as shown in Figure 1.
2.4. Experimental design and procedure
The design of experiment (DOE) for optimization in this study was via central composite design (CCD) using a response surface methodological (RSM) approach, which performed by using Design-Expert Software Version 7.0.0. (Stat-Ease Inc., Minneapolis, MN, USA, 2005). The experimental design can be seen as in Tables 1 and 2. The anaerobic digestion process was operated for 30 days. After the optimization process in the first experiment succeeded, the research continued by conducting validation experiments with optimized conditions in triplicate for 30 days to justify the validity of the model produced.

Table 1. Combination of two variables and five levels of experimental combinations with CCD.

| Variables       | -1.41421 | -1.0000 | 0    | 1    | 1.41421 |
|-----------------|----------|---------|------|------|---------|
| Cow Manure      | -0.000108564 | 21.9669 | 75   | 128.033 | 150     |
| Cow Rumen Fluid | -0.000108564 | 21.9669 | 75   | 128.033 | 150     |

Table 2. Variations of the components inside the reactors.

| Reactor       | Cow manure (mL) | Cow rumen fluid (mL) | Total inoculum (mL) | Microalgae (mL) |
|---------------|-----------------|----------------------|---------------------|-----------------|
| KIR22         | 21.97           | 21.97                | 43.93               | 700.00          |
| KIR12822      | 128.03          | 21.97                | 150.00              | 700.00          |
| KIR22128      | 21.97           | 128.03               | 150.00              | 700.00          |
| KIR128        | 128.03          | 128.03               | 256.07              | 700.00          |
| IR75          | 0.00            | 75.00                | 75.00               | 700.00          |
| KIR15075      | 150.00          | 75.00                | 225.00              | 700.00          |
| K75           | 75.00           | 0.00                 | 75.00               | 700.00          |
| KIR75150      | 75.00           | 150.00               | 225.00              | 700.00          |
| KIR75a        | 75.00           | 75.00                | 150.00              | 700.00          |
| KIR75b        | 75.00           | 75.00                | 150.00              | 700.00          |
| KIR75c        | 75.00           | 75.00                | 150.00              | 700.00          |
| KIR75d        | 75.00           | 75.00                | 150.00              | 700.00          |
| KIR75e        | 75.00           | 75.00                | 150.00              | 700.00          |

K = Cow manure; IR = Cow rumen fluid
2.5. Analytical methods
The biogas volume was observed daily and measured by the liquid displacement method using a 100-mL graduated cylinder. Total solids (TS) were measured at the beginning of the study at each reactor. Standard closed-reflux colorimetric method was used to measure chemical oxygen demand (COD) value, while pH and temperature were measured using pH meter and digital thermometer. The value of chemical oxygen demand (COD), pH, and temperature were measured every three days. The abundance of the microbial consortium was calculated once every week using the total plate count method (TPC) in the nutrient agar medium (NA). The quality of biogas was observed using the flame test method [11].

2.6. Statistical analysis
The regression analysis of the model was performed by using Design-Expert Software Version 7.0.0. (Stat-Ease Inc., Minneapolis, MN, USA, 2005). The coefficient of determination $R^2$ would determine the quality of the resulting regression equation and its significance was tested through F-test.

3. Results and Discussion

3.1. Optimization of cow manure and cow rumen fluid concentration for biogas production
Analysis of cumulative biogas production for 30 days was conducted to determine the effect of cow manure and cow rumen fluid during the anaerobic digestion. The amounts of biogas production for 30 days (Figure 2) were inputted into the design-expert software for further analysis through ANOVA. The results of this analysis were the main data in this study and were used to form a regression model that could be used to optimize biogas production from Oscillatoria sp. biomass. The result data shown in Table 3 were obtained from a total of 13 experimental runs that were generated with different set-up conditions by CCD.

![Biogas Production Chart](image)

**Figure 2.** Cumulative biogas production for 30 days.

Cow manure ($X_1$) and cow rumen fluid ($X_2$) have been shown to significantly affect biogas production ($p < 0.05$). Meanwhile, the interaction between cow manure and rumen fluid ($X_1X_2$) and both quadratic model terms ($X_1^2$, $X_2^2$) did not give a significant effect on biogas production ($p > 0.05$). This result is in conformity with another study proving that if the $p$ value is below 0.05 then the model terms are preferred in representing the reliability of the results [12].
Cow manure was found the most influencing factor on biogas production with the p-value of 0.0122, while the cow rumen fluid has a much smaller effect on biogas production with p-value of 0.0471 which is close to \( \alpha \) value (0.05). The equation of regression model generated from ANOVA was as follows:

\[
Y = -122.36167 + (6.18064*X_1) + (0.972844*X_2) - (0.026844*(X_1*X_2)) - (0.017764*(X_1^2)) - (0.000253*(X_2^2))
\]

In the above equation, \( X_1, X_2 \), and \( Y \) were cow dung, cow rumen fluid, and total biogas production, all of which were expressed in milliliters (mL). The equation above took into account the linear, quadratic, and interaction effects between the factors studied. The actual value of the factors studied was the value that can be input into the equation. The ANOVA result for quadratic model shows that the value of \( R^2 \) obtained was 0.7875 meaning that the model could explain 78.75% of the variation occurring in the response (total biogas production). Furthermore, the p-value of lack of fit was equal to 0.1529 (p > 0.1) meaning that the lack of fit was not significant so the model was fit. Therefore, it's significant variables could be used for further optimization by RSM [13].

The value of adequate precision is used to measure the signal to noise ratio where the desired ratio value is greater than 4. Adequate precision obtained in this study is 8.191 so that it significantly stated the suitability of the model and model could be used to navigate the design space. The relationship between cow manure and cow rumen fluid concentration that seen in 3D plots (Figure 3) showed that the smaller the concentration of cow rumen fluid and the greater concentration of cow manure, it would increase the biogas production.

To ensure that ANOVA assumptions were met, residual analysis should be performed. This analysis was also done to determine the feasibility of the model. The residual normality test (Figure 4) results show that the residual of the quadratic regression model had followed the normal distribution where the resulting residual point was located around the linear line on the residual normality plot, so the residual normality assumption of the quadratic regression model had been met and the model could be used. The result of this residual analysis has corresponded another previous study which stated that a good normal probability plot would show a linear straight line and had no "S" shape. On the other hand, the residual versus predicted response plot (Figure 5) indicates that residuals were randomly distributed and there was no particular pattern, whereas poor results would produce a megaphone-shaped pattern [12].

| Source          | Sum of Squares | df | Mean Square | F Value | p-value (Prob > F) |
|-----------------|----------------|----|-------------|---------|-------------------|
| Model           | 117379.46      | 5  | 23475.89    | 5.19    | 0.0262 significant |
| X\(_1\) – Cow manure | 50804.27      | 1  | 50804.27    | 11.23   | 0.0122 significant |
| X\(_2\) – Cow rumen fluid | 26170.54      | 1  | 26170.54    | 5.78    | 0.0471 significant |
| X\(_1\)X\(_2\) | 22801.00      | 1  | 22801.00    | 5.04    | 0.0597 not significant |
| X\(_1\)\(^2\) | 17365.23      | 1  | 17365.23    | 3.84    | 0.0909 not significant |
| X\(_2\)\(^2\) | 3.53          | 1  | 3.53        | 0.00    | 0.9785 not significant |
| Residual        | 31673.62      | 7  | 4524.80     |         |                   |
| Lack of Fit     | 22100.82      | 3  | 7366.94     | 3.08    | 0.1529 not significant |
| Pure Error      | 9572.80       | 4  | 2393.20     |         |                   |
| Cor Total       | 149053.08     | 12 |             |         |                   |
Figure 3. Effect of cow manure and cow rumen fluid on biogas production in 3D plot.

Figure 4. The residual normality test diagnostic plot.

Figure 5. Residual versus predicted response plot.
3.2. Validation of experiment

The previous experiment produced a regression model that could be used to optimize biogas production. In accordance with the calculation of the regression model, the cow manure and cow rumen fluid concentration that required to produce the optimal biogas respectively were 128.03 mL and 21.97 mL. Based on that optimum condition, biogas production for 30 days was predicted to reach as much as 324 mL. Furthermore, the optimal concentration was used to perform validation experiments. The results of a 30-day validation experiment showed that the cumulative biogas production reached 350.33 mL. Thus, the results of the validation experiments had a slightly higher biogas production compared to the predicted result with a difference of 26.33 mL.

In the validation experiment, there appeared to be a long period of microbial adaptation characterized by the formation of biogas in small amounts during two weeks of anaerobic digestion. This might be due to a decrease in the number of microbes from day 0 to 14 that successfully grown in the nutrient agar medium. The low activity of biogas production occurred (Figure 6) because there was a lag phase of microbial growth that could last for 2-4 weeks [14]. During the initial two weeks of anaerobic digestion, the resulting biogas did not show any methane gas content indicated by the absence of a flame when the resulting biogas was burned [15].

During the 3rd and 4th weeks, there was a significant increase in biogas production (Figure 6). This condition indicated that there had been a log phase so that microbes required adequate nutrition in the reactor which would then be converted into biogas [15]. This occurred because the breakdown of the insoluble substrate at the hydrolysis stage had been successful so there was an organic material available for acidogenic, acetogenic and methanogenic bacteria. Therefore, biogas production became significantly increasing. The biogas production stopped until the degradation process left an undigested portion of the substrate [16]. The condition was supported by an increase in the number of microbes at week 3 to the end of the observation. The biogas produced during these two weeks already contained sufficient methane gas identified by a blue flame when burned [15].

![Figure 6. Cumulative biogas production for 30 days on validation experiment.](image)

K : Cow manure; IR : Rumen fluid;
*This is the result of the first experimental study before the validation experiment

3.3. The effects of other parameters on biogas production

In this study, only pH values were shown to affect the biogas production (Figure 7). While the TS content, temperature value, and decrease in COD concentration had no effect on biogas production. In this study, the TS content varied from 0.20 to 0.82%. However, the results did not have a significant
relationship with biogas production that might occur because the TS value range was too small. But according to another study, the most optimal TS content for biogas production was within the range of 7-9% [17]. During the study, the temperature values were always within the range of 28 – 30.3°C which meant that the reactors were operated in mesophilic temperature (20 - 45 °C) [9]. Temperature fluctuations that occurred during the study did not seem to affect the productivity of biogas, because the temperature fluctuations of 3°C could still be tolerated by the mesophilic bacteria [18].

![Figure 7. The effect of pH value on biogas production on validation experiments](image)

The pH values considered to be tolerable for biogas production are within the range of 5.5 - 8.3. Thus, the pH values at all reactors (5.68 - 8.21) involved in this study were within a range that could still be tolerated [19]. The process of conversion of organic matter into biogas is greatly influenced by the pH value [20]. There is a pattern that is easily seen between pH and biogas production. When the pH value decreases the biogas production decreases and vice versa when the pH value increases then the total biogas production also increases. This can be due to methanogen bacteria known to work optimally when the pH is in the range of 6.8 – 7.2 [20].

Fluctuations that occurred at the pH value indicate that the fermentation and methanogenesis processes continue to recur in the reactor. This proved that the process of acidogenesis and methanogenesis occurs at different times [19]. An increase in pH value can occur because the cow manure that was used had a high level of alkalinity so it could act as a buffer at the time of acid product accumulated. Thus, cow manure can stabilize the pH value of the reactor [18]. The Increased of pH values could also be caused by methanogenic bacteria that successfully consume volatile fatty acids and acetic acid [20]. The breakdown of proteins into ammonium ion also increased the pH value as it could easily form an alkali compounds [19].

4. Conclusion

*Oscillatoria* sp. biomass had proven to be utilized as substrate for biogas production within 30 days. The results of variance analysis showed that cow manure and cow rumen fluid gave a significant linear effect in biogas production within 30 day. Overall, cow manure had a greater impact than cow rumen fluid for biogas production. Optimization using CCD-RSM resulted in the following regression model:

\[
Y = -122.36167 + (6.18064\times X_1) + (0.97284\times X_2) - (0.026844\times (X_1\times X_2)) - (0.017764\times (X_1^2)) - (0.000253\times (X_2^2))
\]
Where $X_1$, $X_2$, and $Y$ were cow dung, cow rumen fluid, and total biogas production (mL). From the regression model, the optimum concentration of cow manure and cow rumen fluid was 128.03 mL and 21.97 mL, with the prediction of 324 mL biogas will be produced. The results of the validation experiments showed that the cumulative biogas production reached 350.33 mL or 8.12% more than the predicted biogas produced.

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