Production and Purification of Biogas from *Chlorella* sp Biomass with Cow Manure and Rumen Microbiomes

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Abstract: Biogas is alternative energy with a high content of methane. Biogas could be produced from organic material using cow manure and rumen as a source for methanogenic microorganisms. This research was dedicated to observing the influence of cow manure and rumen on biogas productivity and the possibility of *Chlorella* sp. as feedstock for biogas production. Use of *Chlorella* sp. as the main feedstock for biogas production is because *Chlorella* sp. can be massively produced, always available, and does not require agricultural land. Aside from being biomass, in this study *Chlorella* sp. was also used to reduce CO2 in biogas so that biogas could be purified in a sustainable process. The experiments were conducted on a 1-L enclosed reactor with 300 ml of methanogenic microbe source and 700 ml of *Chlorella* sp. biomass. For purification experiment, the reactor was connected to a 500-ML purification reactor containing only *Chlorella* sp. Our result showed that the reactor with the non-purification system could produce more biogas than with purification system using 1:1 mixture of rumen and manure producing 416.667 ml biogas, while the cow dung alone produced 270.66 ml of gas. Use of *Chlorella* sp. as a purification agent could successfully increase the methane gas content by 8.27% with the result that the composition of methane gas in biogas became 81.15%.

Abstract: rumen; cow manure; microbiomes; chlorella; biogas; methane purification; CO₂ reduction.

1. Introduction

Energy is one of the essential parts of human life. All this time, the energy source that dominates human needs is sustained by the energies from a non-renewable resource. Based on BP's Statistical Review of World Energy 2016, coal production is expected to last for 115 years, and both oil and natural gas is roughly remaining for 50 years [1]. In addition to being non-renewable, these energies have high CO₂ emissions. During 2010, 84% of the U.S. greenhouse CO₂ gas emissions were from the Industrial revolution and human activities as part of fossil fuel combustion [2]. One way to maintain the energy supply needed is to find other renewable and environmentally friendly energy sources. One form of renewable energy that can be used is biogas.

Biogas has enormous economic potential which can be used for replacement of fossil fuels in power and heat production, and also can be used as gaseous vehicle fuel. The high amount of methane also can replace the natural gas function as a feedstock for producing chemicals and materials [3]. Biogas is a gas produced by anaerobic activities or anaerobic fermentation of organic materials such as animal and human waste, household waste and other organic wastes by methanogenic bacteria [4].

Rumen and cow dung as one form of solid and liquid waste from the livestock industry have a methanogenic bacterial consortium that can ferment carbon (C) into biogas to be used as renewable energy. Beside of being a source of bacterial consortium, rumen and cow manure can be utilized further as the biogas biomass. Nevertheless, organic waste from cattle farming industry has a weakness. First, this waste is difficult to collect if the cattle ranch is in the form of a ranch system. Organic waste from the livestock industry will be collected for biogas production if cattle are cultivated in cages. Organic waste from the cattle farm industry is very dependent on the existence of the cow itself. While the organic waste of the livestock industry from animal slaughter centers, the number is very limited. Limitations are also found in organic wastes from the agricultural sector such as maize, potatoes, and rice that are seasonal, therefore, in this study rumen and cow dung were used as the sources of a bacterial consortium to produce biogas.

With the above considerations, it is necessary to look for other organic materials that can be held at any time, cheap/economical and environmentally friendly. One alternative organic material that meets these criteria is microalgae. Microalgae is a biomass that can be used as raw material for producing biogas which is more attractive because it is easy to produce, requires only water, sunlight and CO₂, does not disturb human land [5], can be produced in both fresh and coastal waters and can be combined with the cultivation of other commodities.
In addition, microalgae, depending on the species, can be used to produce bioenergy such as biogas, biodiesel, bio-butanol, and even fuel for jet aircraft [6]. Microalgae also do not need pesticides and can work as bioremediation agents in contaminated areas [7]. Microalgae are capable of producing biomass containing 50-60% protein, 40-50% carbohydrates, 6-18% lipids and bioactive compounds [8].

Besides being used as biogas biomass, microalgae can also be used as biogas purification agents. The quality of biogas can be improved by reducing the carbon dioxide content present in it and thus will produce more optimal alternative energy. Microalgae have the ability of photosynthesis to assimilate CO₂ for growth, development, and cell reproduction of the microalgae. This process may be used to purify biogas from CO₂.

Our study focused on the effect of manure and rumen liquid contents as a source of methanogenic bacteria on biogas production using Chlorella sp. as biomass and purification agent where the stages of biogas formation and purification were directly in a continuous process by utilizing simple equipment.

2. Methodology

2.1. Sample preparation

Both cow manure and rumen fluids used in this experiment were taken randomly from a slaughterhouse, located on Tangerang City. Fresh cow manure and rumen fluid were mixed with water using a 1:2 ratio. The solid content was then separated from the slurry by a filter cloth.

Chlorella sp. for both biomass and purification was obtained from PTL-BPPT. The strain was cultivated in the BBM medium and incubated for enrichment at the room temperature with several LED lights as the lighting source.

2.2. Experimental apparatus set up

Two series of laboratory tests of 1000 ml biodigesters were operated in a batch system. Both systems used biodigesters made from black polyethylene bottles plugged with tight rubber plugs and valves for daily biogas measurement. The temperature of biodigesters was controlled with an electrically heated water bath and maintained between 28°C to 30°C. Biogas formed was measured by ‘Liquid Replacement System (LRS)’ [9].

The first series of the experiment was used for biogas production without purification. In this series, each biodigester was directly connected to a graduated cylinder for the gas measurement. The biodigester design was equipped with valve outlets for sampling and gas collection. This series was coded as NP.

The second series of the experiment was used for biogas production with purification. Unlike the first series, in the second series, the same model of biodigester as the first one was connected to a photobioreactor for the gas purification. The photobioreactor was made from a clear plastic bottle, equipped with an outlet for sampling and gas collection. The LED light was next used for the purification reactor as the light source. This series was coded as FP. The schematic diagram of a laboratory–scale experiment is shown in Figure 1.

![Figure 1. Schematic diagram of a laboratory-scale experiment for non-purification (left) and with purification experiment (right). 1-sampling pipe, 2- gas collector 3- biogas connector, a-biodigester, b- photobioreactor, c- water bath, d-reversed graduated cylinder.](image-url)

2.3. Anaerobic digestion experiment design and procedure

Approximately 300 ml of cow manure (K), rumen fluid (R) and 1:1 mixed rumen fluid and manure (C) was used individually in biodigester reactor for both of purification and non-purification experiment based of the treatment as bacteria consortium. The consortium substrate was fed into the biodigester, and 700 ml of Chlorella sp. biomass was added as the biomass. The composition of consortium substrate and biomass used in this study.
is presented in Table 1. For the purification experiment (P), 500 ml of Chlorella sp. biomass was used in the photobioreactor. Each treatment for both of purification (P) and non-purification experiment (NP) was performed in triplication. The biogas performance was measured by the gas produced daily and the cumulative volume of gas for 30 days.

Table 1. Composition of consortium substrate and biomass in biodigester reactor Keys: NP= non purification experiment; P= purification experiment; R= rumen liquid; M=manure; C= 1:1 mixed rumen fluid and manure.

<table>
<thead>
<tr>
<th></th>
<th>Manure (ml)</th>
<th>Rumen (ml)</th>
<th>Chlorella sp. (ml)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP R</td>
<td>0</td>
<td>300</td>
<td>700</td>
<td>0</td>
</tr>
<tr>
<td>NP K</td>
<td>300</td>
<td>0</td>
<td>700</td>
<td>0</td>
</tr>
<tr>
<td>NP C</td>
<td>150</td>
<td>150</td>
<td>700</td>
<td>0</td>
</tr>
<tr>
<td>P R</td>
<td>0</td>
<td>300</td>
<td>700</td>
<td>0</td>
</tr>
<tr>
<td>P K</td>
<td>300</td>
<td>0</td>
<td>700</td>
<td>0</td>
</tr>
<tr>
<td>P C</td>
<td>150</td>
<td>150</td>
<td>700</td>
<td>0</td>
</tr>
<tr>
<td>Control+ R</td>
<td>0</td>
<td>300</td>
<td>0</td>
<td>700</td>
</tr>
<tr>
<td>Control+ M</td>
<td>300</td>
<td>0</td>
<td>0</td>
<td>700</td>
</tr>
<tr>
<td>Control+ C</td>
<td>150</td>
<td>150</td>
<td>0</td>
<td>700</td>
</tr>
<tr>
<td>Control-</td>
<td>0</td>
<td>0</td>
<td>700</td>
<td>300</td>
</tr>
</tbody>
</table>

2.4. Analytical methods

Sample from each biodigester was analyzed for total solid (TS) and volatile solid (VS) at the beginning and the end of the experiment. All the TS and VS procedures were performed by the standard procedures [10]. The pH, temperature, and chemical oxygen demand (COD) were analyzed every 3 days. The COD analysis procedure was performed according to the SNI 6989.2-2009 standard. Every 6 days the bacteria abundance inside the biodigester and Chlorella sp. abundance inside the photobioreactor were analyzed using total plate count (TPC) for bacteria and hemocytometer for Chlorella sp. At the end of our experiment, the total carbon dioxide, total methane, and flame test were performed. Total CO2 level in biogas was determined by the titration method [11]. The percentage of other gases in biogas is usually small and insignificant so that methane was determined by subtracting the total biogas with the total CO2.

3. Results and Discussion

3.1. Biogas Production

Based on the experimental results, it can be seen that the production of biogas produced continued to increase (Figure 2). The speed of biogas growth depends on the source of the methanogenic bacteria. The gas was first formed on the 13th day of treatment of manure with no purification (NPK) followed by manure with purification treatment (PK) on the 15th day, the mixed with purification treatment (NPC) on day 20, and the mixed with purification (PC) on the 21st day.

![Figure 2. Biogas production from cow manure, rumen fluid and mixed in purification and non-purification treatment.](image-url)
The most biogas production was produced by NPC treatment with a total average of 416.67 ml, followed by NPK treatment with a total of 270.67 ml, PK treatment of 280.33 ml and PC of 78 ml (figure 3). Growth in the purification reactor was slightly less than that of a non-purification reactor. It could be because the carbon dioxide (CO₂) gas was absorbed by the microalgae so that the resulting gas was less, or because of the lack of pressure that drove the exit gas due to the presence of additional tubes for gas purification causing the addition space for methane gas so that not all gas was transmitted in the measuring tube.

Not all our treatments produced biogas. In NPR and PR treatments gas was not formed until the last experiment day. Both treatments used rumen liquid as a source of methanogenic bacteria. In addition to the treatment, positive controls on the rumen also did not produce gas. The absence of gas in all rumen treatments indicated that these negative results appeared not due to leakage. In addition, the leak test had also been done before and during the treatment to ensure that no leaks occurred in each biodigester.

The absence of biogas in rumen treatment could be due to the fact that rumen liquid usually takes longer period to produce biogas. The less optimal quality of rumen liquids and the pH of the acidic rumen liquids could become the reasons biogas production in rumen treatment became slow and did not appear during the study. Rumen liquids also have a high nitrogen gas content with low carbon content, so the condition was less ideal for microbial development in producing biogas. Treatments using only rumen fluid could not produce gas, but treatments by mixing rumen fluid with cow dung could produce more gas than treatment with cow dung only. This could be because the rumen consortium worked better with the addition of cow dung and could increase the biogas production.

![Total cumulative biogas](image)

**Figure 3.** Total biogas in the purification and non-purification treatment.

Each treatment had its own growth rate. The treatment with impurities had the greatest growth rate. Manure with purification (PK) and manure with no purification (NPK) treatments had a growth rate of 10.451 mL/day and 10.208 mL/day, respectively. The mixed with purification and mixed with no purification (NPC) treatment had a growth rate of 9.9282 mL/day and 3.0159 mL/day, respectively (Figure 4). Although NPC treatment produced a higher amount of gas with rapid growth in the later process, treatment with manure produced the gas days earlier in the test so that the treatment with manure had a higher velocity.

Our results showed that each biogas production in the purification and non-purification treatment produced methane gas as one of the main parts of biogas. It was known from the gas characteristics such as the gas was odorless and had a large flame, was blue and was not quickly going out. Our results were in accordance with Meena and Chippa [12] in 2014 study which stated that the positive test of methane gas was characterized by a blue flame, durable, and odorless gas. The appearance of a large flame also indicated that the biogas of each purification and non-purification treatment using manure and rumen- manure mixture had methane gas content above 45%. The estimation of the figure was obtained from Harahap's research [13] stating that methane gas has a high enough heat content that can be a major component of biogas, and biogas from the anaerobic fermentation process will burn if at least containing 45% methane gas.
3.2. pH

The sample in rumen biodigester (R) had the most acidic pH among all biodigesters, followed by biodigesters with mixed biodigester (C) and biodigester containing manure (K) having the most alkaline pH (figure 4). The pH of NPR treatment ranged from 5.30 to 5.73, while the pH in the PR treatment ranged from 5.41 to 5.71. In the NPC treatment, the pH increase was in the range of 5.60 to 7.05, and in the PC treatment, the pH was in the range of 5.87 to 6.92.

Both treatments with mixed methanogenic bacterial sources, both in purification and non-purification treatments experienced a significant increase in pH in the 7th sample and increased steadily to reach neutral pH conditions. This significant increase was also a sign that methanogenetic bacteria were working to produce biogas, as evidenced by the increase in gas. NPK treatment had a pH range from 6.60 to 7.29, an increase also experienced in PK treatment where the pH range is from 6.67 to 7.20. The pH had included optimal pH for methanogenesis bacteria to form biogas. This was evident from the treatment of NPK and PK that first produced gas in the reactor compared with other treatments.

Based on the results of the data in Figure 5, the treatments using manure and mixed treatment had similarities in which they increased until they reached a neutral pH. The initial non-acidic pH range caused the acidogenic bacteria and methanogenesis to grow and produce biogas as one of their metabolic outcomes, while rumen treatment had an initial acidic pH so that methanogenic bacteria were difficult to grow. As a result of this less optimum pH, the biodigester remained at an acid pH below 6.5 to be one reason why biogas was not formed because the pH range was less optimum for methanogenesis bacteria to produce methane gas. However, if rumen liquid was added to manure like in mixed treatment, it improved the pH condition to be more alkaline and become more optimal for biogas production.

**Figure 4.** Velocity rate of biogas production in purification and non-purification treatment.
3.3. Total chemical oxygen demand (COD)

In Figure 6, the COD results in all treatments showed fluctuating data where there were an increase and decrease in COD value. This increase occurred due to the decomposition of biomass by microorganisms so that microorganisms could multiply. This was in accordance with the statement of Kresnawaty et al. [14] in 2008 which explained that the increase in COD could occur due to the added litters began to degrade.

The treatment of PR had the highest levels of COD and tended to be slow to decline. It was related to the biogas content that was not formed where the decomposition process did not occur optimally so that bacteria still could not form biogas as a result of their metabolism. This was in contrast to the treatment of NPK and PK where there was a sharp decline in the value of COD produced. This decrease was also related to the increasing biogas content. Although the COD data was fluctuating, the value continued to decrease. This showed that the hydrolysis process occurred and the amount of biomass decreased.

3.4. Total solids (TS) and volatile solids (VS)

The result of observation as can be seen from Figure 6a shows that there was a decrease in total solid level from before and after research. This could happen because of the degradation process of organic materials at the hydrolysis stage of biogas.
The Mixed-Purification treatment (PC) sample had the largest total solid decrease of 0.594. Both treatment of the mixed manure-rumen liquid in purification and non-purification systems has a bigger total solid decline compared with rumen treatment and manure. It showed that bacterial consortium in mixed treatment could degrade organic matter more rapidly than other treatments. This was proportional to the rapid increase of pH and the growth of gas which was also more and increasing sharply.

Based on Figure 6b, the lowest value of VS before treatment was owned by the Mixed-No Purification (NPC) sample of 66.710%, while the highest VS value was owned by the Purge-Purification (PC) sample of 76.995%. After the test, the Mixed-No Purification (NPC) sample had the largest solid volatile value of 86.146%, and Manure-Purification (PK) had the smallest solid volatile value of 53.721.

Solid volatile values in this study decreased in each treatment. Volatile solid was a food ingredient for the process of hydrolysis in anaerobic bacteria. The decreasing value of VS indicated a decreasing amount of food in the biodigester. The decrease in VS values was due to the organic solids being overhauled into other compounds to be used at the acidogenesis stage and the methanogenesis stage.

![Figure 7](image_url)  
Figure 7. (a) %Total Solid of biodigester samples. (b) %volatile solid of biodigester samples.

### 3.5. Total carbon dioxide and methane

The CO₂ content of the purification system had a lower value than the CO₂ content of the non-purification system. This can be seen from Table 3 where there was a decrease in CO₂ levels in each system. Data from Table 2 shows that methane gas content had an increasing percentage in purification treatment. This data shows that *Chlorella* sp. was a good biogas purification agent in this experiment. Purification treatment had a percentage of methane gas above 80%, while in non-purification treatments, the percentage of methane gas was below 80%. In treatment with impurities, methane gas increased by 8.27%, while in treatment with mixed rumen liquid-manure methane gas mixture increased by 7.04%. This increase seemed not too big, it could be caused by the condition of *Chlorella* sp. which requires more CO₂ gas so that *Chlorella* sp. in this study was in less than optimal condition. It was also showing the density of *Chlorella* sp. on the measurement of a non-significant growth in the hemocytometer. Lack of ability *Chlorella* sp. for breeding in this study might be due to the lack of CO₂ levels produced. *Chlorella* sp. can absorb CO₂ as much as 251.64 mg / L / day [2], the value was much greater than the amount of CO₂ produced from this research. However, from the results obtained, it was proven that *Chlorella* sp. was able to decrease the CO₂ value of biogas produced.

### Table 2. The total volume of biogas, %methane, and %carbon dioxide.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% CO₂</th>
<th>% CH₄</th>
<th>Total volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumen liquids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non purification</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Purification</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Manure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non purification</td>
<td>27.11</td>
<td>72.88</td>
<td>270.66</td>
</tr>
<tr>
<td>Purification</td>
<td>18.84</td>
<td>81.15</td>
<td>280.33</td>
</tr>
<tr>
<td>Mixed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non purification</td>
<td>23.34</td>
<td>76.65</td>
<td>416.66</td>
</tr>
<tr>
<td>Purification</td>
<td>16.30</td>
<td>83.69</td>
<td>104.00</td>
</tr>
</tbody>
</table>
3.6. Chlorella sp. density

Chlorella sp. in the purification reactor was calculated to know the amount of microalgae present in each treatment reactor. From the data obtained (Figure 8), the microalgae content dropped dramatically in the first week. It was because biogas was still not formed so that Chlorella sp. did not get enough CO\textsubscript{2} supply to breed. Chlorella sp. reproduced in the photobioreactor began to adapt after being in a minimal condition of CO\textsubscript{2}. This was shown from the start of increasing the number of Chlorella sp. on treatment with cow manure (PK), and mixed (PC) after the previous density of Chlorella sp. dropped dramatically. This adaptation of Chlorella sp. was proportional to the increase of biogas in the adjacent period to the growth period of Chlorella sp. Therefore, it could be said that Chlorella sp. in both systems began to multiply with the help of CO\textsubscript{2} gas contained in the biogas so that finally there was the experience of improvements in the middle of the process to the end. However, this increasing amount did not cover the initial amounts incorporated into the purification reactors. Hence it was less than optimal if it was to be used as biomass for the biodigester for biogas production, as it must be kept adding biomass from the outside culture.

![Figure 8. The density of Chlorella sp. in photobioreactor samples.](image)

4. Conclusions

The mixed treatment is the most optimal composition of methane gas production in biodigesters followed by the production of gas from cow manure as the second largest gas producer. The study used rumen not to produce biogas until the end of the study period. However, if rumen liquid is combined with manure, it will boost the production of biogas. The biogas production first emerged from the manure treatment and followed by mixed treatment. For the purification process, Chlorella sp. was able to work as purification agent to reduce CO\textsubscript{2} level from biogas up to 8.27%. This was because Chlorella sp. used CO\textsubscript{2} as an energy source. This experimental design of biogas production and purification was considered to improve the quality of biogas.

It was necessary to optimize the system so that Chlorella sp. could absorb more CO\textsubscript{2}, for example, aerated additional aeration that kept Chlorella sp. to stay alive but not to increase the level of CO\textsubscript{2} in the biogas or not having a negative impact on bacteria due to the presence of oxygen in aeration. A specific test for Chlorella sp. as the biomass for biogas production by using only cow manure and only rumen in isolation of bacteria was also necessary for further study. It was also necessary to run the test for biogas content using gas chromatography to determine the types of materials produced specifically.

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