Biomass Production and Lipid Content of *Leptolyngbya* HS-16 grown in Bubble Column Photobioreactors (BCPBR) with Air Bubbler Pore Variation

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Abstract: Research on the production of biomass and lipid content of *Leptolyngbya* HS-16 grown in a photobioreactor with variations in the number of air bubbles forming holes in the photobioreactor has been carried out. Photobioreactor (PBR) is a system designed to support the life of microorganisms in the system by providing various factors that can be used for growth such as light, carbon dioxide and nutrients. Bubble column photobioreactor (BCPBR) provides aeration for mixing nutrients and a carbon dioxide source for culture. In this study, two types of bubble column photobioreactor (BCPBR) were used with variations in the number of air bubbles, namely 12 (BCPBR1) and 24 (BCPBR2). In addition, TPBR without aeration was used as a control. This study used *Leptolyngbya* HS-16, an indigenous cyanobacteria isolated from Red Crater, Pancar Mount, West Java. *Leptolyngbya* HS-16 was inoculated on a bubble column photobioreactor (BCPBR). The lipids obtained from this strain were 4.41% (BCPBR1) and 1.30% (BCPBR2) after 24 days.

Keywords: Indonesia; Indigenous cyanobacteria; *Leptolyngbya*; Lipid; Photobioreactor

1. Introduction and background

There are two types of energy, non-renewable and renewable energy. With the advancements on science and technology, people are turning their eyes to the renewable energy¹. This also because each day, the non-renewable energy source depleted and one day the supply of non-renewable energy source will run out². Scientists and experts around the world are starting to search for a renewable energy source for sustainable energy needs in the near future³.

As we may know, people nowadays are starting to use a different kind of renewable energy source such as solar energy and wind energy. Beside of that, people also start using biofuel that can be produced from plants biomass like sugarcane and corn⁴. Besides plants biomass, biofuel also can be produced from microorganism biomass⁵. Microorganisms that can be used to produce biomass as a basic material for biofuels are microalgae. Microalgae that can be used are both members of eukaryotic and prokaryotic microalgae. One of the prokaryotic algae is the cyanobacteria.

*Leptolyngbya* is one of filamentous cyanobacteria⁶. *Leptolyngbya* can reproduce by forming vegetative cells called Hormogonia. Hormogonia are short filaments derived from long broken filaments, and function as asexual reproductive cells⁷.

The genus *Leptolyngbya* with strain code Hot Spring (HS)-number 16 is the microorganism used in this study. This indigenous microorganism is an isolate from Red Crater, Pancar Mountain that succesfully isolated by Prihantini⁸ on 2015. *Leptolyngbya* HS-16 is known to contain several types of lipids including saturated fatty acids by 40.56%, monounsaturated fatty acids by 31.04%, branched fatty acids by 25.67% and hydroxy-substituted fatty acids by 2.74%⁹. In addition, *Leptolyngbya* HS-16 can be grown on 80 ppm NPK fertilizer medium and produces a lipid content of 45%¹⁰. Lipid from
Leptolyngbya can be used as feedstock to make biofuel after a series of processes. Microorganism biomass usually produced by inoculating microorganism to open system (open pond) or closed system (Photobioreactor)\textsuperscript{11}.

One of the functions of photobioreactors is to increase the production of microbial biomass by providing aeration or mixing of nutrients. The use of aeration causes every microorganism cell to get the same nutrients. Aeration can also provide carbon dioxide needed by microorganisms, especially microalgae/cyanobacteria.

Cyanobacteria culture requires a way to distribute nutrients in the growing medium in a photobioreactor system. Bubbling or aeration is one way that can be used to stir nutrients, especially for filamentous cyanobacteria\textsuperscript{12}. Pre-research that has been carried out has proven that aeration can be used as a form of stirring for Leptolyngbya HS-16 which has a tendency to be benthic or easy to adhere to surfaces. However, pre-study on the growth of Leptolyngbya HS-16 with aeration as a form of agitation is still not optimal. The aeration provided is still not too evenly distributed because the design of the photobioreactor is still very simple. The research was conducted using a photobioreactor design with a bubble-forming aeration channel. The different variations in the number of bubble-forming holes may also affect the growth of cyanobacteria in this case Leptolyngbya HS-16. Therefore, in this study, two photobioreactors with different number of air bubbles were used. This study aims to determine the effect of differences in the number of air bubbles on the lipid production of Leptolyngbya HS-16.

2. Method and experimental setup

The treatment given to Leptolyngbya HS-16 was growing in two different types of the bubble column photobioreactor (BCPBR) using NPK [Grow More] as the growth medium. Tubular Photobioreactor (TPBR) without aeration was used as a control. Meanwhile, for the treatment using BCPBR with two variations of aeration channels (air bubble pores), each of which has 12 pores (BCPBR1) and 24 pores (BCPBR2). Each pores have 0.2 cm diameter.

Each PBR system is made into 3 units as a form of repetition. The number of units of the PBR system is 9 units. The nine units consisted of 3 units of TPBR without aeration, 3 units of BCPBR1, and 3 units of BCPBR1. The study was conducted for 15 days with data collection on day zero (t0) and continued until day 16 (t1, t2, t3, t4, t5, t6, t7, t9, t10, t12, t13, t14, t15 and t16).

The aeration are provided by using pipe that connected to an air compressor. Aeration are provided to mix the nutrients in the growth media\textsuperscript{13}. Beside mixing the nutrients, aeration also provide carbon dioxide needed by Leptolyngbya HS-16.

The medium used in the study was BG-11 medium and NPK [Grow More] medium with a concentration of 80 ppm. The BG-11 medium is used as an enrichment medium. The enrichment medium is a medium that has nutritional components that can only be used by specific microorganisms\textsuperscript{14}.

Biomass propagation is carried out to make stock cultures, work cultures and starter cultures. The Leptolyngbya HS-16 culture was inoculated into 100 mL of BG-11 medium NIES that had been provided in a 250 mL Erlenmeyer flask\textsuperscript{15}. The Erlenmeyer flask is then stored in an incubation cabinet with a temperature of 35°C\textsuperscript{8}.

Leptolyngbya HS-16 biomass cultivated to NPK 80 ppm growth media with pH amount of 7.2 inside the BCPBR. NPK medium with a concentration of 80 ppm requires 80 mg of NPK in 1000 mL of distilled water. The NPK fertilizer used is NPK [GrowMore]\textsuperscript{16}. Data collected in this research are Leptolyngbya HS-16 biomass weight and lipid content in the end of the observations. Observations of biomass weight last for 16 days. Weighing the weight of Leptolyngbya HS-16 biomass was carried out on the observations t0, t1, t2, t3, t4, t5, t6, t7, t10, t13 and t16. Weighing the biomass of Leptolyngbya HS-16 was started by weighing the sterile 2 mL eppendorf tube. The weight of dry biomass is obtained by drying the pellets in eppendorf in an oven at 40°C for about 6 hours. The dried pellets are then weighed using analytical scales. Lipid content measurements were carried out using the Bligh & Dyer (1959)\textsuperscript{17}, method following the method in Addana 2014\textsuperscript{18}. Below are the equations to get the total lipid percentage\textsuperscript{19}.

\[
\text{% Total Lipid} = \left( \frac{DLP \times \text{mg}}{DCW \times \text{g}} \right) \times 100\% \tag{1}
\]

3. Results and Discussion

3.1 Biomass Weight and Growth Curve of Leptolyngbya HS-16

The data of Leptolyngbya HS-16 biomass weight was collected on (t) t0, t1, t2, t3, t4, t5, t6, t7, t10, t13, and t16.
observation days. The number of starter cultures of *Leptolyngbya* HS-16 added to the test culture was 600 mg in 1500 mL of medium. This was done to make each treatment get a uniform number of *Leptolyngbya* HS-16 cultures.

Figure 2 shows the growth curves of *Leptolyngbya* HS-16 grown on three photobioreactor systems. Based on Figure 2, the average weight of *Leptolyngbya* HS-16 biomass in the BCPBR system with the variation in the number of aeration channeling pores increased on the 4th day (t4) of observation. The increase in growth continued with the variation in the observation days. The number of starter cultures of *Leptolyngbya* HS-16. In nutrients that can be used by *Leptolyngbya* HS-16 biomass in the BCPBR system with the variation in bubble-forming holes can affect microorganism growth in photobioreactor because the bubbles can cause shear stress, and it will break the cells.

### 3.2 Lipid Content of *Leptolyngbya* HS-16

Measurement of the lipid content of *Leptolyngbya* HS-16 was carried out at the end of the observation (t16). Lipid content testing was carried out on 24-day-old cultures. The lipid content of *Leptolyngbya* HS-16 in different treatments had different amounts. Based on the measurement results, it is known that the lipid content of *Leptolyngbya* HS-16 in the BCPBR2 system is 1.30% with a biomass weight of 0.6 mg and a lipid weight of 46.2 mg; while the lipid content of *Leptolyngbya* HS-16 in the BCPBR1 system was 4.41% with a biomass weight of 2.9 mg and a lipid weight of 65.8 mg. Meanwhile, the lipid content of *Leptolyngbya* HS-16 in the TPBR system without aeration was 15.23% with a biomass weight of 3.9 mg and a lipid weight of 25.6 mg. The results of the measurement of lipid content are listed in Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lipid weight (mg)</th>
<th>Biomass weight (mg)</th>
<th>Lipid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 Pores (BCPBR2)</td>
<td>0.6</td>
<td>46.2</td>
<td>1.30</td>
</tr>
<tr>
<td>12 Pores (BCPBR1)</td>
<td>2.9</td>
<td>65.8</td>
<td>4.41</td>
</tr>
<tr>
<td>without aeration</td>
<td>3.9</td>
<td>25.6</td>
<td>15.23</td>
</tr>
</tbody>
</table>

The results are quite small rather than a few research done by another researcher. Singh et.al. (2014) get lipid percentage 16—21% of dry biomass weight. Tsolcha et.al. (2018) get lipid percentage 14.8%. Meanwhile, Maity et al. (2018) get lipid percentage 31.34%. This could happen because there is mucilage production that occur due to shear stress or another environment stress. Mucilage which is a carbohydrate compound can not be dissolved and does not evaporate when the chloroform and metanol compound was applied to dilute cell wall. The mucilage will remain and become one with dry biomass. Mucilage also have function as the cell sheath.
backbone of mats in mats forming microorganisms.  

4. Conclusions  
The average biomass of Leptolyngbya HS-16 produced on BCPBR2 have more amount rather than on BCPBR1. In other hand, the lipid percentage are higher in BCPBR1 rather than on BCPBR2. Consideration in using the different type of BCPBR are important, due to its function in production. More improvement is needed to increase the biomass and lipid production on each of BCPBR.  

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Nomenclature  

<table>
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<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>DLP</td>
<td>Dry lipid</td>
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<tr>
<td>DCW</td>
<td>Dry cell weight (mg/mL)</td>
</tr>
</tbody>
</table>

References  
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