

Effects of aeration intensity as agitation in simple photobioreactors on *Leptolyngbya* (cyanobacteria) growth as biofuel feedstock

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Abstract. Indonesia known as a hotspot of biodiversity, including cyanobacteria biodiversity. One member of cyanobacteria (prokaryotic algae) is *Leptolyngbya*. *Leptolyngbya* HS-16 is an isolate that had been isolated from hot spring in Red Crater of Gunung Pancar, Sentul, Bogor. As mats-producing microalgae, this strain is a very promising source of Biofuel. Biofuel can be extracted from lipid of microalgal biomass. Bioreactor is a method to encourage the growth of microalgal biomass. To get a best result in growth, agitation must be done, to make sure every single cell of microalgae gets the adequate nutrition. The aeration on simple photobioreactors is set to high and low intensity. The high intensity of aerations average amount are 191 bubble/min, while the low intensity one are 117 bubble/min, with a device that could produce smaller bubble size to reduce the aeration-agitation effect. The research was done to acknowledge the effect of aeration intensity to *Leptolyngbya* HS-16.

1 Introduction

There are two types of microalgae, prokaryotic and eukaryotic. One member of prokaryotic microalgae is *Leptolyngbya*, which belong to cyanobacteria. Cyanobacteria is a group of microorganism that utilize photosynthetic process to produce energy and could live upon an extreme area [1]. They could be found at water or soil, whether as solitaire-living microorganism or forming a symbiotic relationship with plants or *lichen-forming* fungi. *Leptolyngbya* is a member of *Synechococcales* order, one order from Cyanobacteria, which is known for their ability to produce mats [2]. One individual of *Leptolyngbya* may be 0.5 to 3.2 micrometer in size [3]. *Leptolyngbya* reproduce by hormogonia, a short-motile filament, that could produce vegetative filaments after a few cell division. In addition to their ability to produce mats and forming filaments for reproduction, *Leptolyngbya* also known as filamentous algae.

Leptolyngbya HS-16 is an isolate that had been isolated from hot spring in Red Crater of Gunung Pancar, Sentul, Bogor [4]. As mats-producing microalgae, *Leptolyngbya* was a promising source of biofuel [5]. Biomass from *Leptolyngbya* could produce lipid and after going through a few process, they could be used as biofuel. Biomass was produced because of growth of the microalgae. Growth requires nutrients as well as another factors, such as light, air and how nutrients are utilized.

A suitable agitation needed, to ensure the growth will run well and evenly [6]. A previous research on suitable agitation on filamentous algae [7] had proven aeration or bubbling, are by far the best methods of agitation to use

for filamentous algae. Aeration is one type of agitation to circulate the nutrients provided by the medium, to be put to good use by filamentous algae.

In the recent years, the race to found a new sustainable source for energy had rose. People started to think how to get environmental friendly renewable energy. Microalgae contains lipid ranging in 20—50%, and with that value, they could replace oil palm, since they have ten times lipid productivity per hectare, higher than oil palm [8]. More over, the cultivation of microalgae does not require agricultural land development.

Photobioreactors are another option for industrial microalgal cultivation systems. Open ponds systems may have low capital and low operational costs, but their lack of control on operational conditions and their capability to sustain only low biomass productivity, are their major drawback. On the other side, there are photobioreactors (PBR), that can support higher photosynthetic efficiency, biomass productivity and biomass concentration [9]. Furthermore, we have an options to increase the performance of PBR, like having low cost design and designing the efficient mass and light transfer [10]

2 Materials and Methods

2.1 Medium and Microorganisms

Medium used are Blue Green 11, recipe obtained from NIES [11] with a few modifications. The medium were made by mixing all the ingredients with Distillated Water, and sterilized by using autoclave, on 121°C temperature,

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for about 20 minutes. Medium later then were used to make *working culture*, *stock culture*, and *starter culture*. For *working culture*, 200 mL culture were inoculated to 400 mL media, as for *starter culture*, 200 mL culture were inoculated to 400 mL media.

Table 1. Blue Green 11 Recipe

| Component | Stock Solution (mg · mL ⁻¹) | Quantity Used |
|---|---|---------------|
| Distilled Water | — | 968.9 mL |
| NaNO ₃ | — | 1.5 g |
| MgSO ₄ · 7H ₂ O | 10 | 7.5 mL |
| K ₂ HPO ₄ · 3H ₂ O | 10 | 4 mL |
| CaCl ₂ · 2H ₂ O | 10 | 3.6 mL |
| Citric acid | 1 | 6 mL |
| Ferric amonium citrate | 1 | 6 mL |
| Na ₂ EDTA-Mg | 1 | 1 mL |
| Na ₂ CO ₃ | 10 | 2 mL |
| Trace Metal Mix | See following recipe | 1 mL |

Table 2. Trace Metal Mix Recipe

| Component | Stock Solution (mg · mL ⁻¹) |
|---|---|
| MnCl ₂ · 4H ₂ O | 10 |
| ZnSO ₄ · 7H ₂ O | 10 |
| NaMoO ₄ · 2H ₂ O | 10 |
| H ₃ BO ₃ | 10 |
| CuSO ₄ · 5H ₂ O | 1 |
| Co(NO ₃) ₂ · 6H ₂ O | 1 |

Microorganisms used in this research had previously been studied [4]. From the study, *Leptolyngbya* HS-16 was known could live and grow on temperatures ranging on 35°C to 50°C. Natural habitats for *Leptolyngbya* HS-16 was warm water body and usually had calm surface.

2.2 Designing Photobioreactors

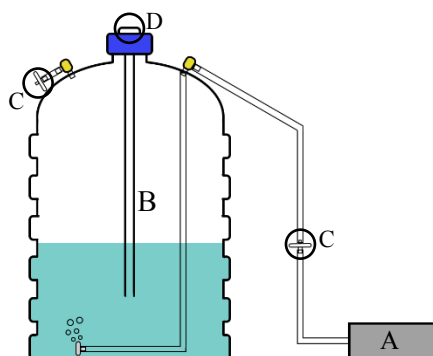


Fig. 1 Initial design for photobioreactors (A : Aerator, B : Acrylic Pipe, C : Sterile Membrane Filter ,D Closure)

Photobioreactors are made out of five litre PET, acrylic pipe, clear PVC hose, and a closure from beverages. All the materials were assembled by using liquid nails and glue. As for the light sources, four tubular lamp were

added to the system, each two on the opposite side. Two photobioreactors were assembled, the one with low intensity of aeration and another one with high intensity of aeration. Aeration provided to photobioreactors was filtered sterilized with sterile membrane silica 0.22 micron to keep the system sterile from contaminants such as bacteria or fungi.

2.3 Cultivating and Harvesting

All measurements were done on 24 hours basis. *Starter culture* of *Leptolyngbya* HS-16 was inoculated into photobioreactors with 1:2.5 ratio. The intensity of aeration was set to Low Intensity (117 bubbles/minutes) and High Intensity (191 bubbles/minutes). Photobioreactors were placed inside a room without contact to sun light. Complementary data such as environment temperature, photobioreactors temperature, pH and light intensity also collected for further research.

Observation were held for 14 days and wet weight were measured 8 times. Sampling for biomass wet weight was done by using 2 mL eppendorf tube and wet weight could be obtained from subtracting eppendorf tube weight with sample to empty eppendorf tube weight.

3 Results and Discussion

3.1 *Leptolyngbya* HS-16 Wet Biomass Weight

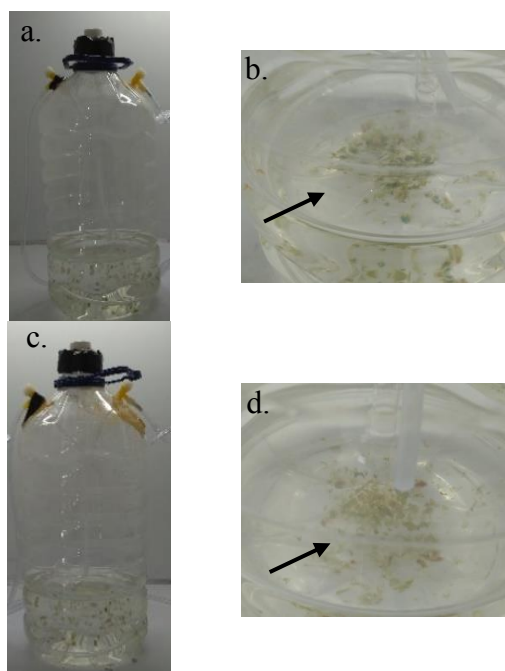


Fig. 2a, b, c, d Photobioreactors on day 3 (T₂) (a : High Intensity PBR, b (arrow) : Culture on High Intensity, c : Low Intensity PBR, d (arrow) : Culture on Low Intensity)

Biomass wet weight of *Leptolyngbya* HS-16 were obtained by subtracting the weight of eppendorf tube with centrifuged sample with empty eppendorf tube weight.

$$Wet\ Weight = W_{1(tube\ with\ sample)} - W_{0(empty\ tube)} \quad (1)$$

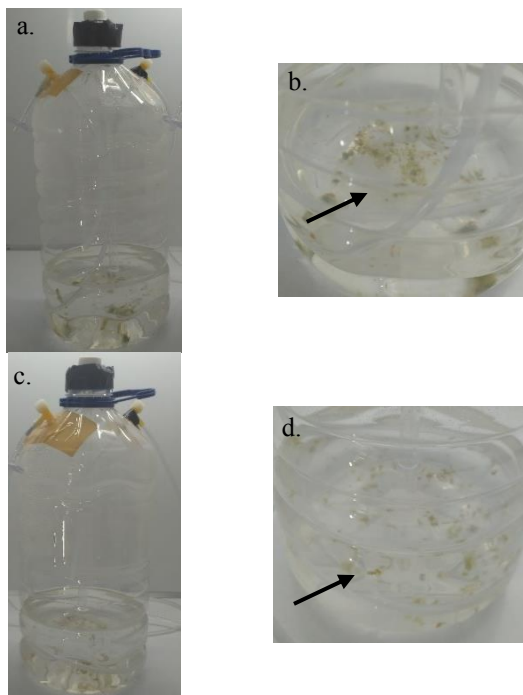


Fig. 3 a, b, c, d Photobioreactors on day 11 (T₁₀) (a : High Intensity PBR, b (arrow) : Culture on High Intensity, c : Low Intensity PBR, d (arrow) : Culture on Low Intensity)

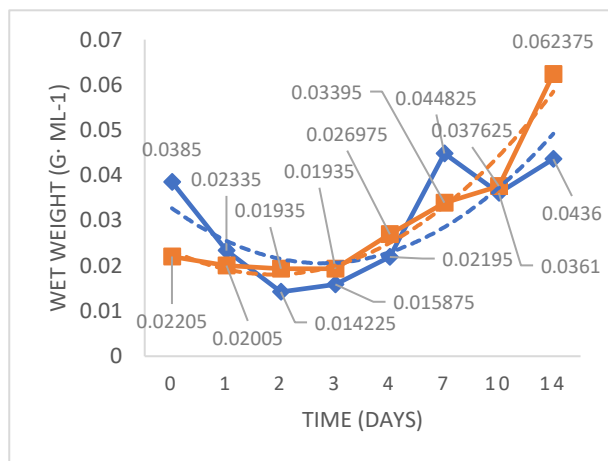


Fig. 4 Average of biomass wet weight (Diamond : High Intensity of Aeration PBR, Square : Low Intensity of Aeration)

From the data shown above, the culture from photobioreactors with high intensity hit the first peak, on day 7 of the observation before declining and hit another peak on day 14, while the other one, photobioreactors with low intensity still showing exponential growth. Also, from the data shown above we can see that, the population from low intensity of aeration have longer log phase, while high intensity aeration, which already hit the peak on day 7, have shorter log phase.

Cyanobacteria, as a bacteria, have four life phases, starting from the lag phase, log phase, stationary phase and death phase. Lag phase are a phase, where bacteria adapt themselves to stay alive in new environment. Log phase are where bacteria, started to grow, while stationary phase are a phase, when nutritions from environment getting low on numbers. Death phase are phase when

there are no more grow, because there are no more or a little number nutrients [12].

3.2 Temperature and Power of Hydrogen (pH)

Data on temperature and pH were recorded from day 0 to day 14. From day 0 to day 14, the pH value are constant on 6. The environment temperature fluctuation are ranged from 27°C to 29°C, as for Photobioreactors temperature are ranged from 26.5°C to 30°C. Photobioreactors temperature may show a little different value, because of light source heat, that made the inside of Photobioreactors a little bit warm rather than the temperature outside. The place where Photobioreactors taking place too, could affect the value of the temperature, since it does not get sun light.

3.3 Aeration

Aeration are a way to agitate or circulate medium, to ensure every microalgae cells gets equal amount of nutrients. Carbon dioxide provide by aeration, affect biomasses growth, mainly on the photosynthetic pathway of microalgae. Carbon dioxide were fixed by rubisco (ribulose biphosphate carboxylase oxygenase) to produce phosphoglycerate. These carbon organic acids are reduced to the sugars and the substrate for starch and oil production. The efficiency of CO₂ capture by microalgae, can vary according to algal physiology, nutrients, and temperature. Under optimal conditions, carbon dioxide capture efficiencies as high as 80% to 99% [13]. Aeration are also the best methods to encourage the growth of *Leptolyngbya* HS-16, because the use of manual stirring or regular stirring could damage the cells, due to hydrodynamic stress [14]. Low Intensity of Aeration appear to have a good effects on the growth of the microalgae. The growth curve from both of aeration intensity show increasing trend, but the one from low intensity show more stable growth curve, rather than another one. This could happen because filamentous algae, are usually found in mats-form on calm water surface [4].

4 Conclusion

On the previous studies of aeration effect to biomass growth, and another studies, aeration does have effect on encouraging the biomass growth by mixing the nutrients and providing CO₂, that take effects on photosynthetic pathways of microalgae. Low Intensity of Aeration have a good effect, since filamentous algae usually forming a mats on calm water surface. Further studies for aeration rates are needed to optimized the photobioreactors systems.

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