

# A low-cost media for *Leptolyngbya* (cyanobacteria) strain growth isolated from Indonesia as a potential biofuel feedstock

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**Abstract.** The study of a low cost media for *Leptolyngbya* growth had been done. Commercial NPK fertilizer was one of the low cost media that commonly used for microalgae growth. The objective of this study aimed to determine the best concentration of commercial NPK fertilizer for *Leptolyngbya* NBP-7A growth as a potential biofuel feedstock. *Leptolyngbya* NBP-7A was isolated from Cisolok hot spring, located in Indonesia. In this study, *Leptolyngbya* NBP-7A was grown in commercial NPK fertilizer with concentration 80 ppm, 240 ppm, and in BBM (Bold Basal's Medium), BBM+NPK 80 ppm as a control. This strain was incubated on 35 °C. Observation were made approximately 14 days with 2 sampling for each media. The average of wet weight *Leptolyngbya* NBP-7A after 14<sup>th</sup> day observation, was obtained at NPK 80 ppm was 5 mg/L, NPK 240 ppm was 4.1 mg/L, BBM was 19.75 mg/L, and BBM+NPK 80 ppm was 14.65 mg/L). The results of 14 days observation showed that the best concentration NPK culture media for growing *Leptolyngbya* NBP-7A was in BBM + NPK 80 ppm. This study showed that commercial NPK fertilizer could be used for *Leptolyngbya* NBP-7A growth as a potential biofuel feedstock.

## 1 Introduction

Microalgae have the potential as used for biofuel production [1]. Despite that, microalgae has several advantages for using them as biofuel production. The advantages of this organisms such as growth faster, easy to be manipulated and used CO<sub>2</sub> as carbon source [2]. One of the microalgae that can be used as biofuel production is cyanobacteria.

Cyanobacteria can be found in soil, rocks and waters [3]. Another source where cyanobacteria can be found is hot spring [4]. Cisolok hot spring is the example of source whereas cyanobacteria can be found. *Leptolyngbya* is one genus of cyanobacteria that can be found in Cisolok hot spring.

*Leptolyngbya* have filamentous as the form of the colony. Characteristic of these organisms have a thin filament with 0,5 to 3,5 wide with simple trichome, and some species have sheath [5]. *Leptolyngbya* can be found in environmental condition with pH 7 to 8,5 [6].

*Leptolyngbya* can also have potential as used for biofuel production. It could be happened because *Leptolyngbya* has ability for production lipid as one of the raw material as used biofuel production [7]. High lipid production was supported by high biomass production. Biomass production depends on nutrients in growth media. Nutrients that have a plays role in biomass production were N (nitrogen), P (phosphorus), K (potassium) [8]. Those nutrients are available in growth media of cyanobacteria.

Many research for search a low cost media had been done. One of low cost media that could be used for microalgae is commercial fertilizer NPK [8]. Commercial fertilizer NPK has been used for cultivation of *Scenedesmus* sp and could produced high lipid contain 28.55% of biomass [10]. Unfortunately, this low cost media has not been yet attempted to grow *Leptolyngbya* NBP-7A. The aim of this study is to determine the best concentration of commercial NPK fertilizer for *Leptolyngbya* NBP-7A growth.

## 2 Materials and Methods

### 2.1 Microorganisms and Growth Medium.

The microorganisms which used in this study was cyanobacteria genus *Leptolyngbya* strain NBP-7A. *Leptolyngbya* NBP-7A was isolated from Cisolok hot spring. Those strains were grown in variants of growth medium. The growth medium consist of BBM and BBM added with NPK 80 ppm as a control, NPK 80 ppm and NPK 160 ppm. BBM was obtained by following the recipe. NPK growth media were obtained by dissolve the commercial fertilizer NPK GrowMore 20:20:20 in 1000 mL aquades. The concentration of growth media depends on how much commercial fertilizer NPK dissolve. For example, 80 ppm was obtained by 80 mg dissolve in 1000 mL aquades. BBM plus NPK was obtained by added

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BBM 1000 mL and dissolved NPK 80 mg, then homogenized with magnetic stirrer.

## 2.2 Cyanobacteria cultivation in variants growth medium.

The first step of cultivation cyanobacteria is inoculated 30 mg biomass of each strain into growth medium. Before cyanobacteria were inoculated into variant growth medium, amount 70 mL of growth medium were added into erlenmayer flask 100 mL. The treatment of each growth media was repeated twice. The strain were incubated at temperature 35 °C.

## 2.3 Measurement the weight of wet biomass Leptolyngbya NBP-7A

Measurement of *Leptolyngbya* NBP-7A biomass were done in 14 days with 9 times of sampling. The sterile eppendorf tube 1.5 mL was measured at analytical measurement tool. Biomass of *Leptolyngbya* NBP-7A was taken aseptically with sterile micropipet amount of 1 mL. Eppendorf tube with biomass inside was sentrifuged for 10 minute with 10.000 rpm. The supernatant was taken out and wet biomass weight was measured with analytical measurement tool. The growth curves was made by comparrison between wet biomass weight as the ordinate axis Y with observed time as absisca X. The growth curves were made by Microsoft Excel.

## 3 Result and Discussion

The study of growth *Leptolyngbya* NBP-7A taken 14 days with 9 times of sampling. The result produced growth curve of *Leptolyngbya* NBP-7A in variant growth media. The growth curve of this study still imperfect, because *Leptolyngbya* NBP-7A was still adapted with the condition of variation growth media. It could be seen at the growth curve of *Leptolyngbya* NBP-7A produce the growth curve unstable. The age of inoculum that used in this study was 1 months old.



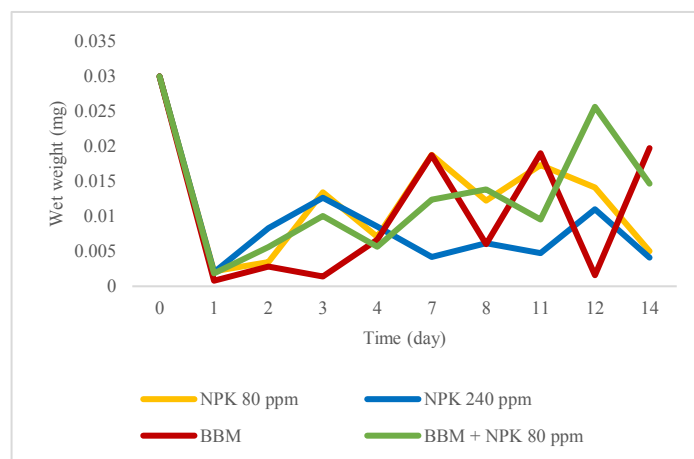
**Fig. 1.** Biomass *Leptolyngbya* NBP-7A in erlenmayer flask 200 mL.

Macroscopic observation of *Leptolyngbya* were observed. Color appereance of *Leptolyngbya* based on Faber Castle standard color. Color appereance of *Leptolyngbya* NBP-7A was sea grass at day 0 (t0). Macroscopic observation of *Leptolyngbya* NBP-7A shown on Fig. 1.

The Average of wet weight biomass of *Leptolyngbya* NBP-7A shown on Table 3, while the growth curve of *Leptolyngbya* NBP-7A shown on Fig. 2. The growth curve of *Leptolyngbya* NBP-7A were made based on their wet weight of biomass.

**Table 1.** The Average of wet weight biomass of *Leptolyngbya* NBP-7A

Time	Wet weight (mg)			
	NPK 80 ppm	NPK 160 ppm	BBM	BBM + NPK 80 ppm
0	30	30	30	30
1	2.15	2	0.8	1.85
2	3.45	8.3	2.8	5.6
3	13.45	12.65	14.2	1.005
4	7.05	8.55	6.65	5.65
7	18.8	4.2	18.75	12.4
8	12.25	6.15	6	13.85
11	17.3	4.75	19	9.55
12	14.1	11	1.59	25.65
14	5	4.1	19.75	14.65



**Fig. 2.** The growth curve of *Leptolyngbya* NBP-7A

After 14<sup>th</sup> day of incubation *Leptolyngbya* NBP-7A were able to grow in NPK growth media with concentration 80 ppm and 160 ppm. Compared to Bold's Basal Medium (BBM) and BBM + NPK 80 ppm as a control, *Leptolyngbya* NBP-7A has the lowest wet weight biomass at both concentration of NPK growth media. Wet weight of *Leptolyngbya* NBP-7A in 80 ppm NPK growth media was 5 mg, and 160 ppm was 4.1 mg.

The wet weight biomass of *Leptolyngbya* NBP-7A in all of growth media were rise from 0<sup>th</sup> until 3<sup>th</sup>, except in BBM + NPK 80 ppm. After 3<sup>th</sup> day observation, the wet weight biomass of *Leptolyngbya* NBP-7A were decreased in all of growth media. At the day observation 7<sup>th</sup>, *Leptolyngbya* NBP-7A produced the growth curve unstable. It could be seen from the figure that *Leptolyngbya* NBP-7A produce wet weight biomass in all of growth media rise at 7<sup>th</sup>, then decreased at 8<sup>th</sup> day, then rise again at 11<sup>th</sup> day, and decreased at 14<sup>th</sup> day of observation. It might be happened because *Leptolyngbya* NBP-7A still adapted with new environmental condition, especially the nutrient in growth media [10]. But, as we can see from the growth curve *Leptolyngbya* NBP-7A, the highest biomass production was in NPK 80 ppm in 7<sup>th</sup> day of observation. Based on the result in 7<sup>th</sup> days of observation, we can harvest the biomass of *Leptolyngbya* NBP-7A in short time period.

*Leptolyngbya* NBP-7A were able to grow in a low-cost media using commercial fertilizer NPK. Concentration of commercial fertilizer NPK affected the grow of *Leptolyngbya* NBP-7A. From the result, it could be seen that *Leptolyngbya* NBP-7A was more adapted with concentration 80 ppm than 240 ppm. It could be happened because the higher concentration of nitrogen contain in growth media of microalgae can caused toxic in microalgae. The higher concentration of nitrogen caused disruption of photosynthetic pigment formation [9]. The highest biomass production at the end of observation was in BBM + NPK 80 ppm. It could be happened because BBM + NPK 80 ppm was rich media. The macronutrient and micronutrient in BBM made *Leptolyngbya* NBP-7A produce more biomass production. Although the growth curve of *Leptolyngbya* NBP-7A in this study still unstable and need more day of observation, because *Leptolyngbya* NBP-7A still adapted with the growth media. The more biomass produced, the higher lipid content was produced and potentially could be used as biofuel feedstock.

## 4 Conclusion

Based on the observation of the 7<sup>th</sup> day and the discussion that had been done, *Leptolyngbya* NBP-7A were able to grow in a low-cost media using commercial fertilizer NPK. The best growth media for *Leptolyngbya* NBP-7A 80 ppm. It could be seen from the result that *Leptolyngbya* NBP-7A produce the highest biomass production at 7<sup>th</sup> day observation. As we can see from the result, we can harvest the biomass of *Leptolyngbya* NBP-7A in short period time. Furthermore, we can exchange the BBM with NPK fertilizer as growth media for *Leptolyngbya* NBP-7A growth.

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