

# Growth of *mastigocladus* (Cyanobacteria) HS-46 isolated from maribaya hot spring, indonesia in medium NPK as a potential biofuel feedstock

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**Abstract.** One of cyanobacteria genera which isolated from Maribaya hot spring located in Indonesia is *Mastigocladus* HS-46 can be used as raw material for biofuel production. *Mastigocladus* is cultured in commercial NPK fertilizer. Commercial NPK fertilizer is one of the low cost medium that commonly used for microalgae growth. The research aims to know the best concentration of commercial NPK fertilizer for *Mastigocladus* HS-46 growth. In this research, *Mastigocladus* HS-46 was grown in commercial NPK fertilizer with concentration 80 ppm, 240 ppm, NPK 80 ppm+BBM, and BBM (Bold Basal's Medium) as a control. This strain was incubated on 35 °C. Observation were made approximately 14 days with 2 sampling for each medium. The average of wet weight *Mastigocladus* HS-46 after 14 day observation was obtained at NPK 80 ppm: 0,019 g/L, NPK 240 ppm: 0.009 g/L, NPK+BBM: 0,014 g/L, and BBM: 0,015 g/L. The results showed that the best NPK medium concentration was 80 ppm for growth *Mastigocladus* HS-46. Commercial NPK fertilizer can be used for growth *Mastigocladus* HS-46 which has potential as biofuel feedstock.

## 1 Introduction

One of the genera of cyanobacteria that can live hot spring, namely *Mastigocladus* sp. *Mastigocladus* HS-46 was isolated from the Maribaya hot spring in Indonesia. *Mastigocladus* HS-46 lives at 42 °C and pH 6 [1]. *Mastigocladus* is potentially as a raw material for the production of biofuels, because biomass *Mastigocladus* contain lipid which can be used as a raw material for the production of biofuels [2]. The benefits of using microalgae including cyanobacteria as a raw material for biofuels, that is in the process of harvesting can be done continuously, the cost of harvesting and transportation relatively low, and also does not require extensive land in breeding [3]. to be able to produce biofuel, it takes high amounts of biomass. Therefore, it needed a growth medium for growing cyanobacteria so it can produce a high amount of biomass that can be used as raw material for the production of biofuels.

Growth medium containing nutrient for the growth of cyanobacteria. The growth medium commonly used for the growth of microalgae including cyanobacteria is Bold Basal's Medium. The Bold Basal's medium has a complete macronutrient and micronutrient [4] to serve as a medium control for the study. Nevertheless, the use of medium BBM to growing cyanobacteria in large scale research that leads to the production of biofuels is not

economically. Therefore, to produce a high biomass needed an economical alternative medium.

In addition to the Bold Basal's Medium, NPK fertilizers can be used as microalgae growth mediums [5]. NPK Fertilizer is an inorganic fertilizer with macronutrient composition consisting of N, P, and K which is essential and commonly used in plants to support its growth [6]. Other than as a plant fertilizer, NPK fertilizer can also be used to grow the cyanobacteria because it has enough content of nutrients for the growth of cyanobacteria.

This study is a basic study to find alternative medium, that is using NPK fertilizer to grow *Mastigocladus* HS-46 in order to produce high biomass. Research is expected to continue in large scale research leading to the biotechnology sector to produce biofuels.

## 2 Method and Materials

### 2.1 Microorganisms and Growth Medium

The microorganisms which used in this study was cyanobacteria genus *Mastigocladus* strain HS-46. *Mastigocladus* HS-46 was isolated from Maribaya hot spring. *Mastigocladus* HS-46 were grown in growth medium BBM as a control medium, and medium NPK

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fertilizer with variations concentrations 80 ppm, 240 ppm, and medium NPK fertilizer mix with BBM (NPK 80 ppm+BBM).

## 2.2 Method

The first step of cultivation cyanobacteria is inoculated 30 mg biomass into growth medium. Amount 70 mL of growth medium were added into Erlenmeyer flask 100 mL. *Mastigocladus* HS-46 was incubated in an incubator at temperature 35 °C and light intensity  $\pm$  3300 lux, with initial pH 6-6.5 with two repetitions.

## 2.3 Measurement the weight of wet biomass *Mastigocladus* HS-46

Measurement of *Mastigocladus* HS-46 were done in 14 days with 10 times of observation. The sterile eppendorf tube 1.5 mL was measured at analytical measurement tool. Eppendorf tube with biomass were sentrifuged for 10 minute with 10.000 rpm. The supernatant were taken out and wet biomass weight were measured with analytical measurement tool.

## 2.4 Preparation and processing of data

This research is descriptive. Observation data include quantitative and qualitative data. Quantitative data in the form of weight biomass (g/L) and the qualitative data in the form of a color culture of *Mastigocladus* HS-46. Data weight biomass will be shown in the form of tables and curves while the color culture of *Mastigocladus* HS-46 will be shown in the photos of microscopic. Growth curve was made by comparing the value of biomass weight as ordinate axis Y by biomass weight counting time as abscissa X. The growth curves were made by Microsoft Excel.

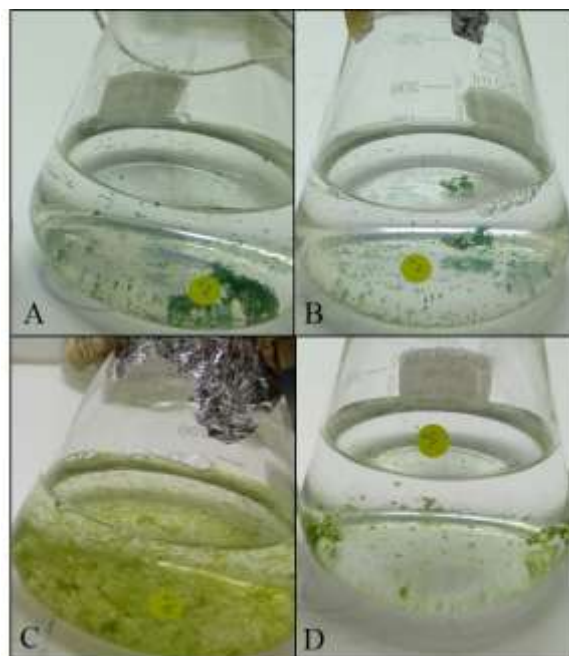
## 3 Results and Discussion

### 3.1 Macroscopic observation *Mastigocladus* HS-46

Macroscopic observations of *Mastigocladus* HS-46 cultures by comparing the culture color on the Faber Castle standard color [7]. Based on the color chart, the color of the starter culture *Mastigocladus* HS-46 is sea green (Fig.1). The color apperance of *Mastigocladus* HS-46 in medium NPK 80 ppm dan 240 ppm was changed from sea green into apple green at day 14 ( $t_{14}$ ). while *Mastigocladus* HS-46 in BBM and NPK+BBM not changed and still colored sea green at day 14 (Fig.2)



**Fig. 1.** Macroscopic sightings starter cultures *Mastigocladus* HS-46 in the Erlenmeyer flask is colored sea green.



**Fig. 2.** Cultures color difference of *Mastigocladus* HS-46 in medium BBM (A), medium NPK+BBM (B), NPK 80 ppm (C), and NPK 240 ppm (D). *Mastigocladus* HS-46 in BBM medium and NPK+BBM medium not changed and still coloured sea green, while *Mastigocladus* HS-46 is grown in NPK medium 80 ppm and 240 ppm, changing colour from green sea to apple green.

The color change that occurs in the *Mastigocladus* HS-46 grown in medium NPK 240 ppm and 80 ppm, probably due to the chlorophyll degradations process. The process of chlorophyll degradation mechanism can occur due to the reaction of phaeophytin formation. Phaeophytin is a form of chlorophyll that loses the  $Mg^{2+}$  ion. The process begins with the presence of  $Mg^{2+}$  ions in the middle of the molecule. Furthermore, the  $Mg^{2+}$  ion will be removed, and replaced by hydrogen ions. Consequently the color expressed becomes yellow [8].

Therefore *Mastigocladus* HS-46 grown in medium NPK 80 ppm and NPK 240 ppm change color to apple green.

### 3.2 Biomass Weight *Mastigocladus* HS-46

*Mastigocladus* HS-46 culture growth was seen in changes in the amount of biomass weight. Measurement of biomass weight of culture was done on day 0 (t<sub>0</sub>), day 1 (t<sub>1</sub>), day 2 (t<sub>2</sub>), day 3 (t<sub>3</sub>), day 4 (t<sub>4</sub>), day 7 (t<sub>7</sub>), day 8 (t<sub>8</sub>), day 11 (t<sub>11</sub>), day 12 (t<sub>12</sub>), and day 14 (t<sub>14</sub>).

**Table 1.** The average weight ratio of *Mastigocladus* HS-46 biomass in medium BBM, NPK 80 ppm, NPK 240 ppm, and NPK+BBM at observation time for 14 days

Time (Day)	Biomass weight (g/L)			
	BBM	NPK 80 ppm	NPK 240 ppm	NPK + BBM
t <sub>0</sub>	0.003	0.003	0.003	0.003
t <sub>1</sub>	0.001	0.001	0.002	0.001
t <sub>2</sub>	0.003	0.003	0.006	0.003
t <sub>3</sub>	0.013	0.003	0.005	0.002
t <sub>4</sub>	0.007	0.003	0.005	0.008
t <sub>7</sub>	0.013	0.005	0.015	0.018
t <sub>8</sub>	0.015	0.013	0.009	0.014
t <sub>11</sub>	0.001	0.013	0.018	0.017
t <sub>12</sub>	0.003	0.014	0.004	0.014
t <sub>14</sub>	0.015	0.019	0.009	0.014

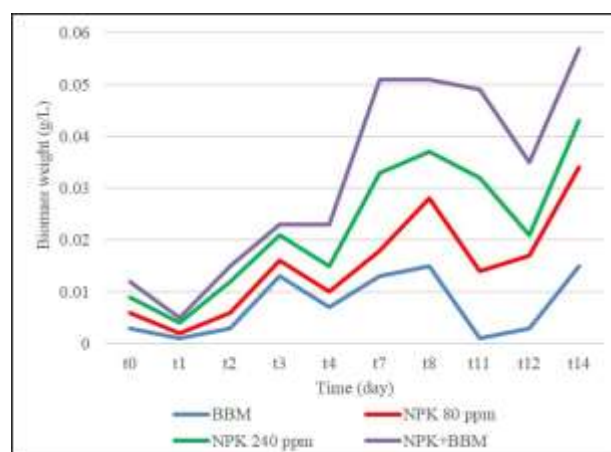
Based on Table 1. the results of the mean weight calculation of *Mastigocladus* HS-46 biomass in all treatments decreased from day 0 (t<sub>0</sub>) to day 1 (t<sub>1</sub>). *Mastigocladus* HS-46 grown in BBM medium increased biomass on day 3 (t<sub>3</sub>): 0.013 g/L; day 8 (t<sub>8</sub>): 0.015 g/L; and day 14 (t<sub>14</sub>): 0.015 g/L.

*Mastigocladus* HS-46 grown in NPK 80 ppm increased biomass on day 8 (t<sub>8</sub>): 0.013 g/L; day 12 (t<sub>12</sub>): 0.014 g/L; and day 14 (t<sub>14</sub>): 0.019 g/L. *Mastigocladus* HS-46 grown in NPK 240 ppm increased biomass on day ke-7 (t<sub>7</sub>) sebesar 0.015 g/L and day 11 (t<sub>11</sub>): 0.018 g/L. Then decreased on day 14 (t<sub>14</sub>): 0.009 g/L. *Mastigocladus* HS-46 grown in NPK+BBM medium increased biomass on day ke-18 (t<sub>18</sub>): 0.018 g/L. Then decreased on day 14 (t<sub>14</sub>): 0.014 g/L.

The result of qualitative observation using growth curve shows *Mastigocladus* HS-46 difference which is grown in variation medium of BBM, medium NPK 80 ppm, 240 ppm, and NPK+BBM. The difference can be seen in Fig.4.

The growth curve of this study still imperfect, because *Mastigocladus* HS-46 were still adapted with the condition of growth medium [5]. The growth curve can increase and decrease again so it has not reached stable. This is because the medium BBM, NPK 80 ppm, and NPK 240 ppm, has a high nitrogen content. Nitrogen contained in the medium is a major nutrient in the growth of microalgae including cyanobacteria. Nitrogen function in biochemical processes, such as the

biosynthesis of nucleic acids (DNA and RNA) and amino acids (protein). Cyanobacteria use sources of nitrogen in the form of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, and NO<sup>-</sup> and also organic molecules in the form of urea and amino acids [9]. The source of nitrogen in the medium BBM from NaNO<sub>3</sub> while the nitrogen source in the NPK medium from amonium (NH<sub>4</sub><sup>+</sup>); nitrat (NO<sub>3</sub><sup>-</sup>); and urea. High levels of nitrogen in the growth medium can lead to the deactivation of pigment production for photosynthesis [5]. Therefore, mediums that have high nitrogen levels take longer to reach a stable phase of growth. The nitrogen content in the BBM medium was 41.17 mg, while the nitrogen content of NPK medium 80 ppm and 240 ppm was 16 mg and 48 mg.



**Fig.4.** Growth curve of *Mastigocladus* HS-46 in BBM medium; NPK 80 ppm; NPK 240 ppm; and NPK+BBM.

### 4 Conclusion

Based on the observation of the 14 day and the discussion that has been done. The medium concentration of NPK 80 ppm is the best concentration of NPK for *Mastigocladus* HS-46 growth, because the average biomass weight *Mastigocladus* HS-46 in NPK 80 ppm on day 14 (t<sub>14</sub>): 0.019 g/L. The low average biomass weight in *Mastigocladus* HS-46 grown in NPK medium 240 ppm on day 14 (t<sub>14</sub>): 0.009 g/L.

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