Biomass production of *Mastigocladus* (cyanobacteria) HS-46 in bold basal medium and npk medium to produce high lipid content

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**Abstract.** The study about the effect of variation concentration medium NPK to the Biomass *Mastigocladus* (Cyanobacteria) had been done. The aim of the study was to known the effect of variance concentration of NPK growth media within the biomass production. The other aim of the study was to determine the best concentration NPK growth media for *Mastigocladus* HS-46 to produce higher biomass with high lipid content. *Mastigocladus* HS-46 was grown in BBM as a control and NPK growth media as working media. The variance concentration of NPK growth media that had been used for this study were 80 ppm, 160 ppm and 240 ppm. *Mastigocladus* HS-46 was incubated on 35 °C with light intensity 1500-3000 lux and initial pH 6.5. The growth of *Mastigocladus* HS-46 was measured by qualitative data based on morphology and color of biomass and quantitative data based on cell diameter size (width cell), biomass weight (mg/mL), and lipid content (%). The result showed that the best concentration for *Mastigocladus* HS-46 growth was in 240 ppm than BBM, NPK medium 80 ppm, and NPK medium 160 ppm. Based on the highest biomass production was 2.09 mg. mL⁻¹ and lipid content was 57%.

**1. Introduction**

Cyanobacteria are microorganisms that can live in both aquatic and terrestrial environments [1]. Some cyanobacteria can live in extreme environments, because they have the ability to adapt to their environment. One form of adaptation of cyanobacteria, which is able to live in high-temperature environments. Cyanobacteria that can adapt to high-temperature environments have thermostability related to metabolic processes [2]. This thermostability allows some cyanobacteria to adapt to high-temperature environments, such as hot springs.

One of the hot springs in Indonesia is the Maribaya hot spring. Maribaya hot springs have water temperatures ranging from 38-42 °C. Prihantini (2015) succeeded in isolating *Mastigocladus* from the Maribaya hot spring. *Mastigocladus* found in Maribaya hot springs can live at a pH of 6 and a...
temperature of 42 °C is referred to as Mastigocladus with strain code HS-46 (Hot Spring number 46) [3]. Taxonomically Mastigocladus is classified into the kingdom Eubacteria, a member of the phylum Cyanobacteria, class Cyanophyceae, and belongs to the nation Nostocales [4].

In addition to the influence of temperature and pH, light intensity affects the growth of Mastigocladus. Light is needed by Mastigocladus for photosynthesis. Photosynthesis in Mastigocladus occurs oxygenically, where H₂O functions as an electron donor [5]. In addition to the influence of environmental factors such as temperature, pH, and light, nutrient components can also affect the growth of Mastigocladus in order to produce high biomass. Then, also can affect to the components contained in Mastigocladus biomass include lipids. The content contained in Mastigocladus biomass is lipid [6].

Lipids are a group of organic components which, among other things, can be used as basic materials for biofuel production [7]. Related to its potential as raw material for biofuels, microalgae including cyanobacteria are able to accumulate lipids in their cells [8]. The advantages of using microalgae include cyanobacteria as raw material for biofuel production, namely that the harvesting process can be carried out continuously and the costs associated with harvesting and transportation are relatively low. In addition, cyanobacteria can reduce competition in productive land uses, because they do not require large areas of land for breeding [9]. The breeding process is carried out by growing cyanobacteria in a growth medium. The commonly used growth medium for microalgae is Bold Basal's Medium (BBM). This medium is widely used, because it has a complete composition of macronutrients and micronutrients [10].

Cyanobacteria can grow in BBM. Prihantini (2015) also grows Leptolyngbya and Nostoc in the BBM medium. Based on the pre-research that has been done, Mastigocladus can grow in BBM medium [3]. However, the use of BBM as a medium for growth of cyanobacteria on a large scale is not economical, because the components of chemical compounds in the BBM are expensive. Therefore, an economical culture medium is needed to produce high biomass. In addition to the BBM, NPK fertilizer can also be used as a growth medium for microalgae including cyanobacteria.

One of the inorganic fertilizers with essential macronutrient compositions of nitrogen (N), phosphorus (P), and potassium (K) is NPK fertilizer. NPK fertilizers are commonly used by plants to support their growth [11]. Besides having sufficient nutrient content for plant and microalgae growth, including cyanobacteria, NPK fertilizer medium is also very economical.

Utilization of NPK 20:20:20 medium to grow Mastigocladus HS-46 has been carried out in the pre-study. The NPK medium in the pre-study used concentrations of 80 ppm and 240 ppm. At the preliminary study could be seen that Mastigocladus HS-46 could grow at all concentrations of the given NPK medium. Based on this information, it can be seen that concentrations of 80 and 240 ppm in NPK media can be used as growth media for Mastigocladus HS-46. Meanwhile, a concentration of 160 ppm of NPK medium was added in the study. This is based on an arithmetic series between 80 ppm and 240 ppm. Therefore, the treatment given in this study used NPK medium with concentrations of 80, 160, and 240 ppm.

The hypothesis of the study was that the administration of NPK medium at concentrations of 80, 160, and 240 ppm had an effect on the mass of Mastigocladus HS-46 biomass. The next hypothesis is that the right concentration of NPK medium can produce high biomass supported by the lipid content produced. This study objective was to determine the concentration effect of NPK medium, namely 80, 160, and 240 ppm on the weight of the biomass supported by the lipid content produced. The research is expected to continue in large-scale research that leads to the biotechnology sector to produce biofuels and organic compounds.

2. Material and methods

2.1. Microorganisms and medium
Mastigocladus HS-46 was microorganisms used in this study. Mastigocladus HS-46 isolate was isolated from Maribaya hot springs (S06°49.892' and E107°39.443'). Mastigocladus HS-46 lived at an ambient
temperature of 42 °C and pH 6. This strain was isolated in a previous study by Prihantini in 2015 [3]. The strain was an axenic culture and was grown and maintained with BBM [12] at pH 6.5.

2.2. Treatment and research design
Mastigocladus HS-46 was grown in medium NPK with different concentrations of 80, 160, and 240 ppm. The study was conducted during 25 days of observation by the number of repeat six times. The number of repetitions is calculated based on Federer's formula, which is (t-1) (n-1) ≥ 15 with (t) the number of treatments and (n) number of replications [13].

2.3. Propagation of mastigocladus HS-46 Biomass
The biomass was propagated by inoculating the Mastigocladus HS-46 culture in a 50 mL Erlenmeyer flask containing 40 mL of BBM medium. Then, the cultures were stored in a 35 °C incubation cabinet. Next, the cultured biomass was transferred to a 250 mL Erlenmeyer flask.

2.4. Preparation of stock cultures and working cultures
Preparation of culture were carried out by preparing a stock culture and working culture. A stock culture was prepared by inserting 180 mL of BBM and 20 mg of Mastigocladus HS-46 culture into a 250 mL Erlenmeyer flask. Stock cultures were grown in an incubation cabinet at 35 °C.

2.5. Macroscopic and microscopic observations of Mastigocladus HS-46
For each data collection on observations on day 0 (t0), t1, t2, t3, t4, t6, t7, t11, t14, t18, t21, t25 macroscopic and microscopic observations were made. Macroscopic observations were carried out with the help of the Faber Castell standard color [14]. The color of the culture was compared with the standard color. Microscopic observations were carried out by observing the shape of the cells using a microscope.

2.6. Inoculation of test cultures
The preparation of the working culture was started by preparing the biomass starter Mastigocladus HS-46. A total of 60 mg of Mastigocladus HS-46 culture was put into a sterile 250 mL Erlenmeyer flask containing 200 mL of BBM. The same treatment was carried out using NPK fertilizer medium. NPK fertilizer used is NPK fertilizer [Grow More][15]. Each treatment was repeated six times. Therefore, the number of test cultures was 24 test cultures. Test cultures were incubated in a 35 °C incubation cabinet.

2.7. Laying of working cultures in incubation cabinets
The working culture was placed in a 35 °C incubation cabinet at. The distance between the working cultures was adjusted based on the measurement of light intensity using a luxmeter (1500-3300 Lux). The placement of the Mastigocladus HS-46 working culture can be seen in figure 1.

2.8. Calculation of biomass weight of mastigocladus HS-46
The calculation of the biomass weight of Mastigocladus HS-46 was carried out 10 times at observations t0, t1, t2, t3, t4, t6, t7, t11, t14, t18, t21, t25. Mastigocladus HS-46 biomass collection was carried out at the same time. The calculation of the weight of the biomass begins with weighing an empty of 2 mL Eppendorf micro tube using an analytical balance [Precisa XT-220 A]. The weight of an empty Eppendorf microtube was then recorded. Furthermore, harvesting and weighing of Mastigocladus biomass was carried out in the following way. Before it is harvested, shaken the test culture flask in advance so that the biomass dispersed. Then immediately, a total of 2 mL of the test culture was taken aseptically using a micropipette, then put into a 2 mL Eppendorf micro tube and centrifuged for 10 minutes at 10,000 rpm [EBA 20 HETTICH ZENTRIFUGEN]. The supernatant formed was taken using a sterile micropipette until only pellets were obtained in the Eppendorf micro tube. The pellets in a 2 mL Eppendorf micro tube were dried using an oven at 40 °C. After that, it was weighed using an analytical balance and the results were recorded. The weight of the biomass can be determined by...
calculating the difference in the weight of the Eppendorf micro-tube containing pellets with the weight of the empty micro-tube [16].

![Figure 1](image1.png)

**Figure 1.** *Mastigocladus* HS-46 working culture laying in culture cabinet. Note:
A. Laying of working cultures as control (BBM); B. Laying of working cultures in 80 ppm NPK medium; C. Laying of working cultures in NPK medium 160 ppm; D. Laying of working cultures in NPK medium 240 ppm

2.9. Lipid content measurement of *Mastigocladus* HS-46
Measurement of *Mastigocladus* HS-46 lipid content was carried out at starter and t25. Lipid content measurement was carried out by the Bligh & Dyer method (1959) using chemicals as solvents [17]. Chemical solvents used are methanol, chloroform, and distilled water. The measurement begins with weighing an empty petri dish and an empty filter paper, then the results are recorded.

*Mastigocladus* HS-46 biomass was taken as much as 3 mL. The biomass was then put into a mortar and pounded using a pestle, gradually added 3 mL of chloroform and 3 mL of methanol. The mixture was put into a 500 mL Erlenmeyer flask, then sonicated for 30 minutes. The sonication process aims to break down the cell walls of *Mastigocladus* HS-46. The sonicated sample was added with 2.5 mL of distilled water and 2.5 mL of chloroform. The sample was sonicated again for 30 minutes, then transferred to a 15 mL centrifugation tube and centrifuged at 6,000 rpm for 25 minutes. The supernatant and pellet were then transferred to a Buchner funnel for filtering. The mixture of lipid and chloroform was transferred to a petri dish and dried in an oven at 105 °C for 2 hours until the weight was constant. Lipid weight was obtained from the difference between the weight of the culture filled petri dish and the empty petri dish. Cell dry weight was obtained from the difference between the culture content filter paper and the blank filter paper. The lipid content of *Mastigocladus* sp. calculated based on the following equation: % Total Lipid = (DLP (g))/ (DCW (g)) x 100 % with DLP: Lipid Dry Weight; DCW: Cell Dry Weight [18].

2.10. Environmental conditions measurement
Measurement of environmental conditions consists of measuring room temperature and light intensity. The incubation cabinet temperature was measured using an air thermometer. Air thermometer placed in the incubation cabinet. Measurement of light intensity using a luxmeter placed in the incubation cabinet.
facing the light source, then the light intensity measured and printed on the luxmeter is recorded. Measurement of environmental conditions was carried out every day during the study.

2.11. Compilation and processing data
Observational data includes quantitative and qualitative data. Quantitative data in the form of cell diameter size (cell width), biomass weight (mg/mL), and lipid content (%). Qualitative data in the form of morphology and color of Mastigocladus HS-46 cells. Data on biomass weight (mg/mL) and lipid content were analyzed and then displayed in tables and curves, while the morphological and color data of Mastigocladus HS-46 culture were shown in microscopic photos.

The growth curve was made to see the growth phases of Mastigocladus HS-46. The Y axis on the growth curve shows the amount of Mastigocladus HS-46 biomass, and the X axis shows the growth time. Data analysis begins with a normality test using the Kolmogorov Smirnov test. If the resulting data is not normally distributed, then the data will be tested with a non-parametric test. The nonparametric test used is the Kruskall-Wallis test [19]. These data were tested to determine whether or not there was an effect of variations in the concentration of NPK medium on the average biomass of Mastigocladus HS-46. The data obtained will be processed using Microsoft Excel software to assist in generating data in the form of tables and graphs.

3. Results and discussion
3.1. Macroscopic observations of Mastigocladus HS-46
Macroscopic observations were made by observing the color of Mastigocladus HS-46 culture. Based on the Faber Castell color table, the color of the starter culture for Mastigocladus HS-46 was Sea Green. The macroscopic appearance of the starter Mastigocladus HS-46 in the BBM can be seen in figure 2.

![Sea Green](image)

**Figure 2.** Macroscopic appearance of starter of *Mastigocladus* HS-46

Macroscopic observations of *Mastigocladus* HS-46 culture were carried out on day 0 (t0), day 1 (t1), day 2 (t2), day 3 (t3), day 4 (t4), day 6th day (t6), 7th day (t7), 11th day (t11), 14th day (t14), 18th day (t18), 21st day (t21), and -25 (t25). On day 1 (t1) to day 14 (t14) there was no color change from all treatments. The macroscopic color change of *Mastigocladus* HS-46 in all treatments occurred on day 18 (t18). The results of the observations can be seen in figure 3.
Figure 3. Color observations of *Mastigocladus* HS-46 culture from day 1 to 25.

In Figure 4 the culture of *Mastigocladus* HS-46 in the medium of BBM, 80 ppm NPK, and 240 ppm changed color in the 18th day. *Mastigocladus* HS-46 culture in BBM changed color from Sea Green almost like Emerald Green. Meanwhile, *Mastigocladus* HS-46 in 80 ppm and 240 ppm NPK medium underwent a color change from Sea Green to Apple Green. *Mastigocladus* HS-46 grown in NPK medium of 160 ppm did not change color and remained Sea Green. Color change to Apple Green may be related to photoinhibition grown in 80 ppm and 240 ppm NPK medium.

Photoinhibition causes inhibition of the photosynthesis process, so that the expressed color looks yellowish green [20]. The process of photosynthesis can be inhibited due to the formation of phaeophytin...
Phaeophytin is a form of chlorophyll that loses Mg2+ ions. The process of formation of phaeophytin begins with Mg2+ ions in the middle of the molecule being released and replaced by hydrogen ions, causing the color expressed to be yellow [21]. Therefore, the color of the culture of *Mastigocladus* HS-46 in 80 ppm and 240 ppm NPK medium underwent a color change from Sea Green to Apple Green.

**Figure 4.** *Mastigocladus* HS-46 macroscopic appearance on 18th day. Note: A. *Mastigocladus* HS-46 in BBM; B. *Mastigocladus* HS-46 in 80 ppm NPK medium; C. *Mastigocladus* HS-46 in 160 ppm NPK medium; D. *Mastigocladus* HS-46 in 240 ppm NPK medium

3.2. **Microscopic observations of mastigocladus HS-46**

Microscopic observations were made by observing the culture preparations of *Mastigocladus* HS-46 under a Leica DM 500 microscope with a magnification of 10 x 40. The microscopic characteristics observed were the shape and width of the cells from each treatment. The shape and width of the cell can be seen in figure 5.

**Figure 5.** The shape and width of the observed cells. Note: A. Branched filament form; B. filament form; 1. Branching cells that will begin to form; 2. Cell width.

Based on the results of observations on the starter culture of *Mastigocladus* HS-46 which was grown in BBM and aged 30 days, it showed that the cells of *Mastigocladus* HS-46 had a round cell shape. Cell width measurements were carried out on 10 *Mastigocladus* cells in each treatment. The average cell width is 9.26 µm. Microscopic observations of *Mastigocladus* HS-46 on the 0th day starter (t0) can be seen in figure 6.
Figure 6. Microscopic appearance of Mastigocladus HS-46 starter on day 0 (t0). Note: a. Branching on Mastigocladus HS-46; b. Round shape of vegetative cells

*Mastigocladus* HS-46 grown in BBM during the 25th observation (t25) had a cell width range of 4.5-4.53 µm. Meanwhile, the size range of *Mastigocladus* HS-46 cells grown in 80 ppm NPK medium was 4.8-4.95 µm. *Mastigocladus* HS-46 grown in 160 ppm NPK medium had a cell width of 6.9-7.47 µm. *Mastigocladus* HS-46 grown in 240 ppm NPK medium had a cell width of 5.7-6.23 µm. The width of the *Mastigocladus* HS-46 can be seen in figure 7. The width of the *Mastigocladus* HS-46 cells in each treatment was a normal cell size, because the width of the *Mastigocladus* vegetative cells was generally 5-10 µm [22].

Figure 7. Cell size of *Mastigocladus* HS-46 during observation

During the observation, no heterocyst cells were found in all treatments. Microscopic appearance on the 25th day can be seen in figure 8. This was due to the high nitrogen content in the BBM and NPK medium used. The nitrogen source in the BBM comes from nitrate (NO$_3^-$) and the nitrogen source in the NPK medium comes from nitrate (NO$_3^-$) and ammonium (NH$_4^+$).

If a growth medium contains a high nitrogen source, heterocysts and nitrogenase enzyme activity are not found [23]. This is because heterocyst cells play a role in free nitrogen fixation from the environment. Nitrogen fixation by heterocysts occurs because heterocysts have nitrogenase enzymes that can reduce free nitrogen (N$_2$) from the air to ammonium (NH$_3$) so that it can be used for protein synthesis in cyanobacteria [24]. The mechanism for the formation of heterocysts occurs when the nitrogen content is low, the hetR gene will be transcribed and encodes the protein 299aa. The hetR gene is a gene involved in the formation of heterocyst cells. Furthermore, 299aa protein plays a role in forming heterocyst cells [25].
During the observation, no akinete also was found in *Mastigocladus* HS-46. This was probably due to the medium used in the study containing sufficient sources of phosphate, so that akinete cells were not formed. The source of phosphate in the BBM comes from KH₂PO₄ and K₂HPO₄ and the source of phosphate in the NPK medium comes from P₂O₅. Phosphate is important for the growth of cyanobacteria, because it plays a role in the biosynthesis of nucleic acids and proteins [26].

The formation of akinete is influenced by the element phosphate. If there is a phosphate deficiency in the growth medium, akinete will be formed [27]. Akinete cells are characterized by thick cell walls and there are granular inside the cells, these granular are cyanobacteria food reserves in the form of cyanophycin polypeptide and cynophycin starch which can support cyanobacteria to survive in extreme environmental conditions [28]. This is supported by previous research conducted by Van Dok and Hart (1996) who grew *Anabaena circinalis* in a medium with a deficiency of phosphate elements, so that akinete was not formed in *Anabaena circinalis* [29]. Therefore, in the study, the formation of akinete and heterocysts in *Mastigocladus* HS-46 was influenced by BBM and NPK media, while cell size was not significantly influenced by BBM and NPK media.

![Figure 8](image_url)

**Figure 8.** Microscopic appearance of *Mastigocladus* HS-46 on the 25th day in each medium. Note: A. Culture in BBM; B. Culture in 80 ppm NPK medium; C. Culture in 160 ppm NPK medium; D. Culture in 240 ppm NPK medium; b. Branch

### 3.3. Biomass weight of mastigocladus HS-46

The biomass weight data was collected for 10 observations at t₀, t₁, t₂, t₃, t₄, t₆, t₇, t₁₁, t₁₄, t₁₈, t₂₁, and t₂₅. The data collection time span is two days and every three days. The time span of data collection was obtained from pre-study. In the pre-study data collection starts from t₀, t₁, t₂, t₃, t₄, t₇, t₈, t₁₁, t₁₂, and t₁₄. Based on the data obtained, until the 14th day the mass of *Mastigocladus* HS-46 biomass was still increasing. Therefore, in the study the time of data collection was extended to 25 days. The extension of the data collection time was aimed at obtaining sufficient *Mastigocladus* HS-46 biomass for lipid data collection so that it could be measured. Lipid data are supporting data from the study.
The results of calculating the average of *Mastigocladus* HS-46 biomass weight in all treatments decreased from day 0 (t0) to day 1 (t1). The results can be seen at table 1. *Mastigocladus* HS-46 grown in BBM experienced an increase in biomass on the 7th day of 0.80000 mg/mL; 18th day (t18) of 1.30000 mg/mL; and day 21 (t21) of 0.40000 mg/mL. Meanwhile, on the last day of observation (t25) there was a decrease in biomass of 0.40000 mg/mL.

The biomass weight of *Mastigocladus* HS-46 grown in 80 ppm NPK medium experienced an increase in biomass on the 3rd day by 0.90000 mg/mL; day 14 (t14) of 0.90000 mg/mL; 18th day (t18) of 3.70000 mg/mL. Meanwhile, on day 21 (t21) and day 25 (t25) there was a decrease in biomass from 1.50000 mg/mL to 0.300000 mg/mL.

*Mastigocladus* HS-46 grown in 160 ppm NPK medium only experienced an increase in biomass on day 11 (t11) by 1.00000 mg/mL. *Mastigocladus* HS-46 grown in 240 ppm NPK medium experienced an increase in biomass on day 3 (t3) of 0.83000 mg/mL; 6th day (t6) of 0.12000 mg/mL. There was a decrease in biomass on the 14th day (t14) of 0.13000 mg/mL and a not too much increase on the 18th day of 0.19000 mg/mL. There was a high increase in biomass on day 21 of 4.08000 mg/mL and on day 25 (t25) there was a decrease in biomass of 2.090000 mg/mL.

**Table 1.** Biomass weight average of *Mastigocladus* HS-46

| Observation (days) | BBM          | 80 ppm NPK Medium | 160 ppm NPK Medium | 240 ppm NPK Medium |
|-------------------|--------------|-------------------|--------------------|--------------------|
| t0                | 0.30000± 0.00000 | 0.30000± 0.00000 | 0.30000± 0.00000 | 0.30000± 0.00000 |
| t1                | 0.10000± 0.00198 | 0.10000± 0.00005 | 0.20000± 0.00009 | 0.36000± 0.00054 |
| t2                | 0.50000± 0.00088 | 0.10000± 0.00033 | 0.20000± 0.00007 | 0.23000± 0.00014 |
| t3                | 0.20000± 0.00009 | 0.90000± 0.00187 | 0.20000± 0.01006 | 0.83000± 0.00143 |
| t4                | 0.10000± 0.00002 | 0.10000± 0.00003 | 0.10000± 0.00003 | 0.60000± 0.00120 |
| t6                | 0.10000± 0.00004 | 0.10000± 0.00008 | 0.20000± 0.000010 | 0.12000± 0.00004 |
| t7                | 0.80000± 0.00165 | 0.10000± 0.00004 | 0.10000± 0.00004 | 0.29000± 0.00036 |
| t11               | 0.10000± 0.00003 | 0.20000± 0.00160 | **1.00000± 0.00154** | 1.60000± 0.00272 |
| t14               | 0.10000± 0.00003 | 0.90000± 0.00180 | 0.20000± 0.000012 | 0.13000± 0.00003 |
| t18               | 1.30000± 0.00267 | 3.70000± 0.000267 | 0.30000± 0.00026 | 0.19000± 0.00011 |
| t21               | **2.00000± 0.00352** | 1.50000± 0.00257 | 0.20000± 0.000020 | **4.08000± 0.00460** |
| t25               | 0.40000± 0.00027 | 0.30000± 0.00012 | 0.30000± 0.00017 | 2.09000± 0.00446 |

The average biomass of *Mastigocladus* HS-46 grown in various mediums was statistically tested to see if the distribution of the data was normal or abnormal. The statistical test begins with a normality test using non-parametric statistical test such as the Kolmogorov Smirnov test. The results of the normality test showed that the average biomass of *Mastigocladus* HS-46 was not normally distributed, so the next test used to determine whether there was a difference in the variation of the medium to the biomass of *Mastigocladus* HS-46, using non-parametric statistics.

The non-parametric statistical test used was the Kruskal-Wallis test. The result of the Kruskal-Wallis test was that there was no difference between the average biomass of *Mastigocladus* HS-46 against different mediums. This may be due to the high standard deviation value of the mean weight of biomass (Table 1) and because the value of the weight of biomass is too low, so that at the time of data analysis the results were not detected. However, qualitatively it showed that there was a difference between the average biomass of *Mastigocladus* HS-46 biomass against different mediums.

The results of qualitative observations using growth curves showed that there were differences in *Mastigocladus* HS-46 grown in various variations of BBM, 80 ppm, 160 ppm, and 240 ppm NPK medium. These differences can be seen in figure 9.
The growth of *Mastigocladus* of HS-46 were grown in BBM through a phase of adaptation of t0 to t11. The exponential phase is reached at t21. Then experiencing the death phase at t25 also can be seen in figure 9.

*Mastigocladus* HS-46 are grown in medium 80 ppm NPK through a phase of adaptation of t0 to t7. The exponential phase starts from t11 to t18. Then experiencing a death phase starting from t21 to t25 can be seen in figure 9.

*Mastigocladus* HS-46 grown in 160 ppm NPK medium probably started the adaptation phase from t0 to t6. There was an insignificant increase at t11. *Mastigocladus* HS-46 grown in 240 ppm NPK medium underwent an adaptation phase starting from t0 to t18. The exponential phase was reached at t21 and decreased at t25.

Based on the growth curve of *Mastigocladus* HS-46 grown in BBM, 80 ppm, 160 ppm and 240 ppm NPK medium tend to have unstable growth curves. The growth curve can increase and decrease again so that it has not yet reached stable. This is because the BBM and NPK medium with various concentrations have high nitrogen content. Nitrogen is the main nutrient in the growth of cyanobacteria. Medium that has a high nitrogen content takes longer to reach a stable phase of growth [30].

3.4. Lipid content of *Mastigocladus* HS-46

Lipid content data is supporting data from the biomass weight data of *Mastigocladus* HS-46. Measurement of lipid content was carried out on the starter and 25th day (t25). Measurement of lipid content using the Bligh & Dyer method (1959) with chloroform, methanol, and aquadest as solvents. Chloroform solvent serves to bind nonpolar lipids, while methanol will bind to distilled water [17].

Table 2. Biomass weight and lipid content of *Mastigocladus* HS-46 on day 25

| Medium    | Biomass weight (mg) | Lipid weight (mg) | Lipid content (%) |
|-----------|---------------------|-------------------|-------------------|
| BBM       | 38.4                | 12.8              | 35                |
| 80 ppm NPK| 22.5                | 7.8               | 45                |
| 160 ppm NPK| 9.3                | 4.2               | 56                |
| 240 ppm NPK| 8.2                | 4.6               | 57                |

The lipid content in the starter culture of *Mastigocladus* HS-46 was 33% of the total weight of the biomass. The results showed that lipid content of *Mastigocladus* HS-46 on the 25th day in the BBM was 35% with a biomass weight of 38.4 mg; medium 80 ppm NPK at 45% with a biomass weight of 22.5
mg; medium 160 ppm NPK by 56% with biomass weight of 9.3 mg; and 57% of 240 ppm NPK medium with a biomass weight of 8.2 mg. The results of the measurement of lipid content at t25 in each medium can be seen in Table 2. Comparison of the lipid content of *Mastigocladus* HS-46 is shown in figure 10.

![Comparison of lipid content of *Mastigocladus* HS-46 on the starter and the 25th day](image)

**Figure 10.** Comparison of lipid content of *Mastigocladus* HS-46 on the starter and the 25th day

Figure 10 shows that the lipid content of *Mastigocladus* HS-46 measured in NPK medium with various concentrations has a high lipid content compared to the control medium (BBM). This is possible because the NPK medium contains ammonium (NH\(_4^+\)) [31-32]. Meanwhile, in the BBM the nitrogen source comes from nitrate (NO\(_3^-\)) which must be converted into ammonium ions (NH\(_4^+\)). All forms of nitrogen in the body will be converted into intermediate compounds in the form of NH\(_4^+\) [33]. The conversion process from nitrate (NO\(_3^-\)) to ammonium ion (NH\(_4^+\)) occurs through two stages of reduction using the enzymes nitrate reductase (Nar) and nitrite reductase (Nir). This process requires ATP, so that the amount of ATP in the cell has already been used for the nitrogen assimilation process [33].

4. Conclusion

Based on the results above, there was an effect of BBM and variations in the concentration of NPK medium on the biomass production of *Mastigocladus* HS-46. Medium concentration of 240 ppm NPK was the most appropriate concentration for the growth of *Mastigocladus* HS-46, seen from the highest biomass weight on day 25. Then it was supported by the highest lipid content on the 25th day, which was 57%.

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