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Enhanced Growth and Lipid Production of Indonesia Indigenous Filamentous *Cyanobacteria* (*Mastigocladus* HS-46) in a Flat Photobioreactor Using Fertilizer Medium

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Abstract: Research on biomass and lipid production from *Mastigocladus* HS-46, an Indonesian indigenous filamentous microalga from Maribaya Hotspring as potential biofuel raw material has been done. This study was done to determine the effect of bean sprout extract various concentrations in 350 ppm NPK media on the biomass weight and lipid percentage of *Mastigocladus* HS-46 cultivated in Flat Photobioreactor (FPBR). Variations of growth media in this study are 350 ppm NPK Fertilizer medium with the addition of 1%, 2%, and 3% bean sprout extract concentrations. Besides, BBM (Bold's Basal Media) was used as a control media. The results showed that *Mastigocladus* HS-46 produced the highest biomass (0.1632 g/mL) and lipid percentage (62%) when cultivated in 350 ppm NPK media with 3% bean sprout extract. Nevertheless, The Kruskal-Wallis test (α=0.05) result showed there is no significant difference in the biomass average weight of this strain, given the addition of various concentrations of bean sprout extract in NPK Fertilizer medium. Therefore, Flat photobioreactor (FPBR) as a growth container and NPK Fertilizer media with addition of bean sprout extract as a growth media are considered suitable to grow *Mastigocladus* HS-46 as a future biofuel raw material.

Keywords: *Mastigocladus*; Filamentous *Cyanobacteria*; Flat photobioreactor; Biomass weight; Lipid percentage

1. Introduction and background

Diversification of national energy sources production is beneficial for guaranteeing energy security, especially for meeting the growing energy needs of society and industry[1)]. One of the energy sources that can be developed is biofuel. Biofuel as a lipid-based fuel can be used in other oil-based energy generators to produce energy without generator engine modification[2)]. Biofuel are produced from biomass lipid extraction, so the discovery of high lipid producing organism is necessary to produce biofuel.

Biofuel raw material can be obtained from the biomass of *Cyanobacteria*. As prokaryotic microorganism, *Cyanobacteria* can reproduce relatively quick, have flexibility in genetic material, and do not require large breeding area and easy to find in all types of habitat[3)]. *Cyanobacteria* can live in extreme temperature environments, such as hot springs and crater. Several genera of *Cyanobacteria* originated from hot springs and crater in Indonesia have the potential to become a source of natural materials for bioenergy, including *Synechococcus*[4)] and *Leptolyngbya*[5)] from Order Synechococcales[6)], so as *Nostoc* and *Mastigocladus*[12)]

from Order *Nostocales*[6]. Previous research about microalgae from Order *Nostocales* showed that *Anabaena variabilis* had 10.5% lipid, *Nostoc commune* had 8.4% lipid, and *Nostoc paludosum* had 10.4% lipid from their biomass[7].

Microalga from Order *Nostocales* that was studied in this study was *Mastigocladus. Mastigocladus* is a multicellular prokaryotic *Cyanobacteria* from the order *Nostocales* with a branched filament morphology[6]. It has a round and cylindrical cell shape[10], ranging from 1.5—10 μm[10][11]. *Mastigocladus* can live as a thermotolerant in warm water habitats (35—60 ºC)[11]. Indonesia's indigenous *Mastigocladus,* which is isolated by Prihantini in 2015 from the waters of the Maribaya hot spring, Mount Tangkuban Perahu, West Java, was named *Mastigocladus* HS-46. It can grow well at 42 ºC and pH 6. The biomass of this strain contains 25.77% saturated fatty acids[12]. Thereore, it is considered potential to be processed further as biofuel[13].

Production of biofuels requires the production of large amounts of biomass in a short time. It can be achieved if microalgae are cultivated in a good photobioreactor system. Photobioreactor (PBR) is a closed-system designed to provide optimal biological (contaminant-free), physical (enough light and agitation), and chemical (balanced nutrient and pH) conditions to microalgae or *Cyanobacteria* for biomass production as a biofuels raw material or other sources[14].

The photobioreactor system has many developments that come from optimizing various factors[15], such as its shape. Several experiments were done to find the optimum photobioreactor shape to cultivate Indonesia's indigenous *Cyanobacteria,* such as Bubble Column Photobioreactor[4][17][18] to cultivate *Synechococcus* HS-9 and *Leptolyngbya* HS-16, and Rectangular Airlift Photobioreactor[19] to cultivate *Synechococcus* HS-9.

Flat photobioreactors (FPBR) are flat-shaped photobioreactors which can be made of glass, acrylic, or plastic. It has a larger surface area to volume ratio to maximize the supply of light and temperature from outside to the system. In addition, the simple shape of the flat photobioreactor can control the hydrodynamic forces of the agitation process caused by introducing aeration into the system[20]. Flat-shaped photobioreactors have been used by Vogel and Bregmann (2017) to grow filamentous microalgae *Spirogyra* sp. [21]. The flat photobioreactor system usually consists of a port for harvesting biomass (microalgal biomass exit), an aeration port (air pump), and a nutrient dose pump[20]. The modification of the flat photobioreactor design used in the study lies in the nutrient delivery port because the growth medium used in the study was directly introduced into the system as a whole.

Beside the PBR shape, nutrient availability in the growth medium should be also considered. One of the media that is often used for *Cyanobacteria* culture is BBM (Bold's Basal Medium)[14]. Unfortunately, the BBM is considered not cost-effective to cultivate microalgae in a large-scaled system. An inexpensive alternative growing medium for growing *Cyanobacteria* is NPK fertilizer medium. The Nitrogen (N), Phosphate (P), and Potassium (K) contained in the NPK medium is crucial compounds needed for the growth of microorganisms, such as microalgae[23]. Nitrogen is an essential element in chlorophyll formation. If there is nitrogen deficiency in cells, the chlorophyll formation is disrupted, resulting in chlorosis[24]. Phosphorus is involved in the formation of ATP and the biosynthesis of nucleic acids [25]. Meanwhile, potassium has a role as an enzyme activator in the process of photosynthesis and cellular respiration[26].

The development of NPK medium as a microorganism growth medium can be carried out by adding micronutrient components obtained from natural media, such as coconut water[28], tea leaf extract[29], soybeans[30], or bean sprout extract[31]. Bean sprout extract contains macronutrients carbohydrates, protein, fat, fiber, fatty acids, and amino acids, as well as micronutrients, vitamins and minerals[32]. Research on bean sprout extract or *ekstrak tauge* (ET) as an alternative growth medium for microalgae has been carried out by Prihantini (2007) in the *Scenedesmus* genus at varying concentrations of 1%, 2%, 3%, 4%, 5% and 6%. The results showed that bean sprout extract produced the highest *Scenedesmus* cell density of 3,981,071 cells/mL at a concentration of 4% bean sprout extract[31]. However, bean sprout extract medium combined with NPK medium from Indonesia's local fertilizer has not been used to cultivate *Mastigocladus* HS-46.

Therefore, this study was carried out to determine the effect of BBM and 350 ppm NPK medium with addition of 1%, 2%, and 3% bean sprout extract to the growth, which was determined by biomass weight, cell size, and lipid percentage of *Mastigocladus* HS-46 cultivated in flat photobioreactor system. Data obtained from this study can be used as initial information for bioprospecting activities of Indonesian indigenous microalgae, *Mastigocladus* HS-46, as a biofuel agent.

2. Methods and experimental setting

2.1. Research design

The research design included the treatment given to *Mastigocladus* HS-46 which was grown in 350 ppm NPK combination medium with various concentrations of bean sprout extract addition, namely 1%, 2%, and 3%, which was dissolved in sterile water from natural springs, FMIPA UI Natural Lab. Growth data was determined by measuring average biomass weight of *Mastigocladus* HS-46 in 350 ppm NPK medium with the addition of 1%, 2%, and 3% bean sprout extract, which were then compared with average biomass weight of *Mastigocladus* HS-46 BBM medium as a control. The study was conducted for 10 observation days, with 3 repeated measurements on 2 photobioreactors for each treatment, so that the total

repetition was 6 times according to the Federer formula (n-1) (k-1) ≥ 15.[33)]

2.2. The Flat Photobioreactor System Preparation

Flat photobioreactor (FPBR) preparation begins with manual assembly of the entire system. The assembled FPBR components consist of a main body, an air distribution system which includes a hose, filter, aeration tank, and a pipe with 6 holes 2 cm apart and a cover body. The system was assembled based on a design modification from Hossain and Mahlia's research[20)]. The container or body of the FPBR was 5 cm wide, 30 cm long, and 20 cm tall. It has additional 3 cm additional length on the bottom right and left for support. The lid was 10 cm long and 5 cm wide. It was equipped with 1.5 x 2 cm additional hinge and 20 cm long aeration distribution port. The FPBR used in this study can be seen in Fig 1.

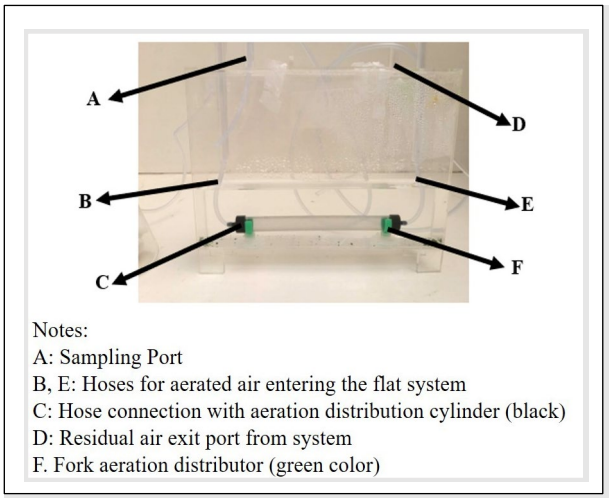

Fig 1. Flat Photobioreactor (FBPR) in this Study

The flat photobioreactor system consists of a sampling port where aeration enters the system, a port for residual air leaving the system, and a cylindrical structure for distributing aeration. After assembly, a system feasibility test was carried out, namely through a leak testing process. After the feasibility test, if no leaks are found in the system, then the photobioreactor is sterilized in stages. Photobioreactor sterilization consists of washing and bleaching, then drying and applying 70% alcohol. The sterilization process is carried out 3 times in a row, then irradiated with UV for 1 hour in a transfer box.

2.3. Collection and Filtration of FMIPA UI Natural Lab. Small Lake Water

Water sampling from the FMIPA UI Natural Lab was done before being used as a solvent of algal growth medium. Water from the Natural Lab small lake, FMIPA UI was taken as much as 15 L and put into a closed jerry can with a size of 20 L during the rainy season, pH 5.5 and ambient temperature 28 ºC. Natural Lab small lake is located in the laboratory area of the FMIPA UI campus, Depok. The water itself naturally contained nutrients, such as 8.86 mg of Total Nitrogen, 0.0064 mg of Phosphorus (in form of P2O5), 3.08 mg of Potassium (in form of K2O), 9.66 mg of Calcium, 1.24 mg of Magnesium, 0.64 mg of Ferrum, and 13.0 mg of Chloride in 100 mL of water.

2.4. Preparation of BBM, NPK medium, and Bean Sprouts Extract

The medium used to grow *Mastigocladus* HS-46 was Bold's Basal Medium (BBM) and 350 ppm NPK medium with various concentrations of bean sprout extract (ET). BBM medium used as the first control medium. The test treatment medium in the study consisted of NPK medium with the addition of various concentrations of 1%, 2% and 3% bean sprout extract which was dissolved in Nature Lab water at FMIPA UI. Each gram of the NPK Medium contained 42 mg Total Nitrogen, 18.3 mg Phosphorus (in form of P_2O_5), and 49.37 mg Kalium (in form of K_2O).

The Bold's Basal Medium (BBM) is made by dissolving the components of chemical compounds, consisting of $NaNO_3$, KOH, HCl, $CaCl.2H_2O$, $MgSO_4.7H_2O$, K_2HPO_4, KH_2PO_4, NaCl, EDTA, $FeSO_4.7H_2O$, H_3BO_3, $ZnSO_4.7H_2O$, MoO_3, $CuSO_4.5H_2O$, $MnCl_2.4H_2O$, and $Co(NO_2).6H_2O$ with certain concentrations and volumes[34)] into 1 L distilled water in an Erlenmeyer flask. All chemicals were dissolved in distilled water using a magnetic stirrer until homogeneous. Then the pH of the medium is adjusted until it reaches a value of 6.6. Then, BBM was sterilized by autoclave at 121°C, 15 lbs pressure, for 15 minutes.

NPK fertilizer in a solid form was weighed first before making the treatment medium for the combination of NPK and bean sprout extract. A total of 350 mg of NPK fertilizer granules that had been mashed with a mortar, were weighed with an analytical balance 3 times in a row so that 3 stocks of 350 mg of NPK fertilizer were obtained, then each of them were dissolved in 1 L of water, which would be stored for the manufacture of 3 treatment medium consisting of 350 ppm NPK medium with 1% (1 mL taken from 100 mL of stock), 2% (2 mL taken from 100 mL of stock) and 3% (3 mL taken from 100 mL of stock) of bean sprout extract.

The stock solution of bean sprout extract was prepared prior for making combination medium of NPK and bean sprout extract. Chemicals consist of NPK 12:12:17 [Kebo Mas] fertilizer, and all chemical components used to make the medium. The green bean seeds used were the YULET variety *Phaseolus radiatus*.

The bean sprout extract solution was made through the stages of seed germination, boiling and sterilization through the tindalization process. Mung bean seeds are soaked for 12 hours and then germinated for 48 hours using clean cotton. As much as 100 g of mung bean sprouts are washed again until clean, then were boiled using 500 mL of boiling distilled water for 1 hour. The

results of the decoction were then filtered with gauze covered with 3 pieces of cotton pads. The bean sprout extract solution was sterilized by tindalization, which was boiled in boiling water at 100 °C for 1 hour in a row 3 times within 24 hours. The last extract solution was sterilized using an autoclave at 121 °C and 15 lbs pressure for 15 minutes. The bean sprout extract solution was then stored as a stock solution for the preparation of NPK and bean sprout extract combination medium.

The stock solution of bean sprout extract was prepared first before the NPK combination medium with bean sprout extract was prepared. The 350 ppm NPK medium with the addition of 1% bean sprout extract was prepared by dissolving 350 mg of NPK fertilizer into 10 mL of bean sprout extract stock solution and then adding it with Natural Lab water until the volume reached 1000 mL. Likewise, for the preparation of NPK fertilizer medium with 2% and 3% bean sprout extract, in the same way by dissolving 350 mg of NPK fertilizer into 10 mL of bean sprout extract stock solution of 20 mL and 30 mL respectively (Modification from Prihantini, 2007)[31].

2.5. Inoculation of *Mastigocladus* HS-46 Test Culture

Preparation of the test culture begins with preparing the starter culture of HS-46 strain. As much as 900 mg of HS-46 strain culture was put into each photobioreactor unit which already contained 1 L of BBM medium. The same treatment was done with a photobioreactor unit containing 350 ppm NPK medium with the addition of 1%, 2% and 3% variations of bean sprout extract, 1 L each. The cultures were incubated in a greenhouse with a temperature range of 30-35 °C and 10,000 lux light intensity.

2.6. Arrangement of Test Culture Placement in a Photobioreactor System

The photobioreactor, which already contains growth medium and test culture, is then placed in the incubation room located in the greenhouse, Department of Biology FMIPA UI. The incubation room can be seen in Fig 2. The incubation site in the greenhouse has a temperature range of 30-35 °C which is equipped with a roof, body and cover of the incubation chamber which is covered with tarpaulin. Research by Prihantini shows that a temperature of 35 °C can be used to grow *Cyanobacteria*[12].

The layout scheme of the photobioreactor system can be seen in Fig 3. The treatment medium in the photobioreactor system consisted of BBM medium as a control (K), 350 ppm NPK medium with 1% bean sprout extract (A), 350 ppm NPK combined medium with 2% bean sprout extract (B) and 350 ppm NPK combined medium with 3% bean sprout extract (C). The whole system is placed horizontally in front of the lamp. The distance between the lamp and the entire photobioreactor system is determined and marked on the incubation room floor. This is intended so that the cultures get the same light intensity. The culture laying scheme also can be seen in Fig 3. It was done according to block randomization design[35]. During the observation period (10 days), environmental parameters such as pH of the media, temperature of PBR system, and light intensity were regularly measured and controlled[12)22].

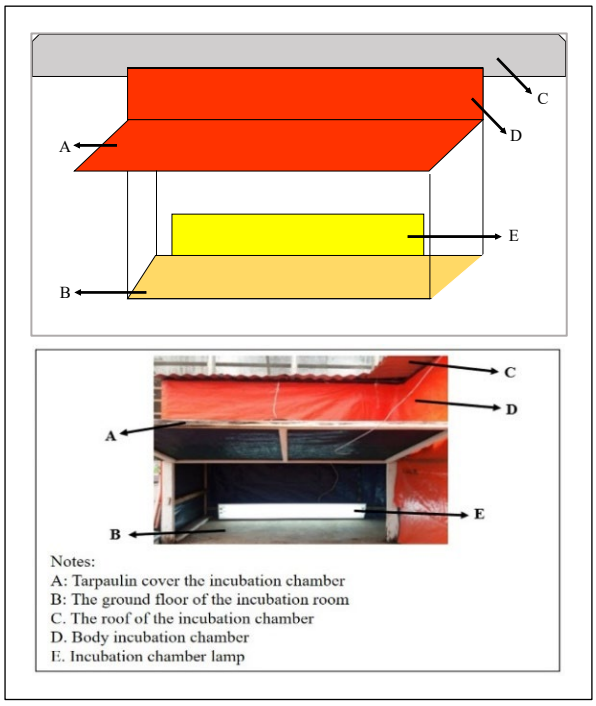

Fig 2. (a) Schematic design of (b) Place of culture incubation in a greenhouse.

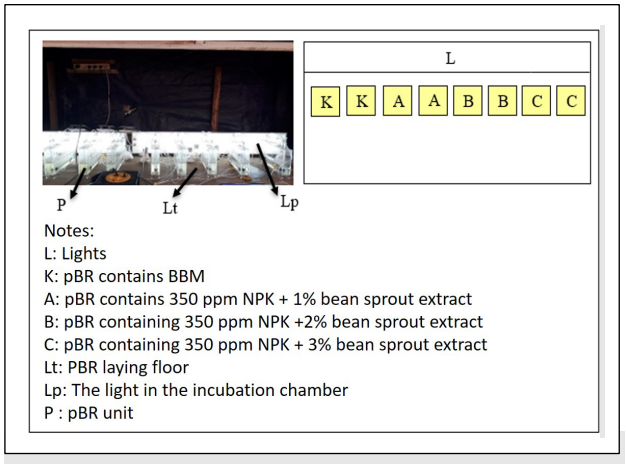

Fig 3. Arrangement of Test Culture Placement in a Photobioreactor System

2.7. Cell Size and Shape Observation of *Mastigocladus* HS-46 culture

Cell size and shape observation were done at the inoculation day (t0), the first day after inoculation (t1), second day after inoculation (t2), third day after inoculation (t3), fourth day after inoculation (t4), seventh

day after inoculation (t7), and tenth day after inoculation (t10). The observation was done under a light microscope with 400x magnification. Cell shapes, the presence of heterocyst, and the presence of akinete were observed. Cell diameter then was measured using the LEICA DM 500 Microscope application, or as known as LAS EZ.

2.8. Biomass Weight Measurement of *Mastigocladus* HS-46

Biomass weight measurement was carried at the inoculation day (t0), the first day after inoculation (t1), second day after inoculation (t2), third day after inoculation (t3), fourth day after inoculation (t4), seventh day after inoculation (t7), and tenth day after inoculation (t10). Biomass measurements were repeated 3 times for each photobioreactor unit. The first step of biomass weight measurement is weighing the empty Eppendorf microtubes with an analytical balance, then the results are recorded. Then, a sterile tube that has been connected to a sterile syringe is prepared. Two milliliters of microalgal sample in each photobioreactor unit was withdrawn with a syringe and put into a hose, then the sample was transferred into a sterile Eppendorf tube which had been weighed beforehand. Then, the biomass in Eppendorf tube was centrifuged for 5 minutes at 10000 rpm. The supernatant was removed using a Pasteur pipette. Then the wet biomass in the Eppendorf micro tube was dried in an oven at 40 ºC for 6 hours. Eppendorf microtubes containing dry biomass were weighed again with an analytical balance. Dry biomass weight is the weight of dry biomass in the micro-tube minus the empty micro-tube weight.

2.9. Measurement of the Lipid percentage from *Mastigocladus* HS-46 Biomass

The lipid percentage from *Mastigocladus* HS-46 biomass was measured at starter conditions (t0) and the last observation day (t10). The extraction method was the Bligh and Dyer (1959) method[36] that had been modified for microalgae[27]. Extracted lipids from the biomass of *Mastigocladus* HS-46 then was calculated using the following formula[37]:

$$\% \text{ Total Lipids} = \frac{\text{DLP (g)}}{\text{DCW (g)}} \times 100\% \qquad (1)$$

2.10. Data Analysis

The results of observations consist of qualitative data and quantitative data. Qualitative data consisted of cell shape of *Mastigocladus* HS-46 cells, presented by picture. Meanwhile, main quantitative data in this study are cell size range (µm), biomass weight (g/mL), and percentage of lipids (%). Quantitative data then were analyzed statistically by Kruskal-Wallis test (non-parametric ANOVA) through Microsoft Excel software (α=0.05). Data testing was carried out with the same objective, namely to determine whether or not there was a difference in the concentration of bean sprout extract in NPK medium on the *Mastigocladus* HS-46 biomass weight. All quantitative data were presented by graphs or tables.

3. Results and Discussion

3.1. Analysis of Cell Size and Shape of *Mastigocladus* HS-46

The results of microscopic observations of the starter culture (t0) of cells can be seen in Fig 5. Meanwhile, the range of cell diameter size of *Mastigocladus* HS-46 cultivated in FPBR in all medium combination can be seen in Fig 6.

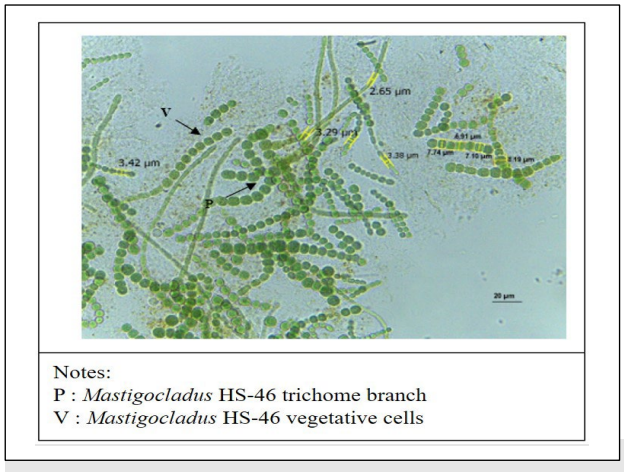

Fig 5. Photomicrograph of *Mastigocladus* HS-46 starter.

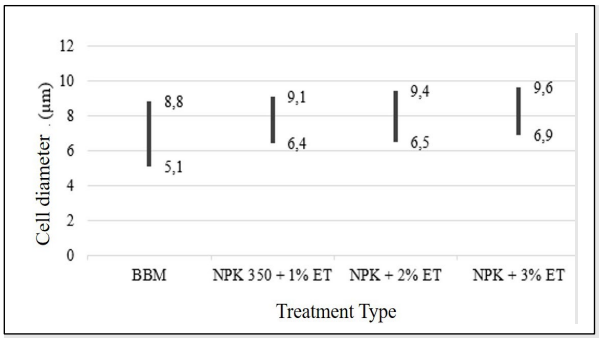

Fig 6. Range of *Mastigocladus* HS-46 cells diameter in all treatment mediums.

Starter cells have a spherical shape with an average cell diameter of 5.8 µm. Meanwhile, the range of cell diameter values of *Mastigocladus* HS-46 in all FBRs were varied, generally ranging from 5,1 to 9,6 µm. The results showed that the greater the concentration of bean sprout extract added, the larger the cell diameter. As seen on the graph, cells in FPBR (C) are quite larger, namely 6.9—9.6 µm.

Difference in cell diameter was influenced by several factors, such as physical or chemical factor. the chemical

content contained in the treatment medium. The growth media in FPBR (C) contained a relatively higher concentration of organic compounds than the BBM medium. Organic compound monomers added from bean sprout extract in NPK medium consisting of glucose, amino acids, fatty acids, and nucleic acids[32] which function as building blocks in the formation of cell structure thereby affecting changes in cell size during growth[23]. Even so, the value of the cell diameter size range in all treatment media is still classified as normal cell size, because *Mastigocladus* has a normal range of cell diameter sizes of 5—10 µm[11]. The shape of the cells was also normal, creating a filament, as described in microalgal identification guide book[6].

Microscopic observations showed no heterocyst cells (Fig 5.) were found in *Mastigocladus* HS-46 biomass. This phenomenon also happened on the previous research, where *Mastigocladus* HS-46 was cultivated in 80 ppm, 160 ppm, and 240 ppm NPK Fertilizer media[27]. Heterocyst cells were not produced due to the relatively high nitrogen content in BBM medium and NPK medium with the addition of bean sprout extract. The nitrogen source in the BBM was found in the form of nitrate ions (NO_3^-)[34]. On the other hand, the nitrogen source in the NPK medium can be found in the form of ammonium (NH_4^+). Meanwhile, the nitrogen source in bean sprout extract can be obtained from organic components such as proteins and amino acids[32]. The BBM itself was rich in N, while NPK Media fortified with bean sprout extract also rich in N. Therefore, heterocyst was not formed by *Mastigocladus* HS-46 strain due to N abundance in the media.

Growth medium with low nitrogen content causes undifferentiated cells to become heterocyst, because heterocyst is the place where nitrogen fixation happens[8]. Results showed that the cells were still at the log phase stage, thus allowing the availability of nitrogen in the medium to be sufficient to support cell growth. Heterocysts in *Mastigocladus* function in facilitating cells in fixing free nitrogen in the environment. Nitrogen fixation in heterocysts occurs because cells have the enzyme nitrogenase which plays a role in the fixation of environmental N_2 into ammonium, so that it can be used by cells to synthesize protein. If the availability of environmental nitrogen is low, especially in the form of ammonium compounds, 2-oxoglutarate accumulates in *Mastigsocladus* cells. The 2-oxoglutarate molecule acts as a carbon skeleton for the formation of ammonium. Excessive accumulation of 2-oxoglutarate signals the transcription of the HetR gene in the cell nucleus. The HetR gene encodes the protein transcriptional factor 299a to activate cell differentiation into heterocysts[38].

Based on microscopic observation (Fig 5.), akinet was also not found. This phenomenon also happened on the previous research, where *Mastigocladus* HS-46 was cultivated in 80 ppm, 160 ppm, and 240 ppm NPK Fertilizer media[29]. Akinet formation can be caused by the lack of phosphate availability in the growth medium[9]. Therefore, it is possible that the availability of phosphate elements in all treatment media is sufficient so that *Mastigocladus* does not produce akinet. Phosphate elements in BBM medium were found in the form of KH_2PO4 and K_2HPO_4 compounds, phosphate elements in NPK medium were found in the form of P_2O_5 compounds, and phosphate elements in bean sprout extract could be obtained from nucleic acid components. Akinet is a dormant cell structure in *Mastigocladus* that allows the genus to survive in extreme environmental conditions[40], because akinetes are only produced when phosphate levels in the environment or growth media are relatively low[9]. Deficiency of elemental phosphate causes reduced formation of ATP energy and decreased production of nucleic acid material, thus causing cell growth to be stunted[38]. Therefore, cells adapt to survive by producing dormant cells in the form of akinet. When the availability of phosphate in the environment is sufficient again, phosphate is used to stimulate germination on the previously formed akinet[11]. Akinet has components of cyanophycin starch and cyanophycin polypeptide which are used as food reserves for *Cyanobacteria* to survive[41].

3.2. The Biomass Weight and Growth Curve Analysis of *Mastigocladus* HS-46

The average weight yield of *Mastigocladus* HS-46 biomass with 2 repetitions in each treatment medium can be seen in Table 1. The results showed that on days 1 to 4, there was a decrease and increase in biomass weight in all treatments. Decreases or increases can occur because *Mastigocladus* HS-46 is experiencing a phase of adaptation to new environmental conditions. The adaptation phase or lag phase occurs when microorganisms are inoculated into a new environment for its growth[42], in this case, a new medium. This phenomenon is also happened to another Indonesia's indigenous microalga *Chlorella* DPK-01 (Chlorophyta) was cultured in BBM and NPK medium in tube-shaped photobioreactor[49]. When cultivated in NPK medium, the adaptation phase of *Chlorella* DPK-01 is slightly longer than in BBM because the starter culture was cultivated in BBM.

Mastigocladus HS-46 biomass weight data is depicted in the form of a growth curve which can be seen in Fig 7. The growth curve shows that the average yield of the biomass on t7 increased in each treatment medium, so that the biomass began to enter the log phase. The log phase is a phase that indicates the cell can divide multiple times due to physiological conditions that have adapted well to the availability of nutrients in the growth medium[51]. A significant increase in biomass weight on day 7 occurred in FPBR (C). The increase in biomass weight continued until t10 in all treatment mediums, except for BBM. The weight of biomass in the BBM decreased on t10, so it took up to 7 days to reach the highest biomass weight in the

BBM, while it took up to 10 days to reach the highest biomass in the 350 ppm NPK medium with the addition of bean sprout extract. Even so, the weight of biomass in FPBR (C) produced the highest weight of biomass from day t7 to t10 when compared to BBM as a control.

Table 1. Dry biomass average weight of *Mastigocladus* HS-46.

T (day)	Dry weight (g/mL)			
	BBM	NPK 350 ppm		
		ET 1%	ET 2%	ET 3%
0	0,0009±0,0000	0,0009±0,0000	0,0009±0,0000	0,0009±0,0000
1	0,0562±0,0372	0,0466±0,0061	0,0406±0,0106	0,0281±0,0307
2	0,0681±0,0052	0,0709±0,0004	0,0599±0,0033	0,0589±0,0014
3	0,0765±0,0191	0,0776±0,0122	0,0815±0,0153	0,0575±0,0030
4	0,0645±0,0033	0,0704±0,0010	0,0729±0,0015	0,0813±0,0093
7	0,1289±0,0467	0,1323±0,0416	0,1481±0,0713	0,1605±0,0144
10	0,1245±0,0076	0,1477±0,0013	0,1521±0,0090	0,1632±0,0018

The length of the growth phase of HS-46 strain is influenced by the availability of nutrients in the growth medium. The time needed for this particular strain to reach the log phase when grown in BBM medium was faster than NPK medium with the addition of bean sprouts extract. During the log phase, cells are physiologically well adapted to the nutritional components in the medium[43]. BBM medium contains inorganic mineral components in the form of ions which are more easily utilized by cells compared to NPK medium which has been given the addition of bean sprout extract which has a more complex nutritional content such as organic components of carbohydrates, proteins and fats[32].

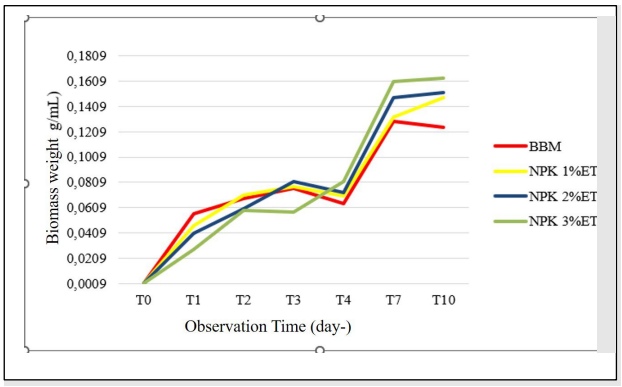

Fig 7. *Mastigocladus* HS-46 growth curve.

The average weight of *Mastigocladus* HS-46 biomass in FPBR Control, (A), (B), and (C), was tested statistically. It was done to determine whether or not there was an effect of treatment on the growth. The Kruskal-Wallis test (α=0.05) result showed there is no significant difference in the biomass average weight of this strain, given the addition of various concentrations of bean sprout extract in NPK medium. This phenomenon is likely to occur because there are still several other variables that affect the growth of *Mastigocladus* HS-46.

3.3. Analysis of Lipid Percentage from *Mastigocladus* HS-46 Biomass

Mastigocladus HS-46 lipid percentage was measured as supporting data for biomass weight. *Mastigocladus* HS-46 lipid percentage was measured at the beginning and end of the research. The results of measuring the lipid percentage of biomass at t10 is shown in Table 2.

Table 2. Percentage of lipid percentage of *Mastigocladus* HS-46 in all treatment mediums at t10.

Treatment Medium		Lipid weight (g)	Biomass weight (g)	Lipid percentage (%)
BBM		0,2080	0,8321	25 %
NPK 350 ppm	ET 1%	0,4080	0,8543	47,8 %
	ET 2%	0,5511	0,9232	59,7 %
	ET 3%	0,5764	0,9301	62 %

Based on the Table 2, there are differences in lipid percentage of *Mastigocladus* HS-46 in each treatment. The highest lipid percentage is found in biomass on FPBR (C), which is 62% of the total biomass compared to the control. It was about 30 times higher than previous study about another microalga from Order *Nostocales,* which is *Nostoc muscorum,* that had only produced 2.43 % lipid after 4 days cultivation in BG-11 media[48]. Meanwhile, this percentage (62%) is slightly higher than another Indonesia's indigenous filamentous microalga, *Leptolyngbya* HS-16, that produced about 45% lipid from its biomass after 25 days cultivation in 80 ppm NPK media[5]. This might happen because the NPK media in this study was fortified by bean sprout extract that contains the micronutrient called biotin[32]. The higher the concentration of bean sprout extract in growth media, the more biotin that can be used by microalga to produce lipid. Biotin acts as a cofactor for the Acetyl-Coa enzyme in the process of lipid biosynthesis[44]. Burkholder and Mcveigh (1945) reported that the concentration of green bean extract that had been germinated increased from 0.2 to 0.78 µg/g after germination, so that the higher the biotin content added from bean sprout extract to the NPK medium, the possibility lipid percentage in cells becomes higher[45]. In addition, the lipid percentage that was previously present in the bean sprout extract affected the value of the total lipid percentage produced by the biomass.

Mastigocladus HS-46 cultivated in FPBR (C) for 10 days produced a higher lipid percentage than starter biomass. This might happen because general principle of lipid formation was related to nitrogen extinction[48]. All Nitrogen in the media were considered to be used by *Mastigocladus* to grow rapidly in 10 days of observation. Besides, this phenomenon also might happen because the lipid percentage in cells can depend on the age of the culture. It is known that the number of lipid bodies in *Mastigocladus* increases with the age of the genus[11]. This

can be also compared with the previous study, where *Mastigocladus* HS-46 cultivated in NPK 240 ppm for 25 days could produce 57% lipid from its biomass[27].

The formation of a mucilage sheath on the cell wall can also affect the final result of the lipid percentage measurement from microalgal biomass. Production of the mucilage sheath in *Mastigocladus* continues to increase with cell development. Mucilage sheath is an organic carbohydrate compound[46] that has a heavier weight and is mixed with the biomass, thereby affecting the dry weight value of the biomass[47].

4. Conclusions

Based 10 days of observation, three conclusions are obtained. First, 350 ppm NPK medium with the addition of various concentrations of 1%, 2%, and 3% bean sprout extract and BBM medium was statistically did not have a significant effect on the weight of *Mastigocladus* HS-46 biomass (Kruskal-Wallis Test, α=0.05). Second, there are differences in total lipid percentage produced by *Mastigocladus* HS-46 biomass in 350 ppm NPK medium with the addition of various concentrations of 1%, 2% and 3% bean sprout extract and BBM medium. 350 ppm NPK medium with 3% bean sprout extract produced the highest biomass weight of 0.1632 g/mL with the highest lipid percentage of 62%. Third, a flat photobioreactor is considered good to grow filamentous *Cyanobacteria*, in this case the genus *Mastigocladus*. By cultivating microalgae in economic media such as NPK Fertilizer Media fortified by bean sprot extract, in a good system, such as flat panel photobioreactor, hopefully we are one step closer to get affordable and clean energy, which can fulfill Sustainable Development Goals (SDGs) number 7.

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Nomenclature

k	the number of treatments according to Federer Formula
n	the number of repetitions according to Federer Formula
DLP	Dry Lipid Weight (g)
DCW	Dry Biomass or Cell Weight (g)
ET	*Ekstrak Tauge* (Bean Sprout Extract)

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