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Effect of Reducing Nitrogen Levels on Growth and Lipid Productivity of Microalgae *Botryococcus braunii* Exposed by UV-C Rays

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ABSTRACT
Microalgae *Botryococcus braunii* is a potential raw material for producing lipids. Mutation using UV-C rays is a method to increase microalgae productivity. Optimum quality of microalgae *B.braunii* could be achieved by UV-C radiation at 1.5 minutes exposure with a continue light irradiance cycle. Reduction of nitrogen levels in nutrients and harvesting time were carried out to determine their effect on lipid productivity and lipid content of microalgae *B.braunii*. Biomass and lipid productivity is largely determined by the growth in the number of cells and cell mass of *B.braunii*. The highest biomass productivity was produced by native *B.braunii* in normal nitrogen medium at 7 days of culture. The highest lipid productivity was produced by UV-C mutated *B.braunii* in low nitrogen medium at 7 days of culture. The highest lipid content was produced by UV-C mutated *B.braunii* in low nitrogen at 20 days of culture. Native *B.braunii* provided the highest biomass productivity. The results of Gas Chromatography analysis showed an increase in the content of free fatty acids (FFA) and Monoacylglycerol (MAG) after mutation treatment and culture in low nitrogen medium.

Keywords: *Microalgae, Botryococcus braunii, nitrogen reduction, mutation, UV-C.*

1. INTRODUCTION

Microalgae are unicellular photosynthetic organisms that have potential as raw material for alternative sources. Microalgae lipids can be used to generate biodiesel. The growth works by utilizing nutrients in the presence of solar energy [1]. Microalgae use water and consume nutrients and carbon dioxide efficiently due to their growth media in aqueous [2].

One of the algae species found suitable for making biodiesel is *Botryococcus braunii* that live in marine water [1]. *B. braunii* containing lipids ranging between 25 to 75 percent dry weight to become one of microalgae with high lipid content [3]. There are 2.1 percent of triacylglycerols, 1.8 percent of diacylglycerols, 1.7 percent of monoacylglycerols, and 41.1 percent of free fatty acids in its lipid. [4] Palmitic acid and oleic acid take over fatty acid types found in the lipid of *B. braunii* [5]. *B.braunii* require a long period of time for cultivation. The low specific growth rate of *B.braunii* becomes resistance in the production of significant amounts of lipids. It is necessary to increase the quantity of biomass along with its levels of hydrocarbon and lipid production [6]. According to Sharma (2012), with optimal growth conditions the amount of biomass produced is large, but the content is relatively small so as to get a high lipid content, microalgae are conditioned under conditions of stress [7].

Mutation or mutagenesis is done to improve the nature of microorganisms. Mutation using UV rays is considered effective, easy to use, and safe for the environment. UV rays are classified based on the wavelength, UV-A (315 to 400 nm), UV-B (280 to 315 nm), and UV-C (100 to 280 nm). UV-C has a higher energy for radiation than UV-A or UV-B., Deoxyribonucleic acid (DNA) has the strong absorption peak at 254 nm that causes more damage to DNA and mutagenesis [6].

In 2017, Sivaramakrishnan and Incharoensakdi induced *Scenedesmus sp.* with UV-C rays 253.7 nm over a span of 0-40 minutes. After 12 days of culture, the biomass and lipid content of *Scenedesmus sp.* respectively increased from 1.9 to 2.4 g/L and from 40 to 55% of the dry cells weight [8]. Sarayloo et al. (2017), found that *Chlorella vulgaris* which was induced by UV-C rays produced biomass and the amount hexadecadienoic acid (C16:2) and linolenic acid (C18:3)

higher than native [9]. Thurakit et al. (2018), reported that UV-C induction in *Botryococcus braunii* increased production of algal biomass 1:32 times of their native with a lipid content reached 34%. The ability to survive of algae is found in the range from 0.66 to 12.54% after exposure to UV-C rays for 3-21 minutes [6].

Microalgae multiply cells and produce biomass compounds by utilizing several essential such as carbon, nitrogen, phosphorus, potassium, and iron through photosynthesis using solar energy [10]. Reducing nutrients such as nitrogen can affect microalgae metabolic system along with photosynthesis and lipid amount. The formation of macromolecules in microalgae is greatly influenced by the presence of nitrogen. Decreased chlorophyll formation due to nitrogen reduction will inhibit the photosynthesis process. In addition, the protein formation process also inhibited, resulting in the formation of carbohydrates and lipids in microalgae will increase Nitrogen reduction in the growth medium of microorganisms provides a stressful environment which inhibits their growth since nitrogen in living cells is a major constituent of nucleic acids and proteins [11]. Reduction of nitrogen levels in the cultivation medium of *Chlorella vulgaris* changes some of lipid composition from free fatty acids to triacylglycerol. The reduction of nitrogen levels also indicates an increase of microalgae lipid productivity [2].

This study aimed to investigate the effect of nitrogen reduction levels on microalgae growth and lipid productivity of native and mutated *B.braunii*. UV-C exposure time, light irradiance cycle, and harvesting time as factors of microalgae growth were investigated of their effect on lipid content and productivity. The investigation was conducted to discover the ideal conditions for achieving high-lipid content and high-lipid productivity for native and mutated *B.braunii*.

2. MATERIALS AND METHODS

2.1. Materials

B.braunii strains were acquired from Balai Besar Perikanan Budidaya Air Payau (BBPBAP) Jepara. Marine water with a salinity level of 21 ppt was added to microalgae strains. Marine water was acquired from Balai Perikanan Budidaya Air Payau Situbondo.

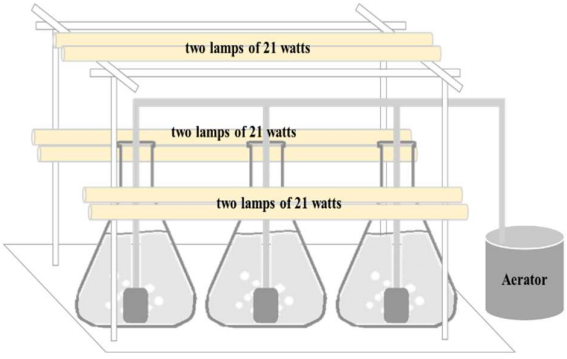


Figure 1 Microalgae Cultivation Installation

2.2. Medium and cultivation condition

Cultivation medium of *B.braunii* was divided into two mediums, normal nutrition medium (normal nitrogen) and low nitrogen medium. The normal nutrition medium of microalgae cultivation was Walne nutrient. The composition of Walne nutrient refered to Isnansetyo & Kurniastuty (1995), per 1 Liter of solvent containing $NaNO_3$, 100 mg; Na_2EDTA, 45 mg; H_3BO_3, 33.6 mg; $NaH_2PO_4.2H_2O$, 20 mg; $FeCl_3.6H_2O$, 1.3 mg; $MnCl_2.4H_2O$, 0.36 mg; Vitamin B1, 0.1 mg; and Vitamin B12, 0.005 mg [14]. The reduction of $NaNO_3$ composition from 100 mg to 0.03 mg was carried out in low nitrogen medium.

Figure 1 shows the installation of microalgae cultivation process. The experiment was carried out in Erlenmeyer 500 mL containing a mixture of 250 mL microalgae strain and 250 mL of marine water. Continuous air supply and circulation in culture medium were handled by an aerator, 2.5 L/min. On two sides and top of the Erlenmeyer, each had two lamps of 21 watts fitted at 3 cm from the Erlenmeyer. The light intensity was installed at 6000 lux (77.08 $\mu E/m^2s$). Every 24 hours, the amount of microalgae cells was counted due to their typical biomass growth increased through 24 hours [1].

2.3. Algae mutation

UV-C light was installed in the blackout chamber to prevent another light influence on the irradiation process, showed in Figure 2. Initial cell of *B. braunii* was counted before the radiation process. Mutation was done by irradiate microalgae to UV-C rays for 1.5 minutes; 3 minutes; and 30 minutes. The distance of the lamp and microalgae was set at 2 cm. Mutated *B. braunii* that survive after irradiation process was counted again.

2.4. Light irradiance cycle examination

Light irradiance cycle was examined by varying light and dark duration. The cultivation was carried out on normal nutrition medium for 10 days. Native and mutated *B. braunii* were cultivated under 12 hours of light / 12

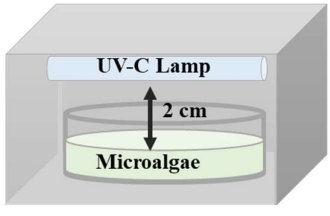


Figure 2 UV-C Irradiation Installation

hours of dark (12:12); 16 hours of light / 8 hours of dark (16:8); and continuous light irradiance for 24 hours (24:0). Cells of microalgae were counted each day after 24 hours.

2.5. Effect of reducing nitrogen levels and harvest time

Native and mutated microalgae were cultivated on normal nutrition medium and low nitrogen medium. Cultivation duration was divided into two types of time. The first type was cultivated for 7 days and the second types was cultivated for 20 days. Each day during cultivation, microalgae cells were counted. After the time passed, microalgae were harvested to extract the lipid.

2.6. Lipid extraction

Microalgae lipids were extracted using Soxhlet extraction method. Microalgae for extraction were centrifuged for 10 minutes at 8500 rpm with a centrifugation temperature of 20°C. Whatman filter paper was used to separate the solid phase from the medium. To achieve complete separation, two sheets of filter paper were used [2]. Wet microalgae were drained through two process. In the first process, wet microalgae were stored for 12 hours in the refrigerator with temperature around 4°C. In the second process, semi-wet microalgae were stored in oven for 2 hours. The oven was set at temperature of 60°C. N-hexane was used as the solvent for 6 hours lipid extraction process of microalgae around. Distillation at 70°C for around 2 hours was used to separate lipids from n-hexane.

2.7. Gas chromatography analysis

Shimadzu GC-2010 gas chromatography from Kyoto, Japan was used to analyse the quantity of free fatty acid and glycerides of microalgae lipids. 1 µL of sample that injected to GC was made from a mixture of 20 mg microalgae lipids and 1 mL ethyl acetate. The flame ionization detector GC was used a non-polar column DB-5HT with 0.1 µm for film thickness and ID 15 m x 0.32 mm. The detector was set at same temperature of injector in 370ºC. Column temperature were set at three steps. The initial temperature was set at 80ºC then increased 15ºC in every minute until reached 305ºC. The second step was to achieve temperature of 335ºC by increasing 5ºC in every minute and maintained the temperature for 5 minutes later. The last step was to achieve temperature of 365ºC by increasing 15ºC in every minute. Nitrogen was used as a carrier gas with a linear rate of 30 cm/s at a temperature of 80ºC with a split ratio of 1:50.

3. RESULT AND DISCUSSION

3.1. Microalgae mutation

3.1.1. Percentage of microalgae death cells after mutation

The mutation treatment was carried out to determine the best UV-C exposure time between 1.5; 3; and 30 minutes to increase lipid productivity of microalgae *B.braunii*. Table 1 shows a cells amount comparison of initial *B.braunii* and after exposure to UV-C rays. Counting chamber method with hemacytometer was used to count only living microalgae cells. The appearance colour of microalgae indicated the condition of the microalgae, either they were dead or not. There were three type colours of microalgae. Dark green microalgae indicated an optimal growth condition. Yellowish green indicated a non-optimal growth condition. Yellow colour was appeared when microalgae was dead.

The mutation treatment showed a decrease in the percentage of living cells at the additional time of exposure to UV-C rays. Mutated cells were used to name the survival cells after irradiation process by UV-C rays' exposure.

Response analysis of mutated *B.braunii* using UV-C rays to exposure time was carried out. On irradiation process by UV-C rays' exposure for 1.5 minutes; 3 minutes; and 30 minutes, *B.braunii* experienced average death rate of 29.31%; 51.55% and 71.81% for run 1, run 2, and run 3. Based on the results obtained, it can be seen that the longer the exposure time to UV-C, the greater the percentage of *B.braunii* cells death. Sivaramakrishnan

Table 1. Cell Amount of Initial and UV-C Mutated *B.braunii*

Exposure Time	Run	Cells amount B.braunii (cell/mL)		Average death cells
		Initial cells	Mutated cells	
1.5 min	1	**99,500,000**	68,000,000	29.31 %
	2		71,000,000	
	3		72,000,000	
3 min	1	**97,000,000**	49,000,000	51.55 %
	2		50,000,000	
	3		42,000,000	
	1		29,500,000	

and Incharoensakdi (2017) reported that increasing the time of UV's exposure from 5 minutes into 40 minutes decreased the rate of survival cells of microalgae from 86.6 percent into 0 percent. The results show that UV radiation over a long period of time is the most lethal. The rate of survival cells decreased after long duration of UV rays' exposure [8]. Zul (2003) explained that cell death occurs due to the penetration of UV rays through the cell membrane and damage to intracellular organelles, which results in damage to protein structures [12].

3.1.2. Microalgae growth and lipid productivity after mutation

The mutated microalgae according to the variable exposure time were cultivated for 5 days and analysed for the number of cells every day. Changes in the number of mutated microalgae cells for 5 days for all variables are presented in Figure 3. From the graph in Figure 3, it shows the growth of *B.braunii* cells that mutated by UV-C rays increased the amount of cells which tends to be constant every day.

Table 2. shows the comparison of biomass concentrations produced by native and mutated *B.braunii*. The variable of 1.5 minutes UV-C exposure time had the highest biomass concentration, followed by native microalgae, 3 minutes and 30 minutes UV-C exposure variables which had the lowest biomass concentration. The high concentration of biomass resulted in a large cell mass as well.

Figure 4 shows that exposure to UV-C rays increased the lipid content of microalgae. The exposure time of 1.5 minutes and 3 minutes had greater lipid content than native microalgae, while the 30 minutes of exposure resulted in lower lipid content than native microalgae, due to the low concentration of biomass produced by microalgae *B.braunii* exposed to UV-C rays for 30 minutes. Microalgae produced biomass in large quantities in normal growth conditions but resulting non-optimal lipid productivity. Under UV rays' exposure, microalgae grown in the stress environment that could produce a high lipid content [7]. However, according to Huerlimann (2010) lipid productivity also depends on the biomass produced from microalgae [13]. The longer the exposure of microalgae to UV-C, the less biomass will be

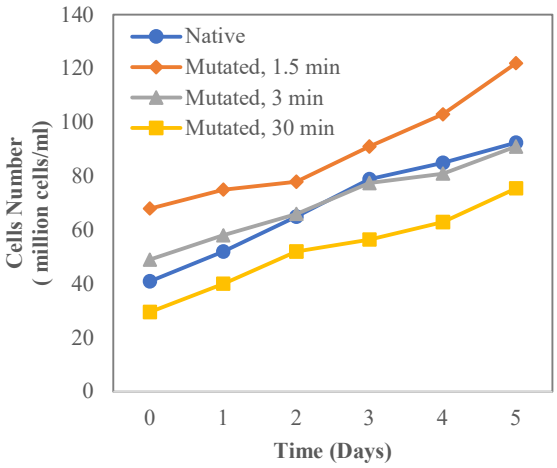


Figure 3 The Cells Growth Curve of Native and Mutated *B.braunii*

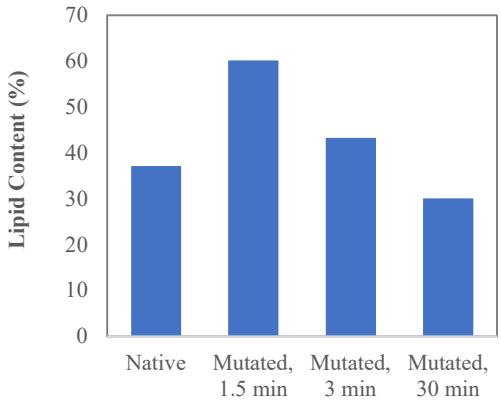


Figure 4 Lipid Content Comparison of Native and Mutated *B.braunii*

Table 2. Comparison of Biomass Concentration and Cell Mass of Native and Mutated *B.braunii*

Algae Condition	Run	Biomass Concentration (mg/ml)	Cell Concentration (sel/ml)	Cell Mass (mg/sel)
Native	1	4.003	90,000,000	4.447×10^{-8}
	2	4.092	92,500,000	4.423×10^{-8}
	3	3.514	94,000,000	3.738×10^{-8}
Mutated, 1.5 min	1	7.084	114,000,000	6.214×10^{-8}
	2	9.194	105,000,000	8.756×10^{-8}
	3	8.759	103,000,000	8.503×10^{-8}
Mutated, 3 min	1	4.164	91,000,000	4.576×10^{-8}
	2	3.578	92,000,000	3.889×10^{-8}
	3	3.230	90,000,000	3.588×10^{-8}

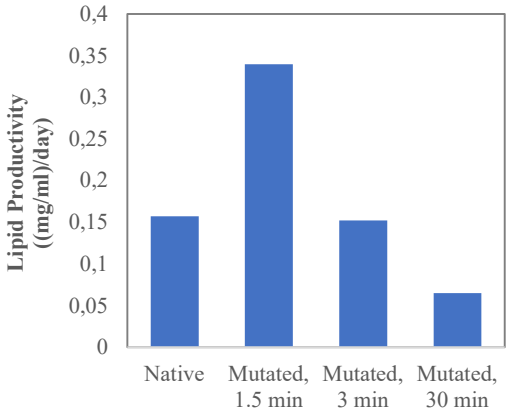


Figure 5 Lipid Productivity of Native and Mutated *B.braunii*

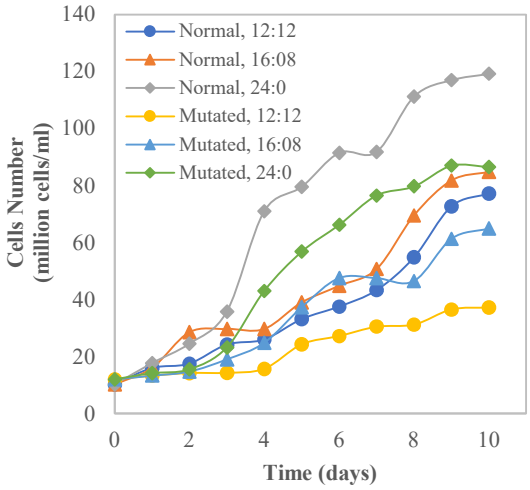


Figure 6 Curve of Native and Mutated *B.braunii* Cells Growth for 10 Days at 3 different Light Irradiance Cycles

produced, and resulting less lipids. Figure 5 shows that microalgae with 1.5 minutes UV-C rays' exposure time produced the highest lipid productivity, and the lowest was produced by microalgae with 30 minutes UV-C rays' exposure time. Native *B.braunii* had lipid productivity that was similar to mutated *B.braunii* after 3 minutes exposure. This shows that the mutation treatment which result optimum quality of microalgae *B.braunii* was 1.5 minutes exposure to UV-C rays.

3.2. Light irradiance cycle examination by microalgae growth

Photosynthetic process requires light energy to synthesis organic compounds from carbon dioxide and water. The duration and light intensity can affect the composition of microalgae biochemical and yield of biomass production [14]. Figure 6 shows a comparison of native and mutated microalgae *B.braunii* in growth for 10 days at 3 different light irradiance cycles (light : dark), 12:12 hours; 16:8 hours; and 24:0 hours. The cells growth of native *B.braunii* increased daily for three variables of the light irradiance cycle. Figure 6 also shows the growth characteristics of UV-C mutants for three light irradiance cycle variables. In the light irradiance cycle variable 12:12 hours, the number of cells increased every day from day 0 to day 10. Nevertheless, mutated *B.braunii* cells growth increased slowly at the initial days and it increased quite rapidly after four days. Likewise, the light irradiance cycle variables 16: 8 hours and 24: 0 hours, these two light irradiance cycle variables also show similar growth characteristics to the 12:12 hours variable. At the initial days, mutated *B.braunii* had a slow cells growth, then the cells growth of mutated *B.braunii* increased quite rapidly after two days.

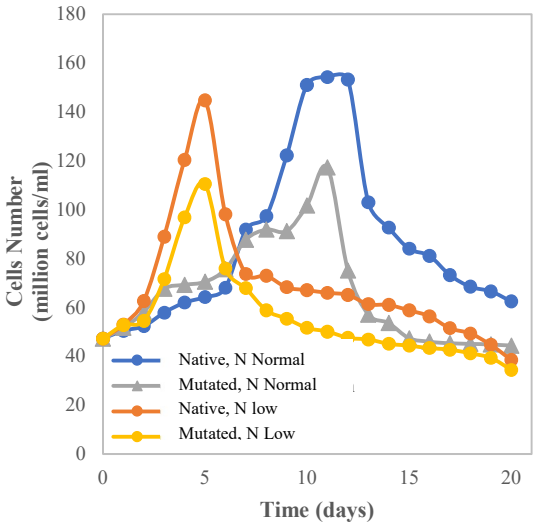
The graph from Figure 6 shows light irradiance cycle of 24:0 hours provided opportunities for native and mutated microalgae *B.braunii* to grow better. This was indicated by an increase in microalgae cells number was more significant in the light irradiance cycle 24:0 hours compared with the light irradiance cycle of 12:12 hours and 16: 8 hours.

3.3. Effect of reducing nitrogen levels and harvest time on microalgae growth

Nitrogen is a functional and structural element that forms proteins in algae cells and accounts for 7-20% of the dry weight of cells. Nitrogen deficiency in algal culture is increased biosynthesis and lipid accumulation. Nitrogen reduction can be considered as stressful environmental that could increase lipid accumulation efficiently. Microalgae can take advantage of nitrate, ammonia, or nitrogen sources. Broadly speaking, lipids and proteins content was strongly influenced by the amount of nitrogen contained in the algae growth medium. Lipid production in cells increased under nitrogen starvation, where the nitrogen content in the growth medium is very small [11].

At this stage, native and UV-C mutated microalgae *B.braunii* were cultured for 7 days and 20 days, then added nutrients with nitrogen levels in different nutrients, 100 mg/L for normal levels of nitrogen and 0.03 mg/L for low nitrogen. Cells number of native and UV-C mutated microalgae were counted each day to provide microalgae cells growth.

In Figure 7 shows the cells growth comparison of native and UV-C mutated *B.braunii* that culture for 7 and 20 days. It can be seen that the addition of nutrients with low nitrogen levels causes *B.braunii* cells growth, both native and mutated, to enter the fourth phase, which is a decrease in the number of cells that is faster than the addition of nutrients with normal nitrogen levels. However, the low nitrogen addition in native *B.braunii*



demonstrated superior cells growth when compared to the growth of mutated *B. braunii* cells. Native and UV-C

Figure 7 Curve of Native and Mutated *B .braunii* Cells Growth for 7 Days and 20 Days in Normal Nutrien Medium and Low Nitrogen Medium

mutated *B. braunii* cells growth for 20 days on the addition of nutrients with normal nitrogen decreased the cells amount on day of 13. The decrease was caused by insufficient of the normal nutrient amounts for cultivate up to 20 days. It is necessary to add more normal nutrients in the cultivation medium. Nitrogen is the primary component of nutrient that effect the microalgae growth. Cultivation medium of microalgae that contain very high concentration of nitrogen can limit photosynthesis by causing a deactivation in pigments production. Therefore, microalgae take longer time to be in stable growth phase when cultivate in high nutrient concentrations [15].

pH has an influence on microalgae growth. Each microalgae species has a minimum value of pH condition that provide survival cells. In this study, pH of the cultivation medium was not controlled, but was still measured every day. The changes in pH for 7 days and 20 days of culture for all variables are presented in Table 3.

Based on Table 3, it shows that pH of the culture decreased for all variables. The decrease in pH for all variables tended to be the same, from pH 8.5 on day 0 to pH 7.2 on day 20. However, this decrease in pH did not significantly affect the growth of microalgae. Even though pH of the culture changed between 6 - 8, *B.braunii* still grew well without any particular obstacles. It has also been reported by Dayananda et al (2007) that production of hydrocarbon and yield of *B.braunii* biomass does not effected significantly by the pH of the cultivation medium in the pH range 6 - 8.5 [16].

3.4. Effect of reducing nitrogen levels and harvest time on microalgae biomass productivity

Microalgae *B. braunii* with normal and low nitrogen levels in the nutrients were extracted and distilled in order to obtain the microalgae lipid. Lipid extraction used dry biomass of native and mutated microalgae. From the dry weight of the microalgae, the biomass concentration was obtained so that the cell mass could be determined.

Table 4 shows that the highest biomass concentration was found in the normal nitrogen variable and 7 days of

Table 3. pH Changes for 7 Days and 20 Days of Culture

Days	pH Culture of Native B.braunii		pH Culture of Native B.braunii	
	Normal Nitrogen	Low Nitrogen	Normal Nitrogen	Low Nitrogen
1	8,5	8,5	8,6	8,5
2	8,5	8,3	8,5	8,5
3	8,3	8,3	8,5	8,5
4	8,3	8,3	8,5	8,3
5	8,3	8,2	8,2	8,3
6	8,3	8,2	8,2	8,3
7	8,2	8,1	8,2	8,3
8	8,2	8,1	8,2	8,2
9	8	8,1	8,2	8,1
10	8	8,1	8	8
11	8	8,1	8	8
12	7,6	8,1	8	8
13	7,6	8,1	8	8
14	7,5	8,1	7,6	8

Table 4. Comparison of Biomass Concentrations and Cell Mass of Native and Mutated *B.braunii* at Various Culture Time and Nitrogen Levels in Nutrients

Algae Condition	Culture TIme	Nitrogen Levels	Biomass Concentration (mg/ml)	Cell Concentration (sel/ml)	Cell Mass (mg/sel)
Native	7	Normal	3.39	91,750,000	3.694×10^{-8}
		Low	3.17	73,750,000	4.298×10^{-8}
	20	Normal	3.17	62,500,000	5.072×10^{-8}
		Low	3.07	38,500,000	7.948×10^{-8}
	7	Normal	3.24	87,750,000	3.699×10^{-8}

culture. While the lowest biomass concentration was found in the low nitrogen variable and 20 days of culture. The biomass concentration obtained was influenced by the growth rate of each variable, where the best growth rate will result in a larger biomass concentration. As shown in the graph in Figure 3, the end cells of native *B.braunii* with variable normal nitrogen and 7 days of culture had the best cell growth when compared to other variables.

Table 4 also shows that the cell mass for each variable when compared between native and mutated *B.braunii*, will get cell mass of native *B.braunii* with normal nitrogen variable at 7 days of culture greater than other variables. While the smallest cell mass was obtained in mutated *B.braunii* with low nitrogen variable at 20 days of culture. Kalla and Khan (2016), explained that reducing nitrogen levels influenced decreasing the growth of cell numbers and microalgae biomass. Microalgae will be in the stress conditions when cultivation in low nitrogen medium, resulting in decreased cell numbers and biomass productivity [17].

3.5. Effect of reducing nitrogen levels and harvest time on microalgae lipid productivity

Based on biomass productivity data, biomass concentration, cell number, and cell mass, the lipid content and lipid productivity of microalgae can be determined. Lipid content can be obtained from the weight of lipids produced from the extraction process and the weight of dry microalgae, lipid productivity can be obtained from lipid content in grams per litre per day and biomass productivity.

The best microalgae growth was native *B.braunii* with normal nitrogen at 7 days of culture. Figure 8 shows that mutated *B.braunii* with low nitrogen at 7 days of culture had the larger lipid content with lower growth than native *B.braunii* with normal nitrogen at 7 days of culture. Native *B.braunii* with low nitrogen at 7 days of culture had lower growth than native *B.braunii* with normal nitrogen at 7 days of culture, but it had higher lipid productivity. Microalgae that provide good growth of cells does not always provide a high lipid content, and poor growth of cells does not always provide a poor lipid content.

In Figure 9, it can be seen that native and mutated *B.braunii* with low nitrogen at 20 days of culture resulted in higher lipid content compared to other variables. This shows that the treatment of reducing nitrogen levels in nutrients at the most optimum culture time can improve the quality of *B.braunii* microalgae as evidenced by the results of the lipid productivity and lipid content of

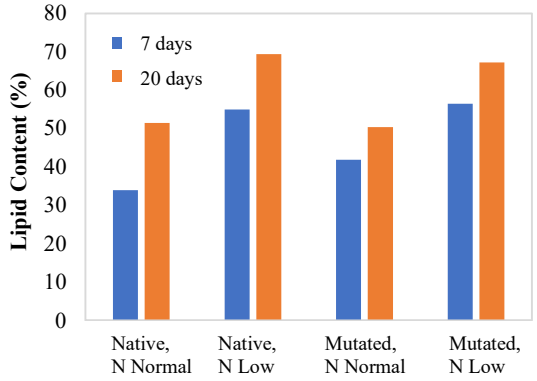


Figure 8 Comparison of Lipid Content of Native and Mutated *B.braunii* at Various Culture Time and Nitrogen Levels in Nutrients

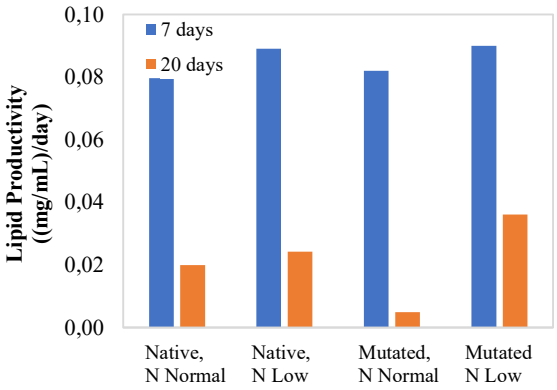


Figure 9 Comparison of Lipid Productivity of Native and Mutated *B.braunii* at Various Culture Time and Nitrogen Levels in Nutrients

Table 5. Gas Chromatography Analysis of Native and Mutated *B.braunii* with Low Nitrogen Levels in Nutrients

Algae Condition	Culture TIme	FFA	MAG	DAG	TAG
		(%)			
Native	7	15.810	19.986	9.096	1.487
	20	22.544	33.289	1.496	0.761
M	7	22.446	38.289	0.631	1.006

B.braunii with a variable reduction of nitrogen levels in nutrients.

The highest lipid productivity was produced by mutated *B.braunii* with low nitrogen 7 days of culture and followed by native *B.braunii* with low nitrogen at 7 days of culture. Large amounts of microalgae biomass that cultivate in normal growth conditions may not have optimal productivity Under normal growth conditions. So that to provide high lipid productivity, microalgae need to be made under stressful environment, in this case by reducing nitrogen levels [18].

The lipid productivity between native and mutated *B.braunii* was not much different, less than 2%. Lipid productivity was determined from the productivity of the biomass times the lipid content, while the lipid content was obtained from the mass of lipids divided by the mass of dry algae. The resulting lipid mass does not always depend on cell mass. In this study, the highest cell mass was native *B.braunii*, but for the highest lipid mass was mutated *B.braunii*. The large lipid mass caused a large lipid content, so that lipid productivity was also large.

Table 5 shows that in low nitrogen levels culture medium, the largest TAG content produced by UV-C mutated *B.braunii* for 20 days of culture. It was seen that mutation with UV-C rays in microalgae *B.braunii* significantly increased the formation of FFA and MAG at 7 days of culture. In microalgae *B.braunii* for 20 days of culture, the differences in the content of FFA, MAG, DAG, and TAG did not have a significant difference between native and mutated microalgae. In native microalgae, the length of culture time showed a significant difference in the resulting FFA and MAG content. An increase of FFA amounts in oil or fat indicates triglycerides hydrolysis. Lipase enzyme action occur the reaction. High amount of FFA also indicate an insufficient condition of storage and processing, such as relative humidity, temperature control and condition of cells tissue. Enzyme source can be derived from the oil or fat extracted tissues or derived from other cell contaminants including microorganisms [19].

4. CONCLUSSION

Microalgae *B.braunii* is a potential raw material for producing lipids. Mutation using UV-C rays is a method to increase microalgae productivity. Reduction of nitrogen levels in nutrients and the length of culture time were carried out to determine their effect on lipid content and lipid productivity of microalgae *B.braunii*.

This study investigated the changes in the growth and lipid productivity of native and UV-C mutated *B.braunii*. Optimum quality of microalgae *B.braunii* could be achieved by UV-C radiation at 1.5 minutes exposure with a continue light irradiance cycle. The concentration of nitrogen in cultivation medium greatly affects *B.braunii* growth and lipid content. Under nitrogen reduction in the cultivation medium, *B.braunii* could not survive in the long duration time of cultivation. *B.braunii* grown in low nitrogen of cultivation medium produced higher lipid content compared to *B.braunii* grown in normal nutrition medium. UV-C rays' exposure on *B.braunii* caused mutations and increased the lipid content of *B.braunii*. After cultivation for 7 days, mutations increased the lipid content of *B.braunii* although not in the significant differences. Native *B.braunii* provides the highest biomass productivity compared to UV-C mutated *B.braunii*, while the highest lipid productivity is produced by UV-C mutated *B.braunii*.

AUTHORS' CONTRIBUTIONS

Thea Prastiwi Soedarmodjo: Conceptualization, Writing - original draft, Writing - review & editing, Visualization **Arief Widjaja:** Conceptualization, Writing - review & editing, Supervision.

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