Microalgae Production *Tetraselmis* sp., *Thalassiosira* sp. and *Porphyridium* sp. with a Combination of EMS & UV-C Mutations Based on Biomass and Lipid Content per Liter of Culture

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Abstract

This study aims to determine the effect of giving both aspects, namely, EMS mutagen and UV-C light on microalgae *Tetraselmis* sp., *Thalassiosira* sp ., and *Porphyridium* sp. so that the comparison can be known on biomass and lipid productivity**.** Both EMS and UV-C mutagen treatments were applied to wild-type and mutated microalgae. Culturing was carried out for 7 days in a photobioreactor with a capacity of 8L. The treatment of mutated microalgae was added with 0.5 M EMS and UV-C irradiation for 3 min. Cell density was calculated from day 1 to 7 in each type of microalgae using a haemocytometer. The results showed that mutagens caused a decrease in cell density in *Tetraselmis* sp., *Thalassiosira* sp., and *Porphyridium* sp. by 37%, 50%, and 2%, respectively, from the wild type. The biomass results showed that mutagens caused a decrease in microalgae *Tetraselmis* sp. and *Porphyridium* sp. by 21% and 13%, but an increase in microalgae *Thalassiosira* sp. by 4% from the wild type. Mutagens also affect the lipid content of microalgae because they significantly increase the lipid content of mutated microalgae *Tetraselmis* sp., *Thalassiosira* sp., and *Porphyridium* sp. by 20.54%, 11.20%, and 20.39%, respectively.

Keywords: *Cell density, mutation, haemocytometer, photobioreactor, increase*

1. Introduction

The consumption of energy and the need for food is increasing along with the increase in the world's population and is characterized by various industrial activities. Currently, various fields of technology have been developed to support the fulfillment of these human needs. One of

them is by utilizing microalgae as an alternative source of protein, carbohydrates, vitamins, and also lipid content (Kawaroe, 2010).

Other Microalgae as a source of biomass is included in the group of eukaryotic and prokaryotic photosynthetic microorganisms, with a simple structure that allows it to grow rapidly (Reynolds, 1990). Microalgae have a lipid content of 20-50% and even up to 80%, making them a potential source of biodiesel. Compared to other plantation crops such as castor and palm oil, microalgae have a high lipid content (Chisti, 2007).

Under normal growth conditions, microalgae produce large amounts of biomass, but the percentage of lipid content produced is relatively small. So it requires treatment or methods that can increase its productivity, genetic manipulation through mutagenesis is the most effective method to increase the productivity of various microorganisms (Augustine, 2015). In addition to paying attention to factors that affect microalgae productivity such as environmental conditions, microalgae breeding methods using seawater media and the addition of nutrients are also things that need to be considered. The use of agricultural fertilizers as nutrients can be easily prepared and save costs in large-scale microalgae cultivation (Febriana et al., 2014).

The types of microalgae that grow and spread in nature have many types and their respective advantages, so to get maximum results it must be known which type of microalgae produces the largest lipids, and this growth is influenced by conditions in microalgae cultures such as CO_2, pH, temperature, salinity, and light intensity (Hadiyanto & Azim, 2012). There are many types of microalgae spread in Indonesian waters, especially Lampung province, including those that have been successfully isolated including *Nannochloropsis* sp., *Tetraselmis* sp., *Dunaliella* sp., *Spirulina* sp., *Nitzschia* sp., *Thalassiosira* sp., *Porphyridium* sp. However, not much data has been presented to support the utilization of these microalgae as a source of biodiesel producing material.

In terms of utilizing microalgae as a source of biodiesel producing material, it is necessary to conduct a study to determine the lipid content and biomass productivity of each type of microalgae, as well as engineering to increase biomass productivity and lipid content, so that an effective type of microalgae can be selected to produce high lipid content and can reduce dependence on non-renewable energy, such as research conducted by Junaidi et al. (2014) using *Synechococcus* sp. microalgae where biomass productivity and lipid content were analyzed. In this study, the biomass productivity value was 0.79 g/L and the lipid value was 0.095 g/L. Thus, *Synechococcus* sp. was found to have low lipid content, but high biomass productivity. Related research also continues to be carried out to increase lipid content by engineering or mutating microalgae as conducted by Soedarmodjo et al. 2021 was conducted to determine the best nutrition for biomass and lipid productivity of *Botrycoccus braunii* microalgae mutated with UV-C light. From this study, it was found that the combination of FM-4 nutrients (urea 126.3 mg/L and TSP 50 mg/L) could reduce biomass productivity (original culture 0.228 gr/L and mutated culture 0.097 gr/L) but increase lipid content levels by 8.96% for wild-type microalgae and 55.11% for mutated microalgae, it can be seen that *Botrycoccus braunii* microalgae can be classified as a type of microalgae that is quite good at increasing biomass productivity and lipid content.

Therefore, it is necessary to conduct further studies to be able to classify other types of microalgae that can be known and improved lipid content and biomass productivity, so that a comparison of microalgae species with the best results can be obtained which can then help classify microalgae species that are good and effective for use as a source of biodiesel producing material. Thus, based on a review of the literature and previous research, this study was conducted using three types of microalgae namely *Tetraselmis* sp., *Thalassiosira* sp., and

Porphyridium sp. to determine the lipid content and biomass productivity in gr/liter of culture produced by the three types of microalgae by mutating them by applying (EMS) and UV-C light, followed by culturing them in a photobioreactor under optimal conditions.

2. Methodology

2.1. Research Procedure

2.1.1. Wild-type Microalgae Cultivation Stage.

The gas flow from the CO2 gas tube and the air pump are combined as shown in Figure 3.2. The CO2 gas flow rate was set at 0.5 L/min for a feed CO2 concentration of 3%. The photobioreactor was filled with microalgae culture with seawater at a ratio of 1:3 and used 136.3 mg/L urea and 50 mg/L TSP fertiliser nutrients for 8L of culture. The culturing process was carried out until day 7 and cell density was calculated with a haemocytometer every day. Harvesting of microalgae in the photobioreactor was carried out until day 7.

2.1.2. Cultivation stage of mutated type microalgae.

A total of 0.5 L of microalgae culture was added with a dose of 0.5 M EMS. Stirring was carried out for 15 minutes and the culture was allowed to stand for 45 minutes. Microalgae that had been given EMS were washed with sterilised seawater for 3 times to remove the remaining EMS on the microalgae. Physical mutation was carried out on EMS mutated microalgae using UV-C light for 3 minutes. The culturing process was carried out until day 7 and cell density was calculated with a haemocytometer every day. Harvesting of EMS and UV-C light mutated microalgae was carried out on day 7.

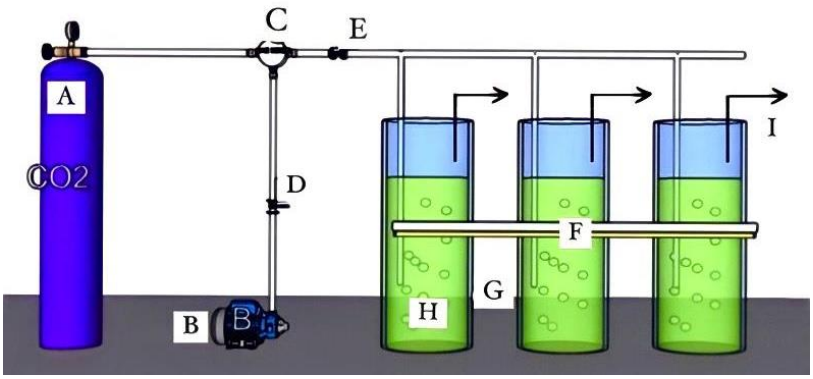

Figure 1. Experimental Setup**.** Where; A. CO_2 gas tube, B. Air blower, C. Gas mixing point, D. Valve, E. Gas flowmeter, F. Tube Light lamp, G. Photobioreactor, H. Stone aerator, I. Output gas.

2.1.3. The Harvesting Stage.

Harvesting of wild-type and mutated microalgae was carried out using the flocculation method using aluminium sulphate flocculant $Al_2(SO_4)_3$ at a dose of 150 mg/L. Manual stirring was carried out using a wooden stirrer for 15 minutes. Then leave it for one hour to separate the microalgae from the medium. Use a satin cloth to separate the microalgae from the media. Satin cloth measuring 60 - 70 mesh as a filter. The filtered microalgae paste is then washed with seawater to remove any residual flocculant on the microalgae.

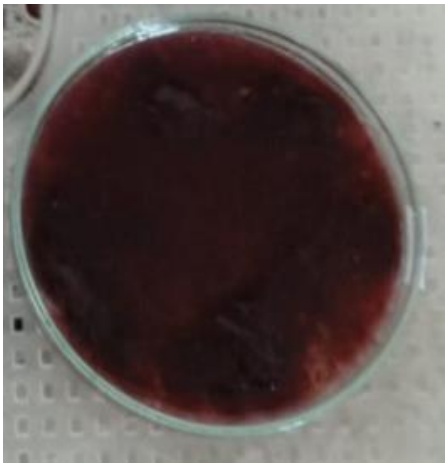

Figure 2. Pasta of the microalgae

2.1.4. Microalgae Extraction Stage.

The microalgae paste that has been obtained is then dried using an oven at 60°C for 2 hours.The dried microalgae were then moved to cool in a desiccator for ± 15 minutes. Dried microalgae were weighed using a digital balance. Extraction on microalgae was carried out by the soxhlet method using 80 ml n-hexane solvent, temperature 70°C for 5.5 hours. Then continued the distillation process for 3 hours at 80 ° C to separate the n-hexane solvent and extracted lipids.

Figure 3. Biomassa of the microalgae.

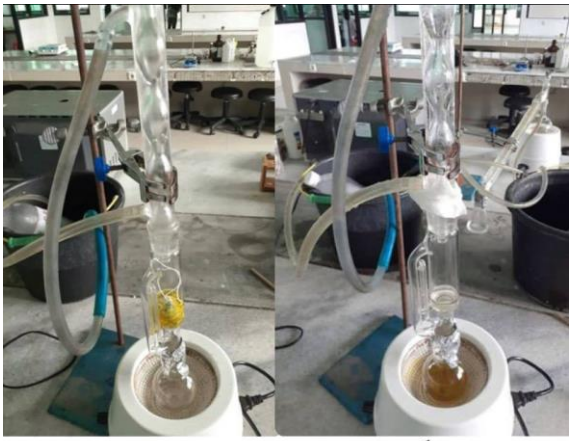

Figure 4. (a) Extraction and (b) Distillation of microalgae.

2.2. Sample Analysis.

2.2.1. Microalgae Extraction Stage.

This was done to determine the growth of microalgae on each day by using a haemocytometer under a microscope, we calculated the cell density as follows, Take a sample using a drop pipette. Dripping the sample about 1 - 2 drops on the haemocytometer. Covered the haemocytometer with a cover glass. Observing with a 100 or 400 times magnification microscope. Counting microalgae cells. Calculating density using equation (1) (Karlson et al., 2010).

$$\text{Total cell count} = \frac{\text{The number of cell}}{\text{Number of squares counted on a hemocytometer}} \times 10^4 \text{ cells/ml} \quad (1)$$

2.2.2. Biomass Calculation.

Microalgae biomass that has been dried using an oven and cooled in a desiccator is weighed using an analytical balance, as follows: Place the empty petri dish into the analytical balance. Recording the weight of the petri dish (Wo). Place the petri dish filled with microalgae biomass (W1) from the drying process into the analytical balance. Record the value on the analytical balance screen. The biomass that has known weight (gr) is stored in a sample bottle, for further processing at the extraction stage. Calculations were carried out, where the biomass (W1) was reduced by the weight of the Petri dish (Wo) which had been weighed previously in order to obtain the net weight of microalgal biomass. The calculation was done using equation (2):

$$A = W_1 - W_0 \quad (2)$$

Where, A is the weight of biomass, W_1 is the weight of biomass + flask weight, and W_0 is flask weight. The weight of microalgae lipids obtained from the extraction and distillation results was determined using an analytical balance. The calculation of biomass productivity of each microalgae species was done by dividing the total biomass of each microalgae species by the total culture used, using equation (3):

$$Biomass\ \left(\frac{gr}{L}\right) = \frac{Total\ Biomass\ (gr)}{8\ L} \quad (3)$$

2.2.3. Calculation of Microalgae Lipid Content.

Calculation of lipid productivity of each type of microalgae is done by dividing the total lipids obtained in each type of microalgae with the culture capacity used (8 L), using equation (4):

$$Lipid\ \left(\frac{gr}{L}\right) = \frac{Total\ Lipid\ (gr)}{8\ L} \quad (4)$$

Furthermore, the Bethien-Diemar method (1963) was used in this study to calculate the lipid content (%) in biomass (Endrawati et al., 2012), using equation (5):

$$\%\ Total\ lipid = \frac{(A-B)}{C} \times 100 \quad (5)$$

Where, A is flask weight + lipid weight after extraction (g), B is flask weight before extraction (g), and C is the weight of dry microalgae (g).

3. Result and Discussion

3.1. The Cell density of Tetraselmis sp.

Figure 5. shows that the growth of *Tetraselmis* sp. is in accordance with normal phytoplankton growth patterns. On day 1 *Tetraselmis* sp. entered the lag phase, the photosynthesis process has occurred but not all *Tetraselmis* sp. cells experienced a significant increase in growth, also because *Tetraselmis* sp. still adapting to the new media, namely water quality conditions and provision of nutrients to the culture, as well as adapting to its metabolism. On days 2 - 7, there was an exponential phase characterized by a rapid increase in cell population up to many times. Furthermore, the stationary phase occurs where there is a balance between growth and death, this phase occurs on day 8 (Hariyati, 2008). Therefore, the microalgae culture process in this study was carried out until day 7 in the hope of getting the maximum number of microalgae cells.

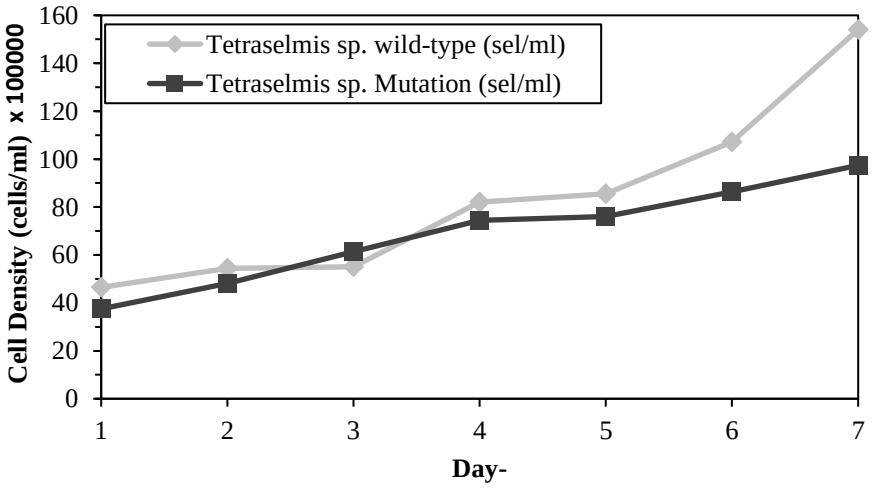

Figure 5. Growth of Wild-type and Mutated *Tetraselmis* sp.

The *Tetraselmis* sp. culture process was carried out in 2 conditions, namely wild-type *Tetraselmis* sp. culture and mutated *Tetraselmis* sp. culture using EMS mutagen at a concentration of 0.5 M as much as 2 drops and UV-C light. In wild-type *Tetraselmis* sp. culture, the lag phase on day 1 had a cell count of 4,647,500 cells/ml and entered the exponential phase on days 2 and 3 cell counts of 5,440,000 cells/ml and 5,505. 000 cells/ml cell division began to experience a significant increase on day 4 at 8,205,000 cells/ml, day 5 at 8,557,500 cells/ml and day 6 at 10,772,500 cells/ml, while the peak of the exponential phase occurred on day 7 at 15,402,500 cells/ml. Meanwhile, for *Tetraselmis* sp. culture with the addition of EMS mutagen and UV-C light, the lag phase on day 1 cells in *Tetraselmis* sp. had a cell number of 3,750,000 cells/ml. Entering the exponential phase, the number of cells of *Tetraselmis* sp. mutation on day 2 to day 5 was 4,812,500 cells/ml, 6,137,500 cells/ml, 7,445,000 cells/ml, and 7,600,000 cells/ml, on day 6 *Tetraselmis* sp. mutation type experienced very high cell division of 8,627,500 cells/ml and on day 7 the number of cells of *Tetraselmis* sp. mutation type reached 9,740,000 cells/ml.

From the data obtained, there is a significant difference between cell density in wild-type *Tetraselmis* sp. and its mutation, because it is influenced by the addition of EMS and UV-C light. The effect in the lag phase causes *Tetraselmis* sp. mutated type to experience less cell density when compared to *Tetraselmis* sp. wild type. Then, at the end of the exponential phase, from day 3 to day 7, wild-type *Tetraselmis* sp. had a significant increase compared to wild-type *Tetraselmis* sp. mutated type. It can be concluded that the addition of chemical mutagen EMS and physical mutagen UV-C light causes m *Tetraselmis* sp. in the culture process to experience slow division so that the cell density is not much higher when compared to wild-type *Tetraselmis* sp. and affects the acquisition of paste and biomass.

3.2.The Cell density of Thalassiosira sp.

It can be seen in Figure 6 that the population density of *Thalassiosira* sp. presented in the form of a graph to compare the population density of wild-type and mutation-type *Thalassiosira* sp. cells during the 7-day cultivation process. The lag phase in *Thalassiosira* sp. occurs on day 1 where there is a process of adaptation of Thalassiosira sp. to the new environment. Furthermore, on days 2 to 7 there is an exponential phase where the process of cell division occurs so that cell density increases which is characterized by a brown color in the photobioreactor which is increasingly dense. Harvesting of *Thalassiosira* sp. is done during the exponential phase because in the exponential phase the cells increase so that the results obtained will be maximized (Johan et al., 2020). On day 8, the stationary phase occurs which is characterized by the amount of nutrients in the media that have been reduced so that the process of growth and cell division can no longer take place (Prihantini et al., 2005).

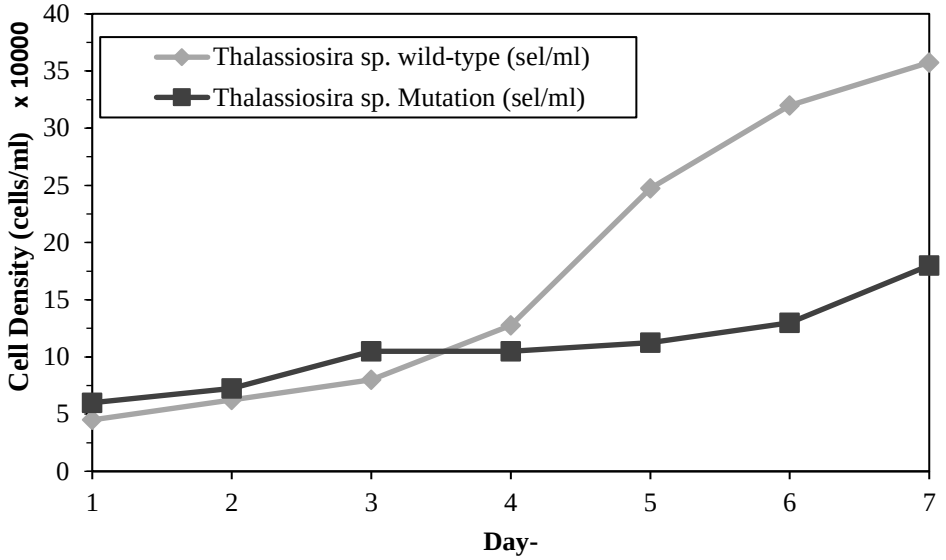

Figure 6. Growth of Wild-type and Mutated *Thalassiosira* sp.

Cell density in wild-type *Thalassiosira* sp. can be seen in the lag phase and exponential phase. On day 1 *Thalassiosira* sp. experiencing a lag phase with a cell number of 45,000 cells / ml, exponential phase on day 2 to 7 takes place normally in accordance with the growth pattern, where cell division occurs which makes the number of cells from day to day is increasing. Cell density *Thalassiosira* sp. from day 2 to day 7 in a row is 62,500 cells/ml, 80,000 cells/ml, 127,500 cells/ml, 247,500 cells/ml, 320,000 cells/ml and 357,500 cells/ml. On day 7 *Thalassiosira* sp. wild type experienced peak population density, this is supported due to optimal culture conditions, namely nutrients, light intensity, and CO_2 requirements are met.

In *Thalassioira* sp. mutated EMS and UV-C light, the lag phase on the first day has a cell number of 60,000 cells / ml, this number is more than the number of wild-type sp. cells, but in the process of growth it experiences a slow addition of cells seen on day 3 where the difference is quite significant. The decrease in the number of cells as much as 2 times occurs due to the mutation process with the addition of chemical mutagens in the form of EMS and physical mutagens UV-C light, where more *Thalassiosira* sp. which is exposed to mutagens experiencing the process of death, compared with *Thalassiosira* sp. which is able to survive against mutagen administration, *Thalassosira* sp. which is able to survive will live well as *Thalassiosira* sp. mutated and then perform cell division much slower in the exponential phase, this is because the mutated microalgae are in a state of stress and inhibit the process of cell division. Exponential phase *Thalassiosira* sp. mutated takes place on day 2 to day 7 with the number of cell densities are 72,500 cells/ml, 105,000 cells/ml, 105,000 cells/ml, 112,500 cells/ml, 130,000 cells/ml and 180,000 cells/ml respectively. Peak exponential phase in mutated *Thalassiosira* sp. occurred on day 7 of the culturing process. The process of adding EMS and UV-C light on *Thalassiosira* sp. causing the wild type has a cell density of 1.98% higher when compared with *Thalassiosira* sp. mutated type.

3.3. The Cell density of Porphyridium sp.

The cell density of wild-type and mutated *Porphyridium* sp. on days 1 to 7 is presented in graphical form as can be seen in Figure 7. In the growth process, this microalgae experienced

a lag phase on day 1 to day 3, both in wild and mutated conditions, with *Porphyridium* sp. wild type having a cell count from day 1 to day 3 of 552,500 cells/ml, 725,000 cells/ml and 772,500 cells/ml respectively, while the number of cells in *Porphyridium* sp. mutated type from day 1 to day 3 of 502,500 cells/ml, 645,000 cells/ml and 652,500 cells/ml respectively. Then the logarithmic phase occurred on day 4 to day 6 where the number of cells increased significantly during the culturing process, where the number of cell densities consecutively from day 4 to 6 were 942,500 cells/ml, 2,200,000 cells/ml, and 3,990,000 cells/ml for the wild type, and 1,200,000 cells/ml, 2,107,000 cells/ml, and 4,060,000 cells/ml for the mutated type. Day 7 is a phase of decline in the number of cell additions, cell density increases but not as significant as in the logarithmic phase in both types of microalgae, namely 4,777,500 cells/ml for wild type and 4,675,000 cells/ml for mutated type. This is the right time for harvesting because the cells reach the maximum number before entering their death phase.

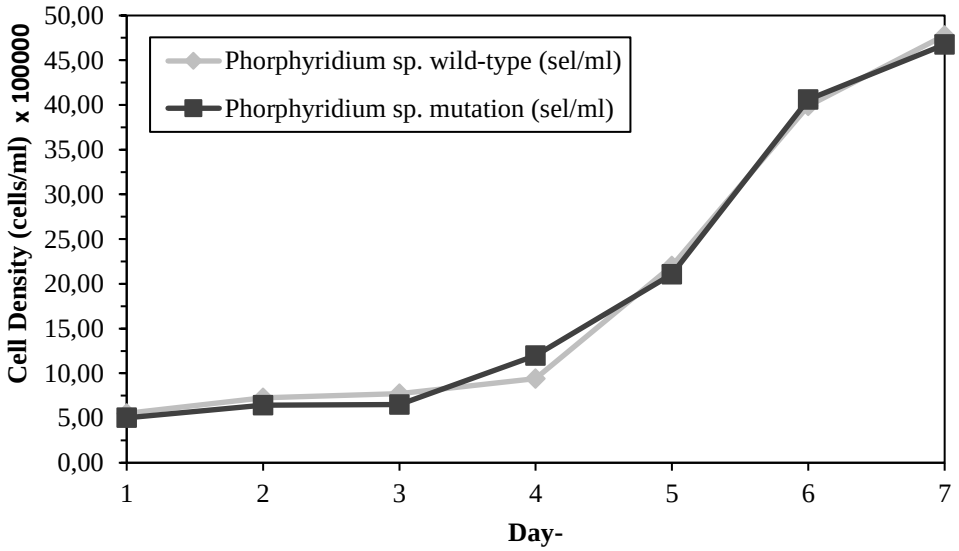

Figure 7. Growth of Wild-type and Mutated *Phorphyridium* sp.

The addition of EMS mutagen and UV-C light to *Phorphyridium* sp. did not have a major effect on the difference in numbers in the process of culturing microalgae in photobioreactors.

3.4. Comparison of the three microalgae cell densities.

Comparison graphs of each cell density in microalgae *Tetraselmis* sp., *Thalassiosira* sp., *Porphyridium* sp. wild type without the administration of mutagens, as well as the mutated type with the addition of EMS and UV-C can be seen in Figures 8. and 9.

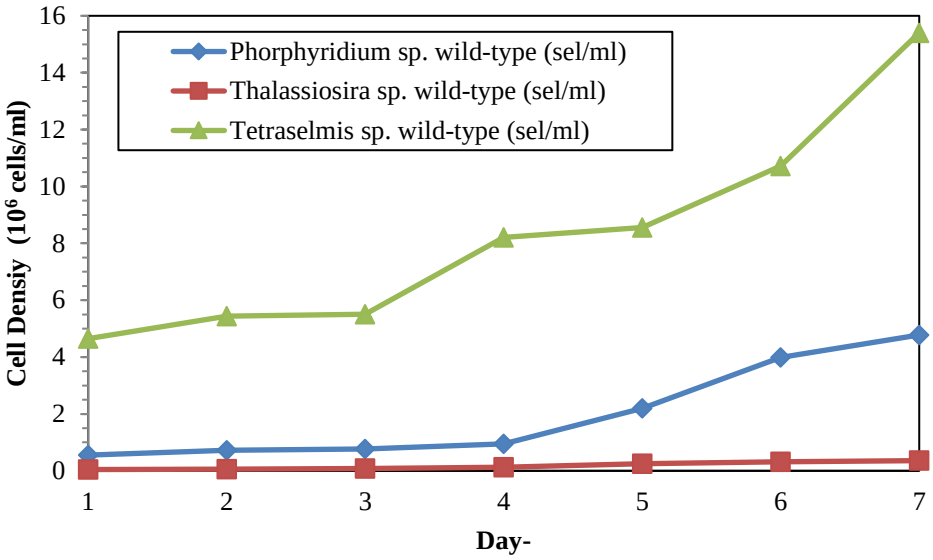

Figure 8. Cell Density Comparison of 3 Wild-type Microalgae (Tetraselmis sp., Thalassiosira sp., Porphyridium sp.).

Figure 4.4 shows the comparison of cell density of 3 types of microalgae (*Tetraselmis* sp., *Thalassiosira* sp., *Porphyridium* sp.) wild type cultured with culturing time for 7 days. In the figure presented, it can be seen that from each type of wild-type microalgae, the longer the number of days of cultivation, the higher the number of cell densities in the microalgae. On the 1st to 7th day, the number of cells from 3 types of microalgae (*Tetraselmis* sp., *Thalassiosira* sp., *Porphyridium* sp.) wild type is increasing and is in the exponential phase, not yet entering the advanced phase, namely the stationary phase and the death phase. However, if a comparison is made of the 3 types of microalgae (*Tetraselmis* sp., *Thalassiosira* sp., *Porphyridium* sp.) wild type, it was found that the microalgae with the highest cell density on day 7 was the microalgae *Tetraselmis* sp. with the number of cells reaching 15,402,500 cells/ml, while for microalgae *Porphyridium* sp. had a cell density of 4,777,500 cells/ml, and in the wild type the lowest cell density was owned by microalgae *Thalassiosira* sp. which was 357,500 cells/ml.

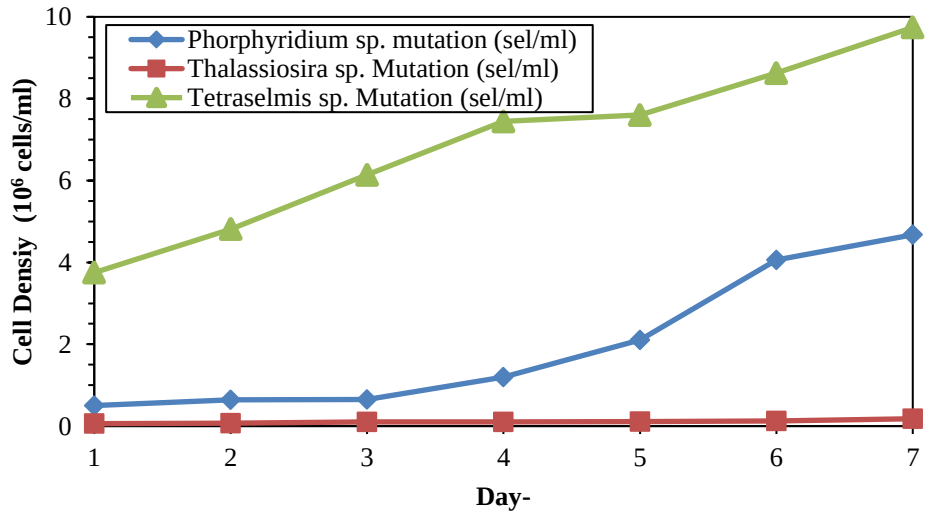

Figure 9. Comparison of Cell Density of 3 Microalgae (Tetraselmis sp., Thalassiosira sp., Porphyridium sp.) of Mutated Type

Figure 9. shows the comparison of cell density of the three types of microalgae (*Tetraselmis* sp., *Thalassiosira* sp., *Porphyridium* sp.) in the mutated treatment. From Figure 9. it can be seen that due to the influence of the length of the day of culturing carried out there is an increase in the number of cell densities of each type of microalgae. Where for 3 types of microalgae (*Tetraselmis* sp., *Thalassiosira* sp., *Porphyridium* sp.) mutated, the order of cell density is the same as the wild type microalgae. The culturing process carried out for 7 days makes the cell density in microalgae increase day by day. However, in Figure 4.5 it can be seen that the 3 types of microalgae (*Tetraselmis* sp., *Thalassiosira* sp., *Porphyridium* sp.) mutated types have a much higher increase in cell density from day 6 to day 7 when compared to wild-type microalgae. The highest cell density in mutated microalgae is owned by *Tetraselmis* sp. with a total cell density on day 7 of 9,740,000 cells/ml, followed by *Porphyridium* sp. with a total cell density of 4,777,500 cells/ml, and the lowest is owned by *Thalassiosira* sp. which is 180,000 cells/ml.

Table 1. shows the equation of growth rate (growth cell) by regression analysis on *Tetraselmis* sp., *Thalassiosira* sp., and *Porphyridium* sp. for 7 days in wild type and mutated.

Tabel 1. Microalgae Growth Rate Equation

Microalgae	Cell growth rate
Tetraselmis sp. wild type	y = 2E+06x + 2E+06 r = 0.8773
Tetraselmis sp. mutated type	y = 966518x + 3E+06 r = 0.9799
Thalassiosira sp. wild type	y = 57857x - 54286 r = 0.9336
Thalassiosira sp. mutated type	y = 17232x + 40357 r = 0.898
Porphyridium sp. wild type	y = 736875x - 953214 r = 0.8418
Porphyridium sp. mutated type	y = 742946x - 994286 r = 0.8614

As can be seen from Table 1, compared to the mutated type, *Tetraselmis* sp., *Thalassiosira* sp. and *Porphyridium* sp. wild type have higher cell growth. If a comparison is made for the three types of microalgae, it is known that the lowest growth rate is owned by *Thalassiosira* sp. mutated type with a linear regression relationship to determine the relationship between growth rate and days of culture with the equation y = 17232x + 40357. While the highest growth rate is owned by *Tetraselmis* sp. wild type and has a linear regression relationship between the growth rate and the length of the culture process is y = 2E+06x + 2E+06. Research conducted by Prasetyo et al. (2022) used regression analysis to determine the relationship between the growth rate of microalgae *C. calcitrans* and different light intensities. From the study obtained regression results y = 0.015x + 14.832 and the value of r = 0.989.

If a comparison is made, *Tetraselmis* sp. in the wild type and mutated are microalgae that have the highest cell density. The increase in cell density is due to the amount of nutrients used by *Tetraselmis s*p. to carry out the growth and reproduction process. This is due to its small

size, supported by a larger cell surface area, the process of nutrient absorption into cell tissue occurs faster (Flynn et al., 2018).

3.5. Biomass productivity

Can be seen in Figure 10. Microalgae cultivation was carried out on an 8L basis in the photobioreactor, after harvesting and drying, the dry weight of microalgae was obtained as much as 6 gr of wild-type *Porphyridium* sp., 5.22 gr of mutated *Porphyridium* sp., 2.27 gr of wild *Thalassiosira* sp., 2.35 gr of mutated *Thalassiosira* sp., 4.31 gr of wild-type *Tetraselmis* sp. and 3.40 gr of mutated *Tetraselmis* sp. mutated. These results have differences in each wild type microalgae and mutated type of EMS and UV light, the effect of mutation treatment on microalgae is not the same in each microalgae on the dry weight or biomass it produces.

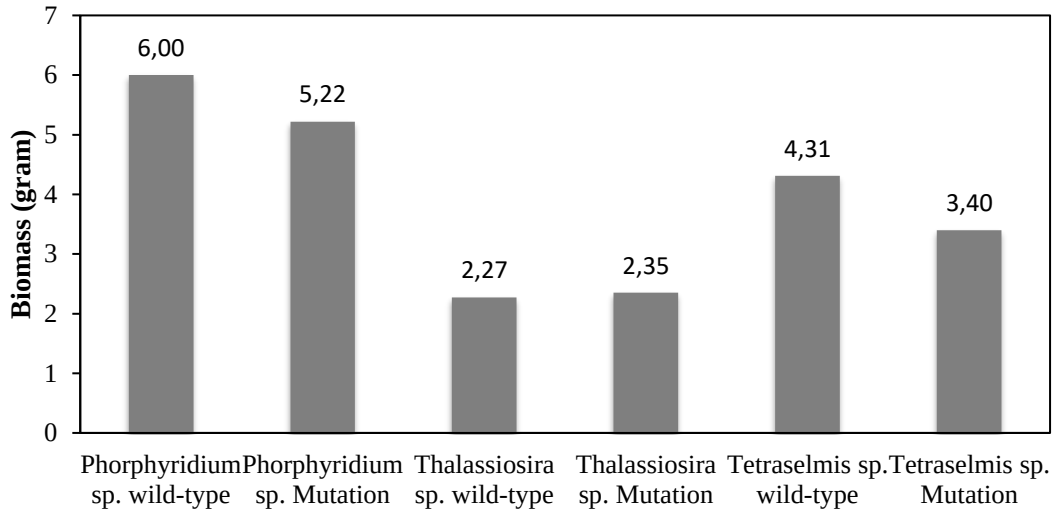

Figure 10. Dry Weight of Each Microalgae Type

As happened in previous research conducted by Beacham et al. in 2015 using mutated *Nannochloropsis salina* microalgae using EMS and UV, it is known that the provision of these mutagens to *Nannochloropsis salina* makes a decrease in growth rates so that the dry weight obtained is less than the culture without mutagen administration. Then, in 2017 a study was conducted by Sarayloo et al., using *Chlorella vulgaris* which was then compared to wild-type and mutated microalgae on dry weight gain and lipid content levels. In the study, it was found that the provision of UV715-EMS25 mutagen greatly affected the dry weight gain, where the dry weight level of wild-type microalgae was 0.96 g/L and increased by 35.4% for the dry weight of mutated *Chlorella vulgaris*, which was 1.3 g/L. Therefore, based on previous research, it can be concluded that the application of EMS mutagens and UV-C light has different effects on each type of microalgae cultured, the final results obtained can be lower or higher when compared to wild-type microalgae. In this study, three types of cultured microalgae produced different dry weights due to the addition of EMS and UV-C mutagens.

The dry weight of wild *Porphyridium* sp. is obtained as much as 0.650 gr/L while *Porphyridium* sp. mutated type has a dry weight of 0.730 gr/L, which is higher with a difference of 0.080 gr/L. In *Thalassiosira* sp. mutated has a higher dry weight with a difference of 0.011 gr / L, which is as much as 0.288 gr / L when compared to the acquisition of dry weight in *Thalassiosira* sp. wild type which only amounted to 0.276 gr / L. From these results, it can be

seen that the effect of EMS mutation and UV-C light has a slight good effect on increasing the biomass of microalgae species *Porphyridium* sp. and *Thalassiosira* sp. While in *Tetraselmis* sp. which is also cultured in a photobioreactor with the same culture conditions as other microalgae, the provision of EMS mutagens and UV-C light before the culturing process has a different effect, namely reducing the amount of biomass produced, compared to the other 2 types of microalgae. The dry weight obtained for the wild type *Tetraselmis* sp. was 0.539 g/L and the mutated *Tetraselmis* sp. produced a dry weight of 0.425 g/L, a difference of 0.114 g/L less than the wild type.

It can be concluded that the provision of EMS and UV-C mutagens has a different effect on each type of microalgae, said to have a good effect here, namely on the acquisition of biomass results from these microalgae. For *Porphyridium* sp. and *Thalassiosira* sp. mutation treatment by adding EMS and UV-C to the culturing process has a good effect with higher biomass yields compared to the wild type. While for *Tetraselmis* sp. mutation treatment gives a less good effect, namely reducing the biomass produced compared to the wild type.

3.6. Lipid productivity

Figure 11. shows the percentage of lipid content in each type of microalgae. Where it is known that each type of microalgae experienced an increase in lipid content due to the treatment of EMS and UV-C mutagens. In the percentage of lipid content of biomass, each type of microalgae experienced an increase in lipid content with different levels. The mutated *Porphyridium* sp. had an increase in lipid content of 0.204 times when compared to the wild type, the mutated Thalassiosira sp. had an increase in lipid content of 0.12 times the wild type, and the mutated Tetraselmis sp. had an increase in lipid content of 0.205 times the wild type. It is known that Tetraselmis sp. has the highest percentage increase due to the EMS and UV-C mutation process compared to other types of microalgae. In previous studies, microalgae with the highest lipid content of 10-67% were found in *Chlorella*, *Dunaliella*, and *Scenedesmus* (Islam et al., 2013; Nascimento et al., 2013). Chisti in Sun et al. (2018) said that microalgae lipid content usually reaches 20-50% of the dry weight of cells and can reach 80% under certain conditions. In this study, the highest lipid content owned by *Tetraselmis* sp. reached 91.18% due to the application of EMS and UV-C mutagens.

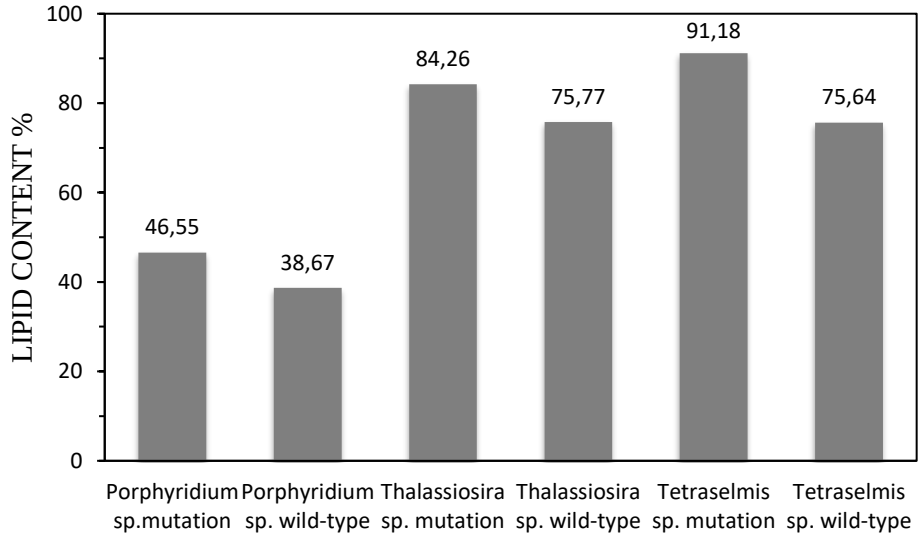

Figure 11. Dry Weight of Each Microalgae Type

The increase in lipid levels in microalgae given mutation treatment is in accordance with previous research. Such as research conducted by Sarayloo et al. (2018) using mutated *Chlorella vulgaris* using EMS and UV-C. In this study, an increase in lipid levels of 67% was obtained, namely lipid levels in wild-type Chlorella vulgaris by 25% and in the mutated type by 42%. Another study was conducted by Chatuverdi & Fujita (2006) using Nannochloropsis sp. microalgae, where in the study it was known that there was an increase in lipid levels due to EMS administration. The increase in lipid levels in microalgae indicates an increase in catalytic activity or synthesis of several enzymes from microalgae Nannochloropsis sp. during the culturing process with the addition of EMS when compared to the original culture without EMS administration. In addition, the increase in lipid levels in each type of microalgae also occurs due to the addition of mutagens, which in this study used EMS and UV-C mutagens, making microalgae exposed to mutagens experience stress. Under optimal growth conditions, microalgae will primarily synthesize fatty acids to promote membrane lipid synthesis. In contrast, under unfavorable conditions (stress), many microalgae switch their lipid biosynthetic pathway to the formation and accumulation of triglycerides in the form of TAG, which plays a role in storing carbon and energy. The increase in total lipid content of microalgae under stress conditions is due to an increase in triglyceride content, especially TAG. This is because lipid metabolism shifts from membrane lipid synthesis to neutral lipids (storage lipids) (Hu et al., 2008). According to Babu et al. (2022) stress occurs because microalgae are in a hostile environment, thus experiencing a shift in carbon flux and changing the pathway of lipid biosynthesis towards the formation and accumulation of neutral lipids in the form of TAG, causing microalgae to survive in adverse conditions and making an increase in lipid production in microalgae.

4. Conclusion

The biomass of mutated microalgae in *Porphyridium* sp., *Thalassiosira* sp., *Tetraselmis* sp. was 0.653 gr/L, 0.294 gr/L, 0.425 gr/L, respectively. The biomass of wild-type microalgae in *Porphyridium* sp., *Thalassiosira* sp., *Tetraselmis* sp., respectively, was 0.539 gr/L, 0.284 gr/L, 0.750 gr/L. The lipid content in the biomass of mutated *Porphyridium* sp., *Thalassiosira* sp., and *Tetraselmis* sp. was 46.55%, 84.26%, and 91.18%, respectively, and in the biomass of wild *Porphyridium* sp., *Thalassiosira* sp., and *Tetraselmis* sp. was 38.67%, 75.77% and 75.64%, respectively. Of the 3 types of microalgae, *Tetraselmis* sp. is the type of microalgae that can produce the largest increase in biomass reaching 26.7% compared to the wild type. The percentage increase in lipid content in the highest biomass is owned by *Tetraselmis* sp. by 20.54% due to the application of EMS & UV-C mutagens.

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