Optimizing Lipid Content and Biomass Production with Combined EMS and UV-C Treatment on 3 Species of Microalgae

Elida Purba[1*] and Kanesia Natalie Yobel[2]

[1,2] Center of Green and Clean Energy, Department of Chemical Engineering, University of Lampung, Bandar Lampung, Indonesia. 35145
[*]elida.purba@eng.unila.ac.id

Abstract. This paper elaborates about the enhancing of lipid content and biomass productivity of the three types of microalgae. The aim of this study is to determine the effect of EMS (ethyl methanesulfonate) mutagen and UV-C on microalgae *Spirulina* sp., *Dunaliella* sp., and *Nitzschia* sp., and comparing their biomass and lipid productivity. Both treatments by EMS mutated microalgae and UV-C light were applied to both wild-type and mutated microalgae. The cultivation was carried out for 7 days in a photobioreactor with a capacity of 8 l. The treatment for mutated microalgae was carried out by adding 0.5 M EMS and UV-C irradiation for 3 minutes. Cell density was calculated from day 1 to 7 on each type of microalgae using a haemocytometer. The results showed that mutagens cause an increase in cell density in *Dunaliella* sp. up to 5.7% and 6.5%. increase in biomass. In *Nitzschia* sp. there was also an increase in cell density up to 16.5% and an increase in biomass of 25.3% from the wild type, however, there was a decrease in cell density and biomass productivity in *Spirulina* sp. Mutagens also affects the lipid content of microalgae because it has a significant effect on increasing the lipid content of mutated microalgae by 1.93-fold in *Spirulina* sp., 0.08-fold in *Dunaliella* sp., and 0.44-fold in *Nitzschia* sp.

Keywords: Microalgae, Lipid content, EMS Mutagen, Biomass productivity, Photobioreactor, Cell density

1 Introduction

The increase in population and the rapid development of technology in the economic and industrial sectors leads to increasing of energy demand. However, the availability of traditional energy sources is increasingly limited. Therefore, a development of alternative renewable fuel sources is urgently needed to overcome the decreasing availability of fossil fuel energy. Many studies have been carried out to overcome the problem of energy demand, one of which is the research by (Ang et al., 2022) to learn about the existing renewable energy technologies, including solar, hydro, wind, bio, geothermal, and hydrogen energy. Meanwhile, research by (Shuba & Kifle, 2018) outlines the potential of microalgae for biofuel production and its practical applications. Additionally,

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research by (Zhang et al., 2022) determines the use of microalgae as a source of biofuel by extracting the lipid content of microalgae.

Microalgae are photosynthetic microorganisms that have a very high growth rate of up to 7–31 times faster than conventional crops such as soybean and rapeseed. The oil productivity of microalgae is much higher than that of palm oil with the same land area (Udayan et al., 2022). The total lipid content of microalgae varies from 1–70% of dry weight and depending on each microalgae species, microalgae growth rate, and culture conditions (Kumar et al., 2021). Due to the diversity of microalgae species, the fatty acid content in microalgae also varies. High productivity aligned with high lipid and fatty acid content makes microalgae a very potential for making biodiesel. In fact, growth enhancement techniques or genetic modification could increase its potential as a renewable energy source in the future (Khan et al., 2018).

With the low lipid productivity of some microalgae, an effort is needed to increase the lipid content of microalgae so that they can be utilized for making biodiesel. Research on increasing microalgae biomass and lipids is still limited, particularly the research on increasing the biomass and the lipids with a new method, that is mutagenesis (Trovão et al., 2022).

Soedarmodjo et al. (2021) investigated *Botryococcus braunii* and mutated it with UV-C light, and it was found that the combination of urea 136.3 mg/l and TSP 50 mg/l nutrients could reduce biomass productivity but it increased lipid levels up to 55.11% for mutated microalgae. Research using *Chlorella vulgaris* was conducted by Sarayloo et al. (2018) with EMS mutation and UV light resulted in an increase in biomass productivity by 35%, an increase in lipid content by 67%.

Theoretically, the cell density in the microalgae remained almost constant or decreased for some time and then increased again when the UV-C mutagen was added. This suggests a significant trend toward increased growth (Soedarmodjo et al., 2021). In addition to direct damage to the photosynthetic system, UV-C slowly damages the cell membrane. The addition of UV-C causes microalgae to produce pyrimidine dimers that inhibit the transcription of psbA and cpc. It induces intracellular and extracellular microcystin degradation, increases sedimentation rate of microalgal cells, and increases reactive oxygen species (ROS) and indirect oxidative damage. However, most DNA damage is repaired within three days (Li et al., 2020). Through the addition of mutagen, the mutated cells were smaller, which may have contributed to the significant reduction in biomass. This is probably due to the random mutagenesis techniques used, which cause physiological and morphological changes in the mutated microalgae (Carino & Vital, 2023).

To date, there is limited information on increasing biomass and lipids of some certain microalgae using combined EMS and UV-C. Therefore, there is a need for investigation for enhancing the biomass and the lipid content for various species of microalgae This study aims to investigate the lipid content and biomass productivity enhancement for three locally available microalgae species: *Spirulina* sp., *Dunaliella* sp., and *Nitzschia* sp. The microalgae underwent treatment with combined EMS and UV radiation and were subsequently evaluated based on cell density, lipid content, and biomass productivity in grams per liter of culture. This comparison was conducted for both wild-type and mutated strains of each microalgae species.

2 Methodology

2.1 Research Procedures

Cultivation of wild-type microalgae. The gas flow from the CO_2 gas tube and the air pump were assembled as shown in Fig. 1. The mixed CO_2-air was fed to the photobioreactor through the mixing point and was measured with the flowmeter. The CO_2 gas flow rate was set at 0.5 l/min for the CO_2 concentration of 3%. The photobioreactor was filled with microalgae culture and seawater at a ratio of 1:3 with 136.3 mg/l urea and 50 mg/l TSP (Soedarmodjo et al., 2021) for 8 liters of culture. The tube light lamp of UV-C was used with an intensity of 5,000 lux.

Cultivation of mutated microalgae. The microalgae mutation process was carried out with addition of 0.5 M EMS (Augustine, 2016) and UV-C light for 3 minutes (Beacham et al., 2015). The culturing process was carried out until day 7 with the same culture conditions as the wild-type microalgae culture. The cell density was measured daily.

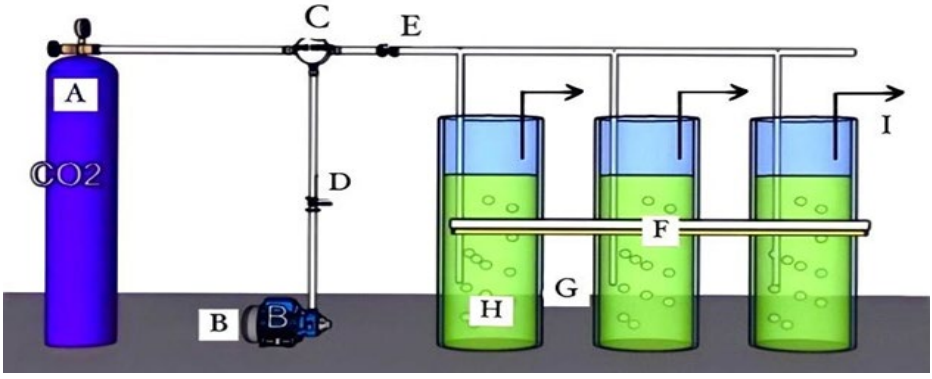
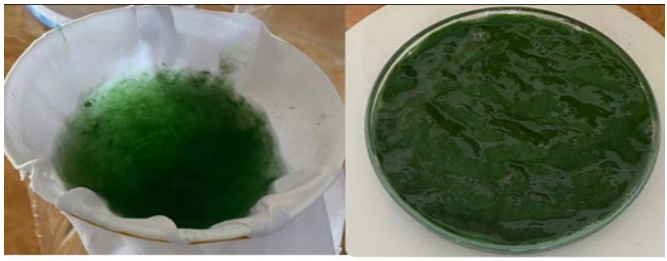


Fig. 1. Experimental Setup**.** Where; (A) CO_2 gas tube, (B) Air blower, (C) Gas mixing point, (D) Valve, (E) Gas flowmeter, (F) Tube Light lamp, (G) Photobioreactor, (H) Stone aerator, (I) Output gas.

Harvesting of microalgae. Microalgae harvesting occurred on day 7, involving the addition of 150 mg/l flocculant, $Al_2(SO_4)_3$ (Hidayati et al., 2015), followed by a settling period of 1 hour. Subsequently, microalgae separation from the medium was achieved by filtration using a 60–70 mesh satin fabric. The resulting microalgae paste was washed with seawater to eliminate any remaining flocculant residue, as depicted in Fig. 2.



a b

Fig. 2. (a) the harvested microalgae, (b) Microalgae biomass in the form of paste.

The Microalgae Biomass Extraction. The microalgae paste was dried using an oven at 60°C for 2 hours before extracting. Dried microalgae was shown in Fig. 3. The extraction was carried out as shown in Fig. 4 using a soxhlet apparatus with 80 ml of n-hexane as a solvent, at a temperature of 70°C for 5,5 hours. The dried microalgae was placed in a fixed porous bag, and the solvent was passed through the dry microalgae. When the extraction process was ended, then the distillation was carried out for 3 hours at 80°C to separate the n-hexane solvent from the lipids.

Fig. 3. Microalgae Biomass.

a b

Fig. 4. (a) Soxlet Extraction process, (b) Distillation for separation of solvent and lipids.

2.2 Analysis

The Cell Density Measurement. The analysis aimed to monitor microalgae growth by measuring cell density. This is aimed to determine the increase in the number of cells every day from day 1 to 7 at 13.00 using a hemocytometer with improved neubauer with the size of 7.5 x 3.5 cm. The sample of microalgae was taken by a dropper pipette, then the sample was placed on the haemocytometer for 2 drops. Then, it was covered with a cover glass. The observation was carried out using a binocular microscope XSZ with 400 times magnification. Calculation of microalgae cell density was performed using the Eq.1 (Karlson et al., 2010):

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

Microalgal Biomass Yield Calculation. The microalgae biomass paste was dried and then was cooled in a desiccator before weighing it using digital balance. The biomass weight was calculated using the Eq. 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.

Calculation of Microalgae Lipid Content. The weight of microalgal lipid obtained from the extraction and distillation was determined using an analytical balance. Then, the calculation was carried out using the Bethien-Diemar method (1963) (Endrawati et al., 2012) to determine the lipid content (%) in the biomass, as follow:

$$\%\ Lipid\ Content = \frac{(A-B)}{C} \times 100 \quad (3)$$

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 Results and Discussion

3.1 The Cell Density of *Spirulina* sp.

Fig. 5 shows the comparison of the culture, both wild type and mutated *Spirulina* sp. on day 2 and 7. On day 2, it can be seen that the microalgae in the photobioreactor was light green and then it turned to dark green on day 7. This color change reflect due the growth process of *Spirulina* sp. where the cell density is much higher on day 7. The cell growth of the *Spirulina* sp. was monitored. As shown in Fig. 6., the cell density on day

7 is much higher than that of in day 2. This indicates that the growth is taking place from day 2 and is also confirmed with Fig. 5 and 6.

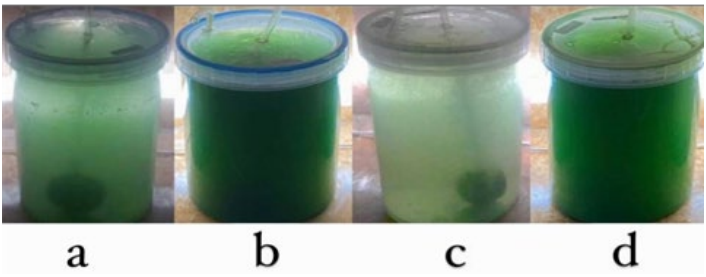


Fig. 5. The comparison of *Spirulina* sp. culture in different days: (a) wild type on day 2, (b) wild type on day 7, (c) mutated on day 2, and (d) mutated type on day 7.

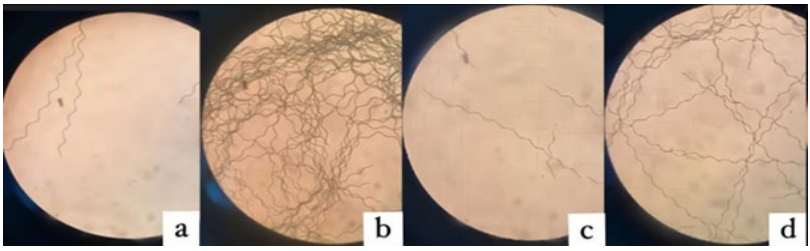


Fig. 6. *Spirulina* sp. cell density: (a) wild on day 2, (b) wild on day 7, (c) mutated on day 2, and (d) mutated on day 7.

Fig. 7. shows the profile of *Spirulina* sp. in terms of cell density for both wild type and mutated treatments. Based on Fig. 7, it can be seen that the longer the duration of culturing, the higher the cell density of wild-type and mutated microalgae on day 1-7.

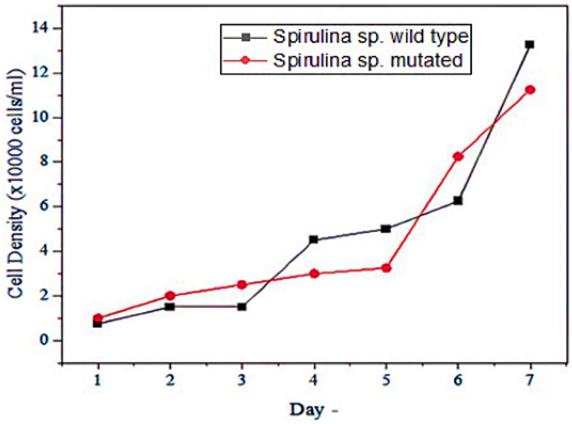


Fig. 7. Growth of wild type and mutated *Spirulina* sp.

The growth of *Spirulina* sp. was found to be aligned with the growth pattern of normal phytoplankton in general. On day 1, *Spirulina* sp. entered the lag phase, then the photosynthetic process took place, but not all *Spirulina* sp. cells experienced a significant increase in growth due to adaptation period to the new media and to the conditions of the water quality and the supply of nutrients to the culture, as well as adapting to its metabolism. An exponential phase occurred on day 2 until 7. This phase was showned by a rapid increase of the cell population up to several fold (Young et al., 2022). Therefore, in this study, the microalgae culture was carried out until day 7 with the expectation of obtaining the maximum number of microalgal cells.

In wild-type *Spirulina* sp. culture, the lag phase on day 1 had a cell number of 0.75 x 10^4 cells/ml and entered the exponential phase on day 2 and 3 and it showed a steady increase in cell number on day 4 to 6, while the peak of the exponential phase occurred on day 7 with a cell density of 13.25 x 10^4 cells/ml. Meanwhile, for the culture of mutated *Spirulina* sp., the lag phase on day 1 had a greater number compared to wild-type *Spirulina* sp. which was 1 x 10^4 cells/ml. The exponential phase occurred on day 2 to 7. On day 7 the number of mutated *Spirulina* sp. cells reached 11.25 x 10^4 cells/ml. These results are consistent with the study conducted by Buwono & Nurhasanah (2018) on *Spirulina* sp. They cultured the microalgae until day 15, showing that days 1 and 2 were an adaptation phase followed by an exponentiation phase. The density of *Spirulina* sp. increased from day 3 to day 7 in this phase. The cell density of *Spirulina* sp. was affected by adding EMS and UV-C. At the end of exponential phase on day 7, wild-type *Spirulina* sp. had 0.18-fold higher cell density than mutated *Spirulina* sp. It is concluded that addition of EMS and UV-C causes microalgae *Spirulina* sp. to undergo slow cell divisions in culture. Therefore, the cell density was not much higher compared to that of wild type *Spirulina* sp. This result is consistent with the research of Soedarmodjo et al. (2021) using *Botryococcus braunii.* In the thermo of cell density, it was found that the UV-C mutant microalgae had lower growth than the wild microalgae strains.

3.2 The Cell Density of *Dunaliella* sp.

Fig. 8. shows the difference in the color of the *Dunaliella* sp. on the photobioreactor. On day 2, the color of the culture looks much lighter. This is because it has just entered the exponential phase. While on day 7, the peak of the exponential phase was reached, which makes the culture color a little darker. However, when is is compared to Fig. 5., for *Spirulina* sp., the colour shows that *Spirulina* sp. has much darker green in comparison to the *Dunaliella* sp.

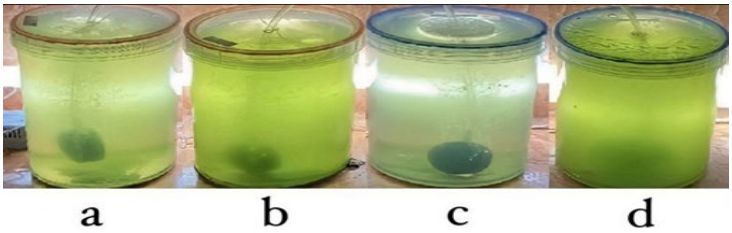


Fig. 8. The comparison of *Dunaliella* sp. culture in different days: (a) wild type on day 2, (b) wild type on day 7, (c) mutated on day 2, and (d) mutated type on day 7.

The profile of the cell density of *Dunaliella* sp. for both treatments, wild and mutation, is shown in Fig. 9. Based on the figure, it can be seen that the longer the day of cultivation, the higher the cell density of both the wild type and mutated microalgae on day 1 to 7.

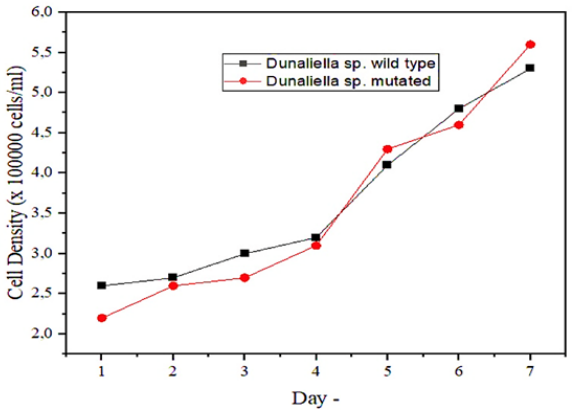


Fig. 9. Growth of wild type and mutated *Dunaliella* sp.

The growth is similar to the growth phase of *Dunaliella* sp. The microalgae has the adaptational and exponential phase in the culturing process. Harvesting of *Dunaliella* sp. was carried out during the exponential phase due to the máximum number of cells (Johan et al., 2020). On day 1, *Dunaliella* sp. underwent a lag phase and gave a cell number of 2.57 x 10^5 cells/ml. Then, the exponential phase on day 2 to 7 takes place normally in accordance with the growth pattern, where cell division takes place causing the increase of number of cells. On day 7 *Dunaliella* sp. wild type underwent peak population density of 5.25 x 10^5 cells/ml. In mutated *Dunaliella* sp., lag phase on day 1 has a cell number of 2.15 x 10^5 cells/ml. This number is much less than the number of cells of *Dunaliella* sp. wild type. Exponential phase of mutated *Dunaliella* sp. took place on day 2 to day 7. Peak exponential phase in mutated *Dunaliella* sp. occurred on day 7 of the culturing process with a cell number of 5.55 x 10^5 cells/ml. The increase in cell density, in line with the research of Wungmool et al. (2019) using *Dunaliella* sp.

They reported that the cell density of *Dunaliella* sp. continued to increase from day 8 to day 9.

The addition of combined EMS and UV-C gives a positive effect on mutated *Dunaliella* sp. The cell density of 0.057-fold higher than the wild-type *Dunaliella* sp was shown. This is in line with research by Sarayloo et al. (2018) using *Chlorella vulgaris* which gave an increase in cell density due to the mutation process with EMS & UV-C. It was found that in the wild type *Chlorella vulgaris* the cell density was 3.94 x 10^7 cells/ml while in the UV715–EMS25 mutant type was 5.31 x 10^7 cells/ml.

3.3 The Cell Density of *Nitzschia* sp.

The difference in color of the *Nitzschia* sp. culture in the photobioreactor on days 2 and 7 is shown in Fig. 10. On day 2, the color of the culture looks more clear because it has just entered the exponential phase. On day 7, the exponential phase has peaked, making the color of the culture darker.

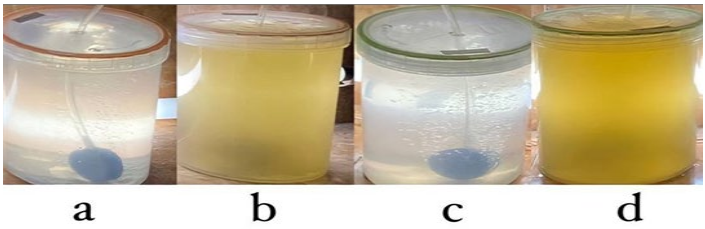


Fig. 10. The comparison of *Nitzschia* sp. culture in different days: (a) wild type on day 2, (b) wild type on day 7, (c) mutated on day 2, and (d) mutated type on day 7.

Fig. 11. shows the profile of *Nitzschia* sp. cell density for both treatments wild type and mutation. Based on Fig. 11., it can be seen that the longer the days of culturing, the higher the cell density of wild-type and mutated microalgae on day 1 to 7.

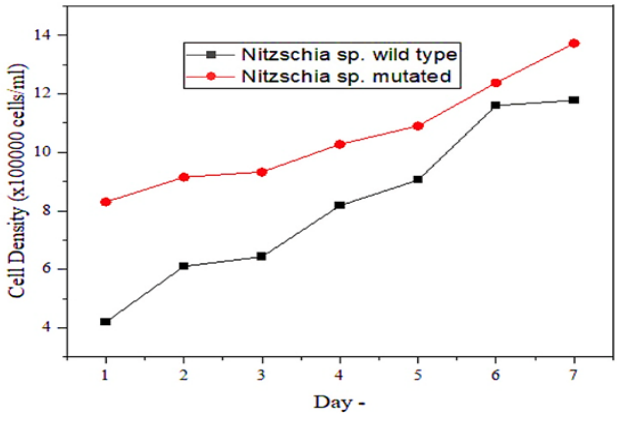


Fig. 11. Growth of wild type and mutated *Nitzschia* sp.

This is aligned with the growth phase of *Nitzschia* sp. Wild-type *Nitzschia* sp. had a cell count of 4.2 x 10^5 cells/ml in the lag phase, while the exponential phase occurred on day 2 where the number of cells increased significantly and continued until day 7. Day 7 was the peak of wild-type *Nitzschia* sp. cell density of 11.775 x 10^5 cells/ml and was the right time for the harvesting process. Mutated *Nitzschia* sp. has a cell number of 8.3 x 10^5 cells/ml in the lag phase and exponential phase has the amount of growth and cell division that increases gradually from day 2 to 7 and the peak cell density of mutated *Nitzschia* sp. occurs on day 7 which is 13.725 x 10^5 cells/ml. The increase in cell density is in accordance with research by Singh et al. (2023) using *Nitzschia* sp., an increase was found in cell density from day 1 to 14.

In the lag phase, mutated *Nitzschia* sp. experienced an increase in the number of cells 0.98-fold more when compared to wild-type *Nitzschia* sp. The addition of EMS & UV-C light makes *Nitzschia* sp. able to adapt to environmental conditions and cells have been mutated can experience rapid cell division, where the number of mutated *Nitzschia* sp. cells on day 7 increased 0.165-fold higher than the wild type *Nitzschia* sp. This is because *Nitzschia* sp. is a type of microalgae that can adapt well and even still live in polluted environmental conditions (Garali et al., 2021).

3.4 Cell density comparison of the three species of microalgae

Fig. 12. shows a comparison of cell density of 3 types of wild-type microalgae. Fig. 12. shows that for each type of wild-type microalgae, the longer the number of days of cultivation, the higher the cell density of the microalgae.

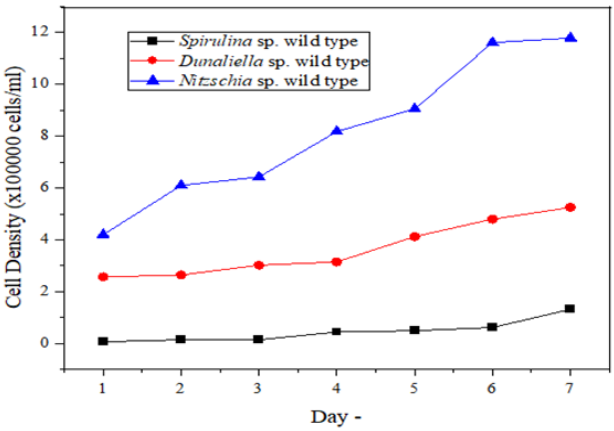


Fig. 12. Cell density comparison of 3 wild-type microalgae (*Spirulina* sp., *Dunaliella* sp., *Nitzschia* sp.).

On day 1 to 7, the number of cells from 3 types of wild-type microalgae increased and was in the exponential phase. The culture is not yet entering the stationary phase and death phase. However, when a comparison is made of the 3 types of wild-type

microalgae, it was found that the microalgae with the highest cell density on day 7 is *Nitzschia* sp. with a cell number of 117.75 x 10^5 cells/ml, while for *Dunaliella* sp. is only 5.25 x 10^5 cells/ml. The lowest cell density was *Spirulina* sp. which was 1.325 x 10^5 cells/ml.

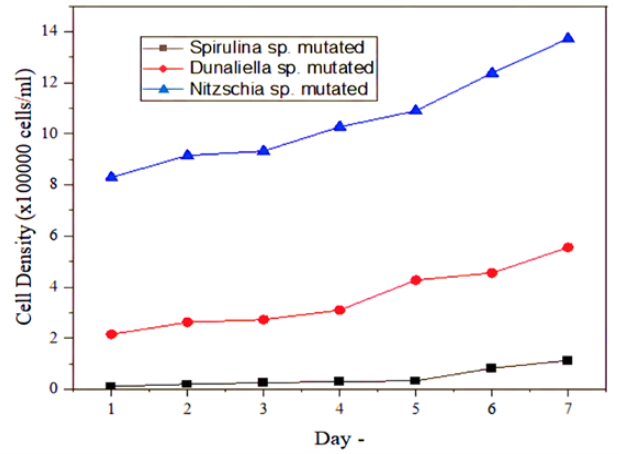


Fig. 13. Cell density of the three mutated microalgae (*Spirulina* sp., *Dunaliella* sp., *Nitzschia* sp.).

Fig. 13. shows the cell density of the three microalgae types in the mutated treatment compared. From the Fig. 13., it can be seen that the number of cells of each type of microalgae increased. The highest cell density in mutated microalgae was found in *Nitzschia* sp. with a total cell density on day 7 of 13.725 x 10^5 cells/ml, followed by *Dunaliella* sp. with a total cell density of 5.55 x 10^5 cells/ml, and the lowest was *Spirulina* sp. with 1.125 x 10^5 cells/ml.

Table 1 shows the growth rate equation by regression analysis of *Spirulina* sp., *Dunaliella* sp. and *Nitzschia* sp. microalgae for 7 days in wild and mutated type.

Table 1. Microalgae growth rate equation.

Microalgae	Cell Growth Rate
Spirulina sp. wild type	**y = 18,036x - 25,357**
Spirulina sp. mutated type	**y =15,714x - 18,214**
Dunaliella sp. wild type	**y = 47,946x + 173,571**
Dunaliella sp. mutated type	**y = 55714x + 133929**
Nitzschia sp. wild type	**y = 129821x + 299643**
Nitzschia sp. mutated type	**y = 86786x + 710714**

As can be seen from the Table 1, compared to the mutant type, the wild-type *Spirulina* sp. and *Nitzschia* sp. had higher cell growth. Meanwhile, for *Dunaliella* sp, the highest cell growth belongs to mutation type. When a comparison is made for the three types

of microalgae, it is known that the lowest growth rate belongs to *Spirulina* sp. mutated-type. Using linear regression to determine the relationship between growth rate and days cultured of y = 15714x - 18214. Meanwhile, the highest growth rate is owned by wild-type *Nitzschia* sp. The wild-type of *Nitzschia* sp. has a linear regression relationship between the growth rate and the length of cultivation, which is y = 129821x + 299643. Research by Prasetyo et al. (2022) used regression analysis to determine the relationship between the growth rate of the microalgae *C. calcitrans* and different light intensities. The regression results showed y = 0.015x + 14.832 and the value of r = 0.989.

In this research, wild type and mutated *Nitzschia* sp. were the microalgae with the highest cell density. The increase in cell density is due to the amount of nutrients used by *Nitzschia* sp. to carry out the growth and reproduction process. However, although Nitzschia sp has higher cell density, in terms of the biomass production, *Nitzschia* sp. gives lowest production. This may be related to the size of cells.

3.5 Microalgae biomass productivity

Fig. 14. shows the dry weight gain for each type of wild-type and mutated microalgae *Spirulina* sp., *Dunaliella* sp., and *Nitzschia* sp. Microalgae culturing was carried out on an 8l basis in a photobioreactor and they were harvested on day 7.

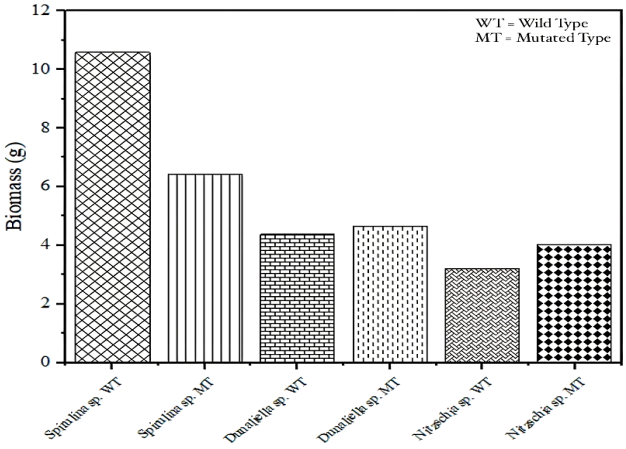


Fig. 14. Dry weight of each type of microalgae.

From Fig. 14. it can be seen that for each type of microalgae there is an increase and decrease in biomass in different types of microalgae due to the mutation process.

The biomass of the mutated *Spirulina* sp. was 6.441 g per 8 l culture or 0.805 g/l, while the wild type *Spirulina* sp. had a 64.72% higher biomass when compared to the mutated *Spirulina* sp. of 10,604 g per 8 l or 1.326 g/l. In *Dunaliella* sp. the addition of EMS and UV-C has little effect of biomass productivity, where mutated *Dunaliella* sp. had a 6.57% higher biomass of 4.673 per 8 l culture or 0.584 g/l when compared to the biomass of wild-type *Dunaliella* sp. which is only 4.385 g per 8 l or 0.548 g/l. *Nitzschia* sp. is the microalgae with the lowest total biomass. The application of EMS and UV-C

before the culturing process was much more influential when compared to the other 2 types of microalgae. The biomass obtained for wild-type *Nitzschia* sp. was 3.213 g per 8 l or 0.402 g/l and mutated *Nitzschia* sp. produced a higher biomass of 25.12% which was 4.026 g per 8 l or 0.503 g/l. Although *Nitzschia* sp. has the highest cell density, after drying the *Nitzschia* sp. paste, it was found that the biomass productivity of *Nitzschia* sp. was the lowest in each type, wild type and mutated. This is due to the size of *Nitzschia* sp. which is the smallest in comparison to the other 2 microalgae, which is 3 micrometers wide range (Bowers et al., 2018).

Research by Beacham et al. (2015) with mutated *Nannochloropsis salina* using EMS and UV, it was found that the addition of these mutagens to *Nannochloropsis salina* made a decrease in growth rates so that the dry weight obtained was less than the culture without mutagen application. The research was done by Sarayloo et al. (2018) using *Chlorella vulgaris* microalgae which was then compared to wild-type and mutated microalgae on dry weight gain and lipid content levels. In this study, it was found that the addition of UV715–EMS25 mutagen greatly influenced the acquisition of dry weight, where the dry weight content 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. Based on previous research, it can be concluded that the addition of EMS and UV-C has a different effect on biomass in each type of microalgae cultured, the final results obtained can be lower or higher when compared to wild type microalgae.

3.6 Lipid productivity of microalgae

Fig. 15. shows the percentage of lipid content in each type of microalgae. It can be seen that each type of microalgae shows an increase in lipid content due to treatment with EMS and UV-C mutagens. On the percentage of lipid content in the biomass, the mutated *Spirulina* sp. shows increase in the lipid content by 1.93-fold compared to that of the wild type. *Spirulina* sp. was found to have the highest percentage of increase due to the EMS and UV-C mutation process compared to other types of microalgae, *Nitzschia* sp. which only increased the percentage of lipid content 0.44-fold and *Dunaliella* sp. which increased 0.08-fold when compared to the wild type.

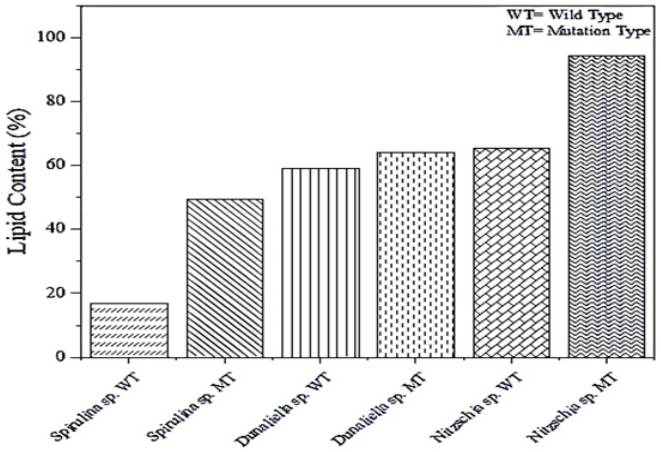


Fig. 15. Lipid content of each type of microalgae.

In previous studies, microalgae with the highest lipid content of 10–67% were found in *Chlorella*, *Dunaliella*, and *Scenedesmus* microalgae (Morales et al., 2021; Udayan et al., 2022). Saputro et al. (2019) said that microalgae lipid content usually reaches 1.9–40% of cell dry weight and can reach 80% under certain conditions. In this study, the highest lipid content is found in the *Nitzschia* sp., that is 94.39% due to the addition of EMS and UV-C.

The increase in lipid levels in mutated microalgae is in accordance with previous research. Research done by Sarayloo et al. (2018) using *Chlorella vulgaris* which was mutated using EMS and UV-C. In this study, an increase in lipid levels of 67% was obtained, lipid levels in wild-type by 25% and in the mutated type by 42%. Increased lipid content due to the addition of EMS and UV-C will cause microalgae to become stressed. Stress is caused by microalgae being in a toxic environment, which shifts the carbon flux and changes the lipid biosynthesis pathway towards the formation and accumulation of neutral lipids in the form of TAG and causes microalgae to survive in adverse conditions and leads to an increase in lipid production in microalgae (Babu et al., 2022).

4 Conclusion

The amount of biomass of mutated microalgae in *Spirulina* sp., *Dunaliella* sp., *Nitzschia* sp. are 0.805 g/l, 0.584 g/l, 0.503 g/l, respectively. There is an increase in biomass of *Dunaliella* sp. and *Nitzschia* sp., but on the other hand, there is a decrease in biomass of *Spirulina* sp. compared to the wild type. Meanwhile, there is an increase in lipid content in the biomass of each type of microalgae with the addition of EMS & UV-C. The amount of lipid content in the biomass of mutated microalgae in *Spirulina* sp., *Dunaliella* sp., and *Nitzschia* sp. are 46.68%, 64.20%, and 94.39%, respectively. So, the highest biomass was obtained in *Nitzschia* sp. and increased by 25.3% in

comparison to wild-type. The highest percentage of increase in lipid content was obtained in *Spirulina* sp. that is an increase of 1.93-fold.

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