The effects of UV-C and HNO₂ mutagen, pH and the use of commercial fertilizers on the growth of microalgae Botryococcus braunii

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Abstract. Botryococcus braunii microalgae is a potential producer of biodiesel as an alternative fossil fuel because it has a high lipid content. This paper was conducted to determine the optimal mutation agent, microalgae resistance to changes in pH and, optimal nutrients from commercial fertilizers for the growth of Botryococcus Braunii. To find the best mutation agent, culture was planted in seawater media with Walne nutrient with UV-C and HNO₂ exposure. The results showed that the optimal mutation agent was a mutation with UV-C exposure expressed by lipid productivity values. Furthermore, to see microalgae resistance to changes in pH, B. braunii was cultured in seawater media with Walne nutrients at various pHs, namely 3-8 with mutations of UV-C rays and without mutation agents. The results show that natural and mutated B. braunii microalgae grow optimally at pH 8. This also shows that mutated microalgae have better adaptability compared to natural microalgae, as evidenced by the number of natural microalgae cells that continues to decrease with a decrease in pH. The final goal is to find the optimal nutrient for growth in B. Braunii from commercial fertilizer. Culture was planted in seawater media with various concentrations of commercial fertilizer, for 7 days of culture, the results showed that each nitrogen source had a different effect on the growth of microalgae

1. Introduction

The Fossil fuels such as petroleum, native gas, and coal are limited and pollute the environment (Sharma, 2012). It is completely necessary to generate fuel from renewable and environmentally friendly sources. Microalgae lipids as a potential alternative can be converted into biodiesel. Marine water microalgae have advantages over other oil-producing plants such as jatropha, sunflower, or oil palm as the growth land does not compete with other plants, has a much higher growth rate, and a better lipid composition as a biodiesel feedstock (Widjaja et al., 2009).

Marine water microalgae cultivation as a lipid producer and then converted into biodiesel will provide high economic benefits. Some of the problems in the cultivation of microalgae as lipid producers are their resistance to pH changes in the culture medium. CO₂ is a source of carbon for microalgae culture while the addition of CO₂ will decrease its pH value along with its reaction with H₂O to produce HCO₃⁻ (Widjaja et al., 2009).

Microalgae is a microorganism that is often examined for its effect on UV-B radiation. Skerratt et al. (1998), examined the effect of UV-B mutations on the lipid content of Antarctic marine phytoplankton
microorganisms. It was found that lipid levels per cell increased when exposed to high levels of UV-B caused by increased concentrations of free fatty acids and might show complex lipid degradation during the high-level UV-B irradiation process. In the research of Xue et al. (2005), it is said that exposure to UV-B light continuously decreases the chlorophyll content, but increases the UV-B-absorbing compound in algae. Decreased photosynthesis in algae, especially at high UV-B irradiation doses, is influenced by both direct effects (effects on photosystem) and indirect (decreased pigment). The decrease in chlorophyll pigments and photosynthesis results in lower biomass. However, algae have evolved and established defense mechanisms to protect themselves from the damaging effects of UV-B radiation.

The media for microalga cultivation generally consists of marine water that is equipped with quality nutrients. Over the past few years, several studies have been carried out to develop media formulas for the cultivation of various microalga species by replacing analytical grade chemicals with agricultural fertilizers as nutrients. In addition to saving costs for large-scale microalga cultivation, media based on agricultural fertilizers are easy to prepare. Besides, the nutritional value of algae produced is also quite high. (Kamal, 2012)

The research aimed to determine the effect of UV-C and HNO₂ mutagens on the growth of Botryococcus braunii, determine the effect of pH on the growth of native Botryococcus braunii microalgae and their mutants and compare the effect of the use of commercial fertilizer on the growth of native Botryococcus braunii microalgae and UV-C mutated.

2. Methodology

2.1. Media Preparation

The standard nutrition medium for cultivation of Botryococcus braunii was a Walne nutrient made by 100 mg/L NaNO₃, 45 mg/L Na₂EDTA, 33.6 mg/L H₃BO₃; 20 mg/L NaH₂PO₄.2H₂O; 1.3 mg/L FeCl₃.6H₂O; 0.36 mg/L MnCl₂.4H₂O; 0.1 mg/L Vitamin B₁; 0.005 mg/L Vitamin B₁₂ in 1 L of solvent. And also nutrition medium from fertilizer mixture of ZA, TSP and Urea, were modified from Development of Cheap and Simple Culture Medium for the Microalgae Nannochloropsis sp. Based on Agricultural Grade Fertilizers Available in the Local Market of Gaza Strip (Palestine) journal.

2.2. Culture

At first, cells of B. braunii was pre-culture in 500 mL standard nutrition medium and incubated batch wisely at room temperature, with airflow aeration 3 L/min. Then, B. braunii was cultivated with a different nutrient variable for 7 days, checked every 24 hours. Other conditions of incubation such as light intensity and temperature were all the same as the corresponding normal nutrition condition.

2.3. pH (Dayananda et al., 2006)

B. braunii microalgae can grow at a pH range of 6 - 8.5. Then pH variable from 3 to 8 are used, citric acid used to make acid environment in media culture

2.4. Mutation

Mutations can be due to physical and chemical influences. Examples of physical mutagens are ionizing irradiation. These molecules can react to the extreme and can damage DNA. In this study, UV-C rays were used as mutating agents. B. braunii was used as the wild type microalgae for UV-C induction. Microalgae were added to petridish, were placed under a UV-C lamp. In random mutagenesis trials, UV-C was exposed to the samples in each petri dish for 1.5 min. Chemical mutations with HNO₂ carried out mutations with the addition of 20 ml HNO₂ on microalgae growing media. With a ratio of 1: 1 for HNO₂ and native microalgae.

2.5. Lipid Extraction (Zhu et al., 2002)

Normally, cells are harvested by centrifugation at 8500 rpm for 5 minutes and washed once with distilled water. The sample was centrifuged at 3000 rpm for 10 minutes. The solid phase is carefully separated
using filter paper where two sheets of filter paper are applied twice to provide complete separation. then the precipitate was roasted at 60°C for 2 hours, then lipid was smoothed with mortal and ultrasonicated for 30 minutes, then extracted soxhlet for 6 hours with 200mL of n-hexane, then separating n-hexane from lipids by distillated for 2 hours at 90°C.

3. Result and Discussion

3.1. Effect of Mutation

To find out the number of cells that survive the mutation process with HNO$_2$, a counting chamber analysis is performed with the results in (Table 1).

| Table 1 Cell counts before and after mutation used HNO$_2$ |
|---------------------------------------------------------|
| Cell count $B$.braunii (cell/mL) | % of Death |
| Before Mutation | 31,988,888 |
| After Mutation | 29,333,333 |
| % of Death | 8.3 |

Physical mutations performed with UV-C rays, the number of initial $B$.braunii cells, number of cells that lived after UV-C exposure and the percentage of deaths presented in (Table 2).

| Table 2 $B$. Braunii cell counts Before and After a mutation using UV-C rays |
|---------------------------------------------------------|
| UV-C Exposure | Run | Number of Cells $B$.braunii (cell/mL) | Average of Death (%) |
|---------------|-----|-------------------------------------|---------------------|
| 1.5 Minutes   | Run 1 | 99,500,000 | 68,000,000 | 29,31 |
|               | Run 2 | 71,000,000 | 71,000,000 | 29,31 |
|               | Run 3 | 72,000,000 | 72,000,000 | 29,31 |

The results in (Table 2) show that there was a decrease in the percentage of cells that lived after UV-C exposure for 1.5 minutes. Cells that are still alive after exposure to UV-C light are called mutated cells. UV-C rays influence growth, survival, pigmentation, metabolism, and photosynthesis of microalgae. An increase in UV-C radiation, in general, will reduce chlorophyll content and decrease photosynthesis (effects on photosystem). This decrease in chlorophyll and photosynthesis content results in lower biomass (Xue et al., 2005). The mutated $B$. braunii is then cultured for up to 5 days and the number of cells is checked by a counting chamber analysis to see the growth of microalgae every 12 hours according to (Table 3).

| Table 3 Comparison of $B$. braunii growth with various mutating agents |
|---------------------------------------------------------|
| Time (Days) | Number of Cells (cell/mL) |
|-------------|---------------------------|
| Native | UV - C | HNO$_2$ |
| 0 | 41,833,333 | 70,333,333 | 29,333,333 |
| 1 | 49,166,667 | 78,333,333 | 26,666,667 |
| 2 | 60,333,333 | 83,000,000 | 35,555,556 |
| 3 | 72,833,333 | 90,666,667 | 40,000,000 |
| 4 | 83,000,000 | 100,833,333 | 48,888,889 |
| 5 | 92,166,667 | 107,333,333 | 57,777,778 |
We can observe the growth of B. braunii microalgae by looking at the value of specific growth in (Table 4) The specific growth value of native microalgae without native treatment is 0.14556 day⁻¹, whereas for UV-C mutated microalgae the specific growth value is 0.08086 day⁻¹ and the HNO₂ mutated microalgae have a specific growth value of 0.11936 day⁻¹. Thus, it can be concluded that native microalgae without mutated treatment have the best cell growth compared to UV-C and HNO₂ mutated microalgae, where B. braunii microalgae mutated by UV-C rays have the lowest growth among other treatment variables. This is consistent with previous research which states that, where there are two responses from algae to UV-C irradiation, forcing algae to develop methods to adapt to UV-C side effects, this causes stunted algal growth (Xue et al., 2005).

3.2. Effect of Lipid Extraction
Lipid extraction is used to determine the lipid productivity of B. braunii. Extraction was carried out for each variable namely native microalgae, UV-C mutants, and HNO₂ mutants. The native weight of B. braunii microalgae was 0.8707 grams, the dry weight of microalgae B. braunii UV-C mutants was 1.8778 grams, the dry weight of B. braunii mutants was 12.1 grams, the data generated in (Table 5).
From the data in Table 5, it can be seen that the dry weight of B. braunii was mutated to UV-C light 1,878 grams. Whereas the dry mass of B. braunii natively is 0.871 grams. This is consistent with existing research in which mutagenesis with UV light on K.marxianus T-2 causes the addition of dry cell mass (Zul, 2003).

Besides, it can be seen that the greatest productivity of biomass is obtained from microalgae B. braunii mutated UV-C which is 0.565 (mg / mL) / day. Then followed by native microalgae with biomass productivity of 0.421 (mg / mL) / day. The lowest biomass productivity was obtained from B. braunii mutated HNO2 microalgae which are 0.074 (mg / mL) / day.

Biomass productivity is strongly influenced by the number of microalgae cells, where irradiation with UV-C has a great effect on changing the number of microalgae cells. There are two responses from algae to UV-C irradiation, first when exposed to UV-C in a short period time causing changes in the structure of DNA, causing algae to die. Second, the long UV-C irradiation time forces the algae to develop a method to adapt to the side effects of UV-C, this causes the growth of algae to grow slowly (Xue et al., 2005). Whereas the greatest value of lipid productivity was also obtained by B. braunii mutated UV-C rays which were 0.340 (g / L) / day. Then followed by native microalgae with lipid productivity of 0.157 (g / L) / day. The lowest lipid productivity was obtained by B. braunii mutated HNO2 which was 0.025 (g / L) / day.

Under normal growth conditions, microalgae produce large amounts of biomass, but lipid productivity is not optimal. So to obtain high lipid content, microalgae need to be under stress conditions, in this case, exposure to UV-C (Sharma et al., 2012). This is following the research of Skerratt et al. (1998), mutations with UV-C irradiation increase the lipid content of Antarctic marine phytoplankton microorganisms. This is due to the increased concentration of free fatty acids and shows complex lipid degradation during the high-level UV-C irradiation process. So based on these data it can be concluded that better microalgae growth does not always produce more lipids and vice versa. From the research that has been done, it can be concluded that to get high lipid productivity it is necessary to do a UV-C mutation of microalgae.

### 3.3. Effect of pH on the Growth of Native B.braunii and UV-C Mutations

pH affects the growth of microalgae. Each microalgae species has a tolerant value or a minimum pH value that allows for its survival. Most microalgae grow in normal pH conditions between 6-8. CO2 is a carbon source for microalgae while the addition of CO2 will decrease its pH value along with its reaction with H2O to produce HCO3- (Widjaja et al., 2009). Microalgae cell walls function as a buffer layer or layer to maintain pH in the microalgae body. At acidic pH (pH <7), microalgae will consume carbon from HCO3- (CO2 dissolved in water) to form a buffer layer that serves to protect itself from environmental conditions, resulting in decreased levels of dissolved CO2 in water so that the pH of the media gradually increases.

At too high a pH, microalgae will consume CO2 directly from the air. In this case, CO2 is very soluble in water, consequently the levels of dissolved CO2 increase and form acidic H2CO3 so that the pH of the media gradually decreases (Nalewajko et al., 1996). UV-C exposure of B. braunii microalgae was carried out to determine its effect on cell growth in media with a pH of 3-8. The results of native B.braunii cell growth at various pH for 7 days are presented in the following Figure 1.
On the B. braunii microalgae, growth curve shows different growth rates. Based on Figure 1 above we can see the growth of native B. braunii cells at pH 3 - 8. It is seen that the lower the pH of the culture media, the slower the growth of B. braunii cells. On culture media pH 6-8, B. braunii microalgae can grow well. On culture media pH 5, B.braunii cell growth increased to day 6 but decreased on day 7. On culture media pH 3 and 4, B.braunii cell growth decreased from day 1 to day 7. According to (Dayananda et al., 2006), B. braunii microalgae can grow at a pH range of 6 - 8.5. This is strongly influenced by the pH of the culture media from the microalgae. (Ammar et al., 2015), explained that in the growth of microalgae culture there are about four growth phases. The first phase is the induction phase or lag phase which slightly increases in growth rate along with cell absorption. In the second phase, the growth rate increases exponentially. This depends on many factors such as species or types of algae, light intensity, and temperature of the media. The third phase is a constant phase where the cell density becomes relatively constant. Finally, the growth rate decreases when cell division decreases due to several factors that affect the growth rate such as factors of nutrient concentration, pH, dissolved CO$_2$, risk of light, and contamination.

Based on research conducted by (Czeslawa et al., 1996), concluded that microalgae cannot grow optimally at pH below 4.8 because the cell wall of microalgae is no longer able to maintain itself to survive. The results of growth of UV-C mutated B.braunii cells at various pH for 7 days are presented in the following Figure 2
Based on Figure 2 above it can be seen that the growth of UV-C mutated \textit{B.braunii} cells at pH 3 - 8. It appears that the lower the pH of the culture media, the slower the growth of \textit{B.braunii} cells. On culture media pH 3 - 8, \textit{B. braunii} microalgae can grow well. In pH 8 culture media, the growth of UV-C mutated \textit{B.braunii} cells is better than that of native \textit{B.braunii}. However, in lower culture media, \textit{B. braunii} microalgae can grow better than native \textit{B. braunii}. Visible on culture media pH 3 and 4, \textit{B.braunii} microalgae UV-C mutated can still grow compared to native \textit{B.braunii} microalgae. (Warmadewi et al., 2017), explains that mutations aim to deal with native changes that will arise at any time with genetic changes that can be inherited from their offspring. In the changes that arise, the mutated traits are more adaptable than the original traits, so the original character allows it to disappear from circulation and bring up new traits. This new property allows \textit{B. braunii} microalgae to be more resistant to low pH medium. From the results obtained it can be proved that the mutated microalgae have more adaptability than native microalgae.

### 3.4 Effect of Commercial Fertilizers Use for Nutrients on the Growth of UV-C Mutated \textit{B.braunii}

Nutrients are important factors in algal biomass production. Most microalgae require macronutrients such as carbon (C), nitrogen (N), hydrogen (H), sulfur (S), potassium (K), magnesium (Mg), and phosphorus (P). Whereas micronutrients are used to increase cell growth and metabolism, the existence of micronutrients cannot be replaced by other substances. Micronutrient requirements also vary based on microalgae habitat (marine water, marine water, freshwater). Some micronutrients include iron (Fe), boron (B), manganese (Mn), vanadium (Va), silicon (Si), selenium (Se), cuprum (Cu), nickel (Ni), and molybdenum (Mo) (Hadiyanto et al., 2012). Fertilizer as a support for normal cell growth requires a minimum of 16 nutrients in them and there must be 3 absolute elements, namely nitrogen, phosphor, and potassium (Andhikari et al., 2004). Nitrogen (N) and phosphor (P) are major nutrients in the growth of microalgae (Zullaikah et al., 2019) At this stage of microalgae, \textit{B.braunii} mutated UV-C cultured for 7 days, with different variables of TSP, ZA and Urea fertilizers in (Table 6) and (Table 7) as follows

| Fertilizer | Concentration (mg / L) |
|------------|------------------------|
|            | A                      | B          | C          | D          |
| ZA         | 150                    | 100        | 300        | -          |
| Urea       | 7.5                    | 5          | -          | 136,316    |
| TSP        | 25                     | 15         | 50         | 50         |

**Table 6** Nutrient Variables of Commercial Fertilizers
Table 7 Nutrient Content of each Variable

| Variable (mgr) | Walne       | A           | B           | C           | D           |
|---------------|-------------|-------------|-------------|-------------|-------------|
| Nitrogen      | 5.632,372,28| 5.634,129,93| 5.633,541,30| 5.635,545,72| 5.635,546,71|
| Carbon        | 1.120,88    | 1.187,88    | 1.162,88    | 1.112,90    | 2.475,69    |
| Phosphor      | 1.99        | 330,89      | 198,53      | 661,78      | 661,78      |
| Oxygen        | 1.726,775,41| 1.731,136,95| 1.729,618,78| 1.735,354,79| 1.729,904,59|
| Hydrogen      | 2.30        | 500,63      | 332,33      | 951,26      | 496,99      |

During culture, the number of cells is calculated every day to determine cell growth that can be known from the color of microalgaes. At optimal growth conditions, microalgaes will be dark green while microalgaes will turn yellowish-green if the growth conditions are not optimal and even tend to turn yellow when dead. So from the calculation of the number of cells that are counted, it can be concluded that the counted microalgaes cells are living microalgaes cells due to the color of dark green microalgaes. In the mutation process with UV-C light, a wavelength of 254nm is used. In the cell calculation, the counting chamber method is used by using a hemacytometer. The results of native B.braunii cell growth and UV-C mutations for 7 days are presented in (Table 8) following.

Table 8 Growth of Microalgaes with UV-C mutations in various nutrient variables

| Days | Number of cells / mL |
|------|----------------------|
|      | A                    | B                    | C                    | D                    | Walne  |
| 0    | 42,000,000.00        | 41,166,666.67        | 41,000,000.00        | 41,666,666.67        | 35,166,666|
| 1    | 46,833,333.33        | 43,500,000.00        | 41,500,000.00        | 29,500,000.00        | 36,833,333|
| 2    | 69,166,666.67        | 64,000,000.00        | 47,000,000.00        | 43,000,000.00        | 37,000,000|
| 3    | 78,833,333.33        | 65,166,666.67        | 51,833,333.33        | 48,666,666.67        | 39,666,666|
| 4    | 90,000,000.00        | 73,333,333.33        | 52,000,000.00        | 37,166,666.67        | 49,833,333|
| 5    | 96,166,666.67        | 77,833,333.33        | 29,666,666.67        | 13,833,333.33        | 52,500,000|
| 6    | 98,500,000.00        | 80,000,000.00        | 18,333,333.33        | 6,000,000.00         | 56,500,000|
| 7    | 101,333,333.33       | 83,500,000.00        | 16,666,666.67        | 4,666,666.67         | 60,500,000|

Figure 3 Growth Curve of UV-C Mutated B.braunii Cells for 7 Days of Culture with Various Types of Nutrients
From (Table 8) and Figure 3 it can be seen that the growth of UV-C mutated microalgae with nutrient "A" has the fastest growth compared to other nutrient variables with the final cell count on the 7th day is 101,333,333 cells/mL. Nutrient that has a fairly good growth next is nutrient "B" where the final cell count on the 7th day reaches 83,500,000 cells / mL. Next can be seen that microalgae with Walne nutrient which is a standard nutrient that is commonly used for B. braunii culture shows results that are not as good as nutrients "A" and "B" with the number of cells on the 7th day is 60,500,000 cells/mL. Then, the growth of microalgae with nutrient "C" tends to decrease with final number of cells is 16,666,667 cells/mL. It also happened with nutrient "D" which has the least number of cells on the 7th day, which is 4,666,667 cells/mL.

In Figure 4 it can be seen that there are differences in color, where the nutrient with variables C and D are yellow but variable A and B are green. Yellow color of microalgae indicates that the microalgae has died while the green one indicates that the microalgae still growing. For the specific growth value of each nutrient can be seen in (Table 9).

| Nutrient | μ (day⁻¹) |
|----------|-----------|
| A        | 0.07267   |
| B        | 0.11265   |
| C        | 0.09000   |
| D        | -0.17792  |
| Walne    | -0.50992  |

From (Table 9) it can be seen that the value of specific growth nutrient "A" give best value at 0.113 days⁻¹. Followed by specific growth of nutrient "B" which has a value of 0.090 days⁻¹. Furthermore, the original nutrient for B. braunii microalgae has specific growth value at 0.073 days⁻¹. Positive values in this specific growth indicate that microalgae have a value of cell concentration that tends to rise each day. Whereas, nutrient "C" has a specific growth value of -0.178 days⁻¹ and the nutrient "D" has a value of -0.510 days⁻¹. This negative sign indicates that microalgae growth tends to go down each day.

In (Table 6) shows that nutrient "A" and "B" have the same composition of fertilizers but they have different concentrations. The maximum cell concentration in nutrient "A" is 1.21 times higher than...
nutrient "B". This can be happened because nutrient "A" has a higher nutrient concentration of nutrient "B".

One of the important nutrients in the growth of microalgae is nitrogen. Nitrogen is an important component of many macromolecules such as DNA, RNA, chlorophyll and protein, known as one of the most important nutrients for microalgae. Nitrogen deficiency causes a decrease in photosynthesis and protein synthesis, but increases lipid and carbohydrate synthesis. Another important nutrient of microalgae is phosphor, because it can influence protein and carbohydrate synthesis in microalgae (Chrismanda et al., 2006). From (Table 6) we can obtain nitrogen content data in each nutrient as in (Table 10) below

| Nutrient | Nitrogen content (mg / L) | μ (day⁻¹) |
|----------|--------------------------|-----------|
| Walne    | 16.47903065              | 0.07267   |
| A        | 3531.790605              | 0.11265   |
| B        | 2354.52707               | 0.09000   |
| C        | 6363.367933              | -0.17792  |
| D        | 6363.351533              | -0.50992  |

From (Table 10) can be seen that the lower nitrogen content affects the value of specific growth, but the amount of nitrogen needed is different for each Microalgae. There is a maximum condition where the nitrogen content of 3531.79 mg / mL contained in the nutrient "A" causes B. braunii to grow optimally.

If the nitrogen content is too low as in "B" and Walne nutrients, the growth of microalgae is less optimal. This is consistent with previous studies, where the low nitrogen variable causes a decrease in the number of cells. Quoting from (Neha Kalla et al., 2016) that the reduction in nitrogen levels has an effect on decreasing the growth of microalgae cells and biomass, where in low nitrogen conditions the microalgae will experience stress conditions thereby causing a decrease in cell number and biomass productivity. This is also consistent with the quote of (Chrismanda et al., 2006) which states that low nitrogen levels in culture media can cause a decrease in cell count. The nitrogen level is related to the loss of the cell's ability to build functional structures associated with these limited amounts of nutrients. In this study the effect of phosphorus was not reviewed because each variable contained similar phosphorus content so that it was only seen from the nitrogen content (N) which is one of the main nutrients in microalgae.

If nitrogen content is too high as in nutrients "C" and "D", microalgae growth is significantly decrease. Very high nitrogen concentrations in microalgae growth media can cause deactivation in the production of pigments needed for photosynthesis. Therefore, high nutrient concentrations take longer to reach a phase of growth stability (Ammar et al., 2015).

In addition to the amount of nitrogen, the growth of B. braunii microalgae is also influenced by the nitrogen source of the microalgae itself. In Fertilizer ZA, nitrogen is obtained from ammonium sulfate compounds. Whereas in urea fertilizer, nitrogen is found in nitrate compounds. Different again with walne nutrient, the nitrogen source is from NaNO₃ compounds.

B. braunii's growth in nutrients "A" and "B" are significantly higher than Walne media, this is due to the nitrogen source in the media "A" and "B" are NH₄⁺ (as ammonium sulfate) and urea (nitrate). Whereas in Walne nutrient, the nitrogen source is form NaNO₃. This nitrogen difference affects the production of chlorophyll in microalgae (Lourenco et al., 2002).

The beneficial effect of ammonium on algal growth is in accordance with some previous studies, where ammonium was found consumed by Isochrysis aff. Galbana is eight times faster than nitrate when they are added together in culture media. Nitrate absorption stops in the presence of ammonium.
because nitrate reductase is inactive when ammonium concentration is present as the main source of nitrogen (Valenzuela Esinoza et al., 1999). It was also observed that the presence of ammonium in nutrient culture would prevent the absorption of nitrates by *Navicula ostrearia*, *Nitzschia ovalis*, and *Amphora coffeaeformis* (Maestrini et al., 1986).

Although the concentration of ZA fertilizer in "C" nutrient is twice than "A", the results show higher cell concentration in nutrient "A" than "C". This might be related to the presence of urea as an additional nitrogen source in the "A" nutrient but not in the "C" nutrient.

This can also be attributed to the very high concentration of ammonium sulfate in ZA fertilizer. In aqueous solutions, ammonium ions (NH$_4^+$) are in equilibrium with non-ionized or free ammonia (NH$_3$) according to the dissociation equation and can be toxic

$$\text{NH}_3 + \text{H}_2\text{O} \rightleftharpoons \text{NH}_4^+ + \text{OH}^-$$

Because of equilibrium, the increase in one of them automatically increases the other. Furthermore, this balance mainly depends on pH. In this study, there was no pH control, so with increasing algal density the pH in the medium would increase due to the assimilation of carbon dioxide. This condition will force the reaction to move to the left, thereby increasing the concentration of ammonia (NH$_3$) which has a toxic effect on algal growth, consequently reducing algal density in the nutrient "C" (Nabris et al., 2012)

In nutrient "D" it can be seen that the growth of *B. braunii* microalgae is very low. This is in accordance with Nabris research in 2012 where the nitrogen in nitrates contained in urea fertilizer cannot replace the nitrogen contained in ammonium sulfate in ZA fertilizer. This is because the nitrogen in the nitrate is difficult to be absorbed by microalgae.

So, it can be concluded that of all the nutrients tested, the best growth of *B. braunii* was achieved in nutrient "A", because the cell concentration achieved in this medium was greater than other culture media. The use of fertilizer at the agricultural level can replace the Walne nutrient that is commonly used for the cultivation of *B. braunii* microalgae.

4. **Conclusion**

Botryococcus braunii microalgae natively and UV-C mutated grow maximally at pH 8, Botryococcus braunii from mutations can grow better at low pH (3-6) and are more resistant to low pH when compared to native Botryococcus braunii, it is shown by the number of mutation Botryococcus braunii microalgae cells that are still experiencing an increase in the number of cells at pH 3-4. Mutated microalgae can adapt to acidic media by reducing acid in the media so that the pH of the media gradually rises, whereas in native microalgae the pH of the media decreases this is because native microalgae cannot adapt to the environment. The highest concentration of lipids was microalgae mutated by UV-C rays of 60,231%. Lipid productivity was found to be the highest in the UV-C mutation of 0.34 (g / L) / day. Nitrogen levels and types of nitrogen in nutrients greatly affect the growth of the microalgae Botryococcus braunii. The highest growth of microalgae cells produced by Botryococcus braunii microalgae with variable nutrient A for 7 days of culture was 101 million cells / mL.

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