International Journal on Advanced Science Engineering Information Technology

Vol.14 (2024) No. 3
ISSN: 2088-5334

Metabolite Compounds of *Euglena* sp. on Mass Cultivation System under $MgCl_2$ and $CaCl_2$ Salt Stress

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***Abstract*—Euglena sp. is rich in primary and secondary metabolite compounds. This cosmopolitan organism can survive extreme conditions such as salt stress because it can increase cell growth rate and metabolism. This mass cultivation research uses an open pond system that is very vulnerable to contamination, so variations in salinity of MgCl2 and CaCl2 are used as treatments because they minimize contamination. The research examines the correlation of salt types to cell growth rate, biomass, monosaccharide profile, paramylon, pigment, and lipid of Euglena sp. Research methods include preparation, medium making, mass cultivation, and measurement of test parameters. Data were analyzed through One-Way ANOVA followed by DMRT, with a 5% confidence level. Based on the research result, the highest value of biomass and paramylon content and productivity at CaCl2 treatment with consecutive values 0.013 ± 0.001, 0.002 ± 0.000, 9.556 ± 0.070, and 0.149 ± 0.019. Monosaccharides analysis produced in the form of sucrose, glucose, fructose, and arabinose amounted to < 10 ppm. CaCl2 treatment produced the highest sucrose of 6251 ppm. The highest chlorophyll-a and carotenoid pigments are located at CaCl2 treatment, respectively $(1.698 \pm 0.051)^b$ and $(0.500 \pm 0.032)^b$. At the same time, the salt treatment has a significant effect on lipid content. To summarize, almost all metabolite compounds were highest in CaCl2 treatment. This research is expected to contribute to developing the Euglena sp. mass cultivation system as a source of bioenergy and biomaterials.**

***Keywords*— Euglena sp.; mass cultivation; metabolite compounds; salinity.**

Manuscript received 21 Dec. 2023; revised 20 Apr. 2024; accepted 19 May 2024. Date of publication 30 Jun. 2024.

I. INTRODUCTION

Nowadays, the development of *Euglena* sp. in the field of biotechnology includes efforts to utilize biological agents that have advantages over other higher plants. *Euglena* sp. is one of the microalgae species from the Euglenophyta phylum that has high potential in producing primary and secondary metabolite compounds. Some of the characteristics possessed by *Euglena* sp. include including unicellular organisms, lacking cell walls, motile, flagellum functioning as a driving tool, having red eye spots to detect the presence of light, and including autotrophic organisms, meaning that they can produce bioactive metabolites that are renewable and environmentally friendly [1]. Based on its nature and way of life, *Euglena* sp. Shows great potential as a cultivable microorganism for large-scale production of diverse valuable metabolites and natural products, with numerous applications. Its complex metabolism resembles that of multicellular organisms, providing opportunities for metabolic engineering to generate various valuable metabolites [2].

Euglena sp. can accumulate large amounts of β-1,3-glucan as much as 20-75% dry weight if cultured with adequate nutrient sources and environmental conditions [3]. Regarding the significance of the monosaccharide profile, *Euglena* sp. has autotrophic and heterotrophic nutritional modes, which means that it can produce its food through photosynthesis or consume other organisms as a source of energy. In this study, *Euglena* sp. was mass cultivated using an open pond system, which is more economical. On the other hand, cultivation is affected by the evaporation process and is highly susceptible to weather and contamination that can affect cell growth rate and secondary metabolite production [4].

Chlorophyll is a green pigment found in oxidized photosynthetic organisms, such as microalgae, which plays a role in the photosynthesis process. Carotenoids have two functions in photosynthesis: light absorption in the visible spectrum, where no chlorophyll is absorbed effectively, and photoprotection in the photosynthetic system. Over 600

carotenoids have been discovered, with lycopene, β-carotene, zeaxanthin, astaxanthin, lutein, and violaxanthin being the predominant forms found in microalgae. These six carotenoids comprise around 90% of the carotenoids in the human body and are commonly consumed as part of our dietary intake [5]. Microalgae can accumulate 20–60% of total lipids per dry cell weight (DCW) depending on the characteristics of the strain and growth conditions [6]. The lipid content in *Euglena gracilis* can be maximized through certain cultivation conditions, such as high nutrient dosage and aerobic environment [7]. Salinity treatment can also increase lipid production in *Euglena* sp., along with the production of other beneficial metabolites [8].

Research by [9] shows that salinity variations can affect growth rates, cell morphology, photosynthesis, and paramylon content in *Euglena gracilis*. Therefore, this research explores the effects of $MgCl_2$ and $CaCl_2$ salt types as oxidative agents on cell growth rate, biomass, monosaccharide profile, paramylon, pigment, and lipid. The general objective of this study is to examine the effects of adding salt types on the cell growth rate and biomass of Euglena sp. The specific aim of this study is to determine the optimal salinity variation for the monosaccharide profile, paramylon, pigment, and lipid of *Euglena sp*.

II. Materials and Methods

A. Experimental Design and Treatment

This research is a factorial experiment using three sample treatments with three replications. The treatment in this study was provided by providing conditions of salinity variation in the form of MgCl2 and CaCl2 at a concentration of 150 mM.

B. Medium Preparation and Mass Cultivation

Euglena sp. *strain* IDN33 isolates obtained from the Dieng Plateau, Central Java, were mass cultivated in sterilized open ponds with a volume of 500 L using $MgCl_2$, $CaCl_2$, and control salt treatments for 12 days without light control. The media used had an acidic pH in the range of 4-5 including 500 g/L ZA fertilizer, 495 g/L Triple superphosphate (TSP), 100 g/L $MgSO_4$, 10 g/L KCl, 50000 µL/L Trace Metal MIX, 50000 µL/L $Na_2MoO_4.2H_2O$, 10000 µL/L Vitamin B1, and 10000 µL/L Vitamin B12.

C. Cell Growth Rate Measurement

A total of 1.5 mL of *Euglena* sp. sample was measured for optical density using UV-Vis Spectrophotometry for 12 days of observation. Absorbance was measured with wavelengths of 680 nm and 860 nm. The data obtained from the measurement values were tabulated in Microsoft Excel, and a growth rate curve was created.

D. Assessment of Biomass Content and Productivity

The dry weight of *Euglena* sp. biomass was determined using a vacuum pump kit. Previously, the glass fiber filter was weighed, and 15 ml of sample was poured over the glass fiber filter and placed on the clamp. Then, the filtering cup was rinsed with distilled water three times. After that, the glass fiber filter was released from the clamp using tweezers and placed in an incubator. Biomass content can be calculated using the following formula according to [10],

$$\text{Biomass (g/L)} = \frac{\text{Final Weight of Sample} - \text{Initial Weight of Sample}}{\text{Volume of Sample Taken}} \quad (1)$$

The calculation of biomass productivity can be calculated using the following formula according to [11],

$$\text{Biomass productivity (g/L)} = \frac{\text{Max Biomass} - \text{Initial Biomass}}{\text{Biomass Peak Time} - \text{Biomass Start Time}} \quad (2)$$

E. Assessment of Paramylon Content and Productivity

The procedure was done with modifications from the research [9]. A 50 mg portion of dried *Euglena* sp. sample was dissolved in 1.5 mL of acetone and vigorously mixed for 5 minutes. Next, the sample was centrifuged at 10000 rpm for 3 minutes to separate and collect the paramylon. Once again, the preceding step was repeated, and the leftover pellet was combined with 1.5 mL of a 1% SDS solution. The mixture was then vortexed. Subsequently, place the mixture in a water bath set at 95°C for 30 minutes. The sample underwent vortexing, centrifugation, and subsequent removal of the supernatant. The sample was thoroughly rinsed with 1.5 mL of distilled water, vigorously mixed, and subjected to centrifugal force, and the liquid portion was removed. The task was completed on two separate occasions. The pellet was dissolved in 1 mL of 0.5 N NaOH and left undisturbed overnight. The quantification of paramylon was conducted using the phenol-sulfuric acid method. This involved adding 0.5 mL of 5% phenol and 2.5 mL of H_2SO_4 to the extracted samples in a conical tube. The mixture was then let stand for 10 minutes and then vortexed. The sample underwent incubation for 20 minutes, during which the absorbance was quantified using a spectrophotometer set at a wavelength of 480 nm. A standard curve was generated using the same approach, but D-glucose was used as a substitute for the samples.

The calculation of paramylon productivity is calculated using the following formula based on previous calculation [11],

$$\text{Paramylon productivity (g/L)} = \frac{\text{Max Paramylon} - \text{Initial Paramylon}}{\text{Paramylon Peak Time} - \text{Paramylon Start Time}} \quad (3)$$

F. Determination of Monosaccharides Profiles by HPLC

Previously, 1 mL of 0.008 N H_2SO_4 solution was pipetted and dissolved into double distilled water pro-HPLC up to 1 liter, then homogenized and sonicated for 15 minutes. Standard sucrose, glucose, fructose, and arabinose solutions were made as much as 10000 ppm (1% v/v). 10 mg of sample was weighed and added until the solvent volume became 1 mL using ultrapure water in an Eppendorf tube. Then vortexed and sonicated for 15 minutes. After that, it was centrifuged at 13000 rpm at 4°C for 10 minutes. 200µL of supernatant was put into a vial and analyzed using HPLC Aminex HPX-87H Ion Exclusion Column and Reactive Index.

G. Assessment of Pigment

A 2 mL culture sample was collected and transferred to a conical tube, followed by centrifugation at 12000 rpm for 5 minutes. The supernatant was removed and replaced with methanol, an organic solvent, thoroughly mixed using a vortex. Subsequently, the spectrophotometer was utilized to measure the absorbance at 480, 652, and 665 nm wavelengths. Using the spectrophotometer absorbance calculation results, we can determine the chlorophyll content, encompassing the total chlorophyll-a, chlorophyll-b, and carotenoid components. Chlorophyll a and b were calculated using the previous

formula reported by [12]. Meanwhile, the carotenoid content was measured and calculated according to [13] method. This may be achieved through the use of the following equation:

$$\begin{gathered}\text{[Chlorophyll-a] (µg/mL)} = \\ \text{-8.0962 x (}A\text{ 652) + 16.5169 x (}A\text{ 665)} \\ \text{[Chlorophyll-b] (µg/mL)} = \\ \text{27.4405 x (}A\text{ 652) − 12.1688 x (}A\text{ 665)}\end{gathered} \quad (4)$$

$$\text{[Carotenoid] (µg/mL)} = 4 \text{ x (}A\text{ 480)} \quad (5)$$

H. Assessment of Lipid

The lipid content was measured utilizing the Bligh and Dyer method. A 5 mL sample of the culture was collected in a 15 mL conical tube and then centrifuged at 1220 xg for 10 minutes using a LW Scientific Ultra-85 centrifuge machine. The liquid portion was removed, and a mixture of 3 mL methanol and chloroform in a 2:1 ratio was introduced to the solid residue. Subsequently, 1 mL of chloroform reagent and 1 mL of distilled water were poured into the conical tube. The lower layer of a yellow hue, which had been formed within a conical tube, was carefully transferred and introduced into a petri dish. Subsequently, the petri dish was placed in an incubator for a duration of one hour, after which its weight was assessed. The lipid content is subsequently quantified using the following formula according to the previous calculation [14]:

$$\text{Lipid content (g/L)} = \frac{\text{Final Petri Dish Weight} - \text{Initial Petri Dish Weight}}{\text{Sample Volume}} \quad (6)$$

I. Salinity Test

Salinity levels were measured using a calibrated refractometer. Three drops of sample were placed on the glass surface of the refractometer. Then, the refractometer cover was closed, and the observation was directed toward the light source to make the results more transparent.

J. Statistical Analysis

Data underwent analysis of variance (ANOVA) and correlation using IBM's Statistical Product and Service Solutions software, specifically Version 26, developed by IBM Corporation in the United States. The analysis was performed in three replications, and the results were presented as the mean (n=3) ± error standard of the dependent treatments. A study was conducted to investigate the differences between treatment means. The Duncan's Multiple Range Test (DMRT) was used to compare the means, with a significance level of $p < 0.05$.

III. Results and Discussion

A. Cell Growth Rate of Euglena sp.

Upon analyzing the research findings presented in Figure 1 below, it is evident that the growth rate of *Euglena* sp. cells commencing from day 0 to day 12 shows that cultivation in the $CaCl_2$ treatment has the highest optical density, followed by the $MgCl_2$ treatment and control. At the beginning of cultivation, starting on day 0, it can be observed that the exponential phase has occurred, where there is a rapid increase in cell optical density. Then on day 6, the stationary phase began in the $MgCl_2$ treatment and control. Meanwhile, the $CaCl_2$ treatment is still experiencing a logarithmic phase because it is still experiencing an increasing cell growth rate. On day 11, cells underwent a growth decline phase, where the cell growth rate decreased in both the $MgCl_2$ treatment and control groups. Nevertheless, the cell growth rate of *Euglena* sp. with the $CaCl_2$ treatment on day 11 still rose and reached the highest cell growth rate value of $(0.3 \pm 0.014)^c$. This $CaCl_2$ treatment began to experience a phase of cell decline on day 12, which can be seen in that the cells are no longer growing, as seen by the absorbance value, which decreased from the previous day. Salinity treatment significantly affects the optical density of *Euglena* sp. culture cells with a value of $p = 0.00$.

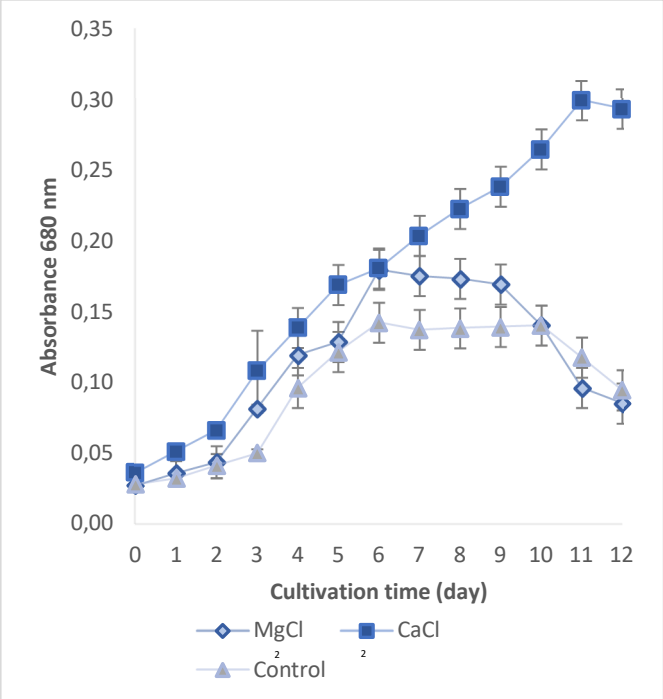

Fig. 1 The growth rate of *Euglena* sp. as affected by salinity variation during twelve days of mass cultivation. The bars represent the standard error of the difference between means.

In the growth curve of this study, starting from day 0, there was an increase in the number of cells until day 6. When entering the stationary phase, cells actively produce primary metabolites to reduce cell division activity. Then, the phase of decreasing growth rate indicates that the cell culture has begun to enter the death phase. The existence of abiotic stresses such as salinity significantly affects the growth rate and survival of *Euglena sp.* cells because salinity acts as an oxidative agent that can accelerate the growth of microalgae by supporting the photosynthesis reaction. This increases the production of metabolites in the cells, which serves as a cell defense mechanism. [15].

B. Biomass Content and Productivity

Based on the results of the study that can be seen in Figure 2 below, it can be seen that the highest successive treatment in the value of total biomass content and productivity lies in the treatment of $CaCl_2$, $MgCl_2$, and control. The value of biomass in the $CaCl_2$ treatment amounted to $(0.013 \pm 0.001)^b$, followed by the $MgCl_2$ treatment, which had a biomass content of $(0.002 \pm 0.000)^a$, and the control treatment had the lowest

value, which was recorded as $(0.001 \pm 0.001)^a$. Meanwhile, the highest to lowest biomass productivity value was in the $CaCl_2$ treatment. Meanwhile, the highest to lowest biomass productivity values for each treatment were $(0{,}002 \pm 0.000)^b$, $(0.0003 \pm 0.000)^a$, and $(0.0001 \pm 0.000)^a$, respectively. The value of biomass content and productivity obtained in each treatment for 12 days of observation show that the treatment of variations in salinity sources has a significantly different effect. The significance value obtained is 0.00 ($p < 0.05$).

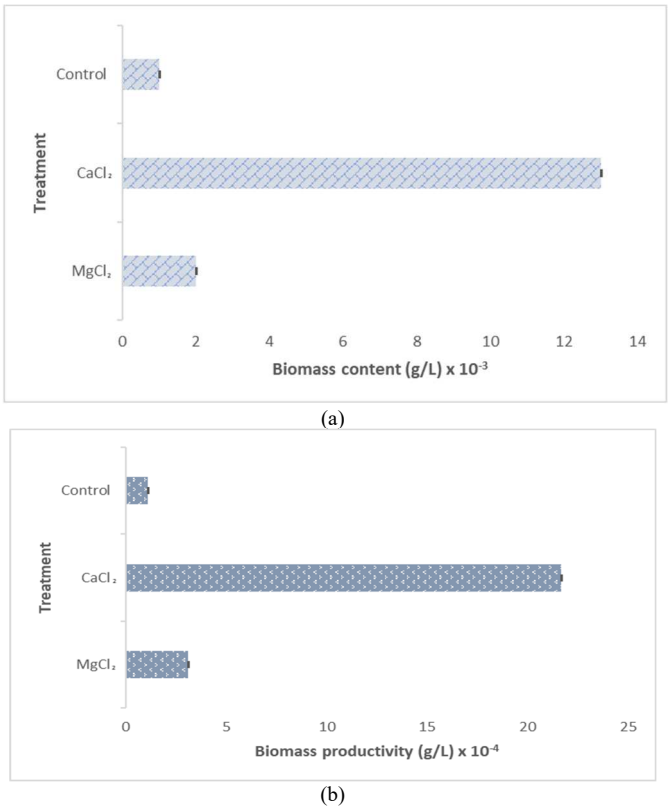

Fig. 2 Biomass value of *Euglena* sp. as affected by salinity variation during twelve days of mass cultivation. a: Biomass *Euglena* sp. b: Biomass productivity. Bars represent the standard error of the difference between means.

In biotechnology, the biomass produced by *Euglena* sp. requires stable conditions. Under stress conditions, such as salinity, microalgae experience an increase in biomass. Study conducted by [16] showed that a treatment with 100mM KCl increased biomass by 1.2 times compared to the control treatment. Light factors including UV exposure, are also external factors that stimulate biomass accumulation and the synthesis of healthy compounds that mainly absorb UV [17]. However, *Euglena* sp. cultivation research is carried out with an open pond system that is free to come into contact with air, making it vulnerable to contamination even though it is easier to operate compared to closed cultivation [18]. Treatment with added salt effectively minimizes contaminants while helping increase biomass content in microalgae [19]. The presence of $CaCl_2$ salt-type treatment can increase the overexpression of carbonic anhydrase in *Euglena* sp. cells to increase its biomass productivity [20].

C. *Paramylon Content and Productivity*

Based on the results of the study that can be seen in Figure 3 below, it can be seen that the highest treatment successively on the value of total content and productivity of paramylon lies in the treatment of $CaCl_2$, $MgCl_2$, and control. The value of paramylon content in $CaCl_2$ treatment amounted to $(9.556 \pm 0.070)^b$, followed by $MgCl_2$ treatment, which has a biomass content of $(9.159 \pm 0.062)^a$, and the control treatment with a value of $(9.109 \pm 0.168)^a$ had the lowest value. Salinity treatment significantly affects the content of *Euglena* sp. cell culture paramylon during 12 days of observation with a value of $p = 0.005$ ($p < 0.05$). While the highest to the lowest values of paramylon productivity for each treatment respectively amounted to $(0.149 \pm 0.019)^b$, $(0.074 \pm 0.014)^a$, and $(0.057 \pm 0.013)^a$. Salinity treatment significantly affects the productivity of *Euglena* sp. cell culture paramylon during 12 days of observation with a value of $p = 0.002$ ($p < 0.05$).

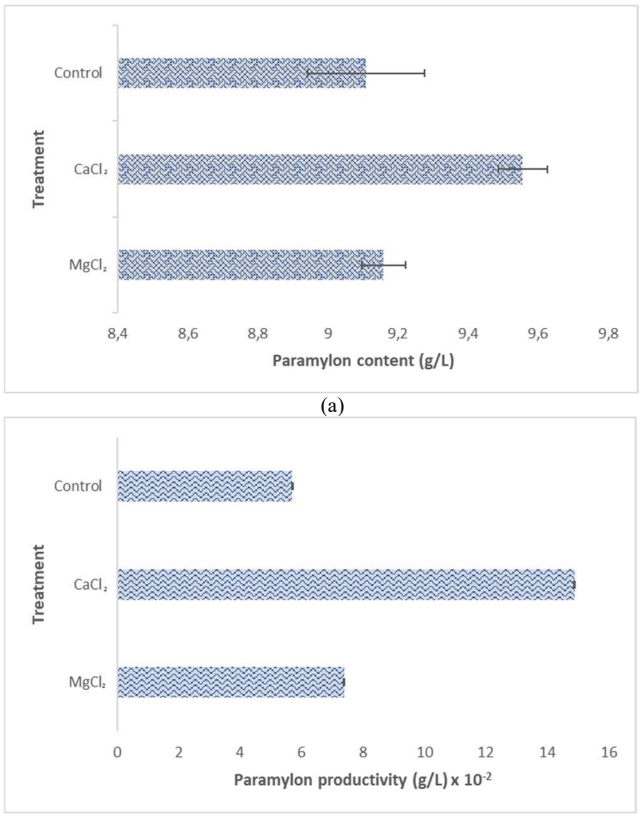

Fig. 3 Paramylon value of *Euglena* sp. as affected by salinity variation during twelve days of mass cultivation. a: Paramylon content. b: Paramylon productivity. Bars represent the standard error of the difference between means.

When Euglena sp. cells are cultivated in extreme environmental conditions. It can synthesize various secondary metabolites, specialized compounds produced in response to environmental changes. They play a key role in the defense mechanism against abiotic and biotic stress via their anti-allergenic, anti-microbial, anti-inflammatory, and antioxidant properties [21]. Two of the secondary metabolite compounds is paramylon and monosaccharide. Paramylon is a glucose polymer that functions as storage, reaching 80% of the dry weight of *Euglena* sp. [22], [23]. *Euglena* sp. stores photosynthetic products from the Calvin cycle in the form of carbohydrates (paramylon) in its cells, which function as the primary cellular energy reserve and as a cell defense against environmental osmotic pressure [15]. Research conducted by

[9], showed that the paramylon content in *Euglena gracilis* using salinity treatment in the form of NaCl with concentrations of 0, 50, 100, and 150 mM showed that the highest paramylon levels could be found in the highest concentration (150 mM) as much as 515.04 ± 76.67%. In comparison, the lowest paramylon levels could be found in the lowest concentration or control (0 mM), which was 100%. It can be seen that the higher the concentration of salinity, the higher the paramylon produced. This study's result aligns with the treatment concentration used in this research.

D. Monosaccharide Profile Analysis

Based on the research results that can be seen in Table 1. below, it can be seen that the highest type of monosaccharide in the $CaCl_2$ treatment lies in the kind of sucrose with a concentration of 6251 ppm. While the monosaccharides glucose, fructose, and arabinose produced concentrations < 10 ppm.

TABLE I
TYPES AND QUANTIFICATION OF *EUGLENA* SP. MONOSACCHARIDES AS AFFECTED BY SALINITY VARIATION DURING TWELVE DAYS OF MASS CULTIVATION.

No.	Treatment	Concentration (ppm)			
		Sucrose ($C_{12}H_{22}O_{11}$)	**Glucose (C6H12O6)**	**Fructose (C6H12O6)**	**Arabinose (C5H10O5)**
1.	$MgCl_2$	< 10	< 10	< 10	< 10
2.	$CaCl_2$	6251	< 10	< 10	< 10
3.	Control	< 10	< 10	< 10	< 10

Euglena sp. cells efficiently convert paramylon, a polysaccharide that serves as a food reserve, into monosaccharides to enhance their metabolic processes. This conversion occurs when the cells have reached their maximal growth rate and depleted their nutrient sources and salinity. The higher sucrose content in the monosaccharide test is interrelated with the specific metabolic pathways of sucrose and its constituent monosaccharides. Sucrose is a disaccharide formed by glucose and fructose. Since both anomeric groups are involved in the glycosidic linkage, sucrose cannot be reduced and exhibits limited chemical reactions. Sucrose plays a vital role in the growth and development of plants as it serves as a raw material for producing essential compounds [24]. The *Calcium chloride* ($CaCl_2$) treatment contains the highest sucrose concentration based on the results of the monosaccharide test on the mass cultivation of *Euglena* sp. in this study. The addition of $CaCl_2$ salt can trigger osmoregulation, where the addition of $CaCl_2$ to the cultivation media can change the osmolarity of the media so that it can affect the growth and survival of *Euglena* sp. cells [25]. Functionally, $CaCl_2$ can also cause the sucrose concentration to increase because, in terms of metabolic pathways, *Euglena* sp. cells can be influenced by various factors, including nutrient availability, light intensity, and temperature. The emergence of changes in these factors can lead to changes in the production and accumulation of carbohydrates, including sucrose [26]. The carbohydrate extraction research on microalgae by [27] states that sucrose has the highest content among fructose and maltose compounds, 0.29 ± 0.06.

E. Pigment Content

Based on the research results that can be seen in Table 2. below, it can be seen that the highest chlorophyll-a pigment content values are successively located in the $CaCl_2$, $MgCl_2$, and control treatments. The chlorophyll-a value in $CaCl_2$ treatment has a value of $(1.698 \pm 0.051)^b$, followed by $MgCl_2$ treatment which has a biomass content of $(0.772 \pm 0.102)^a$, and the lowest value is located in the control treatment with a value of $(0.645 \pm 0.011)^a$. Meanwhile, the highest value of pigment content in chlorophyll-b is successively located on control, $CaCl_2$, and $MgCl_2$ treatment. The value of chlorophyll-b in the control treatment has a value of $(0.706 \pm 0.039)^b$, followed by $CaCl_2$ treatment which has a chlorophyll-b content of $(0.772 \pm 0.102)^a$, and the lowest value is located in the $MgCl_2$ treatment with a value of $(0.458 \pm 0.171)^a$. Then the value of carotenoid pigment content in the $MgCl_2$ treatment is $0.645 \pm 0.011)^a$. Then the highest value of carotenoid pigment content is successively located in $CaCl_2$, control, and $MgCl_2$ treatments. The carotenoid value in the $CaCl_2$ treatment has a value of $(0.500 \pm 0.032)^b$, followed by the control treatment which has a carotenoid content of $(0.285 \pm 0.014)^a$, and the lowest value is located in the $MgCl_2$ treatment with a value of $(0.645 \pm 0.011)^a$. The salinity treatment dramatically influences the content of chlorophyll-a and carotenoid pigments in *Euglena* sp. cell culture during the 12 days of observation with p values of 0.000 and 0.001 ($p < 0.05$). However, salinity treatment did not significantly affect the content of chlorophyll-b pigments in *Euglena* sp. cell culture for 12 days of observation with a value of $p = 0.113$. Table 2 shows the pigment content of *Euglena* sp. as affected by salinity variation during twelve days of mass cultivation. (Different superscript letters in the column indicate significant differences at $p < 0,05$).

TABLE II
CHLOROPHYLL A, B AND CAROTENOID TOTAL OF *EUGLENA* SP. UNDER SALT STRESS COMPARED TO CONTROL GROUP

Treatment	Chlorophyll-a (ųg/mL) ± SD*	Chlorophyll-b (ųg/mL) ± SD*	Carotenoid (ųg/mL) ± SD*
$MgCl_2$	$(0.772 \pm 0.102)^a$	$(0.458 \pm 0.171)^a$	$(0.217 \pm 0.070)^a$
$CaCl_2$	$(1.698 \pm 0.051)^b$	$(0.524 \pm 0.044)^{ab}$	$(0.500 \pm 0.032)^b$
Control	$(0.645 \pm 0.011)^a$	$(0.706 \pm 0.039)^b$	$(0.285 \pm 0.014)^a$

The photosynthetic performance of *Euglena* sp. cell culture can be significantly impacted by salt stress, hence exerting a crucial influence on its growth and survival. The alterations observed in the architecture of the thylakoid membrane and the pigment-protein complexes have the potential to play a role in the adaptive response of *Euglena* sp. cells to salt stress, as suggested by [9]. According to [28], the study demonstrates that $CaCl_2$ has the maximum efficacy in enhancing the levels of chlorophyll and carotenoids. This is attributed to the good impact of $CaCl_2$ on the content of chlorophyll and carotenoids in microalgae, hence leading to an increase in paramylon productivity. Magnesium plays a crucial role in the synthesis of chlorophyll molecules, enabling the efficient process of photosynthesis and the absorption of light energy [29].

F. Lipid Content

Upon analyzing the study's findings, as depicted in Figure 4 below, it can be seen that the highest treatment in total lipid content is in the treatment of control, $CaCl_2$, and $MgCl_2$. The value of lipid content in the control treatment amounted to

$(0.021 \pm 0.000)^b$, followed by the $CaCl_2$ treatment, which had a lipid content of $(0{,}0008 \pm 0{,}000)^a$, and the lowest value was located in the $MgCl_2$ treatment with a value of $(0.0005 \pm 0{,}000)^a$. Based on the lipid content values obtained from each treatment over 12 days of observation, the treatment of variations in salinity sources has a significantly different effect. The significant value obtained is 0.016.

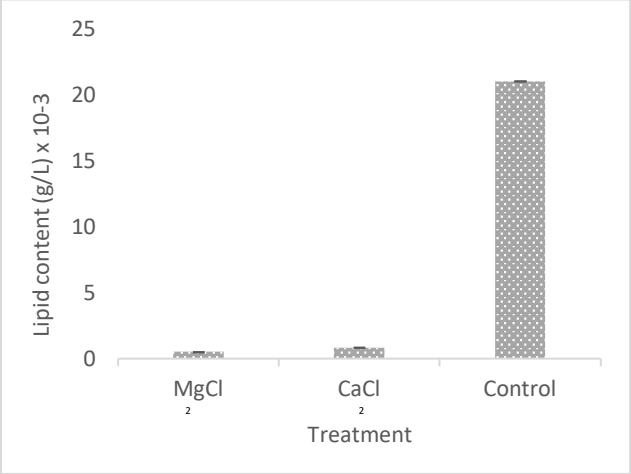

Fig. 4 Lipid content of *Euglena* sp. as affected by salinity variation during twelve days of mass cultivation. Bars represent the standard error of the difference between means

The lipid content of the treatment group is lower than that in the control group, depending on the specific salt concentration used. Consequently, two potential outcomes arise, the impact of salt stress may lead to a reduction in lipid content, or conversely, it may increase. In a study conducted by [28], it was observed that the augmentation of Ca^{2+} levels in the culture medium resulted in elevated lipid content in *Chlorella vulgaris* when subjected to salinity conditions. Additionally, the presence of enhanced Ca^{2+} levels facilitated the survival of microalgae in conditions of salt-induced stress. The Ca^{2+} ion is involved in regulating microalgal triacylglycerol metabolism, a process closely linked to lipid accumulation [30].

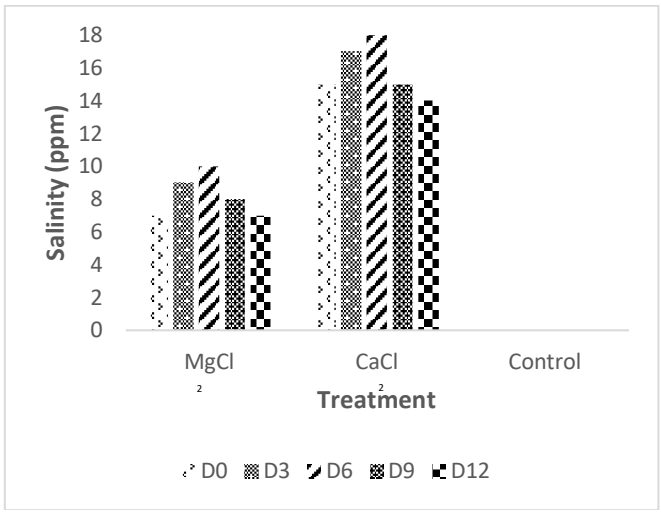

Fig. 5 Salinity levels of *Euglena* sp. as affected by salinity variation during twelve days of mass cultivation.

G. Salinity Level

Upon analyzing the research findings displayed in Figure 5, it becomes evident that the salinity value of the $MgCl_2$ treatment reaches its peak at 10 ppm on day 6, but subsequently decreases to 7 ppm by day 12. Meanwhile, the $CaCl_2$ treatment had the highest salinity level on day 6 at 18 ppm and decreased on day 12 to 14 ppm. Then, the control treatment for 12 days of observation had no salinity content (0 ppm).

The difference in salinity between the salt $MgCl_2$ and $CaCl_2$ can also produce differences in the metabolite results obtained. The higher salinity level as the cultivation time increases is due to the sedimentation of $MgCl_2$ and $CaCl_2$ salts on the surface of the culture tanks, causing the salinity level to increase. Naturally, mass cultivation conditions in an open environment automatically experience a high evaporation process, so water often evaporates. Water is always added so that the volume of water in the culture basin is constant at 500 L. Salinity stress can induce ionic and osmotic imbalances followed by oxidative damage caused by Reactive Oxygen Species (ROS), which can lead to loss of efficiency of the photosynthetic process [9].

IV. Conclusion

In conclusion, this research showed that salt stress treatment, in general, successfully affected cell growth rate, biomass, monosaccharide profile, paramylon, and pigment in the mass cultivation of *Euglena* sp. The $CaCl_2$ treatment had the highest cell growth rate and biomass productivity by 20 times compared to the control treatment. The highest monosaccharide profile concentration was found in sucrose type, as much as 6251 ppm in $CaCl_2$ treatment, while increasing paramylon productivity by three times compared to the control treatment. The highest chlorophyll-a and carotenoid pigments are located in the $CaCl_2$ treatment as much as $(1.698 \pm 0.051)^b$ and $(0.500 \pm 0.032)^b$. Meanwhile, the salts of MgCl2 and CaCl2 significantly affect lipid content.

Acknowledgment

The authors thank Universitas Gadjah Mada, especially the Biotechnology Laboratory of the Faculty of Biology, for providing research facilities.

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