pISSN 2302-1616, eISSN 2580-2909
Vol 11, No. 1, June 2023, pp. 102-113
Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v11i1.35221

Different carbon source alternative medium improves *Euglena* sp. growth and paramylon production

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ABSTRACT. *Euglena* sp. is a type of microalgae are widely recognized for producing an important compound called paramylon. If *Euglena* sp. is grown in a medium containing a carbon source, it can produce more paramylon. The culture medium alternative must be optimized to maximize biomass production and specifically targeted metabolites. This study aims to compare the effects of glucose and glutamic acid as carbon sources on the growth rate, biomass, and paramylon (β-1,3-glucan) content of *Euglena* species. The treatment that carried out as the different organic carbon sources were glucose and glutamic acid. About one g/l of each treatment were added to Cramers and Myers (CM) culture medium to see how they affected growth rate, biomass, and paramylon content in *Euglena* sp. culture. The optical density based on absorbance was used to calculate the density of the cells, biomass was known by measuring the dry weight, and paramylon content produced was analyzed using the phenol-sulfuric acid method. The CM medium treatment with glucose added (CM+Glucose) had the highest specific growth rate, biomass, and paramylon content, with values of 2.902±0.338 (OD680/dx10-1), 0.476±0.023 g/l, and 2.416±0.129 mg/ml *Euglena* sp. can be utilized to produce paramylon on an industrial scale, so it is necessary to carry out further identification process regarding the species of *Euglena* sp. local strain, and it is hoped that there will be further research on other possible methods to increase the paramylon content in *Euglena* sp., such as using organic waste to replace the organic carbon source in the medium.

Keywords: Carbon source; culture collection, *Euglena* sp., growth rate, paramylon content

Article History: Received 12 January 2022; Received in revised form 18 March 2023; Accepted 5 May 2023; Available online 30 June 2023.
How to Cite This Article: Uliesther N, Nurafifah I, Rohmawati I, Putri RAE, Erfianti T, Suyono EA. 2023. Different carbon source alternative medium improves *Euglena* sp. growth and paramylon production. *Biogenesis: Jurnal Ilmiah Biologi*. vol 11(1): 102–113. doi: https://doi.org/10.24252/bio.v11i1.35221.

INTRODUCTION

Euglena sp. is a genus of microalgae that contains high-value compounds such as vitamins, 20 amino acids, polyunsaturated fatty acids (PUFAs), α-tocopherol, β-1,3-glucan (paramylon) and many more. In addition, *Euglena* sp. is easy to grow and has a high metabolic capacity. Thus, *Euglena* sp. has been widely used as a protein-producing microorganism, producing high-value metabolites, and synthesizing natural products in the microalgae biotechnology industry (O'Neill *et al.*, 2017; Wang *et al*., 2018). In Indonesia, special research on *Euglena* sp. indigenous strain is still very minimum. There is not even a stock culture of *Euglena* sp. Indonesian local strains in InaCC (Indonesian Culture Collection). Whereas *Euglena* sp. can be easily found in various locations such as ponds, rice fields, and even in extreme environments.

A high-value compound in *Euglena* sp. is β-1,3-glucan (paramylon). Paramylon is produced by euglenoids, which are then deposited as granules in the cytosol and used as a carbon source. Paramylon is a compound that has attracted the attention of many researchers because it has the ability as an immunostimulant and has antimicrobial activity (Gissibl *et al*., 2019). *Euglena gracilis* can accumulate significant amounts of the reserve polysaccharide paramylon, a β-1,3-glucan, that can account for more than 80% (w/w) of the dry weight (DW, biomass dried to a constant weight without oxidation) (Sun *et al.*, 2018). Additionally, numerous studies have demonstrated that paramylon has hepatoprotective, anti-diabetic, and anti-hyperglycemic properties that protect and treats the liver from damage. *E. gracilis* can accumulate up to 90% of its dry weight in paramylon (Tanaka, 2017; Gissibl *et al*., 2019). It is known that paramylon also present in other euglenoids species such as *E. spirogyra, Lepocinclis acus, Phacus curvicauda, E. viridis, Monomorphina pyrum*,

etc., but the structure and chemistry of paramylon has been well characterized in only a single species, *E. gracilis* as the most widely used species for producing paramylon (Feuzing *et al.,* 2022).

The nutritional content of *Euglena* sp. might be increased by optimizing growth-influencing parameters like nitrogen levels, light intensity, temperature, dissolved CO_2 concentration, culture procedures, and salinity (Sudibyo *et al*., 2018). The success of microalgae growth depends on the growth medium. As a result, it is critical to adjust the medium to promote cell growth and the creation of targeted metabolites. Furthermore, optimization can enhance production by cutting the time needed for cultivation and raising production efficiency (Ivusic *et al*., 2015). For example, various nitrogen levels were known to induce stress in microalgae *Isochrysis galbana*, resulting in decreased cell density at increased nitrogen levels. When compared to the control of normal nitrogen composition in the medium, the produced metabolites such as saturated fatty acids (SFAs), and monounsaturated fatty acids (MUFAs) increased along with the decrease and increase of the nitrogen amount (Zarrinmehr *et al*., 2020). According to Ivušić *et al*., (2022), when cultivated under photoheterotrophic or heterotrophic growth conditions in a growth medium with the appropriate carbon source, *E. gracilis* can produce significantly more paramylon than when grown in a photoautotrophic culture condition. When microalgae use organic substances as a carbon source while requiring light as an energy source, this process is known as a photoheterotrophic culture condition (Hasan *et al*., 2019). Different carbon sources are known to change the composition of phenolic compounds produced by *E. gracilis* (Bernard & Guéguen, 2022).

Euglena sp. can commonly be found in ponds and rice fields; it can also survive in harsh environments, such as rivers that have been contaminated with heavy metals from mining operations (Pavlečić *et al*., 2018). Unfortunately, studies about *Euglena* species are still rare in Indonesia. There is not even a stock culture of Indonesian indigenous strains of *Euglena* sp. in Indonesian Culture Collection (InaCC). The cultivation success of *Euglena* sp. depends on the growth medium's composition. Therefore, this study aimed to compare the effects of glucose and glutamic acid as carbon sources on the growth rate, biomass, and paramylon (*β*-1,3-glucan) content of *Euglena* species as indigenous strains from Indonesia. This study reveals that *Euglena* sp. can grow on artificial media with the best carbon sources, making it a productive and affordable bioprocess for the large-scale production of paramylon.

MATERIALS AND METHODS

Microorganism and medium. *Euglena* sp. strain IDN 2 was isolated from the sample taken from a peat swamp near Telaga Warna, Dieng (7°12'58.3"S, 109°54'36.9"E) by the Microalgae research team of the Faculty of Biology, Universitas Gadjah Mada. The isolation processes began by adding the water sample with Cramer and Myers (CM) medium concentrated five times to ensure that only *Euglena* sp. could grow. Then, single-cell isolation was carried out under the microscope using a dilution and manual micropipette. The cell was then cultured in Biologix 96 well plate, upscaled into 48 well plates, and later into 12 well plates, 15 ml, 50 ml, and 500 ml tubes. Each process requires at least a month while ensuring that each culture does not get contaminated. The CM medium that was utilized to cultivate *Euglena* sp. $(NH_4)2SO_4$, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}2H_2O$, $Fe_2(SO_4)_3{\cdot}7H_2O$, $MnCl_2{\cdot}4H_2O$, $CoSO_4{\cdot}7H_2O$, $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, $Na_2MoO_4{\cdot}2H_2O$, vitamin B1, and vitamin B12 (Kim *et al*., 2021). About 1 g/l of glucose and glutamic acid were introduced to CM medium with an organic carbon source treatment, respectively. The completed medium was then autoclaved in TOMY ES 215 autoclave at 121°C for 15 min to disinfect it.

Microalgae cultivation. The culture of *Euglena* sp. was added to a 500 ml culture vial that included 300 ml of CM medium with two distinct carbon sources: glucose (1 g/l, pH 3.4) and glutamic acid (1 g/l, pH 3.4). pH 3.4 was chosen as the general pH in which *Euglena* sp. can live and produce metabolites efficiently. In addition, 300 ml of CM medium without organic carbon was mixed with 200 ml of *Euglena* sp. for the control treatment. The initial culture was controlled to have a cell density of around 6×10^4 cells/ml. The cell density was measured with the manual haemocytometer

method. Each treatment has three replications. Photoheterotrophically, at 28°C, and with white illumination, *Euglena* sp. was grown. The two-week observation period was completed.

Measurement of cell density. Using a Genesys 150 UV-Vis spectrophotometer and a wavelength of 680 nm, optical density based on absorbance was used to calculate the density of the cells. First, 1 ml of material was obtained, placed in a cuvette, and quantified using a spectrophotometer. As an empty solution, aquadest is employed. Furthermore, specific growth rate (μ) was calculated using the following formula $\mu = 0.693/t_d$. Then, based on the formula below, the doubling time (T_d) computation was calculated by the formula (Humphrey *et al.*, 2019):

$$T_d = \frac{\ln 2\ (\Delta t)}{\ln N_t - \ln N_0} \quad .$$

Note:
Δt = Time interval
Nt = Number of cells acquired during final phase of exponential phase
N0 = Number of cells acquired during the beginning of exponential phase

Biomass measurement. Biomass from *Euglena* sp. was known by measuring the dry weight. Previously, the weight of the empty tube was weighed using a Mettler Toledo AL204 analytical balance. Then, 10 ml of culture samples were inserted into the tube. Then, for 15 min, the samples will be centrifuged at 4000 rpm. After that, the supernatant was discarded to obtain the pellets. Afterwards, the samples were dried in an oven B-One OV-65 at 40°C overnight until the tube reached a constant weight. The tube with the pellets then measured. The biomass measurement was replicated three times, and the results were averaged. Biomass was known through the following formula (Schagerl *et al.*, 2022):

$$\text{Biomass}\ \left(\frac{g}{L}\right) = \frac{(W_n) - (W_0)}{(V)}$$

Notes:
W_n = Final weight of the tube and the pellet (g)
W_o = Initial weight of the empty tube (g)
V = The amount of sample volume (ml)

While biomass productivity was measured by measuring the difference between the biomass in the final day of measurements and biomass amount in the starting day of measurements per the amount of day the measurements conducted. Biomass productivity was calculated through the following formula (Zhu *et al.*, 2016b):

$$\text{Biomass productivity}\ \left(\frac{g}{L} \cdot \text{day}^{-1}\right) = \frac{X_{tn} - X_{t0}}{t_n}$$

Notes:
Xtn = Biomass on the final day of measurements (g/l)
Xt0 = Biomass on the initial day of measurements (g/l)
tn = Time interval (day)

Extraction and measurement of paramylon concentration. *Euglena* sp. culture of 10 mL was taken, and they were centrifuged at 4000 rpm for 15 min. To get the pellets, the supernatant was discarded. The pellet was incubated in a water bath at 37°C for 30 min after dissolving in 5% (w/v) Na_2EDTA and 1% (w/v) SDS solution. The SDS-Na_2EDTA treatment was repeated once again except for the incubation part, and the paramylon was subsequently rinsed with distilled water twice. In 2 ml of NaOH, paramylon pellets were dissolved (Fan *et al.*, 2022). The paramylon content produced was analyzed using the phenol-sulfuric acid method (Chen *et al.*, 2023). In a test tube, the extraction solution was mixed with 5% phenol and H_2SO_4. The test tube was allowed to stand in a standing position for 10 min. Then, the solution was homogenized for 30 s before being incubated for another 20 min at room temperature. The wavelength of 490 nm was used during spectrophotometer analysis. Standard curves were created using the previous method by replacing the samples with pure glucose. Paramylon productivity was calculated through the following formula (Zhu *et al.*, 2016b):

$$\text{Paramylon productivity } (\tfrac{g}{L}/\text{day}) = \frac{\Delta x}{\Delta t}$$

Notes:
Δx = Difference in paramylon on day t1 and day t0
Δt = time interval (day)

Data analysis. Microsoft office excel 2013 was used to process calculation data in the form of cell growth, biomass, and paramylon content. Graphs were used to display statistical data. In addition, the differences between treatments analyzed using ANOVA calculation and tested further with Duncan's Multiple Range Test (DMRT) using SPSS ver. 26.

RESULTS AND DISCUSSION

***Euglena* sp. cell growth**. Photoautotrophic growth indicates that microalgae can harvest energy from sunlight and absorb atmospheric CO_2. On the other hand, photoautotrophic growth results in slow cell growth, low biomass, and higher harvesting costs. The significant limitations of photoautotrophic cultivations are overcome by heterotrophic microalgae cultivation using organic carbon sources (Gim *et al*., 2013). Based on Fig. 1, it can be seen the growth curve of *Euglena* sp. on various carbon sources in the medium for 14 days of observation. The highest absorbance value was found in the CM+glucose treatment, followed by the CM+glutamic acid treatment, and the lowest absorbance value was found in the CM (control) treatment. Cultures in the CM+glucose (1 g/l) treatment had the highest absorbance value on the 11th day, with an absorbance value of 1.017±0.118. CM+glutamic acid (1 g/l) had the highest absorbance value on the 10th day with an absorbance value of 0.760±0.046, and CM (control) had the highest absorbance value on 11th day with an absorbance value of 0.686±0.014. The concentration of glucose had a significant impact on biomass yield of *C. vulgaris*, for example, the addition of glucose proved to improve biomass yield and quality of biomass raw material (Yun *et al*., 2021).

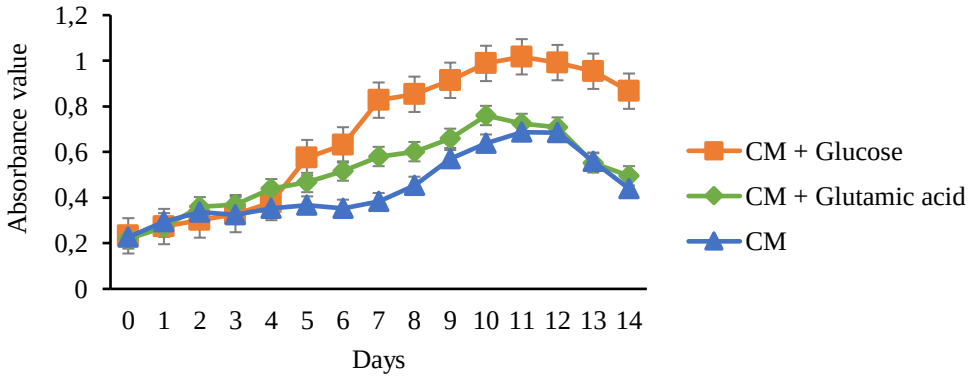

Fig. 1. Growth of *Euglena* sp. on various carbon sources in the medium for 14 days of observation

External carbon can potentially improve microalgal growth performance in hetero- or mixotrophic cultures. The ability of microalgae to grow in such cultures, however, is primarily determined by microalgal species, organic carbon source type, and environmental factors (Engin *et al*., 2018). Sucrose, glucose, glycerol, fructose, and acetates, as well as CO_2, have been used to boost microalgae biomass (Alkhamis & Qin, 2013; Razzak *et al*., 2013; Battah *et al*., 2014; Lin & Wu, 2015). The growth data of microalgae culture can be used to calculate the specific growth rate of each treatment. Specific growth rate can be used to understand the adaptation of the culture towards various medium modifications (Trenkenshu, 2019). The amount of specific growth rate for each treatment was presented in Fig. 2.

Table 1. Effect of various carbon sources in the medium on biomass and paramylon production by *Euglena* sp.

	Average biomass production (g/l)	Average paramylon production (mg/ml)	Paramylon per cell biomass (mg/g-cell)
CM + glucose	$0.476^{b} \pm 0.023$	$2.416^{c} \pm 0.129$	$6.738^{c} \pm 0.295$
CM + glutamic acid	$0.455^{b} \pm 0.022$	$1.771^{b} \pm 0.123$	$4.404^{b} \pm 0.311$
CM (control)	$0.398^{a} \pm 0.018$	$1.096^{a} \pm 0.128$	$3.056^{a} \pm 0.228$

Fig. 2 shows the specific growth rate of *Euglena* sp. on various carbon sources in the medium for 14 days of observation. The highest specific growth rate value (μ) was found in the CM+glucose treatment, followed by the CM (control) treatment, and the CM+glutamic acid treatment with almost the same value. The highest value (μ) was found in the CM+glucose treatment with a value of 2.902±0.338 ($OD680/dx10^{-1}$). Cell growth in *Euglena* sp. was observed based on cell density. Fig. 1 shows that the treatment with CM+glucose variations had the highest average absorbance after 14 days of observation. The lowest mean absorbance was found in the treatment with CM medium without additional organic carbon sources. CM medium originally did not contain any amount of carbon. Therefore, during the culture process, the main source of the carbon was only diluted CO_2. Carbon was an important component to the creation of the cell. Then, the lacking of carbon in the control CM medium might result in lower biomass and paramylon produced by the cells. The presence of two energy sources, namely organic carbon, and light, distinguish photoheterotroph cultures, with the assumption that photosynthesis and organic carbon assimilation can occur concurrently. As a result, the growth rate and biomass produced are greater than those produced by photoautotrophic and heterotrophic cultivation. Numerous studies have been published on improving microalgal biomass and biomolecules, including physiologic and environmental optimization (Ramanna *et al*., 2014; Liu *et al*., 2022), dietary and physical stress (Chen *et al*., 2021), utilization of organic waste (Solovchenko *et al*., 2020), alterations to cultivation vessels or systems (Zhu *et al*., 2015), and more. Microalgae can be grown in photoautotrophic, heterotrophic, and mixotrophic modes with various carbon sources. Microalgae phototrophic growth is a natural growth mode that uses CO_2 as a carbon source when exposed to light but has low biomass. Heterotrophic microalgae cultivation (dark mode with organic or inorganic carbon sources) can boost growth rate and, thus, biomass productivity. Marudhupandi *et al*. (2016), used a heterotrophic method to cultivate *Nannochloropsis salina* using two different kinds of carbon sources, such as glucose and sodium acetate. The specific growth rate of *Euglena* sp. obtained from CM+glucose was also higher than that of CM+glutamic acid and CM (control) treatments in this study. These carbon source shows direct impact on specific growth rate because glucose is the main carbon source in cells, which is also the elemental metabolite for the energy-producing process of glycolysis. Glutamic acid, on the other hand, supports cell growth due to its capacity to biosynthesize amino acids and nucleic acids. Differences in organic carbon, which affects microalgae growth, caused differences in specific growth rates (Fig. 2).

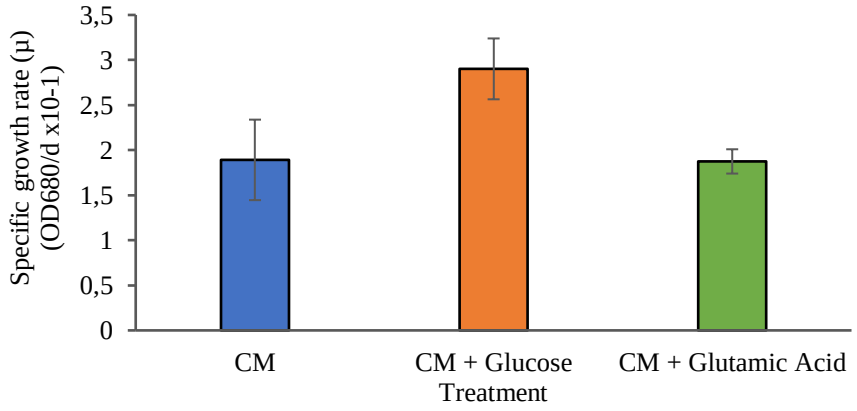

Fig. 2. Specific growth rate (μ) of *Euglena* sp. on various carbon sources in the medium for 14 days of observation

Carbon was known to be used by microalgae to synthesize carbohydrate, protein, lipid, and whole cells, which most common carbon source was in the form of CO_2 and CO_3^{2-} (Morales *et al.*, 2017). Glucose, as the main product of photosynthesis by microalgae, will increase along with the growth of microalgae. The higher glucose content, the lower the free water in the culture (Zhao *et al.*, 2015). The addition of new simple carbon source in the CM medium that not yet contained any carbon source proved to increase the growth rate, paramylon, and biomass production in the culture. Glutamic acid that was also a source of carbon, it also increases growth rate, paramylon and biomass production, yet not significantly due to its complex nature.

The results of statistical tests using one-way analysis of variance (ANOVA) at a confidence level of 5% revealed that the treatment of variations in carbon sources in the medium had a significant and significant effect on *Euglena* sp. growth rate. The obtained significance value was 0.00 (P 0.05). The significance value was less than the 0.05 confidence level used. Cell density ANOVA analysis show significance of 0.014 with calculated F of 9.384. While for cell count, the significance reaches 0.000 with calculated F 80.777. The cell count for every treatment resulted in significant differences in every treatment. Cell density DMRT test shows significant differences in every treatment compared to one another. Then, it can be concluded that the treatment of glucose and glutamic acid really influenced cell count and cell density of the culture. Glucose treatment is significantly different with glutamic acid treatment, and it shows that glucose was able to increase the cell density and cell count of the *Euglena* sp. culture.

Biomass of *Euglena* sp. The nutrient content and environmental factors in medium cultures are important factors for obtaining high biomass productivity of microalgae. Using specific nutrients in the presence of environmental control around culture can alter production costs and affect biomass growth or composition (Morales-Sánchez, *et al*., 2017; Soni *et al*., 2017). Based on Fig. 3, the highest biomass was found in the CM+glucose treatment, in which the highest biomass in the CM+glucose and CM (Control) treatment was obtained on day 12, while in the CM+glutamic acid treatment, the highest biomass value was obtained on day 12-10. The highest average biomass was 0.607±0.011 g/l, 0.833±0.011 g/l, and 0.743±0.029 g/l for each treatment (CM, CM+glucose, and CM+glutamic acid).

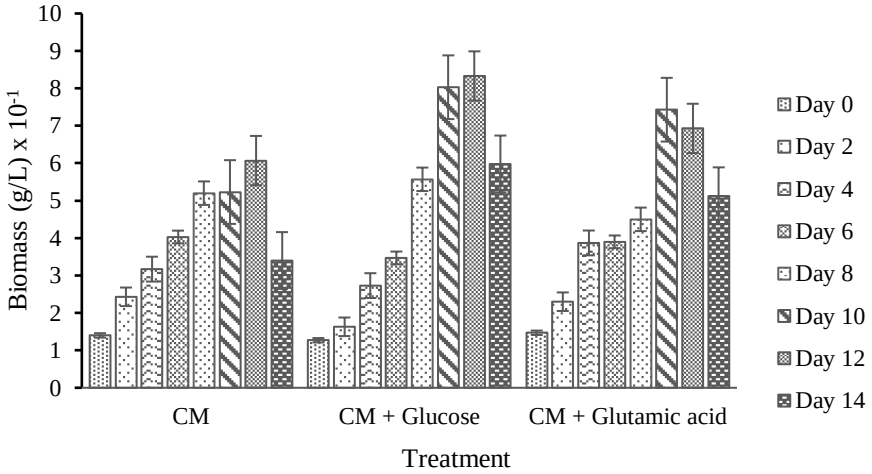

Fig. 3. Biomass of *Euglena* sp. (g/l) on various carbon sources in the medium for 14 days of observation

Microalgae biomass increases exponentially along with the time, even though at day 14 it shows the decrease of biomass amount, indicating the culture was in stationary or even death phase. The decrease might be happened due to the rate of death cells or lysis cells was higher than the amount of growing or doubling rate. The exponential increase of the biomass shows that microalgae was in the growing state. During the logarithmic phase, the amount of cell division increase, as well as the number of healthy cells. As the number of cells increase, the higher the amount of biomass present in the culture (Krishnan *et al.*, 2015). In the beginning of the culture phase, the biomass amount tended

to be low as microalgae still in the phase of adapting with their environments, especially the one with glucose and glutamic acid treatments, as usually *Euglena* sp. was cultured in media without additional carbon. In the same day, it shows that the amount of biomass produced was different in every treatment. For example, in the day 12, the highest biomass amount shown by the treatment of CM+glucose, followed by CM+glutamic acid, and then control (CM only). It proved that glucose was easier to broken down and used by microalgae compared to glutamic acid, as it was able to increase the biomass of microalgae that correlated with number of cells and weight of cells faster than another treatment. Carbon, as the main component of glucose is also the main components of microalgae whole cells (Thiviyanathan *et al.*, 2023).

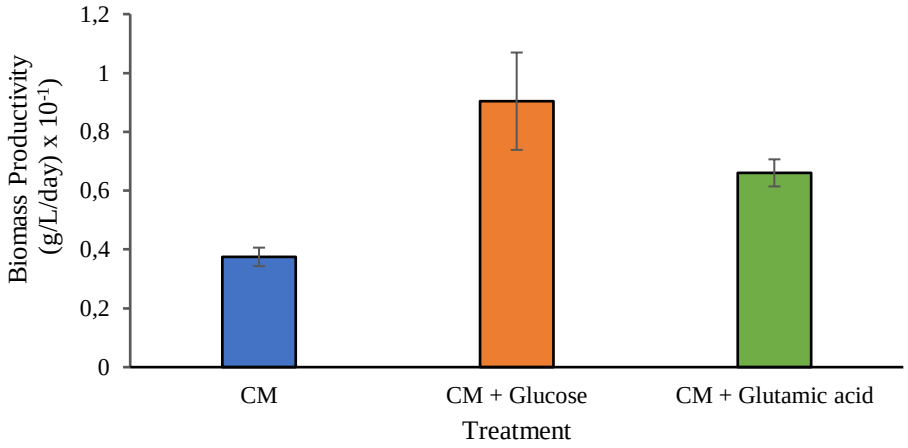

Fig. 4. Biomass productivity of *Euglena* sp. (g/l/day) on various carbon sources in the medium for 14 days of observation

Fig. 4 shows the biomass productivity of *Euglena* sp. (g/l/day) on various carbon sources in the medium for 14 days of observation. Based on the graph, the CM+Glucose treatment had the highest biomass productivity, followed by CM+Glutamic acid treatment, and the lowest biomass productivity was found in the CM treatment (control). The value of paramylon productivity for each treatment starting from the highest (CM+Glutamic, CM+glucose, and CM) was (066±0.005 g/l/day, and 0.090±0.017 g/l/day and 0,037±0,003 g/l/day respectively. Fig. 3 & Fig. 4 show that treatment with additional organic carbon sources CM+glucose and CM+glutamic acid resulted in higher biomass content and productivity than CM itself (control). This is because *Euglena* sp. has a source of organic carbon and light energy that can be used as an energy source to produce ATP and NADPH, allowing for faster cell growth in culture. All cells use glucose as an energy source and substrate for various biochemical reactions (Navale & Paranjape, 2016). Glucose is more effective than glutamic acid in increasing biomass content because it is the simplest monosaccharide and is ready for use by *Euglena* sp. Other carbon sources, such as glutamic acid, require a more complicated metabolic process before they can produce ATP for microalgae growth. Before it could be used in metabolic processes, glutamic acid had to be converted. Although the dose of organic carbon source was the same in each treatment, the results differed due to the metabolic processes (Gim *et al.*, 2013). Glutamic acid is involved in the biosynthesis of amino acids and nucleic acids that result in the growth of the cells (West, 2017). Analysis of variance (ANOVA) using one way method at a confidence level of 5% that was used for statistical tests indicated that the treatment of variations in carbon sources in the medium significantly affected *Euglena*'s average biomass. Although the significance value was 0.01 ($P < 0.05$), the significance value was smaller than the confidence level used at 0.05. Biomass productivity shown significance of 0.002, with calculated F 20.696. Biomass amount shown significance of 0.011 along with calculated F of 10.538. Biomass amount shows not significant difference in the treatments of CM+glutamic acid and CM+glucose, yet shows significant difference to control treatments. Biomass productivity amount shows significant differences between each treatment.

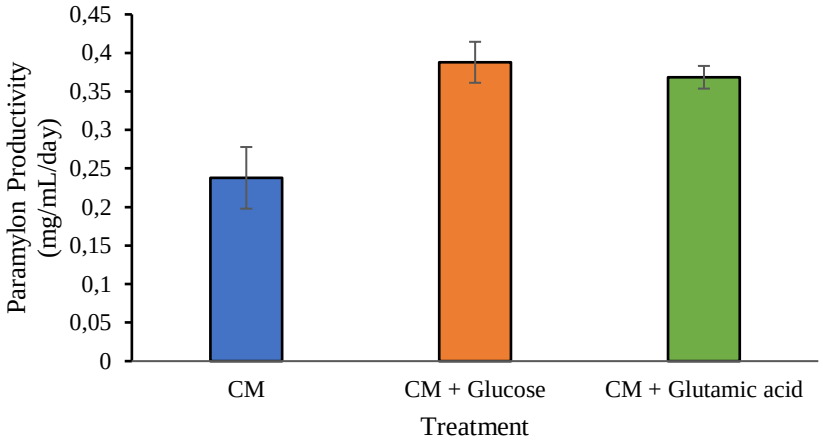

Fig. 5. The content of paramylon (mg/ml/day) on various carbon sources in the medium for 14 days of observation

Paramylon content in *Euglena* sp. culture. The biologically active function (such as immune support) determined by the linear β-1,3-glucan structure makes *Euglena* paramylon a valuable commercial product (Evans *et al*., 2019). The β-glucan content of yeast has been determined to be less than 15% of its dry weight and is intracellular, making isolation an energy-intensive process due to the presence of a tough cell wall (Zhu *et al*., 2016a). When grown with adequate carbon sources, *E. gracilis* can accumulate large amounts of β-1,3-glucan in 20-75% of its dry weight (Muchut *et al*., 2018). Fig. 5 shows the effect of variations in carbon sources in the medium on the paramylon content (mg/ml) of *Euglena* sp. culture. Based on the graph, the CM+glucose treatment had the highest paramylon content, followed by CM+glutamic acid treatment, and the lowest paramylon content was found in the CM treatment (Control). The maximum value of paramylon content for each treatment starting from the highest (CM+glucose, CM+glutamic acid, and CM) was 5.272±0.353 mg/ml, 4.489±0.036 mg/ml, and 2.474±0.054 mg/ml respectively (Fig. 6).

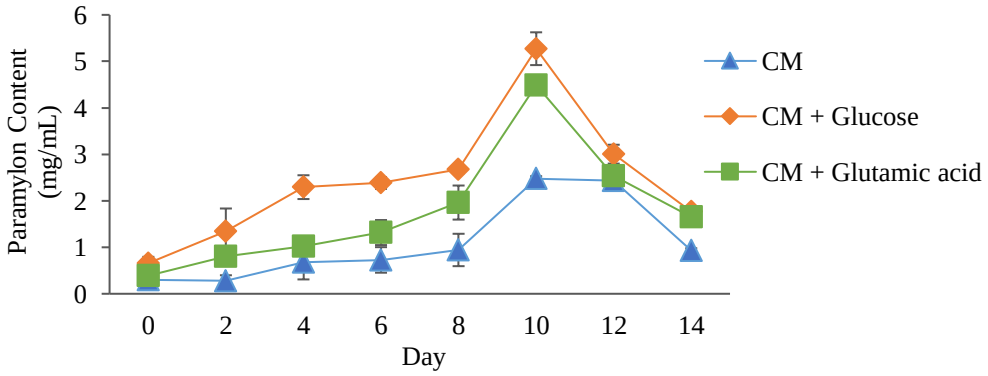

Fig. 6. Paramylon productivity of *Euglena* sp. (mg/ml) on various carbon sources in the medium for 14 days of observation

Fig. 6 depicts the test results for *Euglena* sp. paramylon content, which show that paramylon content was positively correlated with cell growth, with changes in paramylon content representing changes in the cell growth phase. From day 0 to day 2, the paramylon content tended to not increase significantly. This value represented the adaptation phase (lag phase), in which cells were still adapting to medium conditions with new nutrients. Furthermore, from day four to day ten, the content paramylon increased rapidly in the culture of *Euglena* sp. in CM medium that was mixed with glutamic acid, and cells began to divide rapidly so that the paramylon content continued to increase. This value represented the exponential phase (log phase), and so on. Paramylon content consistently increased from the beginning of the observation period. It reached its maximum value on the 10^{th} day of observation, where the 10^{th} day was the end of the exponential phase (log phase). Next, when viewed from the graph, the paramylon content immediately decreased drastically when it entered the

stationary and death phases. This was because, in these two phases, the cell growth rate had reached its maximum value, and both nutrients and organic carbon added to the medium had been exhausted. Thus, *Euglena* sp. cells began to convert paramylon (polysaccharide), which served as a food reserve, to monosaccharides, which were then used in the metabolic process of *Euglena* sp. for cell survival. As a result, the value of paramylon content in culture shows a continuous decrease, both in the stationary and death phases (Ogawa *et al*., 2022). During exponential growth, *Euglena gracilis* was known to synthesize larger quantities of paramylon. Mitochondria would swollen and vesiculated with matrix that involved in paramylon synthesis at the beginning of the exponential growth (Jeon *et al.*, 2019). The culture of *Euglena* sp. will synthesize additional organic carbon for cell division and plastid formation in the early phase of cultivation so that cells do not accumulate paramylon during the adaptation phase (lag phase). Cells would accumulate large amounts of paramylon during the exponential phase (log phase), significantly increasing paramylon content during this phase. It was known that no additional paramylon was accumulated after entering the stationary phase. Microalgae have attracted attention as a promising source for carbon dioxide (CO_2) utilization and, recently, as raw materials for functional foods. *E. gracilis*, a fast-growing microalgal species, can produce value-added materials, including immune-functional paramylon (β-1,3-glucan). Higher amounts of paramylon can accumulate under heterotrophic and mixotrophic conditions in the presence of organic carbon than under photoautotrophic conditions (Lewis *et al*., 2020).

Paramylon content in the culture depended on the growing conditions and the organic carbon source added to the culture. Research conducted by Kim *et al*. (2021) explained that paramylon content in the culture was highly dependent on the growing conditions and the organic carbon source added to the culture. Grimm *et al*. (2015) stated that glucose could increase the paramylon content the highest compared to other organic carbon sources. Kim *et al*. (2020) discovered that glucose, the most beneficial carbon source, promotes paramylon production and biomass growth in *Euglena gracilis*. However, the amount of carbon source added to the medium may differ depending on the strain. According to this study, the CM+glucose treatment yielded the highest paramylon productivity, followed by the CM+glutamic acid treatment (Fig. 6).

The treatment of variations in carbon sources in the medium significantly affected the paramylon productivity of *Euglena* sp. 0.000 ($P<0.05$). Calculated F shows 81.715 for paramylon productivity. However, the significance value was less than the confidence level used of 0.05. Paramylon results show significant differences to each other. This data shows that adding glucose as an organic carbon source to the medium increases *Euglena* sp. culture's paramylon content. Paramylon productivity amount shows that there are no significant differences between CM+glutamic acid and CM+glucose, yet it shows significant different with control treatment. It shows the treatments of CM+glutamic acid and CM+glucose, didn't really influence paramylon productivity. Yet, the addition of organic carbon really influenced the paramylon productivity compared to the culture that was not added by external organic carbon. It shows that the presence of extra organic carbon really able to increase paramylon productivity of the culture.

CONCLUSION

The addition of glucose organic carbon and glutamic acid into Cramer and Myers (CM) medium for *Euglena* sp. culture can be an alternative method to increase the growth rate of cells, along with biomass and paramylon produced, based on the results of this study. The specific growth rate of *Euglena* sp. in CM medium supplemented with glucose is 2.902±0.338 (OD680/dx10-1), the biomass is 0.476±0.023 g/l, and the highest paramylon content is 2.416±0.129 mg/ml. *Euglena* sp. can be utilized to produce paramylon on an industrial scale, so it is necessary to carry out further identification process regarding the species of *Euglena* sp. local strain, and it is hoped that there will be further research on other possible methods to increase the paramylon content in *Euglena* sp., such as using organic waste to replace the organic carbon source in the medium.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Education, Culture, Technology Research, and Higher Education of Indonesia for the research grant 984/UN1/FBI/KSA/PT.01.03/2021 at March, 5th 2021. This manuscript was a part of the first author's thesis.

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