Biosaintifika 15 (1) (2023): 36-47 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Enhancement of Microalgal Metabolite Production through *Euglena* sp. Local Strain and Glagah Strain Consortia

Fatona Nur[1], Tia Erfianti[1], Dea Putri Andeska[1], Renata Adaranyssa Egistha Putri[1], Istini Nurafifah[1], Brilian Ryan Sadewo[2], Eko Agus Suyono[1*]

[1]Faculty of Biology, Gadjah Mada University, Indonesia
[2]Department of Chemical Engineering, Faculty of Engineering, Gadjah Mada University, Indonesia
*Corresponding E-mail: eko_suyono@ugm.ac.id

Submitted: 2022-08-22. Revised: 2022-12-27. Accepted: 2023-02-28

Abstract. *Euglena* sp. is green microalgae in an acidic environment (pH 2.5-3.5). *Euglena* sp. has recently been developed widely in industry because of its capability to produce lipids that can be utilized to synthesize biofuel. Microalgae is a potential source of biodiesel, especially in the form of a consortium culture. One of the microalgae consortium cultures that have been explored is the nature consortium microalgae of Glagah strain. The Glagah consortia were isolated from Lagoon in the Glagah Beach, Kulonprogo, Yogyakarta. This study aimed to determine the total production of biomass, lipids, carbohydrates, and proteins of mixed culture of Glagah strain consortium and *Euglena* sp. as biodiesel substrate. The biomass test was measured using the dry weigh method using a filtration vacuum pump kit, lipids were measured using the Blight & Dryer method by adding chloroform and methanol as solvents, carbohydrates were measured using the Sulfur Phenol Acid method by adding Phenol and sulfuric acid (H_2SO_4), and proteins were measured using the Bradford method by adding SDS and Bradford's solution. The total production and productivity of biomass, lipids, carbohydrates, and proteins showed that the mixed culture of Glagah strain consortium with *Euglena* sp. was higher than the Glagah strain consortium. It reached 0.410 g/L; 0.253; 0.856 g/L; and 0.623 g/L. Therefore, it could be concluded that the mixed culture of the Glagah strain consortium with *Euglena* sp. could increase the production of biomass, lipids, carbohydrates, and protein up to two times that of the Glagah strain consortium so that this mixed culture treatment could be used as a reference in microalgae cultivation for biodiesel.

Keywords: Biodiesel, Concortia cultivation, Euglena sp.

How to Cite: Nur, F., Erfianti, T., Andeska, D. P., Putri, R. A. E., Nurafifah, I., Sadewo, B. R., & Suyono, E. A. (2023). Enhancement of Microalgal Metabolite Production through *Euglena* sp. Local Strain and Glagah Strain Consortia. *Biosaintifika: Journal of Biology & Biology Education*, *15*(1), 36-47.

DOI: http://dx.doi.org/10.15294/biosaintifika.v15i1.41895

INTRODUCTION

One of the alternative renewable energy sources recognized for its financial viability and eco-friendly qualities is biodiesel. Microalgae are microscopic organisms that can produce lipids or oil, which has significant biodiesel production. Examples of microalgae species that have been optimized are; *Navicula* sp., *Spirulina platensis*, and *Chlorella vulgaris* (Insan et al., 2018). Besides their potential as biodiesel, microalgae also can produce valuable compounds such as protein, lipids, carbohydrates, antioxidants, and others (Zulkarnain et al., 2021). Further research is necessary to address the increasing demand for biodiesel; one possibility is to select a potential consortium. *Euglena* sp. is green microalgae that have been widely applied in industry, especially for bioenergy production. On the other hand, to increase the production of microalgal biomass, the use of mixed culture or consortia is one promising for enhancing the productivity of microalgae. Glagah strain consortium is a microalgae consortium obtained from Glagah Beach Lagoon, Kulonprogo, Special Region of Yogyakarta. It is a local microalgae consortium comprising six identified species, including *Cyclotella polymorpha, Clyndospermopsis raciborskii, Golenkinia radiata, Corethron criophilum, Chlamydomonas* sp., *Syracosphaera turquoise (Suyono et al., 2016)*. Glagah strain consortium also contains various types of bacteria that are positively associated with microalgae. Some bacteria that can support the symbiosis completely with microalgae in the Glagah strain consortium include *Corynebacterium ulcerans, Corynebacterium bovis, Bacillus cereus, Bacillus megaterium, Pediococcus parvulus* and *Staphylococcus vitulinus (Suyono et al., 2018).* In addition, bacteria in the culture can protect microalgae cells from a toxin from different species of microorganisms (Pradana et al. 2017). The Glagah strain consortium's mixed culture of microalgae and bacteria provides the potential for

rapid growth and improved biomass production. The presence of growth-promoting substances secreted by bacteria was thought to have an impact on the increase in growth and biomass in the Glagah strain consortium. The bacteria will obtain organic carbons in exchange, enabling their growth (Goli et al., 2016). The lipid content of the Glagah strain consortium's microalgae culture ranges from 1.25% to 13.58%, making it potentially suitable for use as biofuels. 3.42 g/L of dry weight is produced in seawater culture media (Suyono et al., 2015). A microalgae species called *Euglena* sp. might future become a source of biofuel. In comparison to another microalga, *Euglena* sp. is recognized to have higher biomass and cell size. Additionally, it has a variety of nutrients, like paramylon (Watanabe et al., 2013). *Euglena* sp. biomass is also recognized to have approximately 39-61% of proteins, 14-18% of carbohydrates, and 14-20% of lipids (Um and Kim, 2009).

In the previous research, mixed culture between Glagah strain consortium and *Chlorella zofingiensis* is known to have a higher growth rate than single cultivation *C. zofingiensis*. In the mixed culture, the growth rate reached 0.513/day, while in single cultivation of *C. zofingiensis* the growth rate only reached 0.265/day. As a result, biomass productivity from the mixed culture treatment reached up to 31.85 mg/L/day, whereas in single cultivation, biomass productivity only reached around 15.98 mg/L/day (Suyono et al., 2016). Also, mixed cultures can increase the amount of lipid produced. It reached up to 7.76 mg/L in monoculture, compared to 4.39 mg/L of lipid in single cultivation (Gonçalves et al., 2016). Thus, mixed culture treatment was known to increase the dry weight growth and lipid content produced from the culture. Therefore, this research was conducted to determine the effect of mixed culture of Glagah strain consortium with *Euglena* sp. on the production and productivity of total biomass, lipids, carbohydrates, and proteins along with its potential as biodiesel sources.

Low growth rates, low metabolite levels, and bacterial contamination are challenges faced by monoculture microalgae cultures. The implication of this study was to increase the productivity of microalgae and avoid contamination through co-culturing consortium with single culture of *Euglena* sp. The culture system developed by the consortium is a possible solution to the issues with monoculture (Kurnianto and Suyono, 2021). Both the productivity of the biomass and the metabolites that result can be increased using the consortium's culture system. In addition, microalgae release carbon into the water, which bacteria utilize to develop. Glycolate is the specialized carbon source that bacteria require. During photosynthesis, microalgae create glycolate. Extracellular polymeric substances (EPS), which diatoms produce, are another type of carbon. Diatoms release EPS into biofilms. Research has shown that the -proteobacteria group bacteria have increased in the presence of released EPS. On the other hand, some microalgae require the vitamins biotin, cobalamin, and thiamin as their growth factors. But they are unable to generate vitamins. Prokaryotic organisms, which are symbiotic with microalgae and include bacteria, are the type of organisms that may create vitamins. Vitamins are organic substances and metabolites that an organism requires but cannot manufacture by itself. Auxotroph vitamins are the name for these creatures (Rahmawati et al., 2020). This research will be very useful to industrial application in the near future, through elaborating consortia and single culture of microalgae, it showed that the biomass content, and metabolites such as lipid increased instead of culturing single culture of microalgae.

METHODS

Cultivation of mixed culture

Glagah strain consortium and *Euglena* sp. were used in this research. Glagah strain consortium was obtained from the Laguna Beach Lagoon, Kulon Progo, Special Region of Yogyakarta. *Euglena* sp. was isolated from various water sources in the Special Region of Yogyakarta. CM media (Cramer & Myers, 1952) was used for cultivation. Cell density with 1:1 ratio of Glagah strain consortium and *Euglena* sp. was used in mixed culture. Single cultivation was used as a control. All treatments were cultured under aseptic laboratory conditions in 500 mL of capacity for each culture, pH 6 medium., 30°C room temperature and 25,000 lux light intensity. The culture was done in a photoautotrophic condition.

Microalgae Cell Density

The number of cell densities for single cultivation of the Glagah strain consortium, mixed culture of the Glagah strain consortium with *Euglena* sp., and single cultivation of *Euglena* sp. were counted using a hemocytometer every day during 7 days of observation.

Biomass Analysis

Biomass analysis was done using the filtration method every day until the end of cultivation. First, 10 mL of sample was run through the Filtration Vacuum Pump Kit equipped with the dry glass microfiber filter (GF/C) paper to separate the biomass from the medium. Then, the paper was dried 100°C oven for 2 hours. Finally, dried biomass (DW) was measured using an AL204 analytical balance.

Modeling Growth Kinetic

Modeling Growth Kinetic of monoculture Glagah, mixed Culture Glagah *Euglena* sp. and monoculture *Euglena* sp. A dynamics approach to the biomass growth of microalgae by kinetic modeling needs to be developed for predicting the performance and optimization of photobioreactor operating conditions (Galvão et al., 2013). Logistic and Gompertz models of the two non-linear models were suitable for the rapid population growth of organisms such as microalgae (Lam et al., 2017). Due to it is not limited by substrate type and consumption, the Logistic and Gompertz model was the simplest and can be used for general microalgae growth rate.

In this study, Logistic and Gompertz models were used to predict monoculture Glagah, mixed Culture Glagah-*Euglena* sp., and monoculture *Euglena* sp. The Logistic model predicts the number of stable populations using the maximum growth rate per day as its parameter. The Logistic model was calculated using the following formula. X is cell density, X0 is the initial cell density, Xmax is the maximum cell density, and μmax is the maximum specific growth rate (Phukoetphim et al., 2017; Hanief et al., 2020)

$$\frac{dX}{dt} = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)X \qquad (1)$$

$$X = \frac{X_0 \exp(\mu_{max}\, t)}{1-\left[\frac{X}{X_{max}}(1-\exp(\mu_{max}\, t)\right]} \qquad (2)$$

Gompertz model is also used to determine the cell population of the exponential phase. However, the parameters used in this model are more complex, including maximum cell production (*rm*) and lag time (tL).

Gompertz Model

$$X = Xo + \left[X_{max}.\exp\left[-\exp\left(\left(\frac{r_m.\exp(1)}{X_{max}}\right)(t_L - t) + 1\right)\right]\right] \qquad (3)$$

The determination of the model was carried out using the following formula where SSR is sum square residual and SST is sum square total (Phukoetphim et al., 2017; Hanief et al., 2020).

$$R^2 = \left(1 - \frac{SSR}{SST}\right) \qquad (4)$$

Lipid Content Analysis

The total lipid content was analyzed every day using Bligh & Dyer (1959) until the end of cultivation (Day-7). First, 5 mL of the sample was collected and centrifugated at 3300 rpm for 10 minutes to separate the biomass from the medium. 2 mL methanol and 1 mL chloroform, were added to the pellet. 1 mL chloroform and 1 mL aqua dest were added later. The second centrifugation phase used 4000 rpm of speed at 10°C temperature. Then the solution was split using a centrifuge until there were three layers. The bottom layer was taken and incubated in the oven for 24 hours at a temperature of 30°C.

Carbohydrate Content Analysis

The analysis of carbohydrate content was carried out using the Phenol Sulfuric Acid method (Dubois et al., 1956) every day until the end of cultivation. This method was done by adding 5% phenol and concentrated sulfuric acid (H_2SO_4). First, 10 mL of the sample was obtained and centrifugated at 3300 rpm for 15 minutes. Next, the remaining pellet was added with 1 mL H_2SO_4 and 0.5 mL 5% phenol. The mixture was then incubated for 30 minutes at room temperature. After that, absorbance readings were performed using a spectrometer at a wavelength of 490 nm.

Protein Content Analysis

The analysis of protein content was conducted using the Bradford method every day until the end of cultivation. This method was carried out by adding 1 mL of SDS solution to the supernatant collected from the separation process using a centrifuge at 3300 rpm for 15 minutes. Pellets collected from 2 mL samples were added to 1 mL 10% SDS. Then incubation at 95°C was followed by incubation at 4°C each for 5 minutes. Incubation samples were taken and added Bradford's solution (Bradford, 1976). After that, absorbance measurement was performed using ELISA Reader Biotech with a wavelength of 595 nm.

Productivity of Biomass, and Primary Metabolites

Productivity of biomass and primary

metabolites was measured according to Zhu et al. (2016). Biomass productivity and primary metabolites productivity ($g^{L-1}.d^{-1}$) = $(N_2-N_1)/(t_2-t_1)$, where N_1 and N_2 were defined as the biomass and metabolite products at time 1(t_1) and time 2 (t_2), respectively. Growth rate (μ) was estimated using the formula = Ln $(N_2/N_1)/(t_2-t_1)$.

Statistical analysis

The collected data were analyzed using one-way ANOVA at $p<0.05$ using IBM SPSS statistics 26 version. When the treatment effect was significant, the means were separated using Duncan's multiple range tests (DMRT).

RESULTS AND DISCUSSION

Microalgae Cell Density

Cell density is one of the most essential measurements to analyze the growth rate of microalgae. In this study, microalgae cell density has been measured using a hemocytometer under microscope observation. By analyzing the number of cell density, we can understand the growth phase of *Euglena* sp., Glagah Consortia as well as the mixed culture of Glagah and *Euglena* sp.

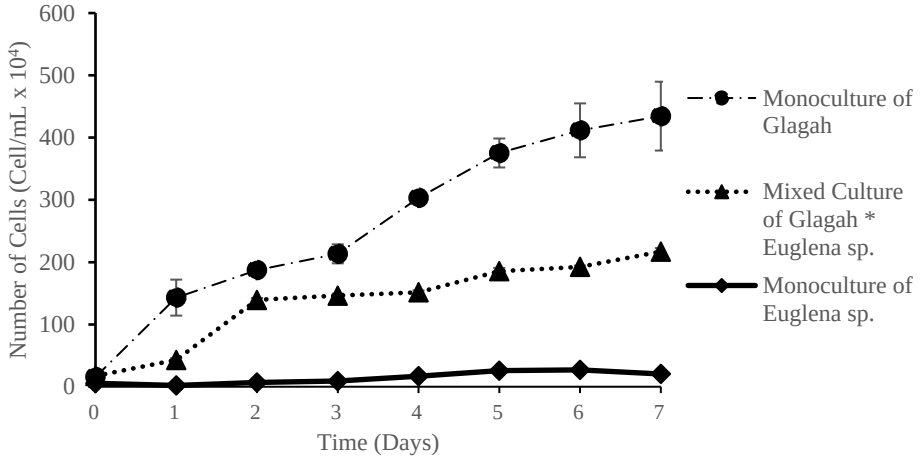

Figure 1. Total cells (cell/mL) of Glagah strain consortium (---◆---); monoculture of Euglena sp. (···▲···) and mixed culture of Glagah strain consortium and Euglena sp. (—■—) until 7 days of cultivation.

Figure 1. shows the number of microalgae cells during 7 days of cultivation. The highest cell number among the three cultures observed was shown by single cultivation of the Glagah strain consortium on day 7, which was 434.5x104 cells/mL. The mixed culture of the Glagah strain consortium and *Euglena* sp. had a higher cell growth (217x104 cells/mL) compared to the single cultivation of *Euglena* sp. (27.1x104 cells/mL). Previous research by Asiandu et al. (2022) showed that the number of *Euglena* sp. single strain reached about $87x10^4$ cells/mL under 100% of tofu waste, this result was affected by the cultivation medium that contain essential nutrients supplied by the waste. On the other hand, the number of cells in the single cultivation of the Glagah strain consortium and mixed culture treatment continued to increase the number of cells. In contrast, the single cultivation of *Euglena* sp. started to decrease on day 7 of the observation. Research by Maharani et al. (2020) also indicated that the number of cells of the Glagah consortium reached $38.5x\ 10^5$ cells/mL under 7.000 lux cultivation light. It indicates that light intensity also affect the growth of the Glagah consortium. In addition, the Glagah strain consortium is a microalgae consortium consisting of 3 genera. It is known to be associated with bacteria that can produce vitamin B12, organic and inorganic nutrients which possibly support microalgae growth (Novoveská et al., 2016). In the mixed culture treatment, *Euglena* sp. provides vitamins B1, B12, C, E, and minerals to enhance the growth of microalgae (Gissibl et al., 2019). Therefore, the species in a mixed culture treatment may be capable of adapting well to each other. Characteristics such as the size and complexity of reproductive forms affected microalgal growth rates. When species compete for resources, the smaller species will grow faster. This fast growth rate occurs due to the surface ratio of each species, which provides a good volume that facilitates the

assimilation of nutrients and carbon available in the culture medium (Phatarpekar et al., 2000). According to research by Kazamia et al. (2012), the interactions formed in mixed culture treatment are more complex, with many ecological niches filled so that the use of resources becomes optimum.

Biomass

The dry weight or biomass of *Euglena* sp. and Glagah consortia have been measured to study biomass production during cultivation. Biomass is the most vital parameter that should be measured in this study. Through biomass production, we can develop other valuable co-products from microalgae. The information regarding the biomass content of *Euglena* sp., the Monoculture of Glagah Consortia, and the Mixed culture of both *Euglena* sp. and Glagah consortia can be seen in the Figure 2 below.

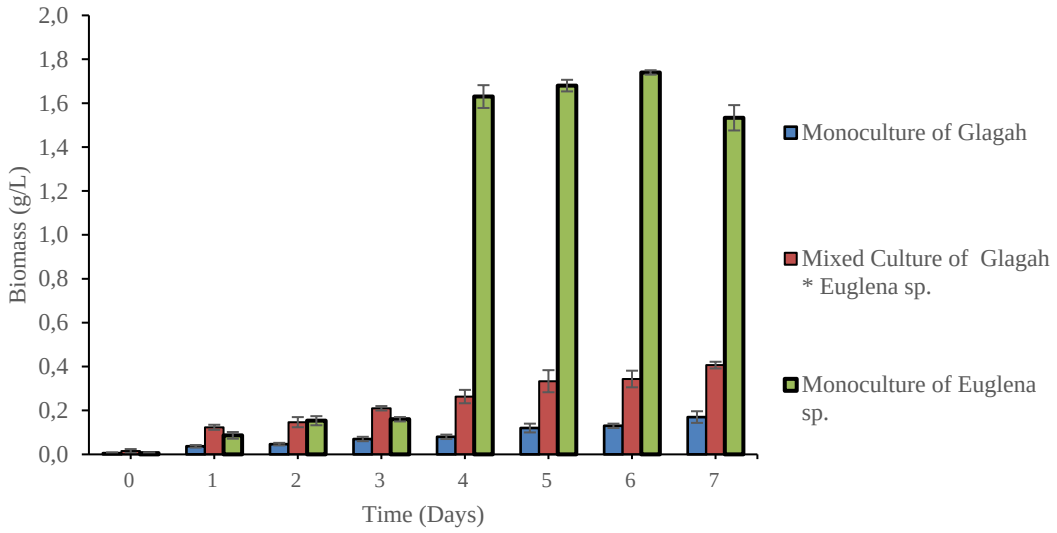

Figure 2. Total biomass (g/L) of Glagah strain consortium until 7 days of cultivation.

Figure 2. shows the highest biomass content generated by single cultivation of *Euglena* sp. on day 6 (1.73 g/L). The mixed culture of the Glagah strain consortium and *Euglena* sp. had higher biomass content (0.41 g/L) than the Glagah strain consortium (0.17 g/L). Among all treatments, the lowest biomass content was shown in the single cultivation of the Glagah strain consortium. Based on the number of cell densities, the single cultivation of the Glagah strain consortium had the highest density of the mixed culture treatment and *Euglena* sp. single cultivation. This explains that the results of photosynthesis are mainly used for cell propagation. These results also align with the research by Jiménez et al. (2003), who stated that in cells with relatively fast metabolic rates, the energy produced is used for cell multiplication through asexual division. The research conducted by Koreiviene et al. (2014) also proved that mixed culture treatment could increase biomass content. The mixed culture treatment shows higher biomass content than the Glagah strain consortium because the medium's nutrient absorption is more efficient, so the biomass produced is more heightened than the Glagah strain consortium.

Growth Kinetic Model

To understand microalgal development and to improve cultivation conditions, growth kinetic models are required. There are many models have been developed to predict the growth of microalgae. In this study we used the Logistic and Gompertz models as can be seen in Figure 3.

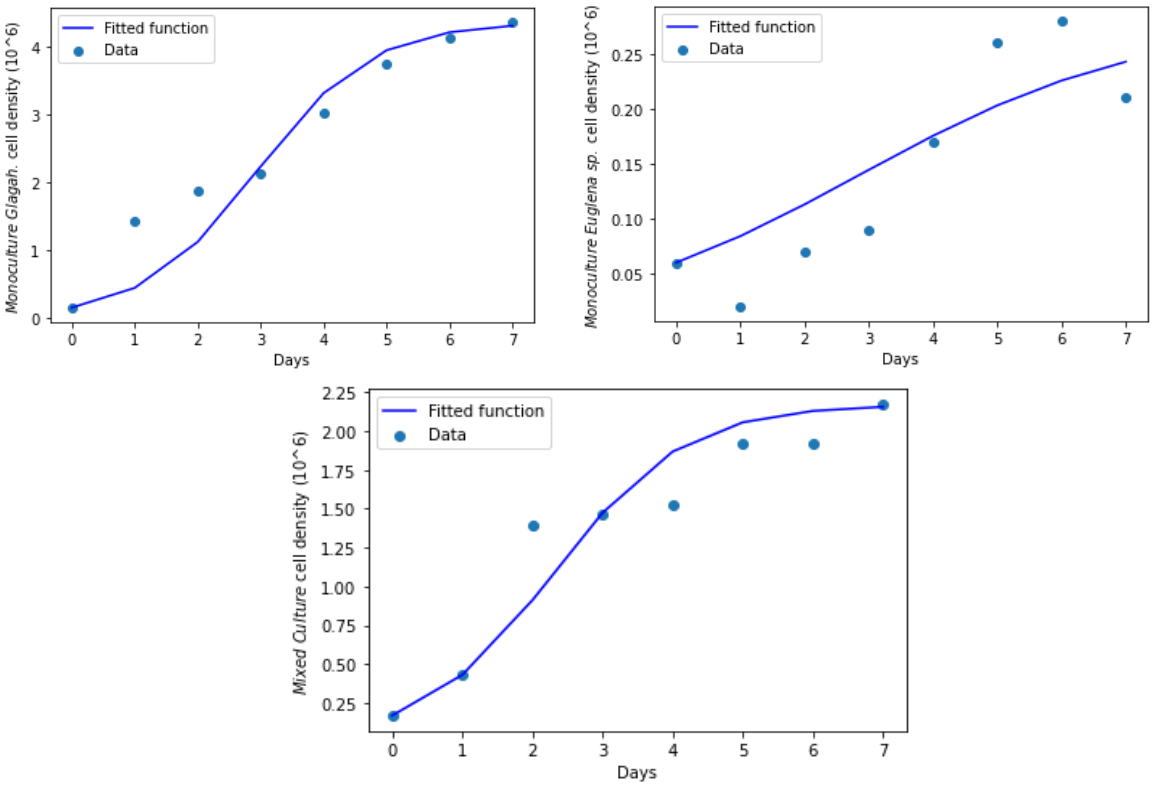

Figure 3. Fitting of Logistic Model (Cell Density); (a) Monocultur of Glagah Strain; (b) Monoculture of *Euglena* sp.; (c) Mixed Culture (Glagah-*Euglena* sp.)

Based on logistic modeling (Figure 3), the maximum specific growth rate (μmax) of monoculture Glagah, mixed Culture Glagah-*Euglena* sp., and monoculture *Euglen*a sp. were 1.1063/day, 1.0722/day and 0.4549 respectively. Consequently, the R2 error were 0.89, 0.89, and 0.76 for monoculture Glagah, mixed Culture Glagah-*Euglena* sp., and monoculture *Euglena* sp., respectively.

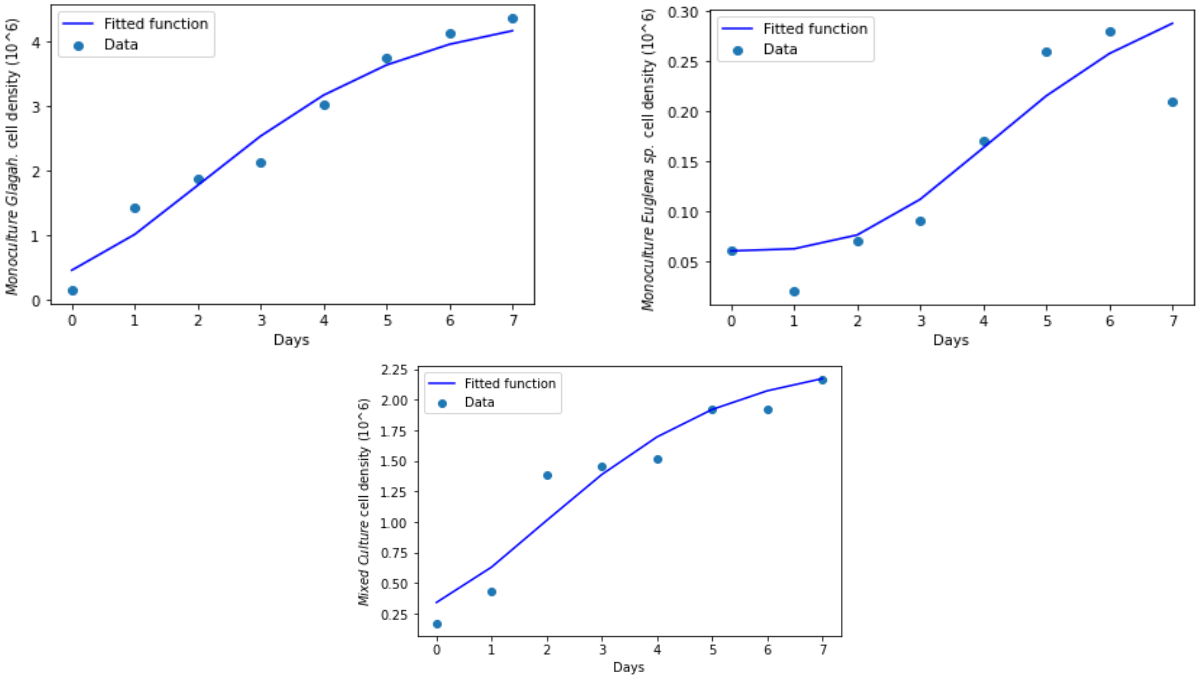

Figure 4. Fitting of Gomperz Model (Cell Density) (a) Monoculture Glagah (b) Monoculture *Euglena* sp. (c) Mixed Culture (Glagah-*Euglena* sp.).

For the Gompertz model, the maximum cell production rate (r*m*) of monoculture Glagah was 7.8910 x 105 cells/mL. The maximum cell production rate (*rm*) of Mixed Culture Glagah and *Euglena* sp. was 3.9390 x 105 cells/mL. The maximum cell production rate (*rm*) of *Euglena* sp. was 0.5420 x 105 cells/mL. The lag time (tL) monoculture Glagah, mixed Culture Glagah-*Euglena* sp., and monoculture *Euglena* sp. were 0/day, -0.1/day, and 2,1/day, respectively. Each of the R square error values of mixed Culture Glagah-*Euglena* sp. and monoculture *Euglena* sp. were 0.96 and 0.92, 0.84.

Tables 1 and 2 showed the results of the Logistic and Gompertz model fitting of monoculture Glagah, mixed Culture Glagah-*Euglena* sp., and monoculture *Euglena* sp.

experimental growth data. The Gompertz model fits the microalgae growth curves better than the Logistic model for three microalgae species. The values of the coefficient of determination R^2 established the goodness of fit of the Gompertz model over the Logistic model in the study.

Table 1. Growth Rate Parameter of Logistic Model

Parameter	Monoculture Glagah	Mixed Culture Glagah and *Euglena* sp.	Monoculture Euglena sp.
μ_{max}	1.1063	1.0722	0.4549
R^2	0.89	0.89	0.76

Table 2. Growth Rate Parameter of Gompertz Model

Parameter	Monoculture Glagah	Mixed Culture Glagah and *Euglena* sp.	Monoculture *Euglena* sp.
rm	7.8910 x 10^5	3.9390 x 10^5	0.5420 x 10^5
tL	0.0	-0.1	2.1
R^2	0.96	0.92	0.84

Lipid Production

Lipid production from microalgae is considered a value-able component because lipids and fatty acids found in microalgae can be used as the basis for biofuel. In this study, lipid content has been measured using Bligh and Dyer's method using methanol and chloroform as solvents to extract microalgal oil inside their cells. The result of lipid content can be seen in the figure below.

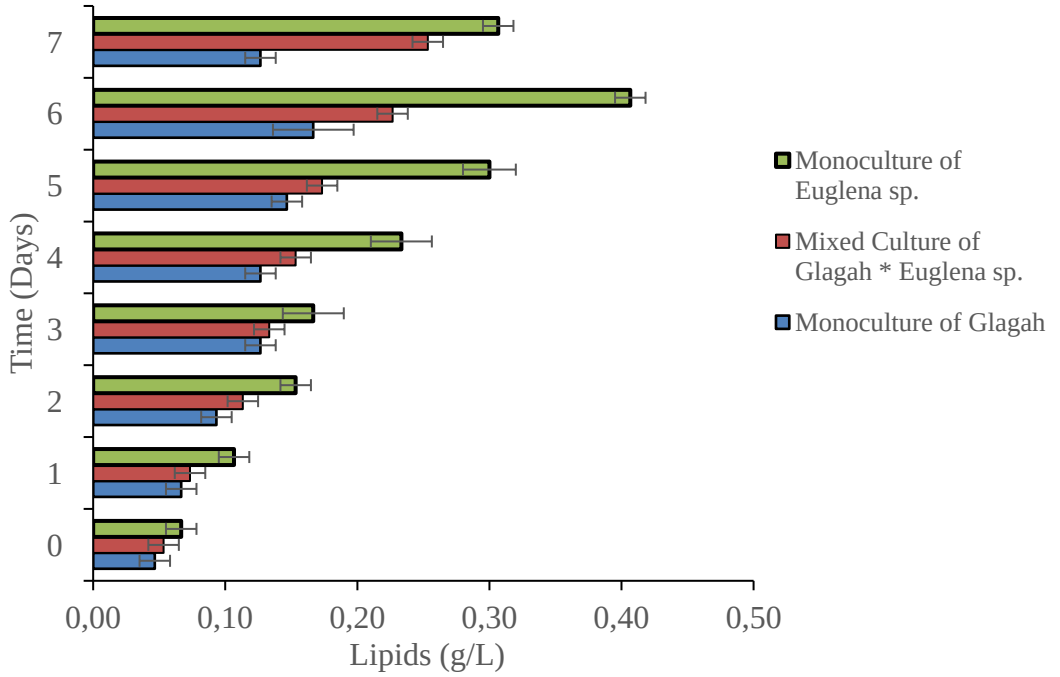

Figure 5. Total lipids (g/L) of Glagah strain consortium until 7 days of cultivation.

Figure 5. Shows that the highest lipid content in *Euglena* sp. single cultivation reached 0.407 g/L on day 6, then started to decrease on day 7 of the observation. On the other hand, the mixed culture treatment produced 0.253 g/L lipid content on day 7. The lowest lipid showed by single cultivation of the Glagah strain consortium was 0.127 g/L on day 6. Previous study by Asiandu et al. (2022) also stated that the lipid production of monoculture *Euglena* sp. reached 1.27 mg/mL under a wastewater medium. On the other hand, mixed culture increased the lipid content from the first day to the maximum point, then started to decrease on the last day of observation. Mixed culture has greater species diversity than the single the Glagah strain consortium cultivation. Therefore, communities with higher species diversity tend to have higher productivity levels than monocultures or single cultivations with less diverse species under the same medium conditions. In addition, the use of nutrients in mixed cultures will be much better due to the ecological niches in mixed cultures that consist of many species (Kazamia et al., 2012). These conditions trigger mixed culture treatment of the Glagah strain consortium and *Euglena* sp. to accumulate higher lipid content when compared to the single cultivation of the Glagah strain consortium.

Carbohydrate Production

The carbohydrate content is a primary metabolic compound found in microalgae. A variety of biofuels, including bioethanol, biobutanol, biomethane, and biohydrogen, can be synthesized from microalgal carbohydrates. Phenol sulfuric acid method was used to measure the carbohydrate content of *Euglena* sp., the Monoculture of Glagah consortia, and the mixed culture of Glagah and *Euglena* sp. (Figure 6).

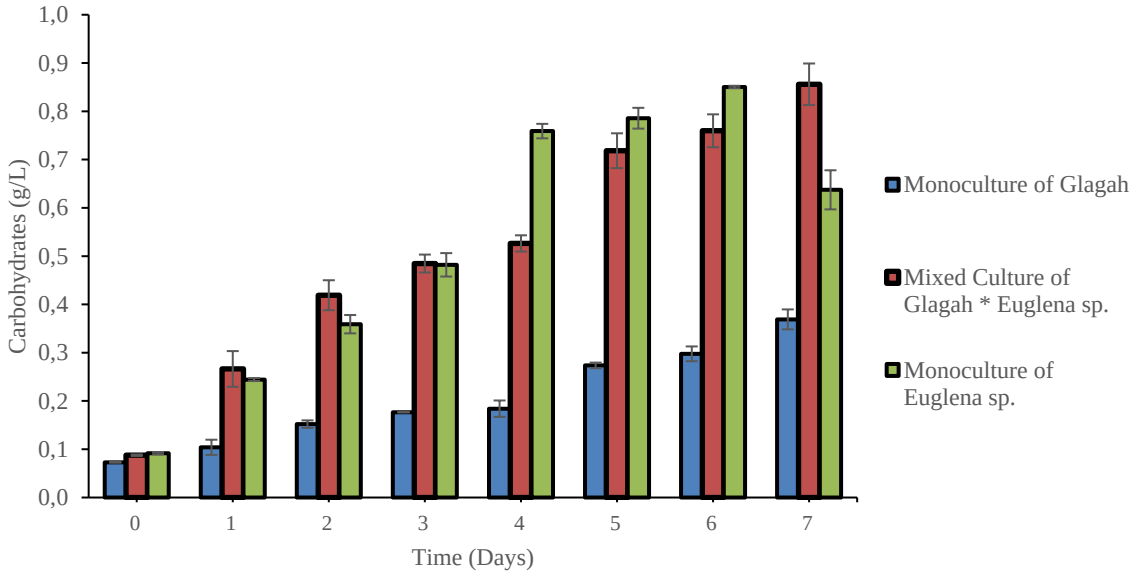

Figure 6. Total carbohydarates (g/L) of Glagah strain consortium until 7 days of cultivation.

Figure 6. This shows that the highest carbohydrate content was in the mixed culture treatment, which reached 0.856 g/L on day 7, followed by *Euglena* sp. single cultivation, which reached 0.850 g/L on day 6. Meanwhile, the lowest carbohydrate content was found in the single cultivation of the Glagah strain consortium, 0.369 g/L on day 7. The carbohydrate content in the mixed culture treatment steadily increased until the last day of observation. The carbohydrate content in the treatment is in line with the biomass and cell number. The increase in cell number results in an increase in biomass content and carbohydrate content. Carbohydrate is contained in cell biomass and is strongly related to the number of cells. The carbohydrate content between mixed culture treatment and single cultivation of *Euglena* sp. do not show any significant differences. The research confirms these results by Sayegh & Montagnes (2011), which stated that in cells with slow cell growth, carbohydrates, and lipids are. Previous research by Jiménez et al. (2003) also showed a similar thing. In cells with a slow metabolic rate, more energy from photosynthesis is stored as carbohydrates. The light intensity factor also causes the high carbohydrate content in culture treatment due to the relationship between light and chlorophyll. The primary function of chlorophyll is to capture light (photons) used in photosynthesis. The result

of photosynthesis is the acquisition of carbohydrates as food reserves.

Protein Production

Microalgae are being researched as novel sources of a variety of highly valuable products, such as proteins for the food and feed industries. However, protein content differs depending on the type of alga, the conditions of the culture, and the harvesting time. Protein content in this study has been measured following the Bradford method. The protein content shown in Figure 7 below.

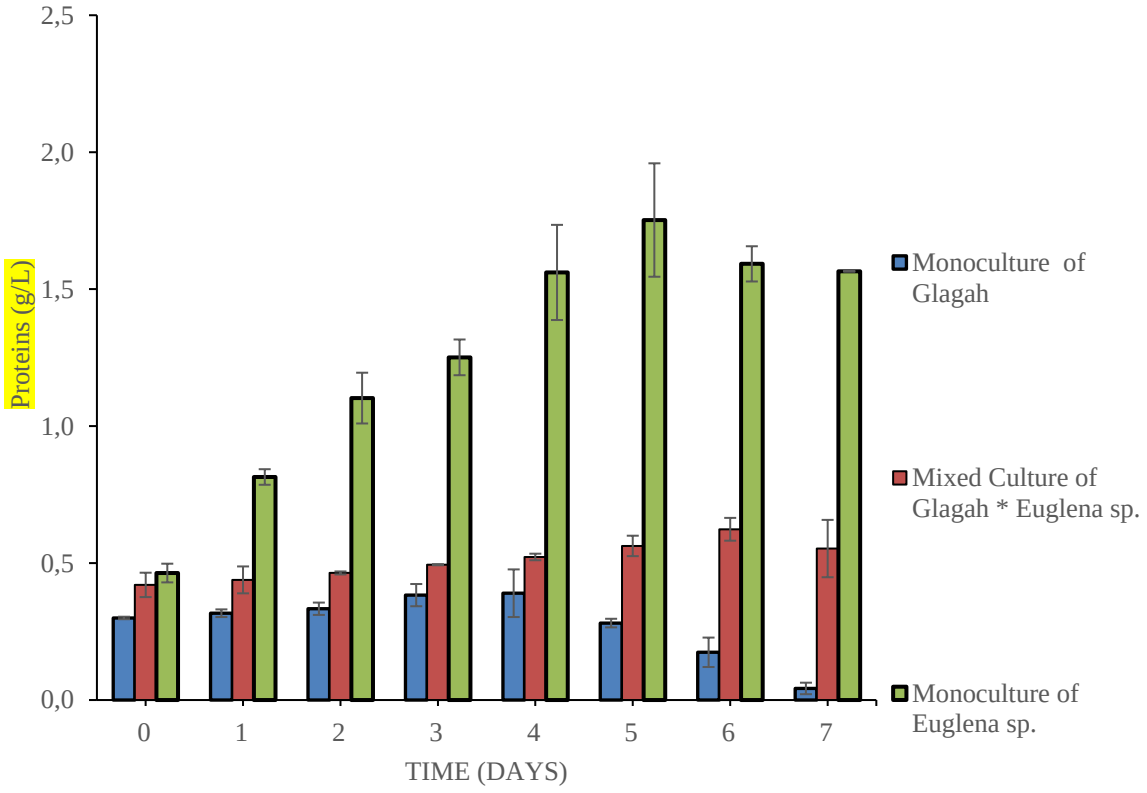

Figure 7. Total proteins (g/L) of Glagah strain consortium until 7 days of cultivation.

Figure 7. The highest protein production in *Euglena* sp. single cultivation reached 2.028 g/L on day 5. The mixed culture treatment showed the second highest protein production with a protein content value of 0.623 g/L on day 6. The lowest protein shown in single cultivation of the Glagah strain consortium was 0.355 g/L on day 4. There was an increase in the protein content in the single cultivation of *Euglena* sp. until day 5, but then it decreased until day 7. Meanwhile, the protein content of the mixed culture treatment did not experience an increase and began to decline on day 7. The single cultivation of the Glagah strain consortium also increased until day 4 and started to decrease from day 5 until day 7 of observation. The protein production in microalgae does not depend on cell concentration. However, protein production will increase when cells are in an exponential phase. The results show that protein production in all cultures tended to follow the study (Aslam, 2017). Research by Johnston & Carell (1973) showed that cells in older cultures contain around half of the maximum protein content per cell.

Biomass, Lipid, Carbohydrates, and Protein Productivity

Mixed culture treatment showed a higher productivity level in single cultivation of Glagah strain consortium. The biomass productivity of mixed culture reached 0.043 g/L/day, while the lipids reached 0.029 g/L/day, carbohydrates reached 0.110 g/L/day, and protein reached 0.030 g/L/day. The mixed culture treatment had the highest carbohydrate productivity compared to another metabolite (Table 1).

Tabel 3. Productivity biomass, lipids, carbohydrates, and proteins of Glagah strain consortium; monoculture of *Euglena* sp. and mixed culture of Glagah strain consortium and *Euglena* sp. (Nur, 2021).

Treatments			
Metabolites	Monoculture of Glagah Strain Consortium (g/L/day)	Mixed Culture of Glagah Strain Consortium *_Euglena_ sp. (g/L/day)	Monoculture of *Euglena* sp.
Biomass	(0.020±0.004)[a]	(0.043±0.003)[b]	(0.27±0.002[c]
Lipids	(0.020±0.005)[a]	(0.029±0.000)[b]	(0.057±0.000)[c]
Carbohydrates	(0.042±0.003)[a]	(0.110±0.006)[b]	(0.126±0.0005)[c]
Proteins	(0.033 ±0.005)[a]	(0.029±0.004)[a]	(0.257±0.046)[b]

*)Numbers followed by the same letter in the same row show results that are not significantly different at the 95% level (α 0.05).

The results of the total production and productivity of biomass and metabolites showed that the mixed culture of Glagah strain consortium with *Euglena* sp. was higher than Glagah strain consortium. The productivity of biomass, lipid, carbohydrates, and protein reached 0.043, 0.029, 0.010, 0.030 g/L/day, respectively (Nur, 2021). Based on Table 3. The productivity test on the metabolite content of the three treatments shows that the mixed culture treatment can increase the productivity of the metabolite content in the cells compared to single cultivation of Glagah strain consortium. However, this mixed culture treatment showed a lower productivity rate compared to the single cultivation of *Euglena* sp. The mixed culture treatment does not produce the maximum metabolite content. In terms of making the metabolite content of *Euglena* sp., it indicates more potential than mixed culture treatment.

CONCLUSION

Based on the completed research, it can be concluded that the co-culture of *Euglena* sp. and the Glagah Consortium was able to significantly enhance several parameters as compared to the Glagah monoculture, particularly in biomass and lipid content that is essential to develop biofuel in the future research. To achieve higher yields compared to its monoculture, the protein and carbohydrate content still needs to be optimized using a comparative ratio between the Glagah consortium and *Euglena* sp.

ACKNOWLEDGEMENT

The authors would like to thank the funding for the realization of this study provided by the Indonesian Ministry of Education, Culture, Research, and Technology, also the Pertamina Research Technology and Innovation Center. This manuscript is a part of the first author's thesis.

REFERENCES

Aslam, A. (2017). Protein quantification of mixed microalgae consortia under different concentration of Coal fired flue gas. *International Journal of Biosciences (IJB), 10*(2), 72–80. https://doi.org/10.12692/ijb/10.2.72-80

Bligh, E. G., & Dyer, W. J. (1959). A Rapid Method of Total Lipid Extraction and Purification. *Canadian Journal of Biochemistry and Physiology*, *37*.

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, *72*, 248–254. https://doi.org/10.1016/j.cj.2017.04.003

Cramer, M., & Myers, J. (1952). Growth and photosynthetic characteristics of euglena gracilis. *Archiv Für Mikrobiologie*, *17*(1–4), 384–402. https://doi.org/10.1007/BF00410835

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, *28*(3), 350–356. https://doi.org/10.1021/ac60111a017

Eko Agus Suyono, Winarto Haryadi, Muhammad Zusron, Matin Nuhamunada, Sri Rahayu, & Andhika Puspito Nugroho. (2015). The Effect of Salinity on Growth, Dry Weight and Lipid Content of the Mixed Microalgae Culture Isolated from Glagah as Biodiesel Substrate.

Journal of Life Sciences, *10*(5), 229–233. https://doi.org/10.17265/1934-7391/2015.05.006

Galvão, R. M., Santana, T. S., Fontes, C. H. O., & Sales, E. A. (2013). Modeling of Biomass Production of Haematococcus pluvialis. *Applied Mathematics*, *04*(08), 50–56. https://doi.org/10.4236/am.2013.48a008

Gissibl, A., Sun, A., Care, A., Nevalainen, H., & Sunna, A. (2019). Bioproducts From Euglena gracilis: Synthesis and Applications. *Frontiers in Bioengineering and Biotechnology*, *7*. https://doi.org/10.3389/fbioe.2019.00108

Goli, A., Shamiri, A., Talaiekhozani, A., Eshtiaghi, N., Aghamohammadi, N., & Aroua, M. K. (2016). An overview of biological processes and their potential for CO2 capture. *Journal of Environmental Management*, *183*, 41–58. https://doi.org/10.1016/j.jenvman.2016.08.054

Gonçalves, A. L., Rodrigues, C. M., Pires, J. C. M., & Simões, M. (2016). The effect of increasing CO2 concentrations on its capture, biomass production and wastewater bioremediation by microalgae and cyanobacteria. *Algal Research*, *14*, 127–136. https://doi.org/10.1016/j.algal.2016.01.008

Hanief, S., Prasakti, L., Pradana, Y. S., Cahyono, R. B., & Budiman, A. (2020). Growth kinetic of Botryococcus braunii microalgae using Logistic and Gompertz Models. *AIP Conference Proceedings*, *2296*(November). https://doi.org/10.1063/5.0030459

Insan, A. I., Hidayah, H. A., Widyartini, D. S., & Article, H. (2018). *Biosaintifika*. *10*(2), 439–447.

Jiménez, C., Cossío, B. R., Labella, D., & Niell, F. X. (2003). The feasibility of industrial production of Spirulina (Arthrospira) in Southern Spain. *Aquaculture*, *217*(1–4), 179–190. https://doi.org/10.1016/S0044-8486(02)00118-7

Johnston, P. L., & Carell, E. F. (1973). Vitamin B12 and the macromolecular composition of euglena: II. recovery from unbalanced growth induced by vitamin B12 deficiency. *Journal of Cell Biology*, *57*(3), 668–674. https://doi.org/10.1083/jcb.57.3.668

Kazamia, E., Aldridge, D. C., & Smith, A. G. (2012). Synthetic ecology - A way forward for sustainable algal biofuel production? *Journal of Biotechnology*, *162*(1), 163–169. https://doi.org/10.1016/j.jbiotec.2012.03.022

Koreiviene, J., Valčiukas, R., Karosiene, J., & Baltrenas, P. (2014). Testing of Chlorella/Scenedesmus microalgae consortia for remediation of wastewater, CO2 mitigation and algae biomass feasibility for lipid production. *Journal of Environmental Engineering and Landscape Management*, *22*(2), 105–114. https://doi.org/10.3846/16486897. 2013.911182

Kurnianto, D., & Agus Suyono, E. (2021). Effect of inoculant density on the growth of glagah consortium culture. *IOP Conference Series: Earth and Environmental Science*, *860*(1). https://doi.org/10.1088/1755-1315/860/1/012033

Lam, M. K., Yusoff, M. I., Uemura, Y., Lim, J. W., Khoo, C. G., Lee, K. T., & Ong, H. C. (2017). Cultivation of Chlorella vulgaris using nutrients source from domestic wastewater for biodiesel production: Growth condition and kinetic studies. *Renewable Energy*, *103*, 197–207. https://doi.org/10.1016/j.renene.2016.11.032

Maharani, D. K., Ardi, A., Syahrin, A. A., Samudra, T. T., Pradana, Y. S., Budiman, A., & Suyono, E. A. (2020). The effect of photoperiod on lipid production of mixed culture Arthrospira maxima Setchell et Gardner and microalgae consortium of glagah isolate. *AIP Conference Proceedings*, *2260*. https://doi.org/10.1063/5.0016187

Novoveská, L., Franks, D. T., Wulfers, T. A., & Henley, W. J. (2016). Stabilizing continuous mixed cultures of microalgae. *Algal Research*, *13*, 126–133. https://doi.org/10.1016/j.algal.2015.11.021

Nur, F. (2021). *Mixed cultures of Glagah Strain Consortium with Euglena sp. for Optimization of Biomass, Lipid, Carbohydrate, and Protein Production as a Source of Biodiesel*. Gadjah Mada University.

Phatarpekar, P. V., Sreepada, R. A., Pednekar, C., & Achuthankutty, C. T. (2000). A comparative study on growth performance and biochemical composition of mixed culture of Isochrysis galbana and Chaetoceros calcitrans with monocultures. *Aquaculture*, *181*(1–2), 141–155. https://doi.org/10.1016/S0044-8486(99)00227-6

Phukoetphim, N., Salakkam, A., Laopaiboon, P., & Laopaiboon, L. (2017). Kinetic models for batch ethanol production from sweet sorghum juice under normal and high gravity fermentations: Logistic and modified Gompertz models. *Journal of Biotechnology*, *243*, 69–75. https://doi.org/10.1016/j.jbiotec.2016.12.012

Puja Asiandu, A., Puspito Nugroho, A., Saifun Naser, A., Ryan Sadewo, B., Donny Koerniawan, M., Budiman, A., Juniarti Siregar,

U., Tri Suwanti, L., & Agus Suyono, E. (2022). The Effect of Tofu Wastewater and pH on the Growth Kinetics and Biomass Composition of Euglena sp. *Current Applied Science and Technology*, *23*(2), 1–16. https://doi.org/10.55003/cast.2022.02.23.010

Rahmawati, B., Ilmi, M., Budiman, A., & Agus Suyono, E. (2020). Screening of IAA Production on the Interaction of Microalgae and Bacteria in the Glagah Consortium. *Biosciences, Biotechnology Research Asia*, *17*(1), 45–52. https://doi.org/10.13005/bbra/2808

Sayegh, F. A. Q., & Montagnes, D. J. S. (2011). Temperature shifts induce intraspecific variation in microalgal production and biochemical composition. *Bioresource Technology*, *102*(3), 3007–3013. https://doi.org/10.1016/j.biortech.2010.10.011

Suyono, E. A., Fahrunnida, Nopitasari, S., & Utama, I. V. (2016). Identification of microalgae species and lipid profiling of Glagah consortium for biodiesel development from local marine resource. *ARPN Journal of Engineering and Applied Sciences*, *11*(16), 9970–9973.

Suyono, E. A., Retnaningrum, E., & Ajijah, N. (2018). Bacterial symbionts isolated from mixed microalgae culture of Glagah strains. *International Journal of Agriculture and Biology*, *20*(1), 33–36. https://doi.org/10.17957/IJAB/15.0326

Um, B. H., & Kim, Y. S. (2009). Review: A chance for Korea to advance algal-biodiesel technology. *Journal of Industrial and Engineering Chemistry*, *15*(1), 1–7. https://doi.org/10.1016/j.jiec.2008.08.002

Watanabe, T., Shimada, R., Matsuyama, A., Yuasa, M., Sawamura, H., Yoshida, E., & Suzuki, K. (2013). Antitumor activity of the β-glucan paramylon from Euglena against preneoplastic colonic aberrant crypt foci in mice. *Food and Function*, *4*(11), 1685–1690. https://doi.org/10.1039/c3fo60256g

Zhu, J., Chen, W., Chen, H., Zhang, X., He, C., Rong, J., & Wang, Q. (2016). Improved productivity of neutral lipids in Chlorella sp. A2 by minimal nitrogen supply. *Frontiers in Microbiology*, *7*(APR), 1–11. https://doi.org/10.3389/fmicb.2016.00557

Zulkarnain, M. I., Rahayu, H. T., Herida, A. P., Erfianti, T., Kusumaningrum, H. P., Zainuri, M., Endrawati, H., Widowati, I., Haryanti, W. D. U., & Mahendrajaya, R. T. (2021). Improvement of the potency of microalgae Nannochloropsis and Chaetoceros through antioxidant analysis and optimization of DNA isolation. *Journal of Physics: Conference Series*, *1943*(1). https://doi.org/10.1088/1742-6596/1943/1/012080