ANSF APPLIED AND NATURAL SCIENCE FOUNDATION

Journal of Applied and Natural Science
15(1), 15 - 22 (2023)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Enhacment of biomass, carbohydrates, lipids, and proteins content using co-culture of Glagah consortium and *Lipomyces starkeyi*

Ni Made Sri Winasti
Faculty of Biology, Universitas Gadjah Mada, Bulaksumur, Yogyakarta, 55281, Indonesia
Dita Aulia Yulyanita
Faculty of Biology, Universitas Gadjah Mada, Bulaksumur, Yogyakarta, 55281, Indonesia
Ahmad Saifun Naser
Faculty of Biology, Universitas Gadjah Mada, Bulaksumur, Yogyakarta, 55281, Indonesia
Eko Agus Suyono*
Faculty of Biology, Universitas Gadjah Mada, Bulaksumur, Yogyakarta, 55281, Indonesia

*Corresponding author. Email: eko_suyono@ugm.ac.id

Article Info
https://doi.org/10.31018/jans.v15i1.4018
Received: August 12, 2022
Revised: January 3, 2023
Accepted: January 12, 2023

How to Cite
Winasti, N. M. S. *et al.* (2023). Enhacment of biomass, carbohydrates, lipids, and proteins content using co-culture of Glagah consortium and *Lipomyces starkeyi*. *Journal of Applied and Natural Science*, 15(1), 15 - 22. https://doi.org/10.31018/jans.v15i1.4018

Abstract
Microorganisms have a high potential as biofuel sources. Co-culture of microalgae and yeasts can result in high lipid production as a modification treatment. The goal of this study was to see how the co-culture of the Glagah consortium (diversity of associated microalgae and bacteria from Glagah Lagoon, Yogyakarta) and *Lipomyces starkeyi* affected the production of biomass, lipids, proteins, and carbohydrates. The culture was performed under airtight conditions on a shaker at 127 rpm, with a light intensity of 27.75 mol/m^2/s and a temperature of 30°C. The culture was subjected to a dark: light (6:18) treatment. Biomass was measured by dry weight, lipids by the Bligh and Dyer method, proteins by the Bradford method and carbohydrates by the phenol-sulfuric acid method. On day 3, *L. starkey* culture produced the most biomass, yielding 2.21 g/L with a productivity of 0.49 g/L/day. On day 4, the highest lipids produced from co-culture treatment yielded 1.03 g/g with a productivity of 0.21 g/L/day. The highest protein yield was obtained from *L. starkeyi* culture treatment on day 4, yielding 0.60 g/g with a productivity of 0.12 g/L/day. On day 6, co-culture produced the total carbohydrates, yielding 4.78 g/g with a productivity of 0.68 g/L/day. The co-culture treatment produced the highest lipids and carbohydrates production (1.03 g/g and 4.78 g/g) and productivity (0.21 g/L/day and 0.68 g/L/day), while *L. starkeyi* culture produced the highest total biomass and protein production (2.21 g/L and 0.6 g/g) and productivity (0.49 g\L\day and 0.12 g/L/day). In microalgae culture, CO_2 generally given directly through the aeration process. In this study, the source of CO_2 was yeast, whereas yeast also obtained O_2 from microalgae in the consortium for their metabolic process. This mutualism symbiosis will help in providing benefits in reducing the costs for the cultivation process, especially in optimizing the production of biomass an lipids.

Keywords: Biomass, Carbohydrates, Co-culture, Glagah consortium, Lipids, *Lipomyces starkeyi,* Primary metabolites, Proteins

INTRODUCTION

Biofuels can be produced from a wide variety of raw materials. Commonly used ingredients such as vegetable oil derived from grains, palm oil, peanuts, radish, sunflower, coconut, etc. Biofuels can also be produced from animal fats such as cooking oil waste. Biofuel products produced by alcohol are called Fatty Acid Methyl Esters (FAME) (Knothe *et al.*, 2010). Microalgae are known as raw materials for biodiesel generation (Zullaikah *et al.,* 2018). Microalgae can be used as a source of various products, including biofuels and other chemicals. Lipids produced by microalgae are considered the most valuable components of biomass that can be used in biodiesel production (Aresta and Dibendettp, 2019). Indigenous microalgae from Glagah Beach can produce biodiesel which is quite high (Suyono *et al.,* 2015). *L. starkeyi* is yeast which is a good candidate for SCO (Single Cell Oils) because its dry cells can accumulate as much as 70% of SCO (Bonturi *et al.,* 2015). *L. starkeyi* induces lipid synthesis and storage when excess carbon and other nutrients are depleted. During this oleaginous phase, cell division slows down, but carbon assimilation continues and lipid production is stored in the form of triacylglycerides (TAG) (Mcneil and Stuart, 2018). Lipids are a form of microalgae cell osmoprotectant to prevent intracellular osmolarity imbalances that can harm microalgae cells. As a local strain, the Glagah consortium has the potential to produce higher lipids as biodiesel because the environmental conditions are adequate for microalgae to grow faster with higher biomass (Suyono *et al.,* 2015).

Microalgae biomass comprises various organic groups such as carbohydrates, proteins, and lipids. The most energy-rich components are lipids (37.6 kJ/g), then protein (16.7 kJ/g), and carbohydrates (15.7 kJ/g) (Cai *et al.,* 2007). Carbohydrates can be used as raw materials for biofuels, such as bioethanol, biobutanol, and biohydrogen (Markou *et al.,* 2012). CO_2 is formed through the process of photosynthesis, the light reaction and the dark reaction. In the light reaction, sunlight is converted by photosynthetic pigments of microalgae as energy to split water into protons, electrons, and O_2. Electrons and protons are used to form NADPH and ATP which are used as metabolic components. In the dark reaction, CO_2 is converted to carbohydrates through the Calvin cycle using energy from NADPH and ATP (Taiz and Zeiger. 2010).

Microalgae are considered an important source of protein. *Arthrospira platensis* contains 50-70% protein, *Chlorella vulgaris* contains 38-58%, *Nannochloropsis oculate* 22-37%, *Porphyridium cruentum* 8-56%, and *Haematococcus pluvialis* 45-50% protein based on dry weight. The amino acid profiles of proteins extracted from microalgae were generally similar and had a consistent ratio of essential and nonessential amino acids (Safi *et al.,* 2014). The quality and quantity of protein in the extract depend on the effectiveness of cell lysis and the structural morphology of the microalgae cell wall (Hayes *et al.,* 2017). The present aimed to identify the effect of the Glagah Consortium and *Lipomyces starkeyi* co-culture on biomass, lipids, proteins, and carbohydrates production and productivity.

MATERIALS AND METHODS

Source of Glagah Consortium and *L. starkeyi*

Glagah Consortium was isolated from Glagah Beach Lagoon in the southern part of the Special Region of Yogyakarta, Indonesia, which was obtained by sampling around the Brackish Water Lagoon at Glagah Beach and used plankton net to filter microalgae from brackish water. *Lipomyces strakeyi* (InaCC Y584) was obtained from the Indonesian Culture Collection (InaCC) of the Indonesian Institute of Sciences .

Co-culture treatment of Glagah consortium and *L. starkeyi*

Consortium and yeast were successfully grown on Bold's Basal Medium Modified C/N 16 as the starter, carried out with a ratio of medium and culture 1:1 for Glagah consortium and 10: 1 for *L. starkeyi*. Both were cultured for 3 days in a shaker with 127 rpm at 30°C and 27.75 µmol/m2/s of light intensity. In this study, three treatments were carried out, Glagah consortium culture, *L. starkeyi* culture and co-culture between the Glagah consortium and *L. starkeyi*. In these three treatments, the number of cells used was 6.38 x 10^5 cells/mL. Each treatment was cultured with the same medium and treatments as before. The cultures were given photoperiod treatment of light and dark with a ratio of 16:8 for 6 days and every 24 hours, a sample was taken for the measurement of biomass, lipids, proteins, and carbohydrates.

Measurement of biomass

Measurement of biomass used Filtration Vacuum Pump Kit. The fiberglass filter was ADVANTEC GF/C. Culture samples were taken 10 mL and poured over the fiberglass filter ADVANTEC GF/C. The culture biomass will be left on the fiberglass filter ADVANTEC GF/C, while the supernatant will enter the Erlenmeyer. The biomass was dried at 30ºC for 24 hours. Biomass measurements were repeated 3 times and the results were averaged.

Biomass (g/L)= Total weight - initial fiberglass filter ADVENTEC GF/C/ sample volume …. 1

Lipids measurement

The measurement of lipids content in the sample was carried out during the observation using the Bligh and Dyer method. 5 mL samples were taken and then centrifuged for 10 minutes at 3300 rpm. The pellets were taken, added 2 mL of methanol and 1 mL of chloroform, and homogenized using a vortex. After homogeneous, the pellet was added with 1 mL of chloroform and 1 mL of distilled water and then homogenized again. Centrifuged for 10 minutes at a speed of 4000 rpm and a temperature of 10ºC. Three layers were formed and the yellow color in the bottom was taken and placed on a petri dish that had previously been dried and weighed. The chloroform was put in an oven at 30ºC then evaporated until only neutral lipids remained in the petri dish. The total lipid weight was obtained using the following formula.

Lipid content (g/g)+ Final weight of the cum -empty cup weight/ sample volume …… 2

(Bligh and Dyer, 1959)

Proteins measurement

The protein content of the sample was measured by the Bradford method. The absorbance was measured at a wavelength of 595 nm using an ELx800 Absorbance Microplate Reader. The protein content in the sample was measured by taking 2 mL of the sample and putting it into a 2 mL microtube and then centrifuged it at 3000 rpm for 10 minutes. The pellet was taken and added with 1 mL of 10% SDS solution. Then the samples were incubated in an oven at 95ºC for 5 minutes and then transferred to 4ºC for 5 minutes. Samples were taken 8 µL and put into a microplate 500 L. Then the sample was added with 200 L of Bradford's reagent. The sample read by ELx800 Absorbance Microplate Reader at a wavelength of 595 nm (Walker, 2002)

Carbohydrates measurements

Measurement of carbohydrates content was carried out for 6 days used Phenol-Sulfuric Acid method. To measure the carbohydrates content in the sample, 10 mL of sample was taken and put into a conical tube and then centrifuged at 3300 rpm for 15 minutes. The pellet was taken, then 1 mL of concentrated sulfuric acid and 0.5 mL of 5% phenol was added then the sample was incubated for 30 minutes at room temperature, then 2 mL of the sample was put into a cuvette and measured spectrophotometrically at a wavelength of 490 nm (Nielsen, 2009).

Data analysis

One-Way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was used to determine significant differences between treatments.

RESULTS AND DISCUSSION

Microalgae and yeast co-culture have a mutually beneficial relationship concerning of gas exchange. Microalgae produce O_2 for yeast respiration, while yeast providing CO_2 for microalgae photosynthesis. This is the reason that the co-culture is potentially profitable as another source of biofuel production in the future because it is considered effective in energy and cost savings (Rakesh and Karthikeyan, 2019). In this study, there were three treatments such as Glagah consortium culture, *L. starkeyi* culture which were the controls in the study and Glagah consortium and *L. starkeyi* co-culture as the main study. This research was carried out by optimizing the modification of the medium and its cultivation technique which minimized the energy costs required. Costs are reduced by replacing the use of bubbling, which is normally used shaker. In addition, photoperiodization treatment was given to save the use of lamps and maximize metabolic results. Using Bold's Basal Medium Modified C/N 16, the culture was also stirred on a shaker at 127 rpm. The medium used in this culture was Bold's Basal Medium modified C/N 16, the carbon source (C) used was glucose. Carbon sources have an important role in regulating metabolism and lipid production in microalgae (Bashir *et al.,* 2019) and yeast (Turcotte *et al.,* 2010). While the nitrogen source used comes from yeast extract.

Microalgae species in Glagah consortium based on Suyono *et al.* (2016) included *Cyclotella* sp., *Cylindrospermopsis* sp., *Golenkinia* sp., *Syracosphaera* sp., *Corethron* sp. and *Chlamydonomas* sp. The two other species of microalgae, *Scenedesmus* sp. and *Desmodesmus* sp. Glagah consortium also contains various types of bacteria that are positively associated with microalgae. The bacterial included the phylum of Acidobacteriota, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Fusobacteria, Gemmatimonadetes, Planctomycetes, Proteobacteria, Spirochaetes, Verrucomicrobia, and two other phyla that have not been identified. Each phylum has its own function in the growth of microalgae, but the composition of bacteria in cultures grown together, microalgae in culture showed faster growth than the bacterial, otherwise when Vancomycin was added in culture caused a decrease in growth rate in the cultured consortium (Ardi *et al.,* 2020). Based on Pradana (Pradana *et al.,* 2017), bacteria inside microalgae cell can protect the cell from toxins from different species of microalgae in consortium.

The criteria for strains suitable for co-culture include growing quickly, having a high lipid content, growing in extreme conditions, tolerating high contamination and large cell sizes to facilitate biomass harvesting. *L. starkeyi* has the advantage of having a greater capacity than other microorganisms to accumulate lipids and use nutrient sources effectively (Griffths and Harrison, 2009; Kitcha and Cheirsilp, 2014; Arora *et al.,* 2019).

The ratio between microalgae and yeast in culture is also important in culture. Yeasts are generally the dominant species in the early 24-48 hours because their growth rate is faster, while microalgae need a longer duration to grow in the early stages (Griffths and Harrison, 2009; Cai *et al.,* 2007; Cheirsilp *et al.,* 2011; Shu *et al.,* 2013). However, after 24-48 hours, microalgae adapt to the environment and grow faster while the yeast has reached the stationary phase (Griffths and Harrison, 2009; Yen *et al.,* 2015). In this study, the ratio between the Glagah consortium and L. *starkeyi* used was 2:1. This was determined based on the growth phase of the two species that had been calculated previously, the *L. starkeyi* was in an exponential phase on days 1 to 3, while Glagah consortium was in an exponential phase on days 3 to 5. In co-culture treatment, the exponential phase is when cells are actively dividing and cell biomass increases (Krishnan *et al.,* 2015). The exponential phase is the most appropriate phase for subculture treatment because in this phase the cell conditions are in the most optimal condition, so the nutrient content in the cells is very high (Putra *et al.,* 2015). This phase indicates that the cultured cells have adapted successfully.

The ideal light intensity for microalgae culture is between 27 and 108 mol/m^2/s. The light intensity to increase lipid production is 67.5 mol/m^2/s more than that will decrease lipid production (Kitcha and Cheirsilp, 2014; Arora *et al.,* 2019; Cheirsilp *et al.,* 2011). Photoperiodism is needed by microalgae related to the optimization of growth and energy production. Light is used for photochemical processes to produce ATP and dark conditions are carried out to synthesise essential molecules and support microalgae cells' growth (Jiang *et al.,* 2018). During cultivation, microalgae cells appear yellow rather than green due to the heterotrophic mode of cultivation in the presence of organic carbon sources such as glucose or glycerol and due to the density of other strains (Kitcha and Cheirsilp, 2014; Arora *et al.,* 2019) in this study is *L. starkeyi*. In this study, the light intensity used was 27.75 mol/m^2/s, where this light supported microalgae cells to grow and increase lipid production. In addition, photoperiodism was also carried out on cultures with a light: dark ratio of 16: 8 hours. The ratio of C / N 16 was the ideal level for the Glagah Consortium and *L. starkeyi* co-culture, yeasts need carbon and nitrogen was higher than microalgae but on the other hand, the number of high carbon and nitrogen that would block cell microalgae to obtain CO_2 as a primary need in photosynthesis.

Fig. 1 shows that the biomass of the Glagah Consortium culture increased until day 4. On the other hand, the biomass of *L. starkeyi* culture increased until day 3 with the highest amount of biomass compared to other treatments. This could be due to the cell division of *L. starkeyi* faster than microalgae. However, the growth of *L. starkeyi* was slower than usual in a medium with organic salt content. This happened because *L. strakeyi* 'custers effect' caused a limitation of the free O_2 transport mechanism for glucose absorption, which caused the growth of *L. starkeyi* to be slower (Zuccaro *et al.,* 2021). In addition, in microalgae itself, light is the main factor that affects photosynthesis kinetics; the quality and quantity of light determine the amount of energy available for microalgae to carry out their metabolic activities because microalgae absorb light of different wavelengths depending on the type of microalgae. For the distribution of the effects of light on microalgae cultures, photoperiod treatment is required. In dark and light settings, duration per day can actively modify the biochemical composition of cells because the light is a source of stress for microalgae (Pedro, 2015). Based on Sudibyo *et al.* (2017), the red light wavelength is greatest to support cell division and blue light to increase dry weight biomass of microalgae. Broad spectrum light that appears as white light is usually used on microalgae culture to support photosynthesis but is not optimal to increase biomass. Meanwhile, *L. starkeyi* can grow well in dark or light conditions. Photoperiod treatment also affected the biomass produced by the microalgae consortium and *L. starkeyi* coculture. Both species were able to adapt to the modified medium containing sugar and yeast extract also, photoperiod 16:8 treatment helped the photosynthetic efficiency of microalgae.

Based on Fig. 2, lipids increased in co-cultures compared to monocultures in this study was caused by the modification of the medium, which caused the loss of several components of the main medium for microalgae such as the nitrate source in the organic salt $NaNO_3$, which was replaced with yeast extract. On the other hand, pressure also occurred in *L. starkeyi* because the medium contained various types of organic salts, which became the medium for growing microalgae. Nutrient deficiencies and high salt concentrations at higher temperatures and pH can increase triacylglyceride (TAG) synthesis as a self-defense mechanism (Sharma *et al.,*

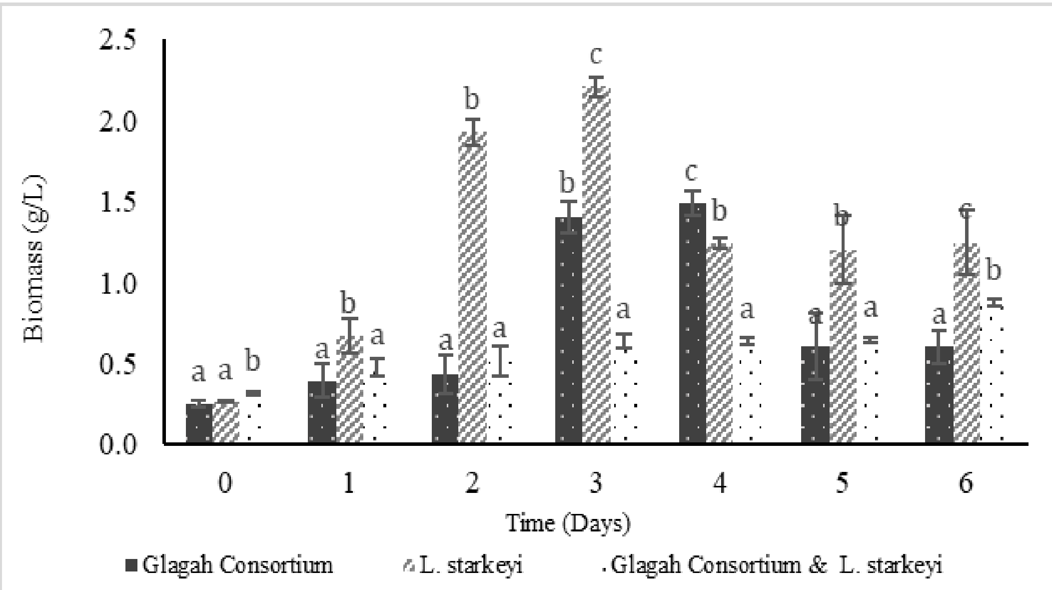

Fig. 1. *Total biomass of Glagah Consortium and L. starkeyi culture (Winasti, 2021)*

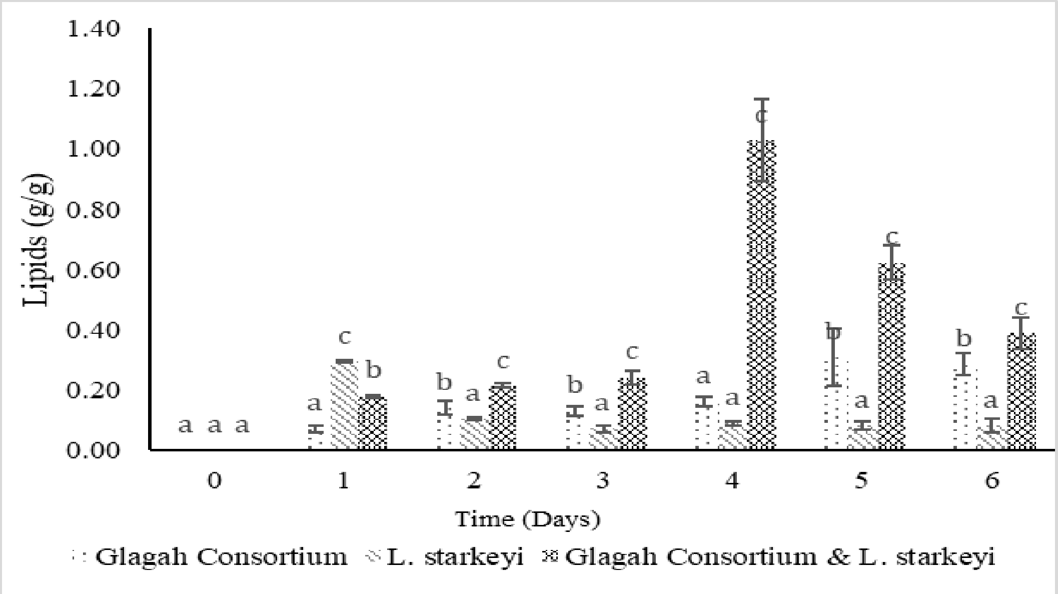

Fig. 2. *Total lipids of Glagah Consortium and L. starkeyi culture (Winasti, 2021)*

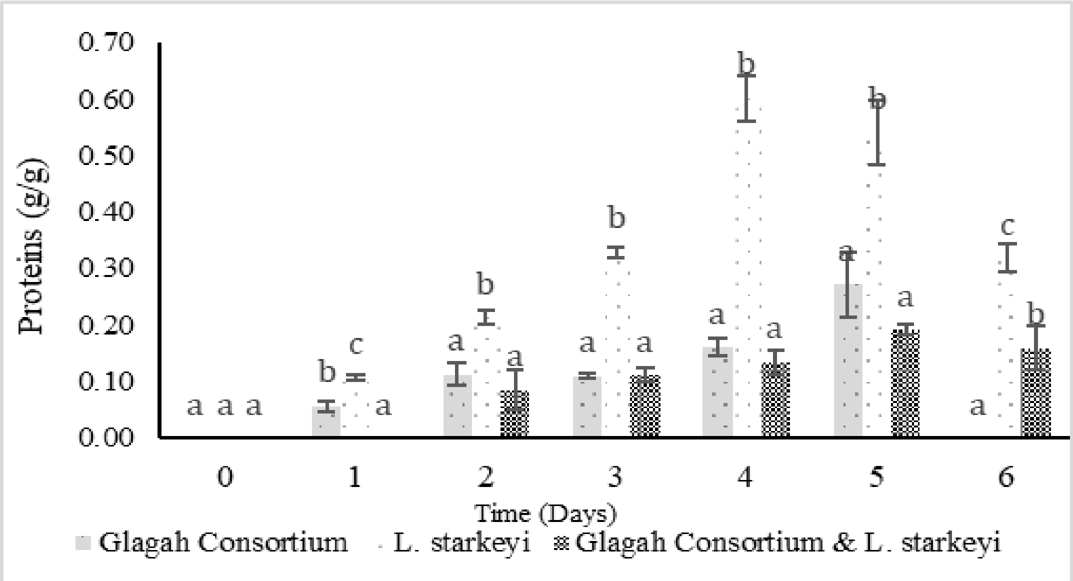

Fig. 3. *Total proteins of Glagah Consortium and L. starkeyi culture (Winasti, 2021)*

2012; Kwak *et al.,* 2016). But with co-culture can reduce the pressure, especially in obtaining nutrients by gas exchange carried out by microalgae and yeast, the synergistic also the amount of dissolved O_2 for yeast respiration (Zhang *et al.,* 2014) and stable pH (Prasad and Shih, 2016). The presence of two interacted species causes increased lipid production and can handle stress with positive interactions.

Proteins that can generally be extracted in microalgae culture are water-soluble peptides and free amino acids, as one of the components that make up cells under optimal conditions. The protein content in microalgae can range from 28-51%. While *L. starkeyi* is one of the yeasts in the order Saccharomycetales, the proteins in this order that were successfully extracted included those involved in metabolism which consisted of many enzymes of carbohydrate and protein metabolism which ranged from 85% of the total protein extracted, while about 15-30% are proteins involved in localization, response to stimuli, and regulation of physiological processes. Other proteins involved in metabolism and secretion, in 96 hours of yeast in this order, can produce up to 8.5 mg/g wet biomass (Moscoso *et al.,* 2013). Fig. 3 shows that *L. starkeyi* culture produced more protein than other treatments, while in the co-culture treatment, the protein produced was lowest but increased until the last day. The total protein produced was directly proportional to the biomass obtained in each treatment because the protein carried out vital activities in cell metabolism.

Fermentation of glucose in medium and carbohydrates produced by the microalgae cells, based on Fig. 4 shows that the number of total carbohydrates in co-cultures increased and the presence of O_2 produced by

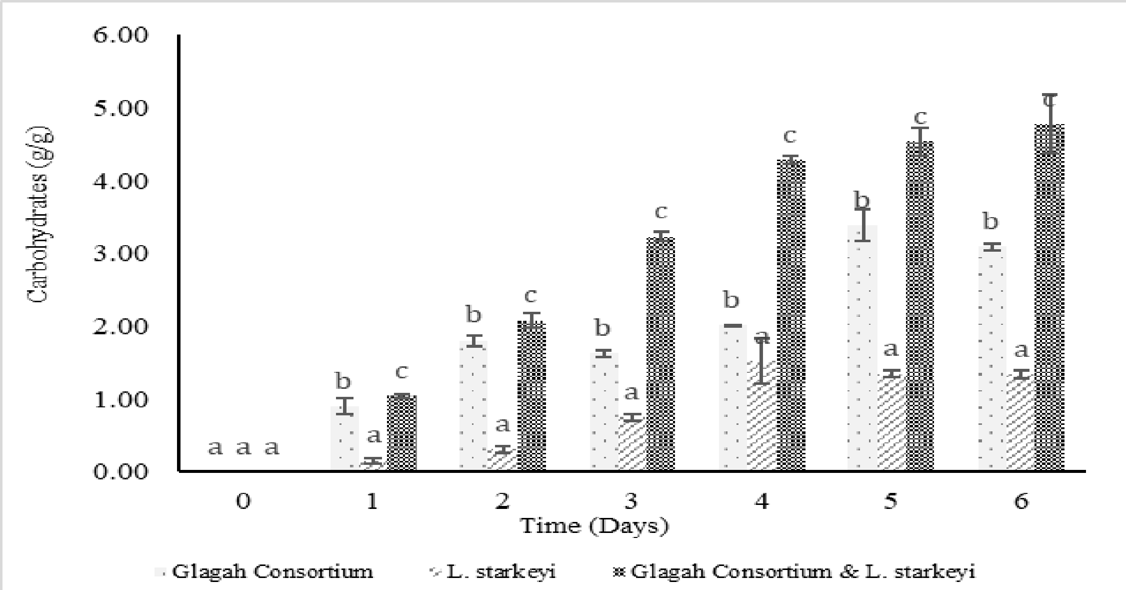

Fig. 4. *Total carbohydrates of Glagah Consortium and L. starkeyi culture (Winasti, 2021)*

Tabel 1. Productivity in biomass, lipids, proteins, and carbohydrates of Glagah Consortium and *L. starkeyi* culture (Winasti, 2021)

Metabolites	Treatments		
	Glagah Consortium culture (g/L/day)	***L. starkeyi* culture (g/L/day)**	**Glagah Consortium and *L. starkeyi* co-culture (g/L/day)**
Biomass	$(0{,}25\pm0{,}015)^{b}$	$(0{,}49\pm0{,}017)^{c}$	$(0{,}08\pm0{,}004)^{a}$
Lipids	$(0{,}06\pm0{,}010)^{a}$	$(0{,}15\pm0{,}002)^{b}$	$(0{,}21\pm0{,}033)^{c}$
Proteins	$(0{,}05\pm0{,}010)^{a}$	$(0{,}12\pm0{,}008)^{b}$	$(0{,}03\pm0{,}002)^{a}$
Carbohydrates	$(0{,}56\pm0{,}037)^{b}$	$(0{,}30\pm0{,}061)^{a}$	$(0{,}68\pm0{,}058)^{c}$

microalgae causing *L. starkeyi* grew quite well in the co-culture. In contrast, the Glagah consortium culture did not obtain a source of CO_2 for photosynthesis, so the number of cells decreased in the last two days of cultivation. *L. starkey*i is a yeast that lives under aerobic conditions (Mcneil and Stuart, 2018); the absence of O_2 also caused cell growth to decrease in the last two days. The decrease in the number of cells in the culture caused the carbohydrate content also decrease due to reduced metabolic activity in the cells.

Based on this research, both biomass and metabolic products obtained from the co-culture of the Glagah consortium and *L. starkeyi* (Table 1) are not all optimal, as evidenced by the lower production and productivity of biomass and proteins compared to monoculture treatment. This can happen because microalgae and *L. strakeyi* cells are difficult to adapt to the medium used. But on the other hand, Bold's Basal Medium modified C/N 16 and culture treatment with shaker can reduce costs in the production of biofuel sources which are still difficult to commercialize because of the high cost of cultivation. Co-culture was carried out for a shorter duration, only 6 days; usually, in microalgae culture, it took up to 14 days for the biomass to be ready for harvest. This shows that although the yield of biomass and metabolism in culture is lower, with lower cultivation costs and shorter duration, this method is more effective in producing biofuel from microorganism sources.

Conclusion

It can be concluded that the biomass and protein produced from the co-culture of the Glagah consortium and *L. Starkeyi* increased up to day 6, but the production and productivity were lower than the monocultures treatment of *L. starkeyi*. Meanwhile, the production of lipids and carbohydrates and the productivity from the Glagah consortium and *L. starkeyi* co-cultures were higher than monocultures. Thus, the co-culture of the Glagah consortium and *L. Starkeyi* has the potential as a source of biofuel.

ACKNOWLEDGEMENTS

This work was part of the first author's thesis project and was financially supported by Universitas Gadjah Mada, Indonesia.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Ardi, A., Safitri, A., Nuhamunada, Budiman, A., Ilmi, M., Gozan, M., Syahrin, A.A., Pradana, Y.S. & Suyono, E.A.

(2020). The effect of vancomycin on the growth and microbial dynamics of microalgae-bacteria consortium isolated from Glagah Beach, Yogyakarta. Ecology, *Environment and Conservation*, 26(2020), 1-7

2. Aresta, M. & Dibendettp, A. (2019). Bioenergy with carbon capture and storage: bioenergy with carbon capture and storage (pp 173-193). Academic Press, Cambridge
3. Rakesh, S. & Karthikeyan, S. (2019). Co-cultivation of microalgae with oleaginous yeast for economical biofuel production. *Journal of Farm Sciences*, 32(2), 125-130. doi.org/10.13140/RG.2.2.10506.41928
4. Arora, N., Patel, A., Mehtani, J., Pruthi, P.A., Pruthi, V. & Poluri, K.M. (2019). Co-culturing of oleaginous microalgae and yeast: paradigm shift towards enhanced lipid productivity. *Environmental Science and Pollution Research*, 26 (17), 16952-16973. doi.org/10.1007/s11356-019-05138-6
5. Bashir, K.M.I., Mansoor, S., Kim, N.R., Grohmann, F.R., Shah, A.A. & Cho, M.G. (2019). Effect of organic carbon sources and environmental factors on cell growth and lipid content of *Pavlolva lutheri*. *Annals of Microbiology*, 69 (2019), 353-368. doi.org/10.1007/s13213-018-1423-2
6. Bligh, E.G. & Dyer, W.J. (1959). A Rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), (912-917). https://doi.org/10.1139/o59-099
7. Bonturi, N., Matsakas, L., Nilsson, R., Christakopoulus. P., Miranda, E.A., Berglund, K.A. & Rova, U. (2015). Single cell oil producing yeasts *Lipomyces starkeyi* and *Rhodosporidium toruloides*: selection of extraction strategies and biodiesel property prediction. *Energies,* 8(6), 5040-5052. doi.org/10.3390/en8065040
8. Cai, S., Hu, C. & Du, S. (2007). Comparisons of growth and biochemical composition between mixed culture of alga and yeast and monocultures. *Journal of Bioscience and Bioengineering*, 104(5), 391-397. doi.org/10.1263/jbb.104.391
9. Cheirsilp, B., Kitcha, S. & Torpee, S. (2011). Co-culture of an oleganious yeast *Rhodoturula glutinis* and a microalga *Chlorella vulgaris* for biomass and lipid production using pure and crude glycerol as a sole carbon source. *Annals of Microbiology,* 62(2012), 987-993. doi.org/ 10.1007/s13213-011-0338-y
10. Cheirsilp, B., Suwarannat, W. & Niyomdecha, R. (2011). Mixed culture of oleaginous yeast *Rhodoturula glutinis* and microalga *Chlorella vulgaris* for lipid production from industrial wastes and its use as biodiesel feedstock. *New Biotechnology*, 28(4), 362-368. doi.org/ 10.1016/j.nbt.2011.01.004
11. Griffths, M.J. & Harrison, S.T.L. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology*, 21(2009), 493-507. doi.org/10.1007/s10811-008-9392-7
12. Hayes, M., Skomedal, H., Skjanes, K., Mazur-Marzec, H., Torunska-Sitarz, A., Catala, M., Hosoglu, M.I. & Garcia-Vaquero, M. (2017). Microalgae-based biofuels and bioproducts (pp 347-368). *Woodhead publishers* . Elesevier, Cambridge
13. Jiang, X., Liu, L., Chen J. & Wei, D. (2018). Effects of Xanthophyllomyces dendrohous on cell growth, lipid, and astaxanthin production of *Chromochloris zofingiensis* by mixed culture strategy. *Journal Applied Phycology*, 30 (2008), 3009-3015. doi.org/10.1007/s10811-018-1553-8
14. Kitcha, S. & Cheirsilp, B. (2014). Enhanced lipid production by co-cultivation and co-encapsulation of oleaginous yeast *Trichosporonoides spathulate* with microalgae in alginate gel bead. *Applied Biochemistry and Biotechnology,* 173(2), 522-534. doi.org/10.1007/s12010-014-0859-5
15. Knothe, G., Krahl, J. & Jerpen, G.V. (2010). The Biodiesel Handbook (pp 1-3). *AOCS Press, Urbana*
16. Krishnan, V., Uemura, Y., Thanh, N.T., Khalid, N.A., Osman, N. & Mansor, N. (2015). Three types of marine microalge and *Nannochloropsis oculate* cultivation for potential source of biomass production, *Journal of Physics*, 622 (2015), 1-7. doi.org/10.1088/1742-6596/622/1/012034
17. Kwak, H.S., Kim, J.Y.H., Woo, H.M., Jin, E.S., Min, B.K. & Sim, S.J. (2016). Synergistic effect of multiple stress conditions for improving microalgal lipid production. *Algal Research*, 19(2016), 215-224. doi.org/10.1016/j.algal.2016.09.003
18. Markou, G., Angelidaki, I. & Georgakakis, D. (2012). Microalgal carbohydrates: An overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. *Applied Microbiology and Biotechnology*, 96(3), 631-45. doi.org/10.1007/s00253-012-4398-0
19. Mcneil, B.A. & Stuart, D.T. (2018). *Lipomyces starkeyi:* an emerging cell factory for production of lipids, oleochemicals and biotechnology applications. *World Journal of Microbiology and Biotechnology*, 34(10): 147. doi.org/10.1007/s11274-018-2532-6
20. Moscoso, J.LG., Obied, W., Kumar, S. & Hatcher, P.G. (2013). Flash hydrolysis of microalgae (*Scenedesmus* sp.) for protein extraction and production of biofuels intermediates. *The Journal of Supercritical Fluids,* 82(2013), 183-190. doi.org/10.1016/j.supflu.2013.07.012
21. Nielsen, S.S. (2009). *Phenol-Sulfuric Acid Method for Total Carbohydrates* (pp 47-53). Springer, Berlin
22. Pedro, I.S.H. (2015). Evaluation of photoperiod effect on the growth and protein content of microalgae (p 4). Ines Soraia Hipolito Pedro, Leiria
23. Pradana, Y.S., Sudibyom H., Suyono, A.E., Indarto & Budiman, A. (2017). Oil algae extraction of selected microalgae species grown in monoculture and mixed cultures for biodiesel production. *Energy Procedia*, 105 (2017), 277-282. doi.org/10.1016/j.egypro.2017.03.314
24. Prasad M.N.V. & Shih, K. 2016. *Environmental materials and waste: resource recovery and pollution prevention* (pp 179-212). Elsevier, London
25. Putra, I.K.R.W., Anggreni, A.A.M.D. & Arnata, I.W. (2015). Pengaruh jenis media terhadap konsentrasi biomassa dan klorofil mikroalga *Tetraselmis chuii.* Jurnal Rekayasa dan Manajemen Agroindustri, 3(2), 40-46. (in Indonesia)
26. Safi, C., Charton M., Ursu A.V., Laroche C., Zebib B., Pontalier P.Y. & Vaca-Garcia C. (2014). Release of hydro-soluble microalgal proteins using mechanical and chemical treatments. *Algal Research*, 3(2014), 55-60. doi.org/10.1016/j.algal.2013.11.017
27. Sharma, K.K., Schuhmann, H. & Schenk, P.M. (2012). High lipid induction in microalgae for biodiesel production. *Energies*, 5(5), 1532-1553. doi.org/10.3390/en5051532
28. Shu, C.H., Tsai, C.C., Chen, K.Y., Liao, W.H. & Huang, H.C. (2013). Enhancing high quality oil accumulation and CO_2 fixation by a mixed culture of *Chlorella* sp. and *Saccharomyces cerevisiae. Journal of the Taiwan Institute of*

Chemical Engineers, 44(6), 936-942. doi.org/10.1016/j.jtice.2013.04.001

29. Sudibyo, H., Yano, S.P. Samudra, T.T., Budiman, A., Indarto & Suyono, E.A. (2017). Study of cultivation under different colors of light and growth kinetic study of *Chlorella zofinginesis* Donz for biofuel production. *Energy Procedia*, 105(2017), 270-276. doi.org/10.1016/j.egypr o.2017.03.313
30. Suyono, E.A., Fahrunnida, Nopitasari, A. & 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
31. Suyono, E.A., Haryadi, W., Zusron, M., Nuhamunada, M., Rahayu, S. & Nugroho, A.P. (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,* 9(2015), 229-233. doi.org/10.17265/1934-7391/2015.05.006
32. Taiz, L. & Zeiger,E. (2010). *Plant Physiology 5th edn* (p 201). Sinauer Associated Inc, Los Angeles
33. Turcotte, B., Liang, X.B., Robert, F. & Soontorngun, N. (2010.) Transcriptional regulation of nonfermentable carbon utilization in budding yeast. *FEMS Yeast Research*, 10(1), 2-13. doi.org/10.1111/j.1567-1364.2009.00555.x
34. Walker, John M. (2002). *The Protein Protocols Handbook* (pp 15-21). Humana Press, New Jersey
35. Winasti, N.M.S. (2021). Ko-kultur Konsorsium Glagah dan *Lipomyces starkeyi* untuk Optimasi Produksi Biomassa, Lipid, Protein dan Karbohidrat (pp 36-43). UGM Library, Yogyakarta
36. Yen, H.W., Chen, P.W. & Chen, L.J. (2015). The synergistic effects for the co-cultivation of oleaginous yeast *Rhodoturula glutinis* and microalgae-*Scenedesmus obliquus* on the biomass and total lipids accumulation. *Bioresource Technology*, 184(2015), 148-152. doi.org/10.1016/j.biortech.2014.09.113
37. Zhang, Z., Ji, H., Gong, G., Zhang, X. & Tan, T. (2014). Synergistic effects of oleaginous yeast *Rhodoturula glutinis* and microalga *Chlorella vulgaris* for enchanement of biomass and lipid yields. *Bioresource Technology*, 164 (2014), 93-99. doi.org/10.1016/j.biortech.2014.04.039
38. Zuccaro, G., Mondo A.d., Pinto, G., Polio, A. & Natale, A.D. (2021). Biorefinery-based approach to exploit mixed cultures of *Lipomyces starkeyi* and *Chlororidium saccharophilum* for single cell oil production. *Energies*, 14(5), 1-22. doi.org/10.3390/en14051340
39. Zullaikah S., Utomo A.T., Yasmin M., Ong L.K & Ju Y.H. (2018). Advances in eco-fuels for a sustainable environment (pp 237-276). Woodhead Publishing Series in Energy, Cambridge