Enhancement 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**

**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/m²/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. starkeyi* 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, CO₂ is generally given directly through the aeration process. In this study, the source of CO₂ was yeast, whereas yeast also obtained O₂ 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 and 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 Dibenedetti, 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 microalgal cell osmoprotectant to prevent intracellular osmolarity imbalances that can harm microalgal 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). CO₂ 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 O₂. Electrons and protons are used to form NADPH and ATP which are used as metabolic components. In the dark reaction, CO₂ 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 platenis contains 50-70% protein, Chlorella vulgaris contains 38-58%, Nannochloropsis oculata 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 microalgal 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 starkeyi (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 10⁸ 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 30°C for 24 hours. Biomass measurements were repeated 3 times and the results were averaged.

Biomass (g/L)= Total weight - initial fiberglass filter AD- VENTEC GF/C/ sample volume  

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 at a temperature of 10°C. 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 30°C 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  

(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 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 95°C for 5 minutes and then transferred to 4°C 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 O₂ for yeast respiration, while yeast providing CO₂ 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., Cylindropermopsis sp., Golenkinia sp., Syracosphaera sp., Corethron sp. and Chlamydomonas 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 (Griffiths 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 (Griffiths 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 (Griffiths 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 be-
between 27 and 108 mol/m²/s. The light intensity to increase lipid production is 67.5 mol/m²/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/m²/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 CO₂ 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. starkeyi* ‘custers effect’ caused a limitation of the free O₂ 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* co-culture. 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 NaNO₃, 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., 2021).
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

**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**

mixed culture strategy. Xanthophyllomyces dendroideus on cell growth, lipid, and productivities. Journal of Bioscience and Bioengineering, 126(3), 137-143.


