The effect of adding bacteria (nitrogen-fixing bacteria, amylolytic, and phosphate solubilizing bacteria) and different culture media on the growth of the microalgae *Chlorella vulgaris*

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Abstract. Microalgae have the potential to be developed. As a source of biofuels, medicines, cosmetics, sources of food and feed, it is necessary to optimize microalgae growth to increase microalgae growth. Bacteria can increase microalgae growth through various mechanisms such as producing CO_2, degrading organic molecules, providing vitamin B12, and preventing oxidative stress. One important factor for the growth of microalgae is culture media. Therefore, it is necessary to do research to find the medium culture that gives the highest density of microalgae cells. The research objective was to determine the effect of the addition of bacteria and culture media on the growth of *Chlorella vulgaris*. Our research used microalgae-bacterial co-culture in Guillard and Gusrina medium. The culture conditions is set 12 hours without an aerator, 12 hours with an aerator; 16 hours of light; 8 dark hours; light intensity 2700 lux. The results of the study showed the co-culture treatment had higher cell density of about 3.8×10^7 cells/mL and Gusrina medium showed the highest cell density at about 4.6×10^7 cells/mL. The addition of bacteria was able to increase the growth of microalgae and Gusrina medium showed a good growth medium for *C. vulgaris*.

1 Introduction

Microalgae are single-celled organisms, which can be found in all aquatic habitats, including oceans, rivers, ponds and wastewater. Microalgae are capable of producing various bioproducts such as polysaccharides, pigments, lipids, proteins, vitamins and other bioactive compounds. In general, microalgae can double biomass in just 24 ours. Even in the exponential phase, microalgae grow is very fast, it can grow twice in 3.5 hours [1].

Algal biomass can be produced on an industrial scale for food and nutritional supplements, and the extract can be used as an ingredient in cosmetics, medicines, and can also be used for biofuel production [2]. The potential of algal biomass as a source of biofuel and bioproducts that can be utilized in various industries, such as food, medicine, cosmetics and animal feed, is in line with the concept of green technology [3]. One species of microalgae that is suitable for cultivation is the *Chlorella vulgaris* [4].

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These various advantages make *C. vulgaris* one of the microalgae that can be considered for cultivation on a large scale or for commercial production purposes [5]. The high potential for the use of microalgae in various industries means that microalgae cultivation can be carried out to meet the needs of microalgae biomass. Ongoing research and technological advances are focused on optimizing cultivation processes, increasing efficiency, utilizing waste, and reducing culture costs [6]. Optimizing the growth of microalgae can be done by utilizing bacteria, namely by carrying out co-culture technology or joint culture between microalgae and several potential bacteria that can increase the growth of microalgae.

In recent years, it has been known that the interaction of bacteria with microalgae has shown the effect of increasing microalgae growth [7]. Co-culture between microalgae and bacteria increases the growth rate of microalgae between 10-70% [8]. Apart from that, the interaction between microalgae and bacteria that has been known so far is the exchange of nutrients between microalgae and bacteria, namely micronutrients such as vitamins [9], and macronutrients such as nitrogen and carbon [10]. One of the bacteria that has been proven to increase the growth of *C. vulgaris* is *Bacillus pumilus* Es4, by providing fixed nitrogen to microalgae [11]. Another study showed that *C. vulgaris*, which was cultured with four different bacteria, showed an increase in growth rate and produced a higher final biomass product, as well as an increase in lipid content from 22.4% to 28% [8].

Optimizing the cultivation process by carrying out joint culture between microalgae and bacteria can increase the productivity of microalgae biomass. In addition, it can reduce commercial scale production costs and the production of bioproducts from algae can be sustainable and environmentally friendly. Indonesia is a country that has rich biodiversity and a large area of water but has not yet explored much of amylolytic bacteria as amylase enzyme producers in the waters. The use of 4 lakes (Ranu Pani, Ranu Regulo, Ranu Grati, and Ngebel Lake) is because the 4 lakes are aquatic ecosystems in Indonesia, especially in East Java [12].

The preliminary study result show that these lakes include eutrophic lakes with a secchi disk depth (brightness) of 75−110 cm, medium microalgae diversity index, moderate dominance, and abundance of high microalgae [13]. So, the potential bacteria resulting from the isolation from the lakes in east java can be used for microalga-bacteria co-culture. The bacteria used are bacteria isolated and identified from lakes in East Java. *Bacillus cereus* bacteria were successfully identified as phosphate-solubilizing bacteria. *Bacillus paramycoides* bacteria as nitrogen-fixing bacteria [14], and amylolytic bacteria [12]. Research by [15] shows that co-culture data between bacterial species and *Chlorella* sp. increasing the number of microalgae cells in co-culture. Thus, co-culture can accelerate the growth of *Chlorella* sp, extend the log and stationary phases, and enhance the environmental carrying capacity.

Furthermore, one of the important factors that needs to be considered in biomass production from microalgae includes cultivating microalgae in a suitable growth medium. This research will conduct trials using different culture media to find out which culture media are suitable for cultivating microalgae. This research used two types of culture media, namely Gusrina media and Guillard media. Gusrina media was chosen because it has the macronutrient and micronutrient components needed by microalgae for their growth.

Apart from that, to date there have been no reports related to studies of the effect of Gusrina culture media on the growth of *C. vulgaris* which is co-cultured with potential bacteria. Meanwhile, Guillard/F2 media was chosen because Guillard/F2 media is a culture medium that is often used for microalgae culture. Based on the report by [16], the elements contained in Guillard media have a more complete composition so that *Chlorella* sp. cells can grow to its maximum potential. This study aimed to determine the effect of adding nitrogen-fixing bacteria, amylolytic bacteria, and phosphate-solubilizing bacteria, as well as differences in culture media on the growth of *C. vulgaris* microalgae.

2 Experimental details

The experimental design used was a two-factor Completely Randomized Design, namely the first factor was the addition of bacteria, consisting of two treatment levels, namely co-culture (microalgae culture with the addition of nitrogen-fixing bacteria, amylolytic bacteria, and phosphate-solubilizing bacteria); and monoculture (microalgae culture without nitrogen-fixing bacteria, amylolytic bacteria and phosphate-solubilizing bacteria). The second factor, namely culture media, consists of two levels of treatment, namely Gusrina and Guillard media. The study consisted of 5 replications and 4 treatment combinations

2.1 Species of microalgae and bacteria

The microalgae used was the *Chlorella vulgaris* species from the algae culture laboratory, Green House of Biology Department, State University of Malang. The bacteria used are bacteria isolated and identified from lakes in East Java. *Bacillus paramycoides* species are nitrogen fixing bacteria and amylolytic bacteria. *Bacillus cereus* bacteria as phosphate solubilizing bacteria.

2.2 Preparation of Gusrina culture media

Gusrina media modified from [17]. Gusrina media was made by mixing the media components in an Erlenmeyer flask, consisting of 22.5 g urea, 15 g Triple superphosphate (TSP), 0.5 g $FeCl_3$, and 500 mL distilled water. Then, the media is sterilized using an autoclave. After completing the autoclaving process, 0.0125 g of vitamin B12 was added.

2.3 Preparation of Guillard culture media

Guillard media modified from [18]. Guillard media is made by dissolving solution A, which consists of 0.5 g $NaH_2PO_{4.2}H_2O$ and 7.5 g $NaNO_3$, into 100 mL of distilled water. Then, dissolve the PS solution components consisting of 0.1 g $CuSO_4.5H_2O$, 0.22 g $ZnSO_4.7H_2O$, 0.1 g $CoCl_2.6H_2O$, 1.8 g $MnCl_2.4H_2O$, and 0.6 g $Na_2MoO_4.2H_2O$ in each 10 mL of distilled water each. Then, make solution B, namely dissolving 0.436 g of Na2EDTA and 0.315 g of $FeCl_3.6H_2O$ in 100 mL of distilled water. After that, take 0.1 mL of each component of the PS solution, then put it into solution B. After that make solution C by preparing 1 g of thiamin HCl, 0.005 g of biotin, and 0.01 g of vitamin B12, then dissolve each substance into 50 mL of distilled water, then take 0.5 mL of each component of solution C, and put it into a new bottle, then add distilled water to 100 mL. The finished Solutions A, B, and C are sterilized using an autoclave.

2.4 Preparation of Nutrient Broth (NB) media

Nutrient Broth (NB) media is made by dissolving 3 g of beef extract and 5 g of peptone in 1000 mL of distilled water, after that cooking until boiling. Then sterilized using an autoclave.

2.5 Making stock of *Chlorella vulgaris* microalgae

Prepare 600 mL of sterile distilled water to which 0.75 mL of Gusrina media has been added. Then 150 mL of *C. vulgaris* culture was added. After that, it was made with the same composition in five other culture bottles. Then wait for the microalgae culture for 7 days of

culture or until it reaches the exponential phase or the cell density increases as indicated by a darker green color.

2.6 Preparation of stocks of nitrogen-fixing bacteria, amylolytic bacteria, and phosphate-solubilizing bacteria

Inoculate 2 doses each of Nitrogen-fixing bacteria, Amylolytic bacteria, and Phosphate-Solubilizing bacteria with an inoculation needle into an erlenmeyer containing 10 mL of NB (Nutrient Borth). Then put in the incubator for 1×24 hours at a temperature of 37^{o}C. Then, bacteria aged 1×24 hours were compared using the McFarland scale, until they reached a cell density of 1.5×10^{8} cfu/mL standard McFarland solution.

2.7 Co-Culture and monoculture treatment of *Chlorella vulgaris* on Guillard media

Prepare a 1 L culture bottle containing 600 mL of sterile distilled water, then add 1 mL of Guillard's medium, then add 75 mL of *C. vulgaris*, then add 25 mL of nitrogen-fixing bacteria, 25 mL of amylolytic bacteria, and 25 mL of phosphate-solubilizing bacteria. After that, the control treatment was *C. vulgaris* monoculture on Guillard media. First, prepare a 1 L culture bottle containing 600 mL of sterile distilled water, then add 1 mL of Guillard's medium, then add 75 mL of *C. vulgaris* and 75 mL of NB medium.

2.8 Co-Culture and monoculture treatment of *Chlorella vulgaris* on Gusrina media

Prepare a 1 L culture bottle containing 600 mL of sterile distilled water, then add 1 mL of Gusrina media, then add 75 mL of *C. vulgaris*, then add 25 mL of nitrogen-fixing bacteria, 25 mL of amylolytic bacteria, and 25 mL of phosphate-solubilizing bacteria. After that, the control treatment was *C. vulgaris* monoculture on Gusrina media. First, prepare a 1 L culture bottle containing 600 mL of sterile distilled water, then add 1 mL of Gusrina media, then add 75 mL of *C. vulgaris*, and 75 mL of NB media.

2.9 Culture Conditions

The culture area is set up by installing a TL lamp as a light source, photoperiod of 16 hours light, 8 hours dark; the aerator machine is set to 12 hours aerator and 12 hours without aerator [19]; The light intensity is set at 2700 lux. A 1 liter glass bottle is arranged on the culture rack, then a hose and aerator stone are attached which will be connected to the aerator tool. The culture treatment bottles that have been arranged are labeled according to each treatment.

2.10 Determination of *Chlorella vulgaris* cell density

C. vulgaris cell density was determined using a Petroff-Hausser counting chamber. Place 1 drop in the center of the counting chamber, then cover with a cover glass, and observe using a microscope at 10×10 magnification. Then, calculate the number of microalgae cell densities in 5 different 1 mm counting chamber blocks, the results of the calculation are entered into the cell density calculation formula, then a growth curve is created.

2.11 Counting bacterial colonies

Dilution was carried out by taking 0.9 mL of sample, then graded dilutions were carried out from 10^{-1} to 10^{-6}. Next, 0.2 mL of sample was taken at a dilution of 10^{-4}, 10^{-5}, and 10^{-6}, put into a petri disk containing PCA media. Then, it was incubated at 37°C for 1×24 hours, then the bacterial colonies that grew on PCA media were counted.

2.12 Data analysis

The data obtained will be analysed using multiple variance analysis, with a significant value (sig.) <0.05, meaning there is a significant difference between groups (independent variables) regarding the dependent variable. Then proceed with the 5% BNT test to find out which treatment gives significantly different results from other treatments.

3 Results and Discussion

Based on the results of the factorial ANOVA statistical test, it shows that the addition of bacteria and the culture media factor show significance values of 0.038 < 0.05 and 0.000 < 0.005, respectively, meaning that there is an influence of the addition of bacteria and culture media on the growth of the *C. vulgaris*.

The co-culture treatment showed the highest growth results compared to the monoculture treatment. Meanwhile, the Gusrina culture media treatment showed higher growth results than the Guillard media treatment. The best treatment combinations were co-culture treatment on Gusrina media, monoculture treatment on Gusrina media, co-culture treatment on Guillard media, and finally monoculture treatment on Guillard media. The results of the analysis of the growth of the microalga *C. vulgaris* can be seen in Fig. 1.

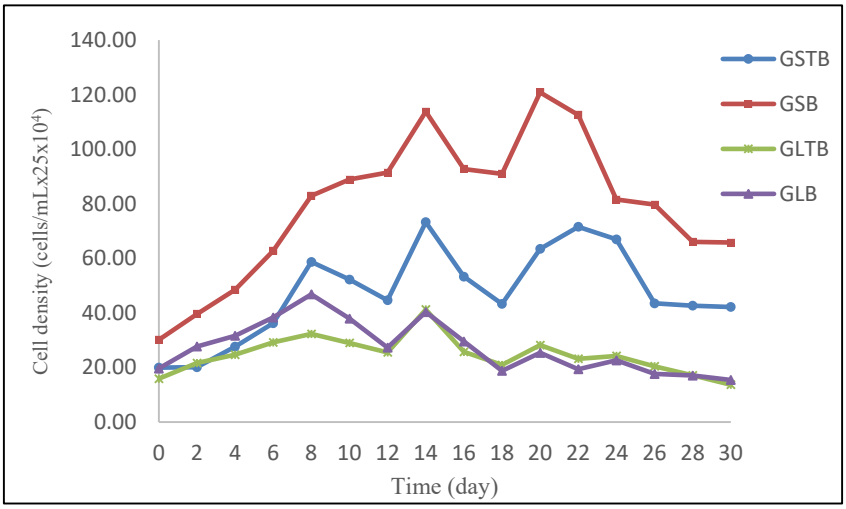

Fig. 1. *Chlorella vulgaris* cell growth rate in each treatment. (GSTB = Gusrina media without bacteria, GSB = Gusrina media with bacteria, GLTB = Guillard media without bacteria, GLB = Guillard media with bacteria).

The growth of *C. vulgaris* starts from the adaptation phase, in the first two days all treatments were still in the lag phase or adaptation phase and did not show a significant increase in growth. The nutritional content of the culture media and environmental conditions that are different from their original environment cause *C. vulgaris* to undergo an adaptation

phase so that it can grow in the next phase. The co-culture treatment on Gusrina media was the treatment that produced the highest number of cells compared to the other three treatments, namely co-culture on Guillard media and monoculture on Gusrina media and Guillard media.

The co-culture treatment on Gusrina media experienced an exponential phase as shown in the graph, which increased from day 4 to day 14 with cell density reaching 2.8×10^7 cells/mL. This increase in cell density was supported by the still high availability of nutrients. Then, after the 14th day, the growth of *C. vulgaris* decreased which was not very significant, and again experienced an increase in growth from the 18th to the 20th day, which was the peak of *C. vulgaris* cell growth, with cell density on the 20th day reaching 3.0×10^7 cells/mL. After the 20th day, cell growth began to decline until the 28th day, and began to enter the stationary phase from the 28th to the 30th day, with the final cell density reaching 1.6×10^7 cells/mL.

The growth of *C. vulgaris* in monoculture treatment on Gusrina media began to experience an increase in growth from day 4 to day 8, with an average cell density reaching 1.4×10^7 cells/mL. Furthermore, after the 8th day, cell growth began to decline until the 12th day. Then, after the 12th day, growth continued to increase until the 14th day, with a cell density reaching 1.8×10^7 cells/mL. After the 14th day there was fluctuation in cell growth, there was an increase and decrease in growth until the 26th day. Then, starting on day 26, cell growth entered the stationary phase until day 30, with the final cell density reaching 1.0×10^7 cells/mL. The highest peak growth of *C. vulgaris* cells was shown on day 14 with a cell density of 1.8×10^7 cells/mL, this result shows that the density of *C. vulgaris* cells cultured with bacteria on Gusrina media is higher than *C. vulgaris* cultures without bacteria on the media Gusrina.

Co-culture treatment on Guillard media showed increased growth from day 2 to day 8, with cell density reaching 1.1×10^7 cells/mL. After the 8th day, the growth rate began to decrease until the 12th day, with a cell density of 6.8×10^6 cells/mL. After day 12, growth again showed an increase until day 14, with a cell density of 1.0×10^7 cells/mL. After the 14th day, cell growth experienced growth fluctuations until the 26th day, then on the 26th day it began to enter the stationary phase until the 30th day, with a final cell density of only around 3.8×10^6 cells/mL. Furthermore, monoculture treatment on Guillard's media began to show increased growth on day 4 to day 8, with cell density reaching 8.0×10^6 cells/mL.

These results show that the number of cells is not higher than the co-culture treatment on Guillard media. Peak growth occurred on day 14, with an average cell density reaching 1.0×10^7 cells/mL. Then, after the 14th day of cell growth experienced growth fluctuations, and after the 24th day the growth rate continued to show a decrease in growth until the 30th day, with an average final cell density reaching 3.4×10^6 cells/mL.

3.1 Effect of addition of potential bacteria on the growth pattern of *Chlorella vulgaris*

The results of this research show that the addition of bacteria to microalgae cultures can increase the growth of *C. vulgaris* microalgae, compared to microalgae cultures without bacteria. In line with research by [20] which reported that bacteria can increase cell density and microalgae biomass. Bacteria are able to provide the nutrients needed by microalgae by remineralizing the organic materials produced by microalgae. The bacterial species used in this research were *Bacillus paramycoides* as nitrogen fixing and amylolytic bacteria, and *Bacillus cereus* as phosphate solubilizing bacteria.

Nitrogen-fixing bacteria are able to convert nitrogen in the atmosphere into ammonium, which is the main source of nitrogen that can be absorbed by microalgae for growth [21]. Phosphorus is an important nutrient after nitrogen for the growth of microalgae. In most

cases, algae can only take up organic phosphorus originating from the hydrolysis of organic phosphorus [22]. Phosphorus availability can be increased by adding phosphate solubilizing bacteria, which can convert organic phosphorus into soluble inorganic forms.

Phosphate solubilizing bacteria will produce phosphatase enzymes which will mineralize most organic phosphorus compounds, and organic phosphorus from damaged algal cells can then be recycled to optimize phosphate availability for microalgae growth [23]. This process has been proven to occur in the bacteria species *Gordonia* sp. and *Burkholderia* sp., which is able to degrade dissolved organic phosphorus into inorganic phosphorus which can be utilized by *Microcystis aeruginosa* for its growth [24].

Our study also use amylolic bacteria to optimize microalgae growth. Amylolytic bacteria will produce amylase enzymes which can hydrolyze starch into various products, including dextrin and small glucose units [25]. The presence of amylolytic bacteria is able to degrade complex compounds into simpler molecules so that they can be absorbed by microalgae to increase their growth. These results are supported by the report of [26] that the bacteria *Pseudomonas* sp., and *Bacillus* sp. showed good amylolytic, proteolytic and lipolytic activity against the growth of microalgae.

The results of this research are also supported by research by [27] who reported that bacteria cultured with microalgae were able to produce hydrolytic enzymes such as lipase, amylase, gelatinase and urease. This exoenzyme activity increases the remineralization process of organic materials by bacteria, which then increases the growth of microalgae [28]. The interaction between bacteria and microalgae is able to create a balance in the nutrient cycle and energy flow, thereby increasing the growth of microalgae [29]. Research by [30] also reported that the biomass of *Botryococcus braunii* increased 1.8 times higher in the presence of endosymbiont bacteria.

In addition, research by [31] also reported that *Chlorella vulgaris* was cultivated together with a consortium of bacteria *Rhizobium* sp., *Hyphomonas* sp., *Terrimonas* sp., *Flavobacterium* sp. and *Mesorhizobium*, showed that the growth rate, cell density and lipid content of *Chlorella vulgaris* were higher compared to microalgae cultures without bacteria. Microalgae and bacteria exchange nutrients including micronutrients such as vitamins and macronutrients such as nitrogen, oxygen and carbon, which can help increase microalgae productivity [32].

During the growth process, microalgae continuously release various metabolites into the surrounding environment [33]. These metabolite products such as carbon, nitrogen, sulfur and iron can be absorbed and reutilized by bacteria to produce inorganic materials that can be utilized by microalgae for their growth [34]. Carbon dioxide that has been converted by bacteria can be utilized by microalgae as the main carbon source for microalgae, then organic material by bacteria can be converted into nitrogen, ammonia, nitrite and nitrate through ammonia and nitrification to provide a nitrogen source for microalgae growth [35]. So, based on the results of this study, it is clear that microalgae cultured with *Bacillus paramycoides* bacteria as nitrogen fixing and amylolytic bacteria, and *Bacillus cereus* bacteria as phosphate soluble bacteria, show better growth and the highest cell density compared to microalgae cultures without bacteria.

3.2 Effect of different culture media on the growth pattern of *Chlorella vulgaris*

The composition of macronutrients and micronutrients in culture media is one of the most important factors influencing the growth parameters and biochemical composition of microalgae [36,37]. The different compositions of Guillard and Gusrina media influence the growth of microalgae. Gusrina media contains urea as a nitrogen source. The use of nitrogen in urea fertilizer is that it can accelerate growth, the formation of proteins and amino acids.

Nitrogen is the most important nutrient that influences growth, biomass production and lipid accumulation of microgae [36].

The nitrogen content in Gusrina media is fully used by the *C.vulgaris* to increase its growth. This statement is supported by the research results of [38] showed that *Chlorella* and *Scenedesmus* microalgae cultured with media containing urea showed a high growth rate compared to other media. Likewise with this research, the urea content in Gusrina media was able to provide a good growth effect compared to Guillard media. In line with the research results reported by [17] showed that the presence of urea content in the culture media was able to increase the growth rate of *Chlorella* sp. Apart from that, research by [39] also showed increased growth and higher biomass production of microalgae in media containing nitrogen.

In addition, Gusrina media contains TSP as a source of phosphate. Phosphorus is another important compound that plays an important role in algal growth, lipid production, fatty acid yield and metabolic processes such as energy transfer, signal transduction and photosynthesis [40,41]. Phosphorus is essential for microalgae cells for the production of cellular components such as phospholipids, DNA, RNA and ATP for metabolic pathways involving energy transfer and nucleic acid synthesis [42]. Thus, by providing sufficient phosphate content in the Gusrina media, it can increase the growth of microalgae cells.

Guillard media also contains various macronutrients and micronutrients. However, the researchers' assumption is that the macronutrient and micronutrient composition of Gusrina media is preferred by microalgae and is more suitable for increasing the growth of *C. vulgaris* microalgae. Apart from that, the growth of microalgae on Guillard media is not optimal, it could be caused by the culture environment on Guillard media which is not always ideal, this can affect the process of nutrient absorption by microalgae. This statement is supported by [43] that phosphorus absorption by microalgae can reach saturation due to limited light, and reduced carbon dioxide and oxygen levels in the culture media.

Microalgae nutritional requirements can be absolute, normal, minimum or optimal. Although many culture media have been designed to cultivate different microalgae, it is very important to continue to have a clear understanding and research on the nutritional requirements for each microalgae species to find out the appropriate culture media for microalgae cultivation techniques on a small and large scale [44]. Based on these results, it can be concluded that the composition of macronutrients and micronutrients in Gusrina media is more suitable and suitable for increasing the growth of the *Chlorella vulgaris* tested.

4 Conclusions

The addition of nitrogen fixing bacteria, amylolytic bacteria, and phosphate solubilizing bacteria effectively increased the growth of *C. vulgaris* microalgae, compared to microalgae culture treatment without bacteria (mono-culture).

The results of this study clearly show the ability of bacteria with various growth enhancing mechanisms to increase the growth of the microalgae *C. vulgaris*. Furthermore, microalgae culture on Gusrina media showed a higher cell density than microalgae culture on Guillard media. Gusrina media is a suitable and suitable medium for cell growth and microalgae biomass productivity, so it can be used for microalgae cultivation on a small or large scale.

We thank the State University of Malang for the research entitled "Optimizing the Growth of Bacterial and Microalgae Consortiums in Co-Culture to Obtain Maximum Energy in Photobioreactors" on number 4. 3. 1709/UN32. 14/KP/ 2020 funded by PNPB UM 2019 under the Center of Excellence for Science and Technology-University Center of Advanced Materials for Renewable Energy (PUI-PT Camry) scheme.

References

1. Sanjaya, E. H. *et al.* Potential of microalgae isolate kelp i (Brawijaya Museum pond) and isolate kelp IV (Selorejo reservoir) as biodiesel feedstock. *AIP Conf. Proc.* **2353**, (2021).
2. da Rosa, M. D. H. *et al.* Macroalgae and Microalgae Biomass as Feedstock for Products Applied to Bioenergy and Food Industry: A Brief Review. *Energies* vol. 16 at https://doi.org/10.3390/en16041820 (2023).
3. Al-zuhair, P. S. Y. P. S. Guest editorial : special issue on ‘“ Microalgae biorefinery : current bottlenecks , challenges , and future directions ”’. *Phytochem. Rev.* **22**, 829–831 (2023).
4. Talebi, A. F. *et al.* Fatty acids profiling: A selective criterion for screening microalgae strains for biodiesel production. *Algal Res.* **2**, 258–267 (2013).
5. Borowitzka, M. A. *Biology of Microalgae*. *Microalgae in Health and Disease Prevention* (Elsevier Inc., 2018). doi:10.1016/B978-0-12-811405-6.00003-7.
6. Kalra, R., Gaur, S. & Goel, M. Journal of Water Process Engineering Microalgae bioremediation : A perspective towards wastewater treatment along with industrial carotenoids production. *J. Water Process Eng.* 101794 (2020) doi:10.1016/j.jwpe.2020.101794.
7. Ramanan, R., Kim, B., Cho, D., Oh, H. & Kim, H. Algae – bacteria interactions : Evolution , ecology and emerging applications. *Biotechnol. Adv.* (2015) doi:10.1016/j.biotechadv.2015.12.003.
8. Cho, D. *et al.* Bioresource Technology Enhancing microalgal biomass productivity by engineering a microalgal – bacterial community. *Bioresour. Technol.* **175**, 578–585 (2015).
9. Teplitski, M. & Rajamani, S. Signal and Nutrient Exchange in the Interactions Between Soil Algae and Bacteria. 413–426 (2011) doi:10.1007/978-3-642-14512-4.
10. Kim, B., Ramanan, R., Cho, D., Oh, H. & Kim, H. ScienceDirect Role of Rhizobium , a plant growth promoting bacterium , in enhancing algal biomass through mutualistic interaction. *Biomass and Bioenergy* **69**, 95–105 (2014).
11. Hernandez, J., Luz, E., Rodriguez, D. J., Rodriguez, Y. & Bashan, Y. Original article Growth promotion of the freshwater microalga Chlorella vulgaris by the nitrogen-fixing , plant growth-promoting bacterium Bacillus pumilus from arid zone soils. *Eur. J. Soil Biol.* **45**, 88–93 (2009).
12. Nisa, I. K., Prabaningtyas, S., Lukiati, B. & Saptawati, R. T. The potential of amylase enzyme activity against bacteria isolated from several lakes in East Java , Indonesia. **22**, 42–49 (2021).
13. Prabaningtyas, S. *et al.* Vertical Distribution of Bacteria in Various Lakes of East Java, Indonesia. *J. Phys. Conf. Ser.* **1093**, (2018).
14. Nafisah, W., Prabaningtyas, S., Witjoro, A., Saptawati, R. T. & Rodiansyah, A. Exploration non-symbiotic nitrogen-fixing bacteria from several lakes in East Java , Indonesia. **23**, 1752–1758 (2022).
15. Prabaningtyas, S. *et al.* The Influence of Dominant Bacteria from Various Lakes of East Java, Indonesia on Chlorella sp. Culture. *IOP Conf. Ser. Earth Environ. Sci.* **276**, (2019).
16. Chilmawati, D. Penggunaan Media Kultur Yang Berbeda Terhadap Pertumbuhan Chlorella sp The Used of Different Culture Medium Effect on The Growth Of Chlorella

Sp. **6**, 71–78 (2010).

17. Hadiyanto, H., Azimatun Nur, M. M. & Hartanto, G. D. Cultivation of chlorella sp. As biofuel sources in palm oil mill effluent (POME). *Int. J. Renew. Energy Dev.* **1**, 45–49 (2012).
18. Yellow-Green Algae. *Van Nostrand's Scientific Encyclopedia* at https://doi.org/10.1002/0471743984.vse7610 (2005).
19. Lirofiatillah *et al.* Effect of differences in the form of photobioreactor prototypes and aeration period on Chlorella sp. Cell growth in co-culture with bacteria. *AIP Conf. Proc.* **2231**, (2020).
20. Xue, L. *et al.* Analysis of growth and lipid production characteristics of Chlorella vulgaris in artificially constructed consortia with symbiotic bacteria. *J. Basic Microbiol.* **58**, 358–367 (2018).
21. Bajpai, R., Prokop, A. & Zappi, M. Algal biorefineries: Volume 1: Cultivation of cells and products. *Algal Biorefineries Vol. 1 Cultiv. Cells Prod.* 1–324 (2014) doi:10.1007/978-94-007-7494-0.
22. Zhu, Y. *et al.* Characterization of organic phosphorus in lake sediments by sequential fractionation and enzymatic hydrolysis. *Environ. Sci. Technol.* **47**, 7679–7687 (2013).
23. Kononova, S. V & Nesmeyanova, M. A. Kononova2002. **67**, 184–195 (2002).
24. Zhao, G. *et al.* The importance of bacteria in promoting algal growth in eutrophic lakes with limited available phosphorus. *Ecol. Eng.* **42**, 107–111 (2012).
25. Zhou, G., Jin, M., Cai, Y. & Zeng, R. Characterization of a thermostable and alkali-stable α-amylase from deep-sea bacterium Flammeovirga pacifica. *Int. J. Biol. Macromol.* **80**, 676–682 (2015).
26. Sureshkumar, S., Jasmin, B., Mujeeb Rahiman, K. M. & Hatha Mohammed, A. A. Growth enhancement of micro algae, Chaetoceros calcitrans and Nannochloropsis oculata, using selected bacterial strains. *Int.J.Curr.Microbiol.App.Sci* **3**, 352–359 (2014).
27. Sandhya, S. V., Preetha, K., Nair, A. V., Antony, M. L. & Vijayan, K. K. Isolation, characterisation and phylogenetic diversity of culturable bacteria associated with marine microalgae from saline habitats of south India. *Aquat. Microb. Ecol.* **79**, 21–30 (2017).
28. Compounds, A. Impact of Microalgae-Bacteria Interactions on the Production of Algal Biomass and. (2016) doi:10.3390/md14050100.
29. Natrah, F. M. I., Bossier, P., Sorgeloos, P., Yusoff, F. M. & Defoirdt, T. Significance of microalgal-bacterial interactions for aquaculture. *Rev. Aquac.* **6**, 48–61 (2014).
30. Tanabe, Y. *et al.* A novel alphaproteobacterial ectosymbiont promotes the growth of the hydrocarbon-rich green alga Botryococcus braunii. *Sci. Rep.* **5**, 1–11 (2015).
31. Cho, D. H. *et al.* Organic carbon, influent microbial diversity and temperature strongly influence algal diversity and biomass in raceway ponds treating raw municipal wastewater. *Bioresour. Technol.* **191**, 481–487 (2015).
32. Khan, M. I., Shin, J. H. & Kim, J. D. The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microb. Cell Fact.* **17**, 1–21 (2018).
33. Kouzuma, A. & Watanabe, K. Exploring the potential of algae/bacteria interactions. *Curr. Opin. Biotechnol.* **33**, 125–129 (2015).
34. Dao, G. H. *et al.* Enhanced microalgae growth through stimulated secretion of indole acetic acid by symbiotic bacteria. *Algal Res.* **33**, 345–351 (2018).

35. Shahid, A. *et al.* Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. *Sci. Total Environ.* **704**, 135303 (2020).
36. Wang, W. *et al.* Medium screening and optimization for photoautotrophic culture of Chlorella pyrenoidosa with high lipid productivity indoors and outdoors. *Bioresour. Technol.* **170**, 395–403 (2014).
37. Lin, T. S. & Wu, J. Y. Effect of carbon sources on growth and lipid accumulation of newly isolated microalgae cultured under mixotrophic condition. *Bioresour. Technol.* **184**, 100–107 (2015).
38. Crofcheck, C. *et al.* Influence of media composition on the growth rate of Chlorella vulgaris and Scenedesmus acutus utilized for CO2 mitigation. *Am. Soc. Agric. Biol. Eng. Annu. Int. Meet. 2012, ASABE 2012* **1**, 532–549 (2012).
39. Yeh, K. L. & Chang, J. S. Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga Chlorella vulgaris ESP-31. *Bioresour. Technol.* **105**, 120–127 (2012).
40. Yang, F., Xiang, W., Li, T. & Long, L. Transcriptome analysis for phosphorus starvation-induced lipid accumulation in Scenedesmus sp. *Sci. Rep.* **8**, 1–11 (2018).
41. Ota, S. *et al.* Deciphering the relationship among phosphate dynamics, electron-dense body and lipid accumulation in the green alga Parachlorella kessleri. *Sci. Rep.* **6**, 1–11 (2016).
42. Atiku, H., Mohamed, R., Al-Gheethi, A., Wurochekke, A. & Kassim, A. H. M. Harvesting of microalgae biomass from the phycoremediation process of greywater. *Environ. Sci. Pollut. Res.* **23**, 24624–24641 (2016).
43. Yaakob, M. A., Mohamed, R. M. S. R., Al-Gheethi, A., Ravishankar, G. A. & Ambati, R. R. Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: An overview. *Cells* **10**, 1–19 (2021).
44. Dhas, A. & Babu, M. Effect of Different Culture Media for the Growth and Oil Yield in Selected Marine Microalgae. **31**, 165–177.