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# Optimasi Media Kultur untuk Pertumbuhan *Chlorella vulgaris* dalam *Co-culture* dengan Bakteri

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## ABSTRAK

Kebutuhan energi bahan bakar di Indonesia terus meningkat, sementara ketersediaan bahan bakar fosil semakin terbatas. Oleh karena itu, diperlukan sumber bahan baku biodiesel terbarukan alternatif, seperti mikroalga *Chlorella vulgaris*. Salah satu strategi untuk meningkatkan biomassa mikroalga adalah melalui budidaya *co-culture* menggunakan bakteri, karena interaksi positif di antara keduanya telah terbukti mampu meningkatkan laju pertumbuhan mikroalga melalui berbagai mekanisme. Sebagai faktor esensial dalam proses budidaya, pemilihan media kultur yang menyediakan nutrisi optimal adalah tahap krusial untuk mendukung pertumbuhan *Chlorella vulgaris* yang maksimal. Penelitian ini bertujuan mengkaji optimasi media kultur dan teknik kokultur bakteri untuk mengoptimalkan pertumbuhan *C. vulgaris*. Kami membandingkan efektivitas dua media kultur (Media Gusrina dan Media Guillard) serta dua taraf penambahan bakteri (monokultur dan kokultur). Budidaya dilakukan fotobioreaktor dengan siklus aerasi 12 jam hidup : 12 jam mati dan siklus penyinaran 16 jam hidup : 8 jam mati. Hasil penelitian menunjukkan bahwa *co-culture* mikroalga-bakteri pada media Gusrina menghasilkan pertumbuhan sel *C. vulgaris* yang paling optimal. Kombinasi bakteri terbaik yang digunakan dalam kokultur adalah bakteri penghasil IAA (*Indole Acetic Acid*), bakteri pelarut fosfat, dan bakteri amilolitik. Kombinasi media dan bakteri ini direkomendasikan untuk pengembangan skala besar biomassa *C. vulgaris* sebagai bahan baku biodiesel.

**Kata kunci:** Bakteri; *Chlorella vulgaris;* media kultur; optimasi

# Optimization Media for the Growth of *Chlorella vulgaris* in Co-Culture with Bacteria

## ABSTRACT

The demand for fuel energy in Indonesia is continuously rising, while the availability of fossil fuels is increasingly limited. Consequently, an alternative renewable biodiesel feedstock, such as the microalga *Chlorella vulgaris*, is urgently needed. One effective strategy to boost microalgal biomass is through co-culture with bacteria, as the positive interactions between the two have been proven to accelerate microalgal growth rates via various mechanisms. As an essential factor in the cultivation process, the selection of a culture medium that provides optimal nutrition is a crucial step to support maximum *C. vulgaris* growth. This study, therefore, aims to evaluate the optimization of both the culture medium and the bacterial co-culture technique to maximize *C. vulgaris* proliferation. We compared the effectiveness of two culture media (Gusrina Medium and Guillard Medium) and two levels of bacterial addition (monoculture and co-culture). Cultivation was performed in a photobioreactor with a 12-hour on : 12-hour off aeration cycle and a 16-hour light : 8-hour dark photoperiod. The results demonstrate that microalgae-bacteria co-culture in Gusrina Medium yielded the most optimal *C. vulgaris* cell growth. The best bacterial

combination utilized in the co-culture consisted of IAA (*Indole Acetic Acid*) producing bacteria, phosphate-solubilizing bacteria, and amylolytic bacteria. This specific combination of medium and bacteria is recommended for the large-scale development of *C. vulgaris* biomass as a sustainable biodiesel feedstock.

**Keywords:** Bacteria; *Chlorella vulgaris;* culture media; optimization

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## INTRODUCTION

Population growth and technological advances have led to an increase in the demand for petroleum-based energy (Gultom, 2018). Meanwhile, gas fuel is expected to run out even faster in 2027 (Wiratmaja & Elisa, 2020). Some efforts that can be made to solve this problem are to use alternative energy, one of which is from microalgae biomass. The macromolecular content in microalgae biomass has been studied to be used as a substitute for fossil fuels or biodiesel (Milano *et al*., 2016). *Chlorella vulgaris* is one of the microalgae that can be used as a biofuel because it contains lipids, besides that its growth is relatively fast and easy (Margono *et al*., 2015). The existence of microalgae in the waters is not alone, but side by side with other microorganisms such as bacteria. The interaction between microalgae and bacteria in aquatic environments occurs naturally (Noerdjito, 2019). These positive interactions can produce growth promoter compounds or compounds that can trigger the growth of microalgae (Fuentes *et al*., 2016). The bacterial strains utilized in this study, which were selected for their potential to accelerate the growth of biofuel-producing microalgae, were initially isolated and explored from four distinct lakes in East Java (Ranu Grati, Ranu Pani, Ranu Regulo, and Ranu Ngebel). Potential bacteria that have been isolated include IAA (*Indole Acetic Acid*) hormone-producing bacteria, phosphate-solubilizing bacteria, amylolytic bacteria, nitrogen-fixing bacteria, and cellulolytic bacteria (Prabaningtyas *et al*., 2019).

The hormone IAA is a growth hormone that can accelerate cell division (Amin *et al*., 2015). IAA hormone-producing bacteria are useful for microalgae as a stimulus for microalgae growth by triggering microalgae cell division (Dao *et al*., 2018; Rahmawati *et al*., 2020). Phosphate also has an important role in the growth of microalgae as a nutrient. In aquatic environments, compound P needs to be dissolved in water in the form of $PO_4^{3-}$ to be absorbed by microalgae (Yaakob *et al*., 2021). Bacteria that can fix nitrogen can convert nitrogen in the environment into ammonium or nitrate forms that can be absorbed by microalgae so that they can accelerate microalgae growth (Radite & Simanjuntak, 2020). Biological nitrogen fixation can only be carried out by prokaryotes, with the help of nitrogenase enzymes (Marcarelli *et al.*, 2022) by catalyzing the conversion of nitrogen ($N_2$) to ammonia ($NH_3$) (Sickerman *et al*., 2017). Amylolytic bacteria are also bacteria that have the potential to help the growth of microalgae cells by hydrolyzing starch or starch into simpler sugars so that microalgae can be used as nutrients (Mawadah & Prabaningtyas, 2024a). Beyond the biological interactions provided by the bacterial consortium, the nutritional content of the growth media must also be optimized.

Nutrient components within the culture medium are a critical factor in microalgal cultivation (Ajayan, 2023). The addition of nutrients to the microalgae culture media is

considered to be the aspect that most influences the quantity of biomass produced by microalgae cultivation (Amalo *et al*., 2019). Optimal application of both macro- and micronutrients is essential for microalgal growth in the culture environment (Ajayan, 2023). Macronutrients such as C, H, N, P, K, S, Mg, and micronutrients including Cu, Fe, Co, Mn, Zn, Mo, Si, are known to enhance microalgal biomass production (Mardalisa *et al*., 2022).

This study compares the use of Gusrina and Guillard media, which possess distinct macro- and micronutrient compositions, yet both are recognized as suitable media for microalgal growth. Previous research indicates that Guillard's medium is superior when compared to Walne's and agricultural fertilizer (Urea) media for microalgal growth (Mardalisa *et al.*, 2022). Despite Gusrina's medium being less frequently utilized in research compared to Guillard's, its positive influence on increasing microalgal growth has been previously documented (Saputri, 2023).

However, studies focusing on cost-effective are limited. This study addresses this gap by comparing the standard Guillard's medium with the practical Gusrina's medium, which is known for its easy accessibility and suitable composition for *Chlorella sp.* growth. Furthermore, this research is novel in its synergistic approach, utilizing a unique local bacterial consortium, isolated from four distinct East Java lakes. Given the broad potential application of *C. vulgaris* as an alternative fuel, it is necessary to conduct a study on the optimization of culture media and this *co-culture* system. The purpose of this study was to evaluate the optimization of both the culture medium and the bacterial *co-culture* technique to maximize *C. vulgaris* proliferation

## RESEARCH METHOD

The research was conducted at the greenhouse and Microbiology Laboratory, State University of Malang. Cultivation was conducted at the greenhouse, while the quantitative analysis of *Chlorella vulgaris* cell density (optical density) and the enumeration of viable bacterial cells (Colony Forming Units or CFU/mL) were performed at the Microbiology Laboratory. This research was carried out from September 2020 to June 2021

### Materials

The tools used in this research include photobioreactor, aerator engine, Erlenmeyer, hotplate, autoclave, culture bottles, measuring cylinder, test tube, incubator, Laminar Air Flow, bunsen burner, ose needle, micropipette 100 µl, micropipette 1000 µl, microtip 100 µl, microtip 1000 µl, McFarland scale, Petroff-Hausser counting chamber, microscope.

The materials used in this study included microalgae *Chlorella vulgaris*, IAA-producing potential bacteria, phosphate-solubilizing bacteria, amylolytic bacteria, nitrogen-fixing bacteria, and cellulolytic bacteria from four distinct lakes in East Java (Ranu Grati, Ranu Pani, Ranu Regulo, and Ranu Ngebel), aquadest, urea, Triple Super Phosphate (TSP), $FeCl_3$, vitamin B12, $NaH_2PO_4.2H_2O$, $NaNO_3$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, $CoCl_2.6H_2O$, $MnCl_2.4H_2O$, $Na_2MoO_4.2H_2O$, $Na_2EDTA$, $FeCl_3.6H_2O$, thiamin HCl, biotin, alcohol 70%, Nutrient Broth.

### Methods

**1.** Gusrina's media production

Gusrina's medium was prepared by first dissolving the inorganic components into 500 mL of Aquadest. The components included 22.5 grams of Urea, 15 grams of Triple Super

Phosphate (TSP), and 0.5 grams of $FeCl_3$. The mixture was heated to boiling, dispensed into an Erlenmeyer flask, and subsequently sterilized using an autoclave. Following sterilization, 0.0125 grams of Vitamin B12 was added aseptically to the medium (Mawadah & Prabaningtyas, 2024).

**2.** Guillard's media production

The composition for making Guillard's media was modified from Guillard & Ryther (1962). Guillard's media was prepared by dissolving solution A, which consisted of 0.5 g $NaH_2PO_4.2H_2O$ and 7.5 g $NaNO_3$, in 100 ml of Aquadest, then dissolved the PS solution components, which consisted 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 Aquadest. Solution B was prepared by dissolving 0.436 g $Na_2EDTA$ and 0.315 g $FeCl_3.6H_2O$ in 100 ml of Aquadest. Then, 0.1 ml of each PS solution component was taken, then put into solution B. Then solution C was made, by preparing 1 g thiamin HCl, 0.005 g biotin, and 0.01 g vitamin B12, then each substance was dissolved into 50 ml of Aquadest. Then, 0.5 ml of each component of solution C was taken, and put into a new bottle, then Aquadest was added to 100 ml. Solutions A, B, and C were autoclaved for sterilization (Guillard & Ryther, 1962).

**3.** Preparation of microalgae stock

The preparation of microalgae stock was carried out by preparing 150 ml of *Chlorella vulgaris* culture; and then adding 0.75 ml of modified media and 600 ml of sterile Aquadest; made with the same composition in five other culture bottles. The microalgae culture is waited for until it reaches the exponential phase or the cell density increases which is indicated by an increasingly dense green color.

**4.** Preparation of bacterial stock.

IAA-producing potential bacteria, phosphate-solubilizing bacteria, amylolytic bacteria, nitrogen-fixing bacteria, cellulolytic bacteria were prepared by dissolving 2 ose of bacteria in 10 ml of Nutrient Broth medium. The Erlenmeyer tube was then tightly closed and placed in the incubator for 1x24 hours. The density of bacterial cells aged 1x24 hours was measured using the McFarland scale. The test tube is filled with bacterial stock and diluted with NB medium until it reaches a cell density of $1.5 \times 10^8$ cfu/ml of 0.5 Mc Farland standard solution.

**5.** Culture treatment.

The experiment utilized six replications per treatment. Cultures were conducted in 1 L culture bottles (7 cm diameter) containing the media compositions specified in Table 1. Photobioreactor conditions were precisely regulated, employing an intermittent aeration cycle (12 hours OFF : 12 hours ON) and a fixed photoperiod (16 hours light : 8 hours dark) (Lirofiatillah et al., 2020).

**Table 1.** Combination treatment of culture media and culture composition

| Treatment | Composition |
|---|---|
| GSB + combination 1 | 600 mL Aquadest + 75 mL *C. vulgaris* + 0.75 mL Gusrina's media + bacteria combination 1 (25 mL each) |
| GLB + combination 1 | 600 mL Aquadest + 75 mL *C. vulgaris* + 0.75 mL Guillard's medium + bacteria combination 1 (25 mL each) |
| GSB + combination 2 | 600 mL Aquadest + 75 mL *C. vulgaris* + 0.75 mL Gusrina's media + bacteria combination 3 (25 mL each) |
| GLB + combination 2 | 600 mL Aquadest + 75 mL *C. vulgaris* + 0.75 mL Guillard's medium + bacteria combination 3 (25 mL each) |
| GSB + combination 3 | 600 mL Aquadest + 75 mL *C. vulgaris* + 0.75 mL Gusrina's media + bacteria combination 4 (25 mL each) |
| GLB + combination 3 | 600 mL Aquadest + 75 mL *C. vulgaris* + 0.75 mL Guillard's medium + bacteria combination 4 (25 mL each) |
| Control GSB | 600 mL Aquadest + 75 mL *C. vulgaris* + 0.75 mL of Gusrina's medium + 75 ml of Nutrient Broth medium |
| Control GLB | 600 mL Aquadest + 75 mL *C. vulgaris* + 0.75 mL of Guillard's medium + 75 mL of nutrient Broth medium |

Notes: GSB + combination 1: treatment of Gusrina's media and combination of IAA bacteria, phosphate-solubilizing bacteria, and cellulolytic, GLB + combination 1: treatment of Guillard's media and combination of IAA bacteria phosphate-solubilizing bacteria, and cellulolytic, GSB + combination 2: Gusrina's media treatment and combination of IAA bacteria, phosphate-solubilizing bacteria, and nitrogen-fixing bacteria, GLB + combination 2: Guillard's media treatment and combination of IAA bacteria, phosphate-solubilizing bacteria, and nitrogen-fixing bacteria, GSB + combination 3: Gusrina's media treatment and combination of IAA bacteria, phosphate-solubilizing bacteria, and amylolytic, GLB + combination 3: treatment of Guillard's media and combination of IAA bacteria, phosphate-solubilizing bacteria, and amylolytic.

**6.** Calculation of the number of cells of *Chlorella vulgaris*.
The number of *Chlorella vulgaris* cells was counted using the Petroff-Hausser counting chamber, every two days for 30 days. Calculation of the number of *Chlorella vulgaris* cells was carried out by measuring the density of *Chlorella vulgaris* cells which were calculated in units per one ml per box of counting chambers, the average multiplied by $25x10^4$ in units of cells/mL.

**Data analysis**

Data were initially analyzed using descriptive statistics to plot the growth curve profiles of *C. vulgaris* cells across all treatments. Inferential testing of the effects of the Culture Medium and Bacterial Combination factors on maximum cell density was performed using a Two-Way Analysis of Variance (ANOVA). Prior to ANOVA, the assumption of variance homogeneity was assessed via the Levene's Test. Where significant effects were identified, a Post-Hoc test (Tukey's HSD) was applied to pinpoint specific differences among the treatment combinations.

## RESULTS AND DISCUSSION

This research employed a factorial experiment in a Completely Randomized Design (CRD). The study utilized two primary treatment factors: Culture Medium (Guillard's and Gusrina's) and Culture Composition (a total of four levels, comprising three bacterial combinations and a non-bacterial control). The three tested bacterial consortia were: Combination 1 (IAA bacteria, phosphate-solubilizing bacteria, and cellulolytic bacteria), Combination 2 (IAA bacteria, phosphate-solubilizing bacteria, and nitrogen-fixing bacteria), and Combination 3 (IAA bacteria, phosphate-solubilizing bacteria, and amylolytic bacteria). Culturing these combinations across both media resulted in eight treatment groups (Figure 1).

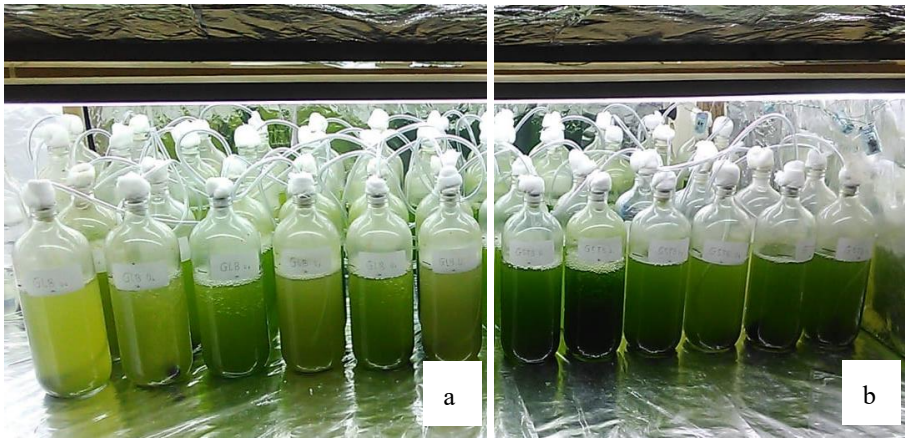


**Figure 1.** Treatment on microalgae culture in experimental unit. a) GLB: Guillard's medium. b) GSB: Gusrina's medium.

The Two-Way ANOVA analysis demonstrated that the overall experimental model significantly accounted for the variation in microalgal cell density ($p < 0.001$), with a high coefficient of determination ($R^2 = 0.843$). Both independent factors, Culture Medium ($p < 0.001$) and Bacterial Combination ($p < 0.001$), had a highly significant effect on cell density. Furthermore, a significant interaction effect was observed between the Culture Medium and the Bacterial Combination ($p = 0.001$). This interaction indicates that the synergistic influence of the bacterial consortia on cell density is highly dependent on the specific culture medium utilized.

The statistical findings were corroborated by the observed growth profiles, with the Gusrina's medium treatment coupled with Bacterial Combination 3 (a combination of IAA-producing, phosphate-solubilizing, and amylolytic bacteria) yielding the optimal growth. This specific combination reached the peak cell density of approximately $2.70 \times 10^7$ cells/mL on the $20^{th}$ day (Figure 2). The growth curve for the optimal Gusrina's medium treatment exhibited a distinctive pattern, commencing with a brief lag phase from Day 0, followed by significant fluctuations up to Day 14. An initial increase in cell count occurred between Day 0 and Day 4, succeeded by a temporary decrease on Day 6. Cell numbers then increased slightly by Day 8, followed by a drastic surge until Day 10, before a decline was observed again until Day 14. After this initial adaptation period, cell growth entered a stabilized exponential (log) phase after Day 14, culminating in the maximum density on Day 20.

Subsequently, cell proliferation entered the death phase after Day 20, showing a decline through Day 30.

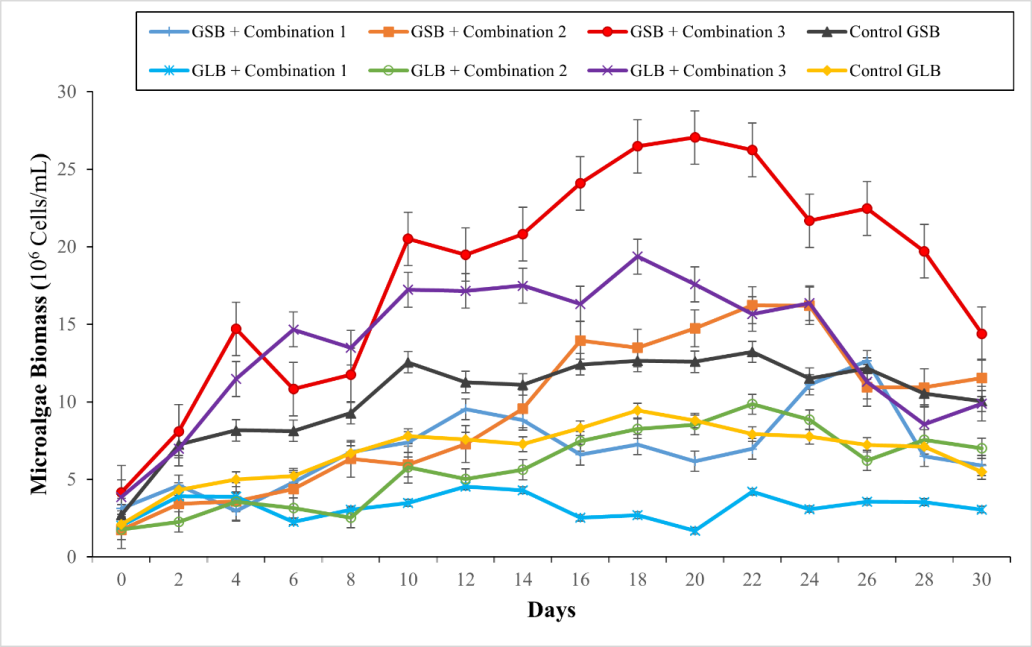


**Figure 2.** *Chlorella vulgaris* cell growth in GSB (Gusrina's medium) and GLB (Guillard's medium)

All other bacterial combination treatments (Combination 1 and Combination 2) also consistently showed better growth kinetics on Gusrina's medium than on Guillard's medium. For instance, Combination 2 (nitrogen-fixing bacteria) peaked later, on the 22$^{nd}$ day, while Combination 1 (cellulolytic bacteria) peaked on the 26$^{th}$ day on Gusrina's medium, demonstrating its lowest point on the 6$^{th}$ day in Guillard's medium. Even Gusrina's medium without bacteria (control) showed a superior growth rate compared to Guillard's treatments, confirming the overall superiority of Gusrina's medium for microalgal culture.

The observed difference in performance is likely attributable to the macronutrient and micronutrient composition of the culture media. The composition of macronutrients and micronutrients in culture media is one of the most important factors affecting growth parameters and biochemical composition of microalgae (Chowdury *et al*., 2020). In Gusrina's media, the nitrogen source used was urea while in Guillard's media, the nitrogen source used was sodium nitrate ($NaNO_3$). In Agwa & Abu's (2016) study, the nitrogen source of urea produced the highest biomass and lipid content compared to $NaNO_3$ and $KNO_3$ in *Chlorella vulgaris* (Agwa & Abu, 2016). This suggests that urea is an effective nitrogen source for the cell growth and biomass production of microalgae (Wu *et al*., 2015; Chen *et al.*, 2020). Furthermore, urea catabolism leads to the final formation of two bicarbonate ions in the culture medium, which can be used as a source of $HCO_3^-$ for microalgae photosynthesis (Rosa *et al*., 2023). This means that the urea utilized in Gusrina's medium serves a dual purpose, providing nitrogen while simultaneously offering a bioavailable carbon source for microalgal assimilation.

Gusrina's media contains TSP as a source of phosphate. Phosphorus is another important compound that plays an important role in algae growth, lipid production, fatty acid

yield, and metabolic processes such as energy transfer, signal transduction, and photosynthesis (Yang *et al.,* 2018). Phosphate content in Gusrina's media can increase the growth of microalgae cells. In Guillard's media, there is also a phosphate content, but the researchers assume that the absorption of phosphate in Guillard's media is not optimal. This is supported by the statement of Yaakob *et al*. (2021) that absorption of phosphorus by microalgae can reach saturation due to limited light, and reduced levels of carbon dioxide and oxygen in the culture media (Yaakob *et al*., 2021).

Gusrina's media is also equipped with micronutrients such as Vitamin B12, which can increase the growth of microalgae. Vitamin B12 is important for microalgae metabolism, but microalgae are unable to produce vitamins, so macronutrients such as vitamins can be added to culture media for microalgae growth (Tandon *et al.,* 2017). Vitamin B12 in culture media can act as a factor stimulating the growth of microalgae (Helliwell *et al*., 2016). The composition of the two media also uses different amounts of $FeCl_3$. In Guillard's media, 3.15 gr/L $FeCl_3$ was used, while in Gusrina's media, 1 ppm $FeCl_3$ was used. Iron acts as a redox catalyst in photosynthesis and nitrogen assimilation, and plays a role in electron transport reactions in photosynthetic organisms, to increase the photosynthetic process of microalgae, the growth rate of microalgae will also increase (Juneja *et al*., 2013).

While the optimized inorganic and micronutrient composition of the culture media provides the necessary foundational support, the ultimate success of the cultivation system relies on the beneficial biotic interactions introduced through the bacterial co-culture. The addition of potential bacteria to microalgae-bacterial cocultures in three culture media optimization studies showed that all combinations of bacteria were able to optimize microalgae growth, with the best growth in combinations of IAA-producing bacteria, phosphate-solubilizing bacteria, amylolytic bacteria. The addition of bacteria can increase the growth of *Chlorella vulgaris* because the presence of these potential bacteria can provide the nutrients needed by microalgae and remineralize organic materials produced by microalgae to optimize the growth of *Chlorella vulgaris*. Microalgae and bacteria synergistically influence each other's physiology and metabolism, and there have been many studies showing the positive effects of microalgae and bacteria symbiosis on microalgae growth (Fuentes *et al*., 2016).

IAA-producing bacteria are bacteria that produce the hormone Indole Acetic Acid (IAA). In the positive microalgae-bacteria interaction, microalgae produce the precursor Tryptophan (Trp). This precursor can be produced by microalgae through Trp synthase and then gives a signal to bacteria to produce IAA hormones (Lin *et al.,* 2022). This hormone acts as a pivotal growth regulator in microalgae and cyanobacteria, simultaneously driving cell proliferation, enhancing the uptake efficiency of critical nutrients (nitrogen and phosphorus), and improving tolerance to stressors by stimulating the accumulation of primary metabolites (Abinandan *et al*., 2019; Fathy *et al*., 2023; Gauthier *et al*., 2020).

Phosphate-solubilizing bacteria are bacteria that can dissolve phosphate. Phosphate (P) is an important maconutrient component and is needed by microalgae for growth, lipid production, and plays a role in microalgae metabolic processes such as photosynthesis (Ota *et al.,* 2016; Yang *et al*., 2018). The P component in organic form cannot be directly absorbed by cells, this component must be hydrolyzed or converted into polyphosphate or orthophosphate to be absorbed by cells (Yaakob *et al*., 2021).

Amylolytic bacteria support the growth of *Chlorella vulgaris* by facilitating nutrient recycling: they hydrolyze starch released from lysed cells during the adaptation phase into simpler sugars, which are then re-assimilated by the microalga for growth (Mawadah & Prabaningtyas, 2024). Nitrogen-fixing bacteria concurrently play a vital role by converting atmospheric or environmental nitrogen ($N_2$) into readily absorbable inorganic forms. These available forms, specifically ammonium ($NH_4^+$) or nitrate ($NO_3^-$), are then utilized by the microalga to ensure optimal nitrogen supply for proliferation (Contreras-Angulo *et al.,* 2019; Radite & Simanjuntak, 2020).

## CONCLUSION

Based on the comparative study conducted using Guillard's medium and Gusrina's medium, the Gusrina nutrient formulation proved to be the most effective in supporting optimal *Chlorella vulgaris* proliferation. Furthermore, the best strategy for maximizing microalgal growth was the application of a synergistic combination of bacteria possessing the capacity to produce IAA (Indole Acetic Acid), solubilize phosphate, and amylolytic activity.

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## REFERENCES

Abinandan, S., Subashchandrabose, S.R., Venkateswarlu, K., & Megharaj, M. (2019). Soil microalgae and cyanobacteria: the biotechnological potential in the maintenance of soil fertility and health. *Critical Reviews in Biotechnology*, *39*(8), 981–998. doi:10.1080/07388551.2019.1654972

Agwa, O., & Abu, G. (2016). Influence of Various Nitrogen Sources on Biomass and Lipid Production by Chlorella vulgaris. *British Biotechnology Journal*, *15*(2), 1–13. doi:10.9734/bbj/2016/21727

Ajayan KV. (2023). Microalgal Cultivation: Cultivation Media and its Supplementary Compounds. *Journal of Food and Agriculture Research*, *3*(2), 145–167. doi:10.47509/JFAR.2023.v03i02.04

Amalo, D., Gaol, M. L., & Beribe, H. D. (2019). Pengaruh Konsentrasi Air Kelapa Terhadap Pertumbuhan Mikroalga *Chlorella vulgaris*. *Jurnal Biotropikal Sains*, *16*(1), 28–39.

Amin, S.A., Hmelo, L.R., Van Tol, H.M., Durham, B.P., Carlson, L.T., Heal, K.R., & Armbrust, E.V. (2015). Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature*, *522*(7554), 98–101. doi:10.1038/nature14488

Chen, J., Jiang, X., & Wei, D. (2020). Effects of urea on cell growth and physiological response in pigment biosynthesis in mixotrophic Chromochloris zofingiensis. *Journal of Applied Phycology*, *32*(3), 1607–1618. doi:10.1007/s10811-020-02114-3

Chowdury, K.H., Nahar, N., & Deb, U.K. (2020). The Growth Factors Involved in Microalgae Cultivation for Biofuel Production: A Review. *Computational Water, Energy, and Environmental Engineering*, *09*(04), 185–215. doi:10.4236/cweee.2020.94012

Contreras-Angulo, J.R., Mata, T.M., Cuellar-Bermudez, S.P., Caetano, N.S., Chandra, R., Garcia-Perez, J.S., & Parra-Saldivar, R. (2019). Symbiotic co-culture of Scenedesmus sp. and Azospirillum brasilense on N-deficient media with biomass production for biofuels. *Sustainability (Switzerland)*, *11*(3). doi:10.3390/su11030707

Dao, G.H., Wu, G.X., Wang, X.X., Zhang, T.Y., Zhan, X.M., & Hu, H.Y. (2018). Enhanced microalgae growth through stimulated secretion of indole acetic acid by symbiotic bacteria. *Algal Research*, *33*, 345–351. doi:10.1016/j.algal.2018.06.006

Fathy, W.A., AbdElgawad, H., Hashem, A.H., Essawy, E., Tawfik, E., Al-Askar, A. A., & Elsayed, K.N.M. (2023). Exploring Exogenous Indole-3-acetic Acid's Effect on the Growth and Biochemical Profiles of Synechocystis sp. PAK13 and Chlorella variabilis. *Molecules*, *28*(14). doi:10.3390/molecules28145501

Fuentes, J.L., Garbayo, I., Cuaresma, M., Montero, Z., González-Del-Valle, M., & Vílchez, C. (2016). Impact of microalgae-bacteria interactions on the production of algal biomass and associated compounds. *Marine Drugs*. MDPI AG. doi:10.3390/md14050100

Gauthier, M.R., Senhorinho, G.N.A., & Scott, J. A. (2020). Microalgae under environmental stress as a source of antioxidants. *Algal Research*, *52*, 102104. doi:10.1016/j.algal.2020.102104

Guillard, R.R.L., & Ryther, J.H. (1962). Studies of Marine Planktonic Diatoms: I. Cyclotella Nana Hustedt and Detonula Confervacea (CLEVE) Gran. *Canadian Journal of Microbiology*, *8*(2), 229–239. doi:10.1139/m62-029

Gultom, S.O. (2018). Mikroalga: Sumber Energi Terbarukan Masa Depan. *Jurnal Kelautan: Indonesian Journal of Marine Science and Technology*, *11*(1), 95. doi:10.21107/jk.v11i1.3802

Helliwell, K.E., Lawrence, A.D., Holzer, A., Kudahl, U.J., Sasso, S., Kräutler, B., & Smith, A.G. (2016). Cyanobacteria and Eukaryotic Algae Use Different Chemical Variants of Vitamin B12. *Current Biology*, *26*(8), 999–1008. doi:10.1016/j.cub.2016.02.041

Juneja, A., Ceballos, R M., & Murthy, G.S. (2013). Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review. *Energies*. MDPI AG. doi:10.3390/en6094607

Lin, H., Li, Y., & Hill, R. T. (2022). Microalgal and bacterial auxin biosynthesis: implications for algal biotechnology. *Current Opinion in Biotechnology*, *73*, 300–307. doi:10.1016/j.copbio.2021.09.006

Lirofiatillah, Prabaningtyas, S., Saptasari, M., Aridowi, D., Marisahaniulfah, M., Listyorini, D., & Suyono, E. A. (2020). Effect of differences in the form of photobioreactor prototypes and aeration period on *Chlorella* sp. Cell growth in co-culture with bacteria. In *AIP Conference Proceedings* (Vol. 2231). American Institute of Physics Inc. doi:10.1063/5.0002527

Marcarelli, A.M., Fulweiler, R.W., & Scott, J. T. (2022). Nitrogen fixation: A poorly understood process along the freshwater-marine continuum. *Limnology And Oceanography Letters*, *7*(1), 1–10. doi:10.1002/lol2.10220

Mardalisa, M., Zalfiatri, Y., & Rahmayuni, R. (2022). The Effect of Culture Media Types on the Growth of Marine Microalgae *Chlorella vulgaris*. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1118). Institute of Physics. doi:10.1088/1755-1315/1118/1/012029

Margono, M., Pranolo, S.H., & Dyartanti, E.R. (2015). Profil Fermentasi pada Produksi Minyak Mikroalga Meggunakan *Nannochloropsis oculata* Dalam Medium BG-11. *EKUILIBIUM*, *14*(2). doi:10.20961/ekuilibrium.v14i2.2050

Mawadah, I., & Prabaningtyas, S. (2024a). 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*. In *BIO Web of Conferences* (Vol. 117). EDP Sciences. doi:10.1051/bioconf/202411701045

Mawadah, I., & Prabaningtyas, S. (2024b). 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*. In *BIO Web of Conferences* (Vol. 117). EDP Sciences. doi:10.1051/bioconf/202411701045

Milano, J., Ong, H.C., Masjuki, H.H., Chong, W.T., Lam, M.K., Loh, P.K., & Vellayan, V. (2016). Microalgae biofuels as an alternative to fossil fuel for power generation. *Renewable and Sustainable Energy Reviews*, *58*, 180–197. doi:10.1016/j.rser.2015.12.150

Noerdjito, D. R. (2019). Interaksi Mikroalga-Bakteri dan Peranannya dalam Produksi Senyawa dalam Kultur Mikroalga. *OSEANA*, *44*(2), 25–34. doi:10.14203/oseana.2019.vol.44no.2.48

Ota, S., Yoshihara, M., Yamazaki, T., Takeshita, T., Hirata, A., Konomi, M., … Kawano, S. (2016). Deciphering the relationship among phosphate dynamics, electron-dense body and lipid accumulation in the green alga Parachlorella kessleri. *Scientific Reports*, *6*. doi:10.1038/srep25731

Radite, S., & Simanjuntak, B. H. (2020). Penggunaan asam humat sebagai pelapis urea terhadap pertumbuhan dan hasil tanaman pakcoy (*Brassica rapa* L.) Humic acid application as urea coating on growth and yield of pakcoy (*Brassica rapa* L.). *AGRILAND Jurnal Ilmu Pertanian*, *8*(1), 72. Retrieved from https://jurnal.uisu.ac.id/index.php/agriland

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. doi:10.13005/bbra/2808

Rosa, R.M., Machado, M., Vaz, M.G.M.V., Lopes-Santos, R., Nascimento, A G. do, Araújo, W.L., & Nunes-Nesi, A. (2023). Urea as a source of nitrogen and carbon leads to increased photosynthesis rates in Chlamydomonas reinhardtii under mixotrophy. *Journal of Biotechnology*, *367*, 20–30. doi:10.1016/j.jbiotec.2023.03.009

Saputri, D. A. E. (2023). Pengaruh perbedaan suhu terhadap pertumbuhan sel Chlorella Vulgaris yang dikultur dengan bakteri pemfiksasi nitrogen dan penghasil IAA (Indole Acetic Acid). Universitas Negeri Malang, Malang.

Sickerman, N.S., Hu, Y., & Ribbe, M.W. (2017). Nitrogenase Assembly: Strategies and Procedures (Vol. 595, pp. 261–302). Methods in Enzymology. doi:10.1016/bs.mie.2017.07.006

Tandon, P., Jin, Q., & Huang, L. (2017, November 28). A promising approach to enhance microalgae productivity by exogenous supply of vitamins. *Microbial Cell Factories*. BioMed Central Ltd. doi:10.1186/s12934-017-0834-2

Wiratmaja, I.G., & Elisa, E. (2020). Kajian Peluang Pemanfaatan Bioetanol Sebagai Bahan Bakar Utama Kendaraan Masa Depan Di Indonesia. *Jurnal Pendidikan Teknik Mesin Undiksha*, *8*(1), 1–8. doi:10.23887/jptm.v8i1.27298

Wu, X., Yan, Y., Wang, P., Ni, L., Gao, J., & Dai, R. (2015). Effect of urea on growth and microcystins production of Microcystis aeruginosa. *Bioresource Technology*, *181*, 72–77. doi:10.1016/j.biortech.2015.01.035

Yaakob, M.A., Mohamed, R.M S.R., Al-Gheethi, A., Ravishankar, G.A., & Ambati, R.R. (2021). Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: An overview. *Cells*. MDPI. doi:10.3390/cells10020393

Yang, F., Xiang, W., Li, T., & Long, L. (2018). Transcriptome analysis for phosphorus starvation-induced lipid accumulation in Scenedesmus sp. *Scientific Reports*, *8*(1). doi:10.1038/s41598-018-34650-x