# Nutritious and Low-Cost Alternate for Marine Microalgae Growth

Nur Sista Maharani[1], Wahyunanto Agung Nugroho[2], Muhammad Hanaul Rofiq[2], Arrahmadiana Estuwilujeng[2], and Yusuf Wibisono[2,3](✉)

[1] Agricultural and Biosystems Engineering, Universitas Brawijaya, Jl. Veteran No. 1, Malang 65145, Indonesia
[2] Bioprocess Engineering, Universitas Brawijaya, Jl. Veteran No. 1, Malang 65145, Indonesia
Y_Wibisono@ub.ac.id
[3] MILI Institute for Water Research, Kawasan Industri Jababeka, Bekasi 17530, Indonesia

**Abstract.** Demand for food consumption is increase rapidly in the coming decades. Microalgae could substitute conventional crop products for food application. Single cell protein from microalgae gain more attention due to low-cost but swift production. During microalgae cultivation, some parameters must be considered, e.g. light intencity, temperature, oxygen dan carbon dioxide, acidity, salinity, and nutrients. Several cultivation media are commonly used in the microalgae cultivation process. There are many uses of microalgae in the food sector, not limited only to feed. So that the use of fertilizer media used during microalgae cultivation must also be considered so that it is not harmful to humans. One of them is using AB Mix hydroponic fertilizer. The purpose of this cultivation is to provide raw materials for microalgae which can then be used for further research. In the microalgae cultivation process, color changes indicate that the density of microalgae cells has increased. If the cell density is high, the resulting color will be more visible. In *Chlorella vulgaris*, the growth increased rapidly on the 10th day compared to Bold's Basal Medium. In *Spirulina sp.* Microalgae, growth increased rapidly on 3$^{rd}$ day compared to Bold's Basal Medium. Meanwhile, the microalgae *Dunaliella salina* showed a gradual increase in growth on 6$^{th}$ day compared to Bold's Basal Medium.

**Keywords:** marine microalgae · cultivation · AB mixed · Chlorela vulgaris · Dunaliela salina · Spirullina

## 1 Introduction

Microalgae are photosynthetic microorganisms that are abundant in Indonesian waters, so they can be said to be a type of potential biodiversity. The cell wall structure of microalgae is very simple and these cells are both prokaryotic and eukaryotic. Microalgae has millions of species, many of which have yet to be identified and cultivated. To survive in water, it needs light, carbon dioxide, $H_2O$, and the major nutrients in the form of nitrogen and phosphorus. Through photosynthesis, microalgae convert these compounds into energy for growth and reproduction. The main components of microalgae cells


Y. A. Yusran et al. (Eds.): BIC 2022, AEBMR 235, pp. 583–594, 2023.
https://doi.org/10.2991/978-94-6463-140-1_58

are lipids, proteins, and carbohydrates. The accessory component of microalgae cells includes phycobilin pigments, chlorophyll, and carotenoids.

Microalgae are found in fresh water and seawater and carry out photosynthesis for their food because they are autotrophs. Microalgae are types of cells that separate alone or in groups. Depending on the type, the size can range from a few micrometers (μm) to several hundred micrometers. Unlike other plants, microalgae are without roots, stems, and leaves. Microalgae can do photosynthesis, produce oxygen, and take in environmental carbon dioxide, to reduce the greenhouse effect and minimize the appearance of global warming [1].

The algal species that are generally used in food, health foods, food supplements, and nutraceutical elds are *Spirulina platensis, Chlorella vulgaris, Daniella salina, Aphanizomenon zilches- aquae, Schizochytrium,* and *Haematococcus pluvialis* [2] but *Spirulina* and *Chlorella* are most prominent due to available expansive exploration, and wide range of composites, especially proteins, that are applied in the food and drug diligence as healthier food products [2]. *Spirulina* is the most well- known and applied due to its macro- andmicro-nutrients including complete and digestible proteins with all essential amino acids and unnecessary amino acids, adipose acids, vitamins and minerals [3]. Chlorella is also a potent asset in mortal health and nutrition due to nutritive contents where it can lower oxidative stress, boosts the vulnerable system, and health- promoting factor by controlling other kinds of diseases similar as injuries, constipation, and anemia, among others [4].

For case, the nutritive values of algal coffers are advanced compared to a sh mess because they contain digestible complete proteins, lipids, and carbohydrates, as well as micronutrients [5]. The algal deduced foods, especially Spirulina, have been traditionally considered and employed as a potent source of proteins for numerous times in some countries, including Japan, Chile, and North America [6]. Lately, scientic studies show that algal coffers are an acceptable and sustainable source of bioactive motes, especially for enhancing the nutritive and functional quality of foods [7].

This research highlights some global challenges hindering the achievement of sustainable development and describes algal coffers inputs in the resolution of these pressing global challenges and achieving sustainable development. Natural coffers are continuing to be depleted while the global population continues to increase [8]. This imbalance is the reason why experimenters are interested in the exploitation and operation of renewable natural coffers for unborn betterment.

Nutrient-rich foods (with all essential nutrients), also nominated as rainbow food or complete food, help help and control colorful conditions due to their physiological conditioning. Taking daily complete food can ameliorate the tone- protection and tone-mending capacity of the body, therefore leading to a healthy body [9]. Pertaining to the available scienti c literature, and some algal deduced products available on the global requests with prominent health creation; algal coffers are one of the implicit natural sources of food with essential nutrients [10], that can contribute to the achievement of the vacuity of nutrient-rich foods with health bene ts, and zero hunger thing. Algal coffers are considered one of the promising and sustainable coffers to be exploited in colorful artificial elds [11], due to their enormous biochemical composition including essential nutrients, functional constituents of food and medicinals [10]. Algal coffers

parade several advantages that makes them part of the presumptive inputs to achieve sustainable development [12].

Because of the mentioned advantages and implicit, algal coffers have bedazzled the attention of numerous experimenters for their unstintingly exploitation and application in resolving some life challenges. The integration of algal- deduced functional foods and nutraceuticals in food security and nutrition would be considered as one volition intervention to promote mortal health and quality of life.

In process of multiplying the culture, the cultivation process is carried out by taking into account several factors such as light intensity, temperature, temperature, oxygen, carbon dioxide, pH, salinity, stirring, and nutrients. Sometimes a small amount of biomass produces the desired product in large quantities, for that, it is necessary to optimize a balanced composition between the amount of biomass and the number of products in the microalgae biomass, one of which is paying attention to the nutrients given to the microalgae. Nutrients are key factors in the production of algal biomass. Most microalgae require macronutrients such as carbon, (C), nitrogen (N), hydrogen (H), sulfur (S), potassium (K), magnesium (Mg), and phosphorus (P). Metabolism. The presence of micronutrients cannot be replaced by other substances.

Currently, several cultivation media are commonly used in the microalgae cultivation process. There are many uses of microalgae in the food sector, not limited only to feed. So that the use of fertilizer media used during microalgae cultivation must also be consider so that it is not harmful to humans. One of them is using AB Mix hydroponic fertilizer.

AB Mix hydroponic fertilizer is often used in hydroponic cultivation that's is divided into two liquids, namely liquid A and liquid A. Liquid A contains macronutrient elements such as N (nitrogen), P (phosphorus), K (potassium), Mg (magnesium), etc. Liquid B contains micro-nutrients such as Fe (iron), Cu (copper), and others.

## 2 Material and Methods

### 2.1 Materials

This research was carried out at the Bioprocess Engineering Laboratory and the Food and Agricultural Product Processing Technology Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya. This research was conducted in August-September 2022. The purpose of this cultivation is to provide raw materials for microalgae which can then be used for further research.

The tools and materials used in this study were 24 1 L glass jars (with a working volume of 700 ml) as a bioreactor, a 4 mm diameter hose as a connector, a diffuser and a pump to provide aeration as well as a carbon source for microalgae, and LED lights as a source. Light. Furthermore, there are 3 species used, namely *Chlorela vulgaris, Spirullina sp.* And *Dunaliela salina*, the three species have brackish water habitats which were obtained from BPBAP Situbondo. The first fertilizer used is BBM (Bold Basal Medium) which will be used as a control in the study, the second is Mix A and Mix B hydroponic fertilizers as materials to be tested to determine the effect of using hydroponic fertilizer media on the growth rate of microalgae.

This study used 50 ml of *Chlorella Vulgaris, Spirulina sp.*, and *Dunaliella salina* cultures, respectively. The variables used were AB mix hydroponic fertilizer media with

the composition of mix A and mix B solutions of 3 ml each. While the control variable used is Bold's Basal Media (BBM).

### 2.2 Cultivation Process

Cultivation of microalgae uses an aerator with 8 W LED lighting (light intensity 1000–4000 lux). Cultivation was carried out for 10 days. The bioreactor with a capacity of 1 L with a working volume of 700 ml is designed using a jar in which two glass jar lids are perforated. The first hole is as input for culture and media and the second hole is for air exchange. The diffuser is placed at the bottom of the reactor which is connected to the pump using a hose and LED lights are installed around the reactor. The design of the reactor can be seen in Fig. 1. While the manufacture of hydroponic fertilizer AB Mix in the form of powder mixed with 1 L of distilled water per solution.

### 2.3 Grow Rate

Observation of the growth rate was carried out at an interval of 24 h for 10 days. Microalgae samples were taken as much as 1 ml and then the number of cells was calculated using a haemacytometer. The formula for calculating the number of cells using a haemacytometer can be seen in Eq. (1). Then the results of the calculation of the number of cells analyzed the growth rate of microalgae.

$$C = \frac{txd}{n} x 10^4 \tag{1}$$

where C is concentration cell (cells/ml); t is total cell in haemacytometer; and d is diluton factor.

## 3 Results and Discussion

### 3.1 Cultivation

In the microalgae cultivation process, color changes indicate that the density of microalgae cells has increased. If the cell count is high, the resulting color will be more visible. On the first day of *Chlorella vulgaris* cultivation, the color of the medium was light green, on the $5^{th}$ and $10^{th}$ days the green was darker. In *Spirulina sp.* the first day still not showing color, on the $5^{th}$ and $10^{th}$ day the color changes from the culture have started to appear in *Dunaliela salina* the color is not very visible from day 1 to day 10 because of the low cell density this arises due to lack of $CO_2$ and light intensity. According to Muchamad et al. [13] there are factors that influence the growth of microalgae, $CO_2$ and light intensity (Fig. 2).

According to Gildantia et al. [14] the green color in microalgae cultivation is produced from chloroplasts containing chlorophyll pigments. In addition, the color change also indicates the utilization of nutrients contained in the AB Mix hydroponic fertilizer media.

**Fig. 1.** Photobioreactor for Microalgae Cultivation

(a) (b) (c)

**Fig. 2.** Color Changes in Cultivation Process: (a) *Chlorella vulgaris* day 1,5,10, (b) *Spirulina sp.* Day 1,5,10, (c) *Dunaliela salina* day 1,5,10

### 3.2 Grow Rate

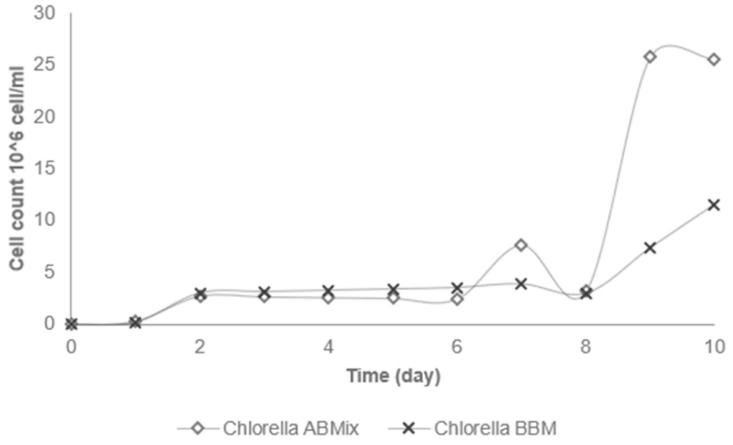
The growth rate of microalgae was observed by counting the number of cells every 24 h for 10 days. Calculation of the number of cells using a haemacytometer which is then calculated in the formula. In general, the growth of microalgae occurs in three different conditions that are phototrophic, heterotrophic and mixed conditions. In phototropic

conditions, microalgae are highly dependent on sunlight as an energy source and $CO_2$ is a carbon source. This condition is known as autotrophic photosynthesis (autotrophs). Under heterotrophic conditions Microalgae growth requires organic carbon as an energy source. Some commonly used organic carbon sources: glucose, acetate and glycerol. Based on previous research, it was shown that the biomass production and lipid content of microalgae that were growing with larger heterotrophic conditions grew under phototropic conditions [15, 16]. The microalgae cell density achieved is superior to the phototropic conditions, so the cost of harvesting is lower. Some types of microalgae are also able to grow in mixed conditions, which are a combination of phototropic and heterotrophic conditions. This type of microalgae can assimilate sunlight and organic carbon as a source of energy either physically simultaneously or alternately.

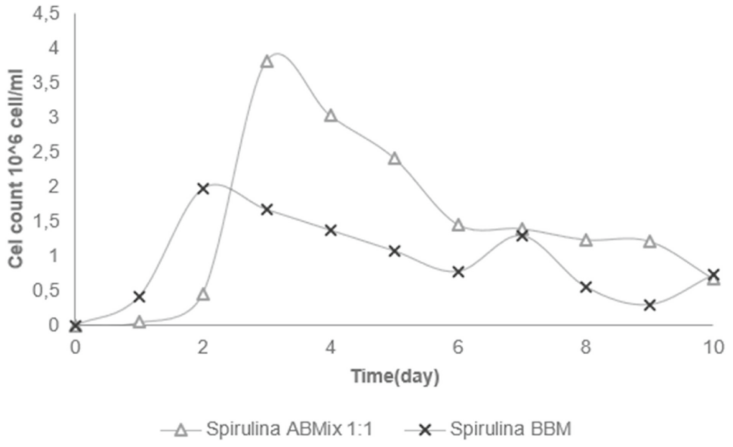
The number of cells in *Chlorela vulgaris* culture with hydroponic fertilizer media on the 1st day was $3.63x10^5$ cells/ml then increased until the maximum cell number on the 9$^{th}$ day was $2.58x10^7$ cells/ml. After that, it decreased until the 10$^{th}$ day as much as $2.56x10^7$ cells/ml. While the number of cells in *Chlorela vulgaris* culture using BBM on the 1$^{st}$ day was $1.62x10^5$ cells/ml and get maximum cell number of $7.4x10^6$ on the 9$^{th}$ day until it decreased on the 10$^{th}$ day as much as $1.16x10^7$ cells/ml. The number of cells in *Spirulina sp.* culture with hydroponic fertilizer media on the 1$^{st}$ day was $4.74x10^4$ cells/ml then it was higher until the maximum number of cells on the 3rd day was $3.8x10^6$ cells/ml. After that, it decreased until the 10$^{th}$ day as much as $6.8x10^5$ cells/ml. While the number of cells in *Chlorela vulgaris* culture using BBM on the 1$^{st}$ day was $4.12x10^5$ cells/ml and get maximum cell number of $1.98x10^6$ on the 2$^{nd}$ day until it decreased on the 10$^{th}$ day as much as $7.3x10^5$ cell/ml. While the number of cells in *Dunaliela salina* culture with hydroponic fertilizer media on the 1st day was $4.6x10^6$ cells/ml then increased until the maximum cell number on the 6th day was $4.26x10^5$ cell/ml. After that, it decreased until the 10$^{th}$ day as much as $1.2x10^5$ cells/ml. While the number of cells in *Chlorela vulgaris* culture using BBM on the 1$^{st}$ day was $4.2x10^5$ cells/ml and get maximum cell number of $2.04x10^5$ on the 6$^{th}$ day until the results of the cell number fluctuated until the 10$^{th}$ day of $1,26x10^5$ cells/ml. The graph of the observed growth rate of microalgae can be seen in Fig. 3.

Microalgae cultivation affected by many important factors. This should be considered because different microalgae require different growing conditions. Microalgae come in various types (strains) specifically to distinguish them from other microalgae. Several types of microalgae can exist under certain conditions. The right conditions are essential for microalgae growth, especially when dealing with other dangerous creatures. Appropriate microalgae culture techniques continue to be carried out, especially to overcome the problem of developing microalgae cell pollution.

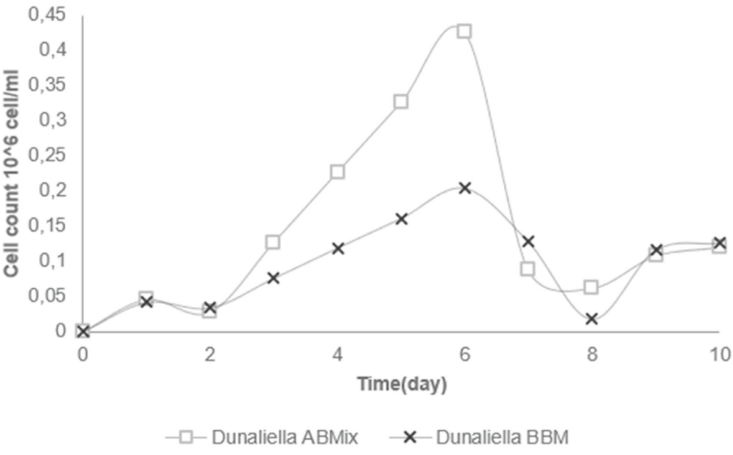
One of the important factors needed by microalgae for their growth is $CO_2$. During metabolism, $CO_2$ is needed so that microalgae can be converted into biomass. Sources of $CO_2$ generally come from industrially produced gases that result from combustion to produce energy.




(a)




(b)




(c)

**Fig. 3.** Microalgae Grow Rate: (a) *Chlorella vulgaris* (b) *Spirulina sp.* c) *Dunaliela salina*

Microalgae growth also affected by physiological properties. This physiological mechanism occurs to affect the absorption of nutrients in cultivation. When microalgae physiology is very good, these conditions can stimulate the production of biomass containing good oils and starches. However, some physiological reactions do not support the presence of microalgae living in ponds. Things can happen because high concentrations of $O_2$ accumulate in the microalgae growth block group [17]. Light saturation is also a problem with microalgae physiology. On top of the microalgae growing on the surface of the pond will get much more sunlight than is needed for the photosynthesis process. Therefore, recent research is accomplished with physiological and genetic alterations to reduce the absorption of light pigments on micromatch [18].

### 3.3 Effect of Hydroponic Fertilizer Use on Microalgae Growth

Based on Fig. 3, it can be seen that there was a fluctuation in the growth of microalgae at a dose of 1:1 compared to using Bold Bassal Medium. In the *Chlorella vulgaris* microorganism, it was found that growth increased rapidly on the $10^{th}$ day compared to BBM. In the microalgae *Spirulina sp.* It was found that growth increased rapidly on $3^{rd}$ day compared to BBM. In the microalgae *Dunaliella salina*, growth increased gradually on $6^{th}$ day compared to BBM. In the microalgae cultivation process, the nutrients needed continuously are N (Nitrogen), P (Phosphate), and K (Potassium) elements [19]. These elements are owned by both BBM and AB Mix Fertilizer, but there are differences in the amount of content and elements as shown in Table 1.

Elements found only in BBM are $NaNO_3$, NaCl, $K_2HPO_4$, $CaCl_2.2H_2O$, $MnCl_2.4H_2O$, $MoO_3$, $Co(NO_3)_2.6H_2O$, KOH, $FeSO_4.7H_2O$, and $H_2SO_4$(conc). The only elements found in Hydroponic Fertilizer are $Ca(NO_3)_2$, $KNO_3$, $(NH_4)2SO_4$, $K_2SO_4$, $ZnSO_4$, $MnSO_4$, $(NH_4)2MO_7O_{24}$. There are several compounds that contain the main elements, namely nitrogen (N), phosphate (P), and potassium (K) as well as supporting elements such as iron (Fe), zinc (Zn), manganese (Mn), and calcium (Ca). The dose of these elements is very influential on the growth of microalgae. In BBM, the main element given to microalgae is 0.3717 g with the main content in nitrogen (N) while in Hydroponic Fertilizer, the element given is 6.8553 g with nitrogen (N) and potassium (K) content. The addition of the dose to the main element increases the growth rate and also increases the doubling time which is in line with the research of Mashadani and Khudhair, 2017 [20]. In Afifah's research [21], the addition of nutrients, especially the main elements, is very important, but too little or too much will inhibit the growth of microalgae. Too few nutrients make the needs of microalgae elements not met so that they quickly enter the dead phase. If there are too many nutrients, it will become contamination. Excess nutrients will have an impact on high levels of ammonia. High levels of ammonia will be toxic.

**Table 1.** Comparison of Hydroponic Fertilizer Content with BBM

| **BBM** | | | | **Hidroponic Fertilizer** | | | |
|---|---|---|---|---|---|---|---|
| **Stock 1 (7 ml)** | | **Stock 2 (0,7 ml)** | | **Solution A (3 ml)** | | **Solution B (3 ml)** | |
| **Nutrient** | **Quantity** | **Nutrient** | **Quantity** | **Nutrient** | **Quantity** | **Nutrient** | **Quantity** |
| $NaNO_3$ | 0,175 g | $ZnSO_4.7H_2O$ | $6{,}174x10^{-3}$ g | $Ca(NO_3)_2$ | 3,528 g | $KH_2PO_4$ | 1,005 g |
| $MgSO_4.7H_2O$ | 0,0525 g | $MnCl_2.4H_2O$ | $1{,}008x10^{-3}$ g | $KNO_3$ | 1,848 g | $(NH_4)_2SO_4$ | 0,366 g |
| NaCl | 0,0175 g | $MoO_3$ | $0{,}497x10^{-3}$ g | Fe-EDTA | 0,114 g | $K_2SO_4$ | 0,108 g |
| $K_2HPO_4$ | 0,0525 g | $CuSO_4.5H_2O$ | $1{,}009x10^{-3}$ g | | | $MgSO_4$ | 2,37 g |
| $KH_2PO_4$ | 0,1225 g | $Co(NO_3)_2.6H_2O$ | $0{,}343x10^{-3}$ g | | | $CuSO_4$ | 0,0012 g |
| $CaCl_2.2H_2O$ | 0,0175 g | $H_3BO_3$ | $7{,}994x10^{-3}$ g | | | $ZnSO_4$ | 0,0045 g |
| | | EDTA | 0,035 g | | | $H_3BO_3$ | 0,012 g |
| | | KOH | 0,0217 g | | | $MnSO_4$ | 0,024 g |
| | | $FeSO_4.7H_2O$ | $3{,}486x10^{-3}$ g | | | $(NH_4)_2MO_7O_{24}$ | 0,0003 g |
| | | $H_2SO_4$(conc) | $0{,}7x10^{-3}$ ml | | | | |

## 4 Conclusion

Color changes in the microalgae cultivation process indicate that the density of microalgae cells has increased. If the cell density is high, the resulting color will be more visible. In the *Chlorella vulgaris* microorganism, it was found that growth increased rapidly on the 10$^{th}$ day compared to BBM. In the microalgae *Spirulina sp.* It was found that growth increased rapidly on 3$^{rd}$ day compared to BBM. In the microalgae *Dunaliella salina*, growth increased gradually on day 6 compared to BBM. Too few nutrients make the needs of microalgae elements not met so that they quickly enter the dead phase. If there are too many nutrients, it will become contamination. Excess nutrients will have an impact on high levels of ammonia. High levels of ammonia will be toxic.

## References

1. D. A. Winahyu, Y. Anggraini, E. L. Rustiati, J. Master, and A. Setiawan, "Studi Pendahuluan Mengenai Keanekaragaman Mikroalga di Pusat Konservasi Gajah , Taman Nasional Way Kambas," *Pros. Semirata FMIPA Univ. Lampung*, vol. 1, no. 1, pp. 93–98, Sep. 2013, Accessed: Sep. 23, 2022. [Online]. Available: https://jurnal.fmipa.unila.ac.id/semirata/article/view/796
2. R. R. A, "Secondary Metabolites from Algae for Nutraceutical Applications," *Nov. Tech. Nutr. Food Sci.*, vol. 1, no. 1, 2017, https://doi.org/10.31031/ntnf.2017.01.000503.
3. M. Soheili and K. Khosravi-Darani, "The Potential Health Benefits of Algae and Micro Algae in Medicine: A Review on Spirulina platensis," *Curr. Nutr. Food Sci.*, vol. 7, no. 4, pp. 279–285, Dec. 2012, https://doi.org/10.2174/157340111804586457.
4. N. Soltani *et al.*, "Biochemical and Physiological Characterization of Tree Microalgae spp. as Candidates for Food Supplement," *J. Appl. Biotechnol. Reports*, vol. 3, no. 1, pp. 377–381, Mar. 2016, Accessed: Nov. 14, 2022. [Online]. Available: http://www.biotechrep.ir/article_69015.html
5. A. Y. Maizatul, R. M. S. Radin Mohamed, A. A. Al-Gheethi, and M. K. A. Hashim, "An overview of the utilisation of microalgae biomass derived from nutrient recycling of wet market wastewater and slaughterhouse wastewater," *Int. Aquat. Res.*, vol. 9, no. 3, pp. 177–193, Sep. 2017, https://doi.org/10.1007/S40071-017-0168-Z/TABLES/8.
6. M. L. Wells *et al.*, "Algae as nutritional and functional food sources: revisiting our understanding," *J. Appl. Phycol.*, vol. 29, no. 2, pp. 949–982, Apr. 2017, https://doi.org/10.1007/S10811-016-0974-5.
7. T. L. Chacón-Lee and G. E. González-Mariño, "Microalgae for 'Healthy' Foods—Possibilities and Challenges," *Compr. Rev. Food Sci. Food Saf.*, vol. 9, no. 6, pp. 655–675, Nov. 2010, https://doi.org/10.1111/J.1541-4337.2010.00132.X.
8. "UN Secretary-General's Scientific Advisory Board to strengthen connection between science and policy," 2013. https://en.unesco.org/news/secretary-general's-scientific-advisory-board-strengthen-connection-between-science-and-policy (accessed Nov. 14, 2022).
9. M. A. Zielińska, A. Wesołowska, B. Pawlus, and J. Hamułka, "Health effects of carotenoids during pregnancy and lactation," *Nutrients*, vol. 9, no. 8. Nutrients, Aug. 04, 2017. https://doi.org/10.3390/nu9080838.
10. O. Pulz, "Photobioreactors: production systems for phototrophic microorganisms," *Appl. Microbiol. Biotechnol. 2001 573*, vol. 57, no. 3, pp. 287–293, 2001, https://doi.org/10.1007/S002530100702.

11. L. Zhu, "Biorefinery as a promising approach to promote microalgae industry: An innovative framework," *Renew. Sustain. Energy Rev.*, vol. 41, pp. 1376–1384, Jan. 2015, https://doi.org/10.1016/J.RSER.2014.09.040.
12. P. Dewapriya and S. kwon Kim, "Marine microorganisms: An emerging avenue in modern nutraceuticals and functional foods," *Food Res. Int.*, vol. 56, pp. 115–125, Feb. 2014, https://doi.org/10.1016/J.FOODRES.2013.12.022.
13. A. Muchammad, E. Kardena, and A. Rinanti, "PENGARUH INTENSITAS CAHAYA TERHADAP PENYERAPAN GAS KARBONDIOKSIDA OLEH MIKROALGA TROPIS Ankistrodesmus sp. DALAM FOTOBIOREAKTOR," *J. Teh. Lingkung.*, vol. 19, no. 2, pp. 103–111, 2013, https://doi.org/10.5614/jtl.2013.19.2.1.
14. E. Gildantia, R. S. Ferniah, A. Budiharjo, A. Suprihadi, M. Zainuri, and H. P. Kusumaningrum, "Identifikasi Spesies Mikroalga dari BBPBAP Jepara secara Morfologi dan Molekuler menggunakan 18S rDNA," *Bul. Oseanografi Mar.*, vol. 11, no. 2, pp. 167–176, May 2022, https://doi.org/10.14710/BULOMA.V11I2.39703.
15. J. Masojídek and G. Torzillo, "Mass Cultivation of Freshwater Microalgae," in *Encyclopedia of Ecology, Five-Volume Set*, Elsevier Inc., 2008, pp. 2226–2235. https://doi.org/10.1016/B978-008045405-4.00830-2.
16. X. Miao and Q. Wu, "Biodiesel production from heterotrophic microalgal oil," *Bioresour. Technol.*, vol. 97, no. 6, pp. 841–846, Apr. 2006, https://doi.org/10.1016/j.biortech.2005.04.008.
17. J. R. Benemann, J. C. Van Olst, M. J. Massingill, J. C. Weissman, and D. E. Brune, "The Controlled Eutrophication Process: Using Microalgae for CO 2 Utilization and Agricultural Fertilizer Recycling".
18. Y. Nakajima and R. Ueda, "The effect of reducing light-harvesting pigment on marine microalgal productivity," *J. Appl. Phycol.*, vol. 12, no. 3–5, pp. 285–290, 2000, https://doi.org/10.1023/A:1008108500409.
19. P. P. Da Silva and L. A. Ribeiro, "Assessing microalgae sustainability as a feedstock for biofuels," *Adv. Bioprocess. Altern. Fuels, Biobased Chem. Bioprod. Technol. Approaches Scale-Up Commer.*, pp. 373–392, Jan. 2019, https://doi.org/10.1016/B978-0-12-817941-3.00019-X.
20. M. K. H. Al-Mashhadani and E. M. Khudhair, "Experimental Study for Commercial Fertilizer NPK (20:20:20+TE N: P: K) in Microalgae Cultivation at Different Aeration Periods," *Iraqi J. Chem. Pet. Eng.*, vol. 18, no. 1, pp. 99–110, 2017, Accessed: Sep. 23, 2022. [Online]. Available: https://www.researchgate.net/publication/336009959_Experimental_Study_for_Commercial_Fertilizer_NPK_202020TE_N_P_K_in_Microalgae_Cultivation_at_Different_Aeration_Periods
21. A. S. Afifah, G. Prajati, Y. Adicita, and Darwin, "Variasi Penambahan Nutrien (NPK cair) dalam Kultivasi Mikroalga Chlorella Sp.," *J. Nat. Enviromental Manag.*, vol. 11, no. 1, pp. 101–107, 2021.