al Kimiya: Jurnal Ilmu Kimia dan Terapan
p-ISSN: 2407-1897, e-ISSN: 2407-1927
Vol. 12, No. 1 (18-25), June 2025/Muharram 1447

BIOMASS CONTENTS OF *Chlorella pyrenoidosa* CULTIVATED IN COBALT-LESS FERTILIZER-ENRICHED SALINE WATER

AWANG WIDYATAMA[1], ELVA STIAWAN[2,5], SARINA HANIFAH[3], SATRIA AQILLA WIDYATAMA[2], ZAIDAN AL FAJRY[2], ATMADI[4], AND ADE HERI MULYAWATI[1*]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Pakuan, Bogor, Indonesia

[2]Department of Chemistry, Faculty of Military Mathematics and Natural Sciences, Universitas Pertahanan RI, Bogor, Indonesia

[3]Department of Chemistry Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Jakarta, Indonesia

[4]Department of Physics, Faculty of Military Mathematics and Natural Sciences, Universitas Pertahanan RI, Bogor, Indonesia

[5]Department of Chemistry, Leiden University, 2300 RA Leiden, The Netherlands

*Corresponding Author email: adeheri.mulyati@unpak.ac.id

Article Information

Received: Sep 23, 2024
Revised: Apr 17, 2025
Accepted: Jun 07, 2025
Published: Jun 30, 2025

DOI: 10.1557/ak..v12i1.39468

Keywords:
Algal Bioprocess, Chlorella pyrenoidosa, Green Microalga, Growth Medium, Microalgal Biomass, UPLC-QToF-MS

Abstract

This research aims to provide some information regarding the metabolite contents of the green microalgae *Chlorella pyrenoidosa* cultivated in designed saline water enriched with affordable commercial fertilizer products in Indonesia. *Chlorella pyrenoidosa* growth conditions and bioactive compounds were obtained through cultivation and extraction processes. The microalga growth in two different conditions was analyzed based on growth curves between microalgae cultivated in a Guillard f/2 medium and the Cobalt-less fertilizer-enriched saline water. The biomass was profiled using UPLC-QToF-MS and DPPH assay. As a result, *Chlorella pyrenoidosa* cultures were normally grown on fertilizer-enriched saline water, but with a lower yield performance than those cultivated in the Guillard f/2 medium. The UPLC-QToF-MS result implies that several peptide and lipid derivatives were detected in the harvested microalgal biomass, with the peptide derivatives as the dominant compounds. The limited antioxidant performance was displayed by the untreated biomass extract. This finding implies that several optimizations are still required in the upstream and downstream stages in culturing *Chlorella pyrenoidosa* by using an uncommon growth medium.

INTRODUCTION

Microalgae have been widely recognized as a promising biological aquatic resource due to their rapid growth, high photosynthetic efficiency, and ability to thrive in diverse environmental conditions. These characteristics make them suitable for a wide range of industrial applications, particularly in the fields of food, cosmetics, and pharmaceuticals. In recent years, considerable research has highlighted the potential of microalgae to biosynthesize numerous high-value compounds, including carotenoids, polysaccharides, vitamins, and lipids [1]. These bioactive substances have demonstrated significant functional roles, such as antioxidant, anti-inflammatory, and antimicrobial effects, making them valuable for health-related and therapeutic applications. The fatty acids are popularly extracted from their lipid-rich biomass. Although microalga-based lipids are quite interesting in terms of their suitability with plant-based consumers' preference [2], challenges still exist to massively provide microalgal biomass. One of the primary obstacles is the high cost and limited affordability of microalgal growth media, which are essential for sustaining optimal growth and metabolite production.

An excellent artificial microalgal growth medium must provide sufficient and balanced nutrients to effectively support microalgal growth and metabolic activity. These nutrients are generally classified into macronutrients and micronutrients, depending on the required quantities for optimal cellular function. Both types of nutrients significantly influence microalgal conditions, particularly in terms of cell density, growth rate, and biomass yield. Several

standardized growth media are commonly used in microalgal cultivation, including Walne, Zarrouk, Conway, and Guillard f/2 media [3-5]. These media typically consist of a precise mixture of inorganic salts and biomolecules dissolved in water, adjusted to specific pH and salinity levels to match the needs of the cultured species. However, a major challenge—especially in large-scale cultivation—is the continuous supply of these fine chemicals, which can be costly and resource-intensive. This limitation has prompted growing interest among researchers in developing alternative nutrient sources or innovative cultivation strategies to sustain microalgal production more efficiently and economically.

An alternative that could be considered is employing commercial agricultural fertilizer to mimic a common microalgal growth medium. Several studies have attempted this approach. In mimicking the Guillard f/2 medium, an effort to utilize fertilizers commercialized in Indonesia has been developed. Several fertilizers marketed in Indonesia demonstrated a prospective profile when employed in the microalgae cultivation. For instance, the utilization of fertilizer products of NPK Mutiara 16-16-16® and Meroke Fitoflex® supplemented by vitamins IPI® displayed fair growth profiles and biomass yields of each *Chlorella pyrenoidosa* and *Botryococcus braunii* culture [6]. However, this approach has not sufficiently demonstrated comparable performance to common microalgal growth media, such as compare to Guillard f/2 medium. Furthermore, the metabolite contents of microalgal biomass ought to be provided to convince that the application of an alternative fertilizer-based medium could not significantly alter the metabolite profiles of the microalgal. Furthermore, the potential dynamic changes of biomass' metabolite content ought to be described to ensure that the utilization of fertilizer-based medium would not eradicate the intended valuable compound.

This work compares the growth conditions of a typical green microalga, *Chlorella pyrenoidosa,* cultivated in the fertilizer-enriched medium and the microalga cultured in the Guillard f/2, as a control. Moreover, the biomass contents from both growth mediums are thoroughly discussed.

EXPERIMENT

Material

The materials are *Chlorella pyrenoidosa* microalgae isolate (purchased from a particular microalgae culture breeding business in Bogor, Indonesia), distilled water, methanol p.a, NPK Mutiara 16-16-16®, Meroke Fitoflex®, three types of vitamins (IPI®), coarse salt, and $Na_2SiO_3.9H_2O$, and 1,1-diphenyl-2-picrylhydrazyl (DPPH). To simplify, the fertilizer-enriched saline water growth medium will be called MMFI, abbreviated as each initial fertilizer and vitamin brand.

Instrumentation

The instruments employed in this study included laboratory glassware (Erlenmeyer flasks, beakers, measuring flasks, graduated cylinders, glass funnels, 1 L glass bottles, and stirring rods), liquid handling tools (Mohr pipettes, drop pipettes, and micropipettes), measurement devices (analytical balance and salinity meter), culture equipment (aerator and autoclave), sample containers (vials), and analytical instruments such as a centrifuge, UV-Vis spectrophotometer, and Ultra Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (UPLC-QToF-MS).

Procedure

Fertilizer-enriched Growth Medium Preparation

The growth medium was developed by adapting the Guillard f/2 formulation, which consists of four separate stock solutions. The adaptation followed a previous work that employed a modified medium without Cobalt content [6]. Solutions A and C of the original Guillard f/2 medium were substituted by Pearl 16-16-16® NPK fertilizer (10 g / 100 mL distilled water) and Meroke Fitoflex® fertilizer (25 g / 100 mL distilled water), respectively. Solution B was substituted by dissolving 5.7 mL of 58% sodium metasilicate nonahydrate ($Na_2SiO_3 \cdot 9H_2O$) in 100 mL of distilled water. Solution D was replaced with a vitamin mixture consisting of 0.1 mL vitamin B12, 1 mL biotin, and 0.4 g vitamin B1 dissolved in 1 L of distilled water. All solutions, except the vitamin mixture, were sterilized by autoclaving at 121°C for 15 minutes and subsequently stored at room temperature for 12 hours. The vitamin solution was added after sterilization to prevent thermal degradation.

Microalgae Cultivation and Growth Profile

The microalgae starter was aseptically cultivated by adding 30 mL of microalgae starter into a Liter of sterilized growth medium that had

been supplemented with a vitamin solution. The cultures were cultivated under 12 : 12 hours of dark and light conditions. The microalgae growth curve was daily determined through its optical density OD), displayed as absorbance using a UV-Vis spectrophotometer at a wavelength of 450 nm [7].

After being cultivated for 21 days, microalgal biomass was harvested in the initial phase before the stationary phase based on the microalgal growth curve using a centrifuge at 4000 rpm for 10 minutes (Christenson and Sims, 2011).

Metabolite Profiling of Biomass using UPLC-QToF-MS

The biomass of *Chlorella pyrenoidosa* was extracted using methanol as the solvent. The extract was then analyzed using UPLC-QToF-MS to obtain qualitative and quantitative data on the metabolites present. The mobile phase used consisted of solvent A (water:formic acid = 99.9:0.1) and solvent B (acetonitrile:formic acid = 99.9:0.1), applied in a gradient elution system with a flow rate of 0.2 mL/min. The resulting chromatograms were processed and interpreted using the MassLynx 4.1 software to determine the molecular weights of detected metabolites. Compound identification was performed based on the previous procedure [8].

RESULT AND DISCUSSION

Growth profile of Chlorella pyrenoidosa

Microalgae growth rate was measured using a UV-vis spectrophotometer related to the OD of the culture. The lower absorbance value implies that the cell numbers that develop in the growth medium are getting fewer [9]. The growth curve is used as a reference to determine the optimal time to harvest microalgae cultures. **Figure 1** displays cultures cultivated in fertilizer-enriched medium and f/2 Guillard medium. Overall, the figure shows a slower growth of microalgae in the fertilizer-enriched medium compared to those cultivated in the f/2 Guillard, especially after 1st week of cultivation.

Growth profile of Chlorella pyrenoidosa

Microalgae growth rate was measured using a UV-vis spectrophotometer related to the OD of the culture. The lower absorbance value implies that the cell numbers that develop in the growth medium are getting fewer [9]. The growth curve is used as a reference to determine the optimal time to harvest microalgae cultures. **Figure 1** displays cultures cultivated in fertilizer-enriched medium and f/2 Guillard medium. Overall, the figure shows a fluctuating increase of growth of fertilizer-enriched medium in 1st week and gradually similarized to f/2 Guillard after 2nd week of cultivation.

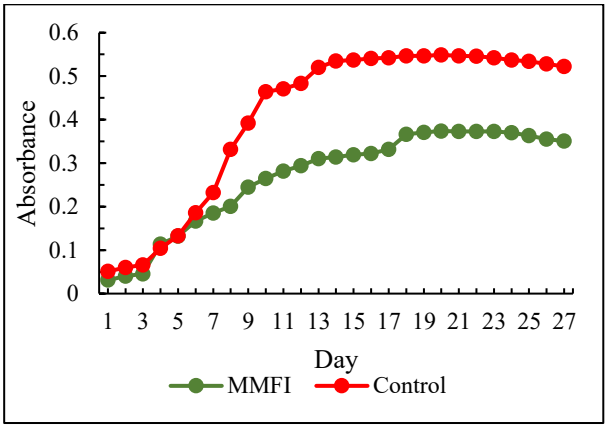

Figure 1. Microalgal growth comparison between cultivation in fertilizer-enriched saline water (coded as MMFI) and Guillard f/2 medium.

Based on the cultivation of *Chlorella pyrenoidosa* microalgae in aerated flasks conducted for 27 days, **Figure 1** depicts that the microalga cultivated in MMFI growth medium has a lower optical cell density than the microalga cultivated in f/2 Guillard as a control. The lower trend of microalgae density within the MMFI medium is considered a consequence of the different starter volume of microalgae seeding. The starter volume used in this work was lower than the previous study. In the non-aerated condition, a ratio of the starter volume to growth medium was 5 mL/100 mL [6], while the ratio in this aerated-flask system was 3 mL/100 mL.

In addition, different N and P contents between Guillard f/2 and MMFI medium may also contribute to this condition as the growth of microalgae *Chlorella pyrenoidosa* is influenced by the content of nitrogen and phosphorus elements in the growth medium [10]. Nitrogen plays a role in the synthesis of various important compounds such as proteins, amino acids, enzymes, nucleic acids, and photosynthetic pigments, thus affecting microalgae cell structure [11], while phosphorus functions to regulate cell growth and metabolism, involved in various cellular processes including energy transfer, signal transduction, macromolecular biosynthesis, photosynthesis, and respiration [12]. In addition to these two elements, other elements are also essential for the nutritional needs of microalgae because microalgae require large amounts of both macro- and micronutrients [13]. Both micro and macro elements for microalgae nutrition have been tried to be provided

in the media. The optimal balance of microalgae growth requires the right nitrate and phosphate levels because both have a vital role in the microalgae growth process [14].

Figure 1 also displays the growth profile of the studied *Chlorella pyrenoidosa*. This microalga has a lag phase on days 1-3. In this phase, these microalgae began to adapt to the dynamic changes of the different growth medium. Afterward is the logarithmic phase, which generally occurs from day 4 to day 19. In this phase, the microalgae population grows rapidly and exponentially. The logarithmic phase is quite interesting in this microalgae cultivation since the microalgae density dramatically increased in the first week of cultivation before it became slower than the control. It was assumed that a particular stress occurred during this phase that impacted on eventual nutrient consumption of some cells in the beginning step of cultivation. On the other hand, the density of microalgal within the f/2 Guillard medium seemed to be more adaptable since those cells had a more dramatic increase, at least until day 10.

However, the actual reason for this circumstance needs to be furtherly investigated. After the logarithmic phase, a stationary phase of the studied microalgae is displayed by the cell density remaining stable on days 20 to 23, followed by the death phase of this microalga culture that occurs between days 24 and 27. In this condition, the microalgal population constantly diminished, and the color of the culture was visually changed from dark green to cloudy-light green. Considerably, the cell density was gradually reduced since competition to obtain the nutrients became strict after the cells reached the optimal density. In addition, the high density of cells may also block light absorption into the medium, which in turn affects photosynthetic activity. Overall, it can be briefly inferred that the growth of *Chlorella pyrenoidosa* on MMFI was relatively slower than in the F/2 Guillard media, especially after the adaptation phase.

Initially, biomass harvesting was planned to be conducted in the exponential phase close to the initial stage of the stationary phase, i.e., at the end of the 2nd week of cultivation, to get as the relatively fresh and as many as possible of viable microalgal cells. However, due to the fluctuating growth of microalgae in MMFI medium, it was decided to collect the biomass in the 3rd week of cultivation. The fresh-wet biomass was harvested through centrifugation instead of filtration to avoid the leached filter paper attached to the biomass during the subsequent metabolite detection. yield of both cultures. The appearances and the yield of harvested biomass can be seen in **Figure 2** and **Table 1**, respectively.

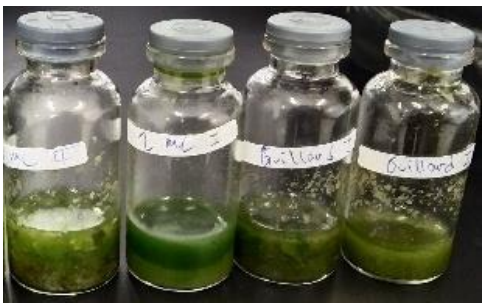

Figure 2. Fresh microalgal biomass from MMFI medium (two vials from left) and Guillard f/2 medium (two vials from right).

Table 1. Biomass yields of harvested *Chlorella pyrenoidosa* cultures.

No.	Growth media	Average biomass (per 15 Liter culture)
1.	MMFI	1.91±0.06 g
2.	Guillard f/2	3.24±0.22 g

Microalgal cultures in the MMFI-cultured microalga produced lower biomass than the microalga within the Guillard f/2 medium. In addition, this yield is also slightly lower than the biomass yield in the previous study [6]. This finding implies that the lower starter volume used in the seeding stage, a lower yield produced in the harvesting stage. Although the yield aspect is not competitive with microalgae within the f/2 Guillard medium, it is still interesting whether the metabolite contents of both biomasses are different or not.

Metabolite Profiles based on UPLC-QToF-MS

Both cultures grown in MMFI and Guillard f/2 media were extracted and subsequently analyzed using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QToF-MS). Chromatographic separation was performed on a C18 reversed-phase column (octadecyl silica) using a gradient elution system The mobile phase used consisted of solvent A (water : formic acid = 99.9 : 0.1) and solvent B (acetonitrile : formic acid = 99.9 : 0.1), applied in a gradient elution system with a flow rate of 0.2 mL/min. The stationary phase was the C18 column. The resulting chromatograms of the two cultures, shown in **Figure 3**, illustrate the distinct metabolite profiles present in each growth condition.

The molecular formula can be predicted based on the mass-to-charge ratio values compared with the integrated database on sites such as ChemSpider, MassBank, Human Metabolome Database, and PubChem. During the interpretation, the predicted molecular formula in the data is reduced by 1 hydrogen atom because 1 hydrogen atom is lost during the separation process through ESI (+) ion shooting [8]. **Table 2** and **Table 3** show the three and two dominant compounds contained in the microalgae *Chlorella pyrenoidosa* cultivated using a fertilizer-enriched saline water and Guillard f/2 media, respectively.

Tables 2 and **3** propose molecular formulas that are manifested by comparing the precise m/z values with theoretical values via mass defect analysis and heuristic rules, including the nitrogen rule as well as hydrogen-to-carbon ratio constraints [15]. In addition, retention time tendency on a C18 reversed-phase column (octadecyl silica) as a stationary phase was considered to guide compound categorization, such as the hydrophobicity of lipopeptide affects the longer elution duration compared to the other less hydrophobic compounds [16,17]. Additionally, the estimation also considers biologically major relevant elements of plant-based biomass, i.e., C, H, N, O, and P, elemental composition predictions were attempted based on observed masses.

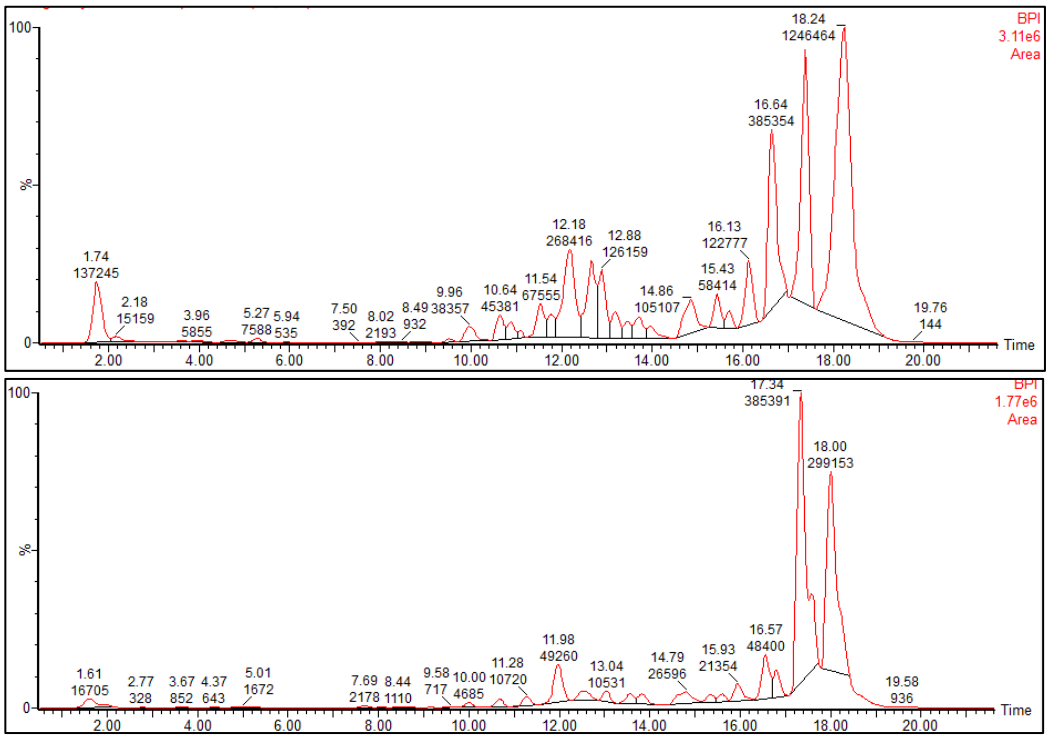

Figure 3. Chromatogram results of UPLC-QToF-MS toward biomass within fertilizer-enriched saline water (MMFI; top) and Guillard f/2 media (bottom).

Table 2. Metabolite Contents of *Chlorella pyrenoidosa* biomass from fertilizer-enriched saline water.

No.	Retention time (min.)	$[M+H]^+$ (m/z)	%Area wide	Estimated Formula/Likely Compound Class
1.	18.24	766.54	34.42	$C_{40}H_{65}N_{11}O_4$/peptides derivative
2.	17.38	764.52	14.25	$C_{40}H_{65}N_{11}O_4$/peptides derivative
3.	16.64	607.29	10.64	$C_{31}H_{46}NO_8P$/ Lysophospholipid (LPE)

In general, the fertilizer-enriched saline water has fewer defined compounds (27 peaks) than the F/2 control media (37 peaks). One of the main factors is considered as the availability of nutrients absorbed from the growth media [18]. Some differences in macronutrients and micronutrients in F/2 Guillard media may also contribute to lower diversity of compounds since some components did not appear, which the precursors or minerals might not be substituted in MMFI as a fertilizer-enriched seawater media, such as Na, Co, EDTA, and Cl. In addition, some elements in MMFI media have levels that are not the same as F/2 Guillard media, which may affect

chemical interactions in the media and microalgae metabolism. These changes could affect the production of different compounds or change the level of certain compounds.

Table 3. Metabolite Contents of *Chlorella pyrenoidosa* biomass from Guillard f/2 Growth Medium.

No	Retention time (min.)	$[M+H]^+$ (m/z)	%Area wide	Estimated Formula/Likely Compound Class
1.	18.00	766.54	30.70	$C_{40}H_{67}N_{11}O_4$/ Hydrogenated analogue of peptides derivative
2.	17.34	764.52	39.55	$C_{40}H_{65}N_{11}O_4$/ peptides derivative
3.	16.57	607.29	4.97	$C_{31}H_{46}NO_8P$/ Lysophospholipid (LPE)

According to the data in **Tables 2** and **3**, the longest retention time in the MMFI medium was 18.00 minutes. This shows a slight difference compared to the f/2 medium, where the corresponding compound eluted at 18.24 minutes. The difference in retention times suggests that the compounds separated in each medium are not identical. In the MMFI medium, the compound is likely $C_{40}H_{65}N_{11}O_4$, identified as a peptide derivative. In contrast, the compound detected in the f/2 medium is likely $C_{40}H_{67}N_{11}O_4$, a hydrogenated analogue of the peptide derivative. The longer retention of $C_{40}H_{67}N_{11}O_4$ suggests that it is more hydrophobic, leading to stronger interactions with the column. Hydrogenation of unsaturated compounds typically reduces polarity by saturating electron-rich functional groups, thereby increasing hydrophobicity [19].

The peak observed at a retention time of 17.38 in the fertilizer-enriched saline water media and at 17.34 in f/2 media showed different % areas, but both correspond to the same compound, as confirmed by comparison with the PubChem reference database. This compound might be identified as $C_{40}H_{65}N_{11}O_4$, a type of carbamate. Although both chromatograms indicate the presence of the same compound, the percentage area of the peak varies significantly between the two, suggesting a notable difference in the compound's abundance. This discrepancy could be attributed to variations in nutrient availability, which may lead to different metabolic responses in the organisms under the two media conditions.

Two peaks observed in the MMFI medium at retention times of 18.24 and 17.38 likely correspond to the same compound, $C_{40}H_{65}N_{11}O_4$, in the form of peptide derivatives. This phenomenon can be explained by the principle that although isomers share the same molecular formula, they can appear as distinct peaks in a chromatogram due to differences in their chemical structures, which influence their interactions with the chromatographic column. Longer retention times suggest that the compound interacts more strongly with the C18 stationary phase, indicating a more non-polar chemical structure. In this context, the polarity of a peptide is influenced by the composition, structure, and distribution of functional groups within its amino acid chain. A similar finding was reported by Winter., et al. [20], who successfully separated six isomeric peptides—each containing a single phosphoserine residue—using UPLC-MS. Their study demonstrated that variations in the stereochemical configuration of peptides can lead to substantial differences in chromatographic behavior, enabling clear separation between isomers.

The MMFI medium, a mixture of commercial fertilizers and non-standard vitamins, likely creates an environment that promotes alternative metabolic pathways, leading to increased production of secondary metabolites such as carbamates. In contrast, the more stable and controlled conditions of the f/2 medium may result in a more regulated metabolic process, limiting the production of these metabolites. We also performed additional profiling to briefly display an overall biomass profile in dealing with DPPH, as inhibitory concentration (IC50), and categorize those antioxidant performances [21]. **Table 4** displays the antioxidant performance of each extract of our microalgal biomass within methanol as a solvent.

Table 4. Antioxidant performances of *Chlorella pyrenoidosa* biomass cultivated in two different media.

No.	Biomass from growth medium	IC_{50} (μg/ml)
1.	MMFI	1339.70
2.	f/2 Guillard	3521.01

Based on **Table 4**, both antioxidant performances of biomass were categorized as very weak [21]. Indeed, this condition requires further investigation since the above performances were roughly measured from a whole metabolite mixture

with diverse characteristics instead of a selected prospective bioactive compound. The diverse compounds within the untreated biomass may have consequences, whether on synergistic or antagonist activities against radicals [22] [23]. Furthermore, subsequent treatment of a particular metabolite of biomass may affect the different antioxidant performance of Chlorella pyrenoidosa. For instance, solid soap fortified with carotenoids from *Chlorella pyrenoidosa* demonstrated a stronger antioxidant performance based on DPPH assay result [24].

CONCLUSION

The *Chlorella pyrenoidosa* isolate grown on our fertilizer-enriched saline water exhibited lower growth performance compared to microalgae cultivated in the common Guillard f/2 medium. Meanwhile, both UPLC-QToF-MS results detected slightly similar peptides and lipid derivatives as the most predominant compounds in the harvested microalgal biomass. Further analysis through additional structure elucidation methods is required to confirm this most abundant biomass metabolite. The findings of this work may enable further optimization of the upstream stage of microalgal bioprocessing with an alternative approach by utilizing some commercial fertilizer products with affordable raw materials instead of expensive fine chemical products.

ACKNOWLEDGEMENT

We thank the Pertamina Foundation for financial support for conducting this work through an appreciation grant of PFSains 2022 and LPPM Unhan RI for providing facilities to complete this work.

REFERENCES

[1] N. W. S. Agustini and A. H. Winarni, "Karakteristik dan aktivitas antioksidan sabun padat transparan yang diperkaya dengan ekstrak kasar karotenoid *Chlorella pyrenoidosa*", *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan*, **12**(1), 1–12, 2017, https://doi.org/10.15578/jpbkp.v12i1.379

[2] E. Stiawan, "Evaluation of the biochemical contents in guillard f/2 and walne growth medium to fulfill the animal-free aspects of microalgal bioprocessing", *Indonesian Journal of Chemical Studies*, **1**(2), 49–53, 2022, https://doi.org/10.55749/ijcs.v1i2.16

[3] A. Sánchez-Bayo, V. Morales, R. Rodríguez, G. Vicente, and L. F. Bautista, "Cultivation of microalgae and cyanobacteria: effect of operating conditions on growth and biomass composition", *Molecules*, **25**(12), 1–17, 2020, https://doi.org/10.3390/molecules25122834

[4] R. Chandarasekaran, M. Ajesh, B. Sundaramoorthy, and M Shalini, "Effect of modified Zarrouk's medium on growth of different Spirulina strains", *Walailak Journal of Science and Technology*, **13**(1), 67–75, 2016.

[5] A. A. da Cruz Coêlho, M. U. Gonçalves Barros, J. H. Cavalcante Bezerra, J. W. Alves da Silva, R. Lafaiete Moreira, and W. R. Lobo Farias, "Growth of the microalgae Tetraselmis tetrathele and nitrate depletion in culture medium Guillard f/2 and Conway", *Acta Scientiarum Biological Sciences*, **35**(2), 163–168, 2013, https://doi.org/10.4025/actascibiolsci.v35i2.13971.

[6] A. Widyatama, Z. Al Fajry, S. A. Widyatama, A. H. Mulyati, and E. Stiawan, "The utilization of two commercial indonesian fertilizers as microalgal growth medium for *Chlorella pyrenoidosa* and *Botryococcus braunii* cultivation", *Jurnal Kartika Kimia*, **7**(1), 19–24, 2024, https://doi.org/10.26874/jkk.v7i1.252

[7] R. Hernandi, A. Dharma, and A. Armaini, "Penapisan, isolasi, dan karakterisasi mikroalga yang berpotensi sebagai sumber biodiesel dari perairan Danau Kerinci, Jambi", *Jurnal Litbang Industri*, **9**(1), 41, 2019, https://doi.org/10.24960/jli.v9i1.4326.41-49

[8] F. N. Maslakhah, R. Mutiah, A. Hakim, R. Aprinda, and A. Suryadinata, "Metabolite profiling bagian akar, batang, daun, dan biji *Helianthus annuus L.* menggunakan instrumen UPLC-MS", *Media Pharmaceutica Indonesia*, **2**(2), 64–81, 2019, https://doi.org/10.24123/mpi.v2i2.1361

[9] R. Sumath and N. Vishnu, "Isolation of fresh water microalgae *Chlorella sp* and its antimicrobial activity on selected pathogens", *International Journal of Advanced Research in Biological Sciences*, **1**(3), 36-43, 2014.

[10] I. Bagus, G. Brahmantara, A. A. M. D. Anggreni, I. Bagus, and W. Gunam, "Pengaruh konsentrasi penambahan sodium

nitrat dan sodium fosfat pada media guillard terhadap konsentrasi biomassa mikroalga *Nannochloropsis sp*.", *Jurnal Rekayasa dan Manajemen Agroindustri*, **3**(4), 73–81, 2015, Available at: https://ojs.unud.ac.id/index.php/jtip/article/view/18702

[11] F. Gao, H. L. Yang, C. Li, Y. Y. Peng, M. M. Lu, W. H Jin, J. J. Bao, and Y. M. Guo, "Effect of organic carbon to nitrogen ratio in wastewater on growth, nutrient uptake and lipid accumulation of a mixotrophic microalgae *Chlorella sp*", *Bioresource Technology*, **282**, 118–124, 2019, https://doi.org/10.1016/j.biortech.2019.03.011

[12] Q. Wu, L. Guo, X. Li, and Y. Wang, "Effect of phosphorus concentration and light/dark condition on phosphorus uptake and distribution with microalgae", *Bioresource Technology.*, **340**, 125745, 2021, https://doi.org/10.1016/j.biortech.2021.125745

[13] A. S. P. Sari, Wisanti, and E. Ratnasari, "Pengaruh pemberian jenis pupuk yang berbeda terhadap laju pertumbuhan populasi dan kadar lemak *Nannochloropsis oculata*", *LenteraBio: Berkala Ilmiah Biologi*, **1**(1), 55–62, 2012.

[14] K. D. W. Dinata B, D. A. A. M. Anggreni, and S. A. Nyoman, "Pengaruh konsentrasi natrium nitrat dan natrium dehidrogen fosfat pada media walne terhadap konsentrasi biomassa dan protein *Nannochloropsis oculata*", *Rekayasa dan Manajemen Agroindustri*, **5**(1), 40–49, 2017, Available at: https://ojs.unud.ac.id/index.php/jtip/article/view/27225

[15] T. Kind and O. Fiehn, "Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry", *BMC Bioinformatics*, **8**(105), 2007, https://doi.org/10.1186/1471-2105-8-105

[16] C. A. Smith, E. J. Want, G. O'Maille, R. Abagyan, and G. Siuzdak, "XCMS: Processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification", *Analytical Chemistry*, **78**(3), 779–787, 2006, https://doi.org/10.1021/ac051437y

[17] A. Roux, D. Lison, C. Junot, and J. F. Heilier, "Applications of liquid chromatography coupled to mass spectrometry-based metabolomics in clinical chemistry and toxicology: A review", *Clinical Biochemistry*, **44**(1), 119–135, 2011, https://doi.org/10.1016/j.clinbiochem.2010.08.016

[18] I. K. Trikuti, A. A. M. D. Anggreni, and I. B. W. Gunam, "Pengaruh jenis media terhadap konsentrasi biomassa dan kandungan protein mikroalga *Chaetoceros calcitrans*", *Jurnal Rekayasa dan Manajemen Agroindustri*, **4**(2), 13–22, 2016, Available at: https://ojs.unud.ac.id/index.php/jtip/article/view/19590

[19] G. Li, Z. Zhao, T. Mou, Q. Tan, B. Wang, and D. Resasco, "Experimental and computational kinetics study of the liquid-phase hydrogenation of C=C and C=O bonds", *Journal of Catalysis*, **404**, 771-785, 2021, https://doi.org/10.1016/j.jcat.2021.09.002

[20] D. Winter, R. Pipkorn, and W. D. Lehmann, "Separation of peptide isomers and conformers by ultra performance liquid chromatography", *Journal of Separation Science*, **32**(8), 1111–1119, 2009, https://doi.org/10.1002/jssc.200800691.

[21] M. Philip, "The use of stable free radical diphenyl picrylhydrazyl (DPPH) for estimating antioxidant activity", *Songklanakarin Journal of Science and Technology*, **26**(2), 211-219, 2004

[22] N. Nenadis and M. Tsimidou, "Observation on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-2-picrylhydrazyl (DPPH) tests", *Journal of the American Oil Chemists Society*, **79**, 1191–1195, 2002, https://doi.org/10.1007/s11746-002-0626-z

[23] K. S. Sonam and S. Guleria, "Synergistic antioxidant activity of natural product", *Annals of Pharmacology and Pharmaceuntics*, **2**(8), 1-6, 2017

[24] W. Agustini and A. H. Winarni, "Karakteristik dan aktivitas antioksidan sabun padat transparan yang diperkaya dengan ekstrak kasar karotenoid *Chlorella pyrenoidosa*", *Jurnal Pasca Panen dan Bioteknologi Kelautan dan Perikanan*, **12**(1), 1-12, 2017, https://doi.org/10.15578/jpbkp.v12i1.379