3rd Malikussaleh International Conference on Multidiciplinary Studies 2022 (3rd MICoMS 2022)

IINTERNATIONAL CONFERENCE PROCEEDINGS 00069 (2022) |DOI: https://doi.org/10.29103/micoms.v3i.234
E-ISSN: 2963-2536

Biomass Production of *Nannochloropsis* sp Cultivated in Combination of Vanname Shrimp Culture Wastewater and Photoperiod as Important Step of Biodiesel Production

Rachmawati Rusydi[1,*] Eva Ayuzar[1], Muliani[1], Munawwar Khalil[1], Indri Widia Wulandari[1], Momika Alfaini[1]

[1] *Aquaculture Department, Faculty of Agriculture, Malikussaleh University, North Aceh-Indonesia*
[*] *Corresponding author. Email: rachmawati.rusydi@unimal.ac.id*

ABSTRACT
Nannochloropsis sp becomes one of potential microalgae which has high lipid content and be explored as biodiesel feedstock. Cultivation of *Nannochloropsis* sp in appropriate manner, low cost, and sustain is the main approach to provide feedstock of biodiesel effectively. By utilizing vannamei shrimp culture wastewater combined to photoperiod, the biomass production of *Nannochloropsis* sp can be resulted optimally. The purpose of this study was to evaluate the combination of vannamei shrimp culture wastewater and photoperiod effectiveness in biomass production of *Nannochloropsis* sp for supporting biodiesel production. The study used experimental laboratory as its method and Factorial Completely Randomized Design as its experimental design. Two factors of treatments namely (1) different percentage of vannamei shrimp culture wastewater and (2) different photoperiod. The levels of first factor were A: Walne, B: 50% of wastewater, C: 75% of wastewater, and D: 100% of wastewater. Then, the levels of second factor were A: 6:18 (L:D), B: 12:12 (L:D), C: 18:6 (L:D), and D: 24:0 (L:D). The result showed that the highest biomass production was achieved by combination 75% of vannamei shrimp culture wastewater and photoperiod 12:12, in amount 12.75 ± 0.212 grams/L. Statistical analysis presented that different media of wastewater and different photoperiod significantly effect on biomass production of *Nannochloropsis* sp ($p<0.05$). Interaction of those two factors also significantly effect on biomass production of that microalgae ($p<0.05$). The recommendation of this study was the combination of vannamei shrimp culture wastewater 75% and photoperiod 12:12 (L:D) for *Nannochloropsis* sp cultivation.

Keywords: Biomass, Biodiesel, *Nannochloropsis* sp, Photoperiod, Vannamei,

1. INTRODUCTION

Nanncochloropsis sp is one type of saline microalgae that has green color and unicellular. It also has mitochondria and membrane surrounded nucleus in its cell [1]. This microalgae has some nutritions such as carbohydrate 16%, protein 52.11%, and lipid 31-68% which is consisted of eicosa pentaenoic acid (EPA) and docosa hexanoic acid (DHA) [2]. Due to its lipid content, *Nannochloropsis* sp becomes one of potential microalga which is explored as biodiesel feedstock.

Biomass production of microalgae becomes the important stage in producing biodiesel. From dried biomass of microalgae, some valuable components can be extracted, such as carbohydrate, protein, and lipid [3]. Furthermore, the quantities of those valuable components are influenced by microalgae's life cycle, nutrients in media, light intensity, pH, temperature, carbon dioxide, and salinity [4]. Then, to support biomass production of microalgae, the cultivation of microalgae is conducted in rich nutrients medium (standard medium) which is expensive and limited. Therefore, some alternative media containing abundant nutrients which are inexpensive, available, and eco-friendly are suggested to alter the standard medium for microalgae.

One of alternative media for cultivating microalgae is vannamei shrimp culture wastewater which has sufficient nutrients, available, and cheap. Vannamei shrimp culture wastewater is resulted from vannamei shrimp culture and contains some important nutrients for microalgae. Based on reference [5], waste solid of vannamei shrimp culture contains 1.92% of organic carbon (C), 0.54% of total nitrogen, and 1.7% of phosphate. Utilization of vannamei shrimp culture wastewater had been done [6] to cultivate *Spirulina* sp. The result showed that vannamei shrimp culture wastewater could increase density and growth of *Spirulina* sp.

Beside the support of nutrients taken from wastewater media, the biomass production of microalgae is also affected by light intensity that is used for photosynthesis process. The duration of light intensity given to organisms is called as photoperiod. A long with light intensity, photoperiod also influences the growth of microalgae and rate of CO2 biofixation [7].

Based on this background, combination of vannamei shrimp culture wastewater and photoperiod implemented in *Nannochloropsis* sp cultivation becomes attractive tobe explored. This study is important to fulfil the information of the factors of *Nannochloropsis's* growth for producing great biomass as biodiesel feedstock. The aim of this study was to evaluate the effect of combination of vannamei shrimp culture wastewater and photoperiod in biomass production of *Nannochloropsis* sp for supporting biodiesel production.

2. MATERIALS AND METHOD

This research was conducted in September to December 2022 in Aquaculture Technology Laboratory, Faculty of Agriculture, Malikussaleh University-North Aceh and Agriculture Laboratory, Syiah Kuala University-Banda Aceh, Indonesia. Meanwhile, *Nannochloropsis* sp starter was obtained from BBPBAP Ujung Batee, Banda Aceh.

The experimental laboratory was used as a method in this research. The experiment purpose was to evaluate the appropriate combination of vannamei shrimp culture wastewater and photoperiod in producing biomass of *Nannochloropsis* sp. There were two factors of experiment, namely 1) different percentage of vannamei shrimp culture wastewater and 2) different photoperiod. The arrangement of treatments is shown in this Table1 below.

Table 1. The arrangement of treatments

Percentage of vannamei shrimp culture wastewater	Photoperiod (light : dark)			
	6:18	12:12	18:6	24:0
Standard medium (Walne)	a b c	a b c	a b c	a b c
50% of vannamei shrimp culture wastewater	a b c	a b c	a b c	a b c
75% of vannamei shrimp culture wastewater	a b c	a b c	a b c	a b c
100% of vannamei shrimp culture wastewater	a b c	a b c	a b c	a b c

2.1. Sterilization of Equipment and Culture Media

The sterilization was conducted by washing containers and tools with soap and rinsing them with freshwater until clean. Then, the containers and tools were sprayed with 70% alcohol and allowed to dry. After drying, the containers were tightly closed using a sterilized container lid, while the tools were wrapped in aluminium foil. The culture containers used were 48 units of 5L jars for running the treatments and 10 units of 5L jars for cultivating the stock of *Nannochloropsis* sp.

The sterilization of wastewater was done initially by filtering wastewater using cloth. Then, wastewater was sterilized by adding chlorine 10 ppm for 24 hours and aerating fully. Then, chlorine in wastewater was neutralized by adding sodium thiosulphate 5 ppm [8].

2.2 Stock Culture of Nannochloropsis sp

Cultivation of stock *Nannochloropsis* sp. was carried out to increase the microalgae starter volume and to ensure the supply of *Nannochloropsis* sp. The medium used in stock culture was Walne fertilizer medium. Cultivation of stock was begun with a volume of 3 Liters of medium and a starter (microalgae inoculant) was given as much as 20% of the medium volume. Cultivation was carried out for 5 (five days) in controlled condition with full aeration and light intensity 2000 lux. Then, on the day 5th, *Nannochloropsis* sp stock was renewed by culturing it in new culture medium with increased medium volume.

2.3 Preparation of Vannamei Shrimp Culture Wastewater as Cultivation Medium

Vannamei shrimp culture wastewater was obtained from CPP Bungkah-North Aceh. The percentages of wastewater used as cultivation media for *Nannochloropsis* sp were in accordance with the treatments. Before using wastewater as medium, it was firstly filtered and sterilized (part 2.1). Furthermore, the volume of each wastewater medium was 3 liters. Then, the starter of *Nannochloropsis* sp was added into medium as much as 20% of medium volume. The wastewater was mixed to saline water in salinity 22 ppt based on the treatments above.

2.4 Nannochloropsis sp Cultivation in Wastewater Media Combined with Photoperiod

Nannochloropsis sp was cultivated in wastewater media, namely 50%, 75%, and 100% of wastewater of vannamei shrimp culture. The cultivations were conducted in controlled condition with full aeration and light intensity 2000 lux. Furthermore, they were also implemented several treatments of photoperiod (light:dark), namely 6:18, 12:12, 18:6, 24:0 for 5 days of cultivation. Then, in day 5th, the biomass of *Nannochloropsis* sp was harvested by flocculation method.

2.5 Harvesting the Biomass of Nannochloropsis sp

The harvesting process was carried out by flocculation method. Sodium hydroxide (NaOH) in amount 1 grams/L was added into volume of cultivation then left for 24 hours. Furthermore, the biomass that already settled was filtered using filter paper. Then, wet biomass was dried in oven in 60°C for 24 hours or until dry and the dried biomass was weighed to measure the produced biomass.

2.6 Data Analysis

Data were analyzed using SPSS software version 21. Analysis of variance used was Factorial Completely Randomized Design with significant level 95%. If there was an influence from the treatments, the analysis was continued by Duncan test. Data were presented in the form of tables and graphs.

3. RESULTS AND DISCUSSION

3.1 Biomass Production of Nannochloropsis sp in Combination of Wastewater and Photoperiod

Biomass production of microalgae is a sustainable and promising feedstock for many purposes of application, such as functional foods, aquaculture, nutraceutical, pharmaceutical, cosmetics, and biofuel [9]. Biodiesel as a type of biofuel, requires feedstocks with high lipid productivity. Some influencing factors for biodiesel production are the factors of microalgae cultivation, lipid accumulation, microalgae harvesting, and lipid extraction [10]. The experiment results are shown by Figure 1 and Figure 2 below.

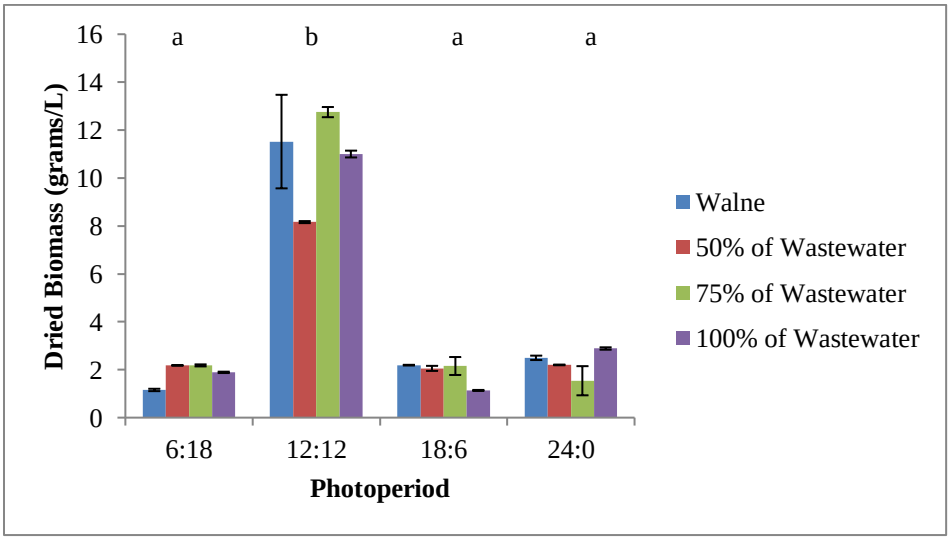

Figure 1. Biomass Production of *Nannochloropsis* sp Cultured in Combination of Vannamei Shrimp Culture Wastewater and Photoperiod

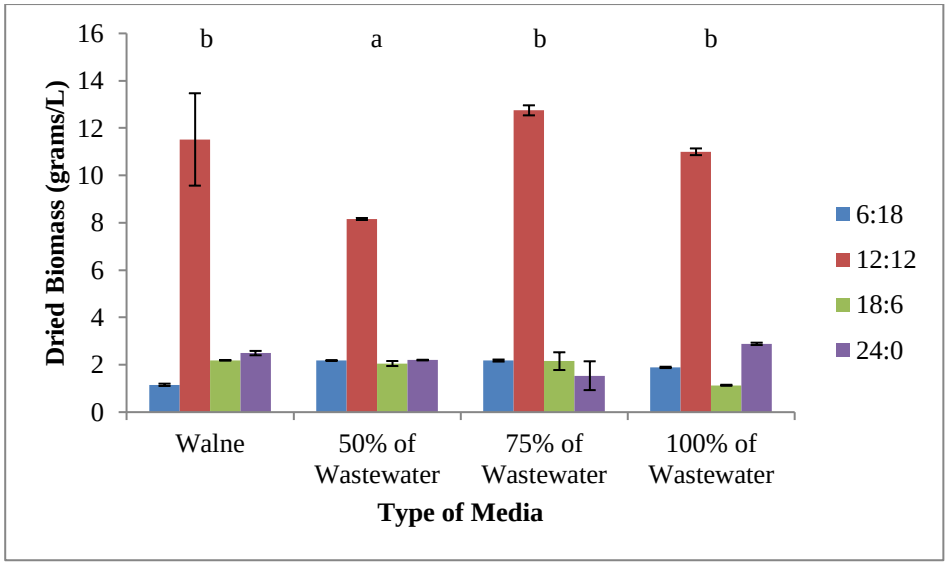

Figure 2. Biomass Production of *Nannochloropsis* sp Cultured in Combination of Vannamei Shrimp Culture Wastewater and Photoperiod

Figure 1 shows that the biomass production of *Nannochloropsis* sp was optimum in photoperiod 12 hours of light and 12 hours of dark in whole types of media. According to statistical analysis, different photoperiod could affect significantly to biomass production of *Nannochloropsis* sp cultivated in several types of media. Based on Duncan analysis, photoperiod 12:12 gave effect differently on biomass production from other photoperiods.

Figure 2 shows that vannamei shrimp culture wastewater was potential tobe cultivation medium for *Nannochloropsis* sp specifically in percentages of 75% and 100% of vannamei shrimp culture wastewater. Vannamei shrimp culture wastewater medium 75% could produce highest biomass of *Nannochloropsis* sp with photoperiod 12:12, in amount 12.75 ± 0.212 grams/L. Then, walne gave better biomass production than vannamei shrimp culture wastewater 100%, in amount 11.52 ± 1.952 grams/L. In general, different media could significantly affect biomass production of *Nannochloropsis* sp ($p<0.05$). Based on Duncan analysis, vannamei shrimp culture wastewater 50% gave effect differently to biomass production of *Nannochloropsis* sp from other media.

Some previous studies showed the potential of vannamei shrimp culture wastewater. The performance of microalgae *Nannochloropsis* sp in white shrimp wastewater cultivation media also gave higher result of cell density, which was 34.5 x 10^4 ind/mL than performance of *Tetraselmis* sp and *Dunaliella* sp [11]. In the other hand, microalgae *Chaetoceros amami* grown in media of water vannamei shrimp farming (50/50) could have good peak phase during cultivation. This media could alter the synthetic fertilizer (walne) in *Chaetoceros amami* cultivation [12].

The studies of photoperiod also have been done by other researchers. The production of microalgae *Verrucodesmus verrucosus* biomass in BG 11 medium and in the 12:12 photoperiod showed greater metabolic adaptation which was proven by shorter lag phase and earlier exponential phase in second day, meanwhile other photoperiods gave longer adaptation phase [13].

4. CONCLUSION

The highest biomass production was achieved by combination 75% of vannamei shrimp culture wastewater and photoperiod 12:12, in amount 12.75 ± 0.212 grams/L. Statistical analysis presented that different media of wastewater and different photoperiod significantly effect on biomass production of *Nannochloropsis* sp ($p<0.05$). Interaction of those two factors also significantly effect on biomass production of that microalgae ($p<0.05$). The recommendation of this study was the combination of vannamei shrimp culture wastewater 75% and photoperiod 12:12 (L:D) for *Nannochloropsis* sp cultivation.

ACKNOWLEDGMENTS

This research was funded by Aksi_Asian Development Bank (ADB), Malikussaleh University. The authors gratefully acknowledge for this financial support, then many thanks to Aquaculture Department, Agriculture Faculty in Malikussaleh University that supported the authors to conduct the experiment.

REFERENCES

[1] Jadid, R., I. Dewiyanti, Nurfadillah. 2017. Addition of Coconut Water in Growth Media of *Nannochloropsis* sp. *Jurnal Ilmiah Mahasiswa Kelautan dan Perikanan*, 2(1): 113-118.
[2] Mirzayanti, Y.W., R.E., Syafutra, Y., Vinataningsih, C., Irawan. 2021. Konversi Mikroalga *Nannochloropsis* sp menjadi Biodiesel melalui Proses Transesterifikasi secara In-Situ. *Buletin Profesi Insinyur*, 4(2): 80-84. http://dx.doi.org/10.20527/bpi.v4i2.113.

[3] Patmawati, B., Ibrahim, I., Setyaningsih, U., Sudadi. 2014. Biodiesel Production from Biomass of Chlamydomonas sp. ICBB 9113 Cultivated in a Cheap Culture Media: Effectivity of different extraction methods. *Widyariset*, 17(2): 269-276.

[4] Loganathan, B.G. 2018. Factors Affecting Growth of Various Microalgal Species. *Environmental Engineering Sciience*, 35(10):

[5] Tangguda, S. & I.A.P, Suryanti. 2017. Pengaruh Limbah Cair Tambak Udang Terhadap Kepadatan Sel dan Laju Pertumbuhan Spesifik *Chlorella* sp. Seminar Nasional Riset Inovatif (SENARI).

[6] Hartami, P., Mauliyani, Erniati, P., Masyithah, , .Kurniawan, N., Suhaila, Muliani, R., Rusydi. 2022. Effectiveness of Spirulina platensis as a bioremediator candidate for vaname shrimp (*Litopenaeus vannamei*) wastewater. *Acta Aquatica: Aquatic Sciences Journal*, 9(1): 54-59.

[7] Gunawan, T.J., Y. Ikhwan, F. Restuhadi, U. Pato. 2018. Effect of Light intensity and Photoperiod on Growth of *Chlorella pyrenoidosa* and CO2 Biofixation. E3S Web of Conferences, 31: 1-7.

[8] Erniati, Erlangga, Hariana. 2012. Pemberian Mikroalga yang Berbeda Terhadap Pertumbuhan *Artemia salina*. *Jurnal Berkala Perikanan Terubuk*, 40(20): 13-19.

[9] Muhammad, G., Md. A. Alam, W. Xiong, Y. Lv, J.L. Xu. 2020. Microalgae Biomass Production: An overview of dynamic operational methods. *Microalgae Biotechnology for Food, Health, and High Value Products*, 415:432.

[10] Zhang, S., L. Zhang, G. Xu, F. Li, X. Li. 2022. A Review on Biodiesel Production from Microalgae: Influencing parameters and recent advanced technologies. *Frontiers in Microbiology*, 13 : 1-20.

[11] Santanumurti, M.B., S. Khanza, Z. Abidin, B. Putri, S. Hudaidah. 2022. The Performance of Microalgae (*Nannochloropsis* sp, *Tetraselmis* sp, and *Dunaliella* sp) on White Shrimp (*Litopenaeus vannamei*) Wastewater Cultivation Media. *Journal of Aquaculture and Fish Health*, 11(1): 1-9.

[12] Febrinawati, N., B. Putri, S.Hudaidah. 2020. Utilization Waste Vanamei Shrimp Farming (*Litopenaeus vanamei*) as a Media Culture Chaetoceros amami. *Jurnal Perikanan*, 10(1): 20-28.

[13] Landa, L.V., H.R.H. De Leon, Y.D.C.P. Luna, S.V. Trujillo, J.M. Acosta, R.B. Hernandez, Y.S. Roque. 2021. Influence of Light Intensity and Photoperiod on the Photoautotrophic Growth and Lipid Content of the Microalgae *Verrucodesmus verrucosus* in a Photobioreactor. *MDPI: Sustainability*, 13: 1-11.