JPPIPA 7(1) (2020)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Utilization Of Rice Waste Water on Biomass and Carotenoid Pigment *Arthrospira platensis*

Anisa Millah Taqiyyah[1*], Yenny Risjani[2], Asep Awaludin Prihanto[3], Gilang Drajat Maulana[4], Karimah[5]

[1]Aquaculture Master Program, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, Indonesia.
[2]Departement of Fisheries and Marine Resources Management, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, Indonesia
[3]Departement of Fisheries Product Technology, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, Indonesia
[4]Aquaculture Master Program, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, Indonesia
[5]Physics Master Program, Brawijaya University, Malang, Indonesia

Received: December 24, 2022
Revised: February 22, 2023
Accepted: February 26, 2023
Published: February 28, 2023

Corresponding Author:
Anisa Millah Taqiyyah
anisamillah10@gmail.com

DOI: 10.29303/jppipa.v9i2.2795

Abstract: *Arthrospira platensis* culture requires macro and micronutrients. The nutrients needed by *Arthrospira platensis* according to Agustina and Herman (2021) are macronutrients consisting of nitrogen (N), phosphorus (P), potassium (K), sulfur (S), iron (Fe), magnesium (Mg), silicon (Si), and calcium (Ca). Micronutrients consist of zinc (Zn), cobalt (Co), molybdenum (Mo), boron (B), and copper (Cu). Rice-washing wastewater contains macro and micronutrients needed for the growth of *Arthrospira platensis*. Almost all of the nutrients needed by *Arthrospira platensis* are found in the wastewater, except for Co. Previous studies have shown that rice-washing wastewater can be used for *Arthrospira platensis* culture, but the growth rate is still higher than Walne fertilizer 0.5 ml/L. Zarrouk fertilizer as a standard growth medium for *Arthrospira platensis* shows that this fertilizer has better growth results than Urea and NPK fertilizers. This research was conducted to determine the effect of rice-washing wastewater as a nutrient source for culture media or a combination of Zarrouk fertilizer and rice-washing wastewater so that it can be used to increase the biomass and carotenoid pigments of *Arthrospira platensis*. This research used a Factorial Completely Randomized Design (CRD) with 12 treatments and 3 replications. *Arthrospira platensis* quality test was carried out by testing dry biomass and carotenoid content. The ANOVA test results showed that this study's treatment had a significant effect on the carotenoid content and biomass of *Arthrospira platensis*. The treatment with the highest effect of biomass and carotenoids was obtained in the treatment of 1.5 ml/L of rice-washing wastewater.

Keywords: *Arthrospira platensis*; Biomass; Carotenoids; Culture Media; Rice Washing; Wastewater.

Introduction

Photosynthetic microorganisms are one of the most promising sources of energy because they are renewable and CO2-neutral. Species belonging to the genus *Arthrospira*, are photosynthetic microorganisms that are in great demand commercially (Salunke et al., 2021). *Arthrospira platensis* is multicellular, filamentous blue-green algae that have many uses in the health care and food fields as a protein and vitamin supplement. This is because *Arthrospira* contains very high amounts of micro and macronutrients (Soni et al., 2019). The blue-green color of this organism is caused by the presence of several photosynthetic pigments such as chlorophyll, carotenoids, phycocyanin, and phycoerythrin. Phycocyanin is responsible for the blue color of organisms (Sow and Ranjan, 2021). *Arthrospira platensis* culture requires macro and micronutrients. The nutrients needed by *Arthrospira platensis* according to Agustina and Herman (2016) are macronutrients consisting of nitrogen (N), phosphorus (P), potassium (K), sulfur (S), iron (Fe), magnesium (Mg), silicon (Si), and calcium (Ca). Micronutrients consist of zinc (Zn), cobalt (Co), molybdenum (Mo), boron (B), and copper

How to Cite:
Taqiyyah, A.M., Risjani, Y., Prihanto, A.A., Maulana, G.D., & Karimah, K. (2023). Utilization Of Rice Waste Water on Biomass and Carotenoid Pigment Arthrospira platensis. *Jurnal Penelitian Pendidikan IPA*, *9*(2), 832–837. https://doi.org/10.29303/jppipa.v9i2.2795

(Cu).

Rice-washing wastewater contains macro and micronutrients needed for the growth of *Arthrospira platensis*. Almost all of the nutrients needed by *Arthrospira platensis* are found in the wastewater, except for Co. Research by Utomo et al., (2020) who used rice-washing wastewater as a source of nutrients in *Arthrospira* sp. culture media. showed that the 1 ml/L treatment produced the highest specific growth rate, but it was higher when compared to the positive control treatment, namely 0.5 ml/L Walne fertilizer. *Arthrospira platensis* cultures generally use Zarrouk fertilizer as a standard microalgae growth medium, but as knowledge develops regarding nutrient content in wastewater, many studies have examined the growth of *Arthrospira platensis* in wastewater media. Research Mulokozi et al., (2019) who compared the growth of *Arthrospira* in Zarrouk, Urea, and NPK media showed that the medium that gave the best results for *Arthrospira* growth was Zarrouk's media. This is because Zarrouk has completed and more balanced macro and microelements. In addition, the carbon, nitrogen, and protein content in *Arthrospira platensis* decreased in the Zarrouk and Urea combination media (Al Mahrouqi et al., 2022).

The use of washed rice water should be encouraged as the practice is part of better water management. Global demand for fresh water in 2025 is projected to increase by 55% (Park, 2013). The increase is due to climate change and an increase in world population, which prompted the United Nations to advocate for more effective water management (Paquin and Cosgrove, 2016), where wastewater is used and reused or recycled for other purposes, rather than being thrown away (Nabayi et al., 2022). The utilization of rice washing wastewater can be proven through research. This study was conducted to analyze the effect of rice-washing wastewater as a source of nutrients for culture media or a combination of Zarrouk fertilizer and rice-washing wastewater so that it can be used to increase the biomass, protein, and carotenoid pigments of *Arthrospira platensis*.

Method

Location and Time of Research

The research was conducted at the Faculty of Fisheries and Marine Sciences, Universitas Brawijaya in August 2022. Test analysis was carried out at the Hydrobiology Laboratory of the Division of Aquatic Environment, Universitas Brawijaya. Sterilization of tools and materials is carried out in the Laboratory of Aquaculture, Division of Fish Disease and Health, Universitas Brawijaya.

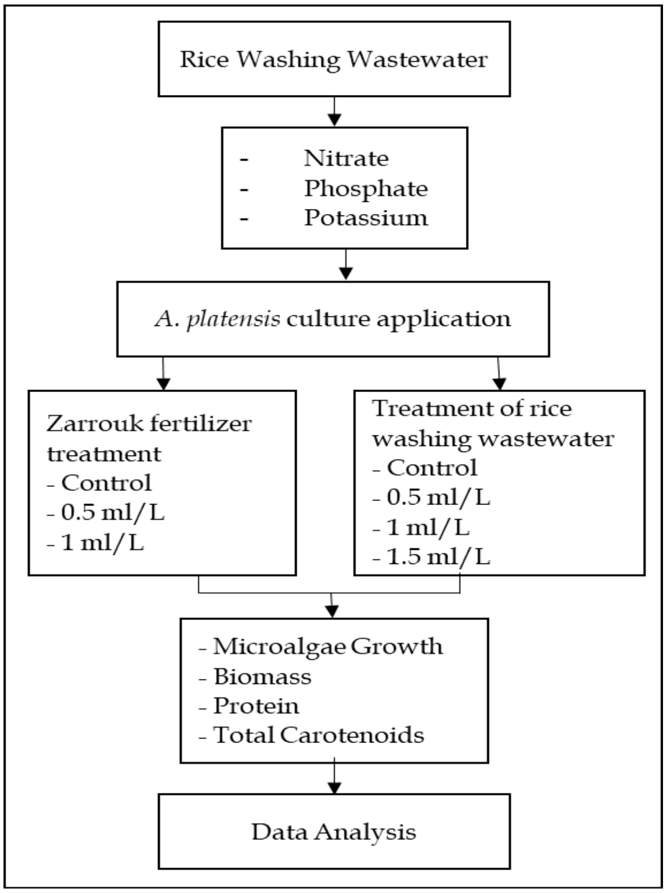

Figure 1. Research flow

Research design

The study had a factorial complete randomized design (CRD) with 12 treatments and 3 replications. The first factor consisted of rice washing wastewater treatment with 4 types of treatment namely B0, B1, B2, and B3. The treatments were control treatments respectively, 0.5 ml/L, 1 ml/L, and 1.5 ml/L. The second factor was Zarrouk fertilizer with 3 types of treatment, namely D0, D1, and D2. The treatments were control treatment, 0.5 ml/L, and 1 ml/L respectively. Rice washing water was obtained according to the method of Utomo et al. (2020), rice is washed with water in the amount of 1:1 the amount of water and rice used. Rice washing is done by squeezing five times. Fish culture wastewater and rice washing waste were filtered using a 100-micron mesh size plankton net filter cloth and autoclaved at 1210C for 20 minutes (Guldhe et al., 2017). The rice-washing wastewater used had nitrate, phosphate, and potassium content of 4.574, 0.798, and 2.339 mg/L respectively.

Cell growth analysis

The density of *Arthrospira platensis* was calculated using the cell concentration calculation method. The number of *Arthrospira platensis* cell units is calculated based on the filaments that are twisted to form a spiral (helix), where one *Arthrospira* cell unit is one wavelength consisting of one valley and one hill (Buwono and Nurhasanah, 2018). The tools used were Haemocytometer 0.1 mm and a microscope. The specific growth rate is calculated using the formula according to Makkasau *et al.*, (2011).

Biomass Analysis

The *Arthrospira* biomass test was carried out according to the method of Janssen et al., (1999).

Protein Analysis

The protein assay method was carried out using the Lowry method with a reagent that detects phenolic groups such as the Folin-Ciocalteu reagent which has been used in determining protein concentrations by Lowry (1951).

Carotenoid Pigment Analysis

The test for carotenoid pigment content was carried out according to the Vo and Tran method (2014).

Data analysis

Data analysis was carried out by ANOVA test and Least Significant Difference (LSD) using the Microsoft Excel version 2013 application.

Result and Discussion

Cell Growth

The growth phase of *Arthrospira platensis* cells was observed within 8 days. The adaptation phase of cell growth in this study occurred on the first and second days of culture. The adaptation phase of *Arthrospira platensis* cells varies. This is caused by the number of nutrients in different media. *Arthrospira platensis* cells entered the log or exponential phase on the third to the 6th day of culture. This is following the research of Hartami et al., (2022). According to Mauretsa *et al*. (2019), sufficient nutrients can facilitate *Arthrospira platensis* in breeding to achieve maximum growth. The highest growth of *Arthrospira platensis* cells was obtained on the seventh day of culture.

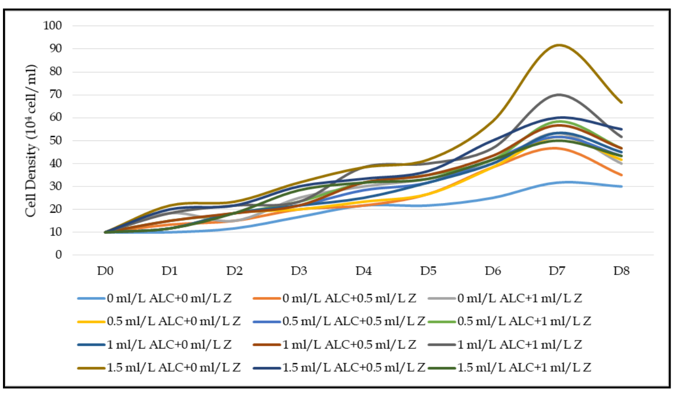

Figure 2. The growth phase of *Arthrospira platensis* cells in rice washing wastewater (RWW) and zarrouk (Z) media.

Arthrospira platensis cells in the study of Hartami et al., (2022) underwent a stationary phase on the seventh day, marked by the density of *Arthrospira platensis* reaching the peak population. *Arthrospira platensis* cell growth decreases or cells die when the nutrients in the culture media are insufficient for cell growth (Mauretsa et al., 2019). The cell death phase of *Arthrospira platensis* in this study occurred on the eighth day of culture. The time required for the growth of microalgae cells can be determined by the specific growth rate, where the higher the growth rate, the faster the microalgae will grow.

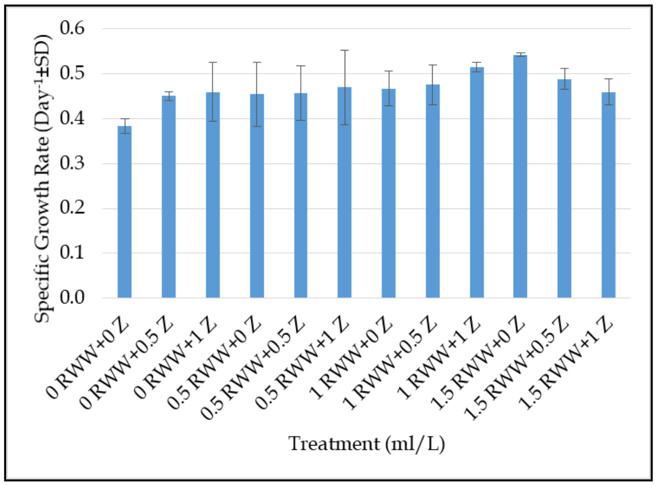

Figure 3. Specific growth rate data (Day^{-1}) of *Arthrospira platensis* during the culture period.

The specific growth rate of *Arthrospira platensis* culture with rice washing wastewater as the main nutrient showed that the highest value was obtained at 0.550 Day-1 in the B3D0 treatment, namely the treatment of rice washing wastewater at 1.5 ml/L. The LSD test on the specific growth rate in Appendix 6 also shows that the treatment has a significant effect. The growth of *Arthrospira platensis* is influenced by the concentration of nutrients in the culture medium. Nutrient concentrations that are unbalanced or suitable can cause non-optimal growth (Mousavi et al., 2022). The elements that limit the growth of microalgae are nitrogen, especially in the form of nitrate and anomia, and phosphorus in the form of phosphate Nogueira et al. (2018), where these elements are present in the culture media. This study showed that the specific growth rate of *Arthrospira platensis* increased with the addition of nutrient sources up to a certain limit.

Biomass

Biomass measurements were carried out on the 7th day, when cell growth reached its peak. The results of the Anova test on *Arthrospira platensis* biomass data showed that the treatment in this study was significantly different from the results of F-count>f-table 5%. The results of the LSD test on the biomass data can be seen in Figure 1. The notation in the figure shows the order of the biomass obtained from the smallest to the largest. Biomass data showed that the treatment with the highest yield of dry biomass and significantly different was obtained in the B3D0 treatment, namely 1.5 ml/L of wastewater.

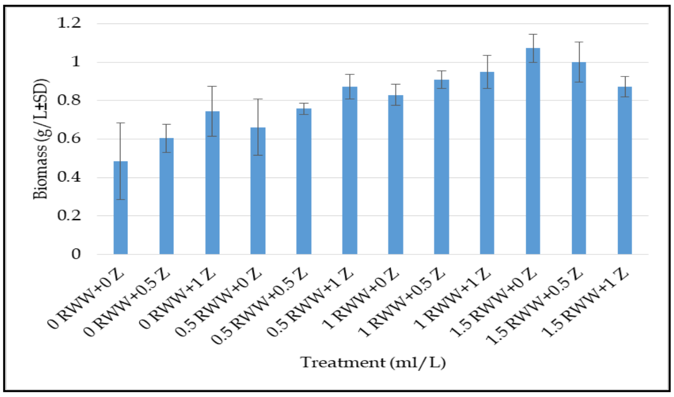

Figure 4. Data on dry biomass measurements (mg/L) of *Arthrospira platensis.*

The decrease in the biomass value of *Arthrospira platensis* can occur due to the high nitrogen content in the media (Kaewdam et al., 2019). Besides nitrogen, the availability of phosphate also affects the biomass of *Arthrospira platensis*. Research by Markou et al., (2021) shows that increasing the concentration of phosphate in the culture media can increase biomass production. Research by Chu et al., (2022) showed that in the nitrate treatment without the addition of phosphate, a phosphate deficiency occurred which caused inhibition of nitrogen adsorption. This has an impact on the yield of biomass culture that is not optimal.

Protein

The microalgae protein test sample was taken during the peak growth phase of *Arthrospira platensis*. The protein test results showed that the highest protein content was obtained in the treatment of 1.5 ml/L of rice-washing wastewater. The LSD test showed that the treatment had a significant effect on Arthrospira platensis protein.

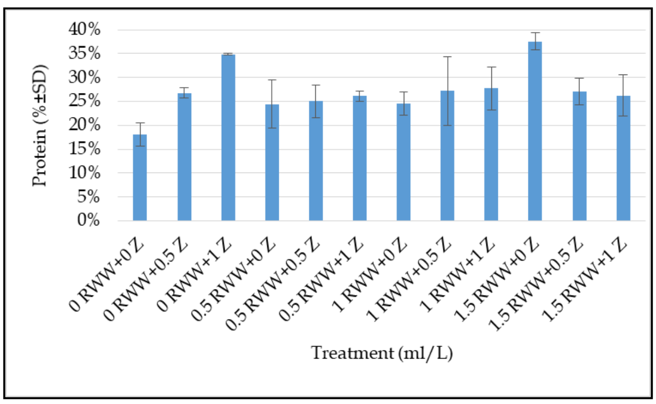

Figure 5. Data of protein test result (%) *Arthrospira platensis.*

The protein content of *Arthrospira platensis* increases with increasing treatment. This is due to an increase in nutrients in the culture media. According to the statement of Kaewdam et al., (2019), an increase in protein biosynthesis occurs due to an increase in the nutrient nitrate, where nitrate is needed for the synthesis of amino acids used to produce protein. In addition, the increase in phosphate can also affect the protein content in *Arthrospira platensis*. The results of Gopi's research (2021) show that the initial conditions of media with limited phosphate content can cause a decrease in protein content.

Carotenoid

Analysis of the carotenoid pigment content test was carried out on the 7th day.

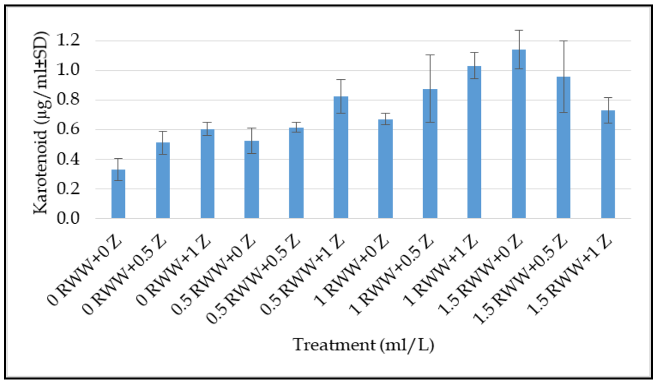

Figure 6. Data on carotenoid test result (mg/L) of *Arthrospira platensis.*

LSD test results on *Arthrospira platensis* carotenoid pigment data showed that the highest content was obtained in the B3D0 treatment, namely 1.5 ml/L wastewater. The content of carotenoids in *Arthrospira platensis* is influenced by the concentration of nitrate in the culture medium. The research of El Baky et al., (2020) showed that in general the total carotenoids of *Arthrospira platensis* are directly proportional to the concentration of nitrate in the culture media, but conditions of culture media that have limited nitrates can produce higher carotenoids than media conditions with optimal nitrates. This can happen because the process of photosynthesis can continue and the resulting compounds such as carbohydrates can be obtained even in cultures with limited nitrates. High availability of nutrients does not guarantee high carotenoid content. This is in accordance with the research of Prabhath et al., (2022) which showed that the high carbon content in the culture media led to low carotenoid content.

Conclusion

Rice washing wastewater can be used as a source of nutrients in Arthrospira platensis culture media to increase the biomass and carotenoid pigments of the microalgae. The best test results for biomass, protein, and carotenoids in this study were obtained in the 1.5 ml/L rice washing wastewater treatment.

Acknowledgements

The author would like to thank Mrs. Nurul, Mrs. Hawa, Ryan, and the staff of the Faculty of Fisheries and Marine Sciences at Brawijaya University, who have provided the facilities and infrastructure to support this research.

References

Agustina, S., dan Herman, S. (2016). Potensi mikroalga sebagai bahan kimia ADI. *Bbkk. Kemenperin.Go.Id*, *3*(1). Retrieved from http://bbkk.kemenperin.go.id/wp-content/uploads/portal/portal2016.pdf#page=19

Al Mahrouqi, H., Vega, J., Dobretsov, S., dan Abdala Díaz, R. T. (2022). The Effect of Medium Concentration and Nitrogen Source on the Productivity and Biochemical Composition of *Arthrospira platensis*. *Biology Bulletin*, *49*(2), 75-84. https://doi.org/10.1134/S1062359022020108

Buwono, N. R., dan Nurhasanah, R. Q. (2018). Study of *Spirulina* sp. Population Growth in The Different Culture Scale. *Jurnal Ilmiah Perikanan dan Kelautan*, *10*(1), 26-33. https://doi.org/10.20473/jipk.v10i1.8202

Chu, R., Hu, D., Zhu, L., Li, S., Yin, Z., & Yu, Y. (2022). Recycling spent water from microalgae harvesting by fungal pellets to re-cultivate Chlorella vulgaris under different nutrient loads for biodiesel production. *Bioresource Technology*, *344*, 126227. https://doi.org/10.1016/j.biortech.2021.126227

El Baky, H. H. A., El Baroty, G. S., dan Mostafa, E. M. (2020). Optimization growth of *Spirulina* (Arthrospira) platensis in photobioreactor under varied Nitrogen concentration for maximized biomass, carotenoids and lipid contents. *Recent Patents on Food, Nutrition and Agriculture*, *11*(1), 40-48. https://doi.org/10.2174/2212798410666181227125229

Gopi, V. (2021). *Potential of Cyanobacterium Spirulina platensis for Eutrophic Water Restoration* (Doctoral dissertation, Université d'Ottawa/University of Ottawa). http://dx.doi.org/10.20381/ruor-26066

Guldhe, A., Ansari, F. A., Singh, P., & Bux, F. (2017). Heterotrophic cultivation of microalgae using aquaculture wastewater: a biorefinery concept for biomass production and nutrient remediation. *Ecological engineering*, *99*, 47-53. https://doi.org/10.1016/j.ecoleng.2016.11.013

Hartami, P., Mauliyani, M., Erniati, E., Masyithah, P., Kurniawan, R., Suhaila, N., ... dan Rusydi, R. (2022). Effectiveness of *Spirulina platensis* as a bioremediator candidate for vaname shrimp (Litopenaeus vannamei) wastewater. *Acta Aquatica: Aquatic Sciences Journal*, *9*(1), 54-59. https://doi.org/10.29103/aa.v9i1.6992

Janssen, M., Kuijpers, T. C., Veldhoen, B., Ternbach, M. B., Tramper, J., Mur, L. R., dan Wijffels, R. H. (1999). Specific growth rate of Chlamydomonas reinhardtii and Chlorella sorokiniana under medium duration light/dark cycles: 13–87 s. In *Progress in Industrial Microbiology*, *35*, 323-333. https://doi.org/10.1016/S0079-6352(99)80124-6

Kaewdam, S., Jaturonglumlert, S., Varith, J., Nitatwichit, C., dan Narkprasom, K. (2019). Kinetic Models For Phycocyanin Production By Fed Batch Cultivation Of The *Spirulina platensis*. *Geomate Journal*, *17*(61), 187-194. https://doi.org/10.21660/2019.61.89205

Lowry, O. H. (1951). Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, *193*, 265-275. https://doi.org/10.1016/S0021-9258(19)52451-6

Markou, G., Eliopoulos, C., Argyri, A., dan Arapoglou, D. (2021). Production of Arthrospira (*Spirulina*) platensis Enriched in β-Glucans through Phosphorus Limitation. *Applied Sciences*, *11*(17), 8121. https://doi.org/10.3390/app11178121

Mauretsa, Z., Zulfahmi, I., dan Rahmawati, L. (2019). Fitoremediasi Limbah Budidaya Ikan Nila, *Oreochromis niloticus* (Linnaeus, 1758) Menggunakan *Spirulina* sp. In *Prosiding SEMDI-UNAYA (Seminar Nasional Multi Disiplin Ilmu UNAYA)*, 3. Retrieved from http://jurnal.abulyatama.ac.id/index.php/semdiunaya

Mousavi, M., Mehrzad, J., Najafi, M. F., Zhiani, R., dan Shamsian, S. A. A. (2022). Nitrate and ammonia: two key nitrogen sources for biomass and phycocyanin production by Arthrospira (*Spirulina*) platensis. *Journal of Applied Phycology*, *34*(5), 2271-2281. https://doi.org/10.1007/s10811-021-02664-0

Mulokozi, D. P., Mtolera, M. S., dan Mmochi, A. J. (2019). Biomass production and growth performance of Momela Lake's *Spirulina* (*Arthrospira fusiformis*) cultured under urea and N: P: K fertilizers as cheaper nitrogen sources. *International Journal of Biological and Chemical Sciences*, *13*(2), 861-869. https://doi.org/10.4314/ijbcs.v13i2.23

Nabayi, A., Teh, C. B. S., Tan, A. K. Z., & Tan, N. P. (2022). Consecutive Application Effects of Washed Rice Water on Plant Growth, Soil Chemical Properties, Nutrient Leaching, and Soil Bacterial Population on Three Different Soil Textures over Three Planting Cycles. *Agronomy*, *12*(9), 2220. https://doi.org/10.3390/agronomy12092220

Nogueira, S. M. S., Souza Junior, J., Maia, H. D., Saboya,

J. P. S., dan Farias, W. R. L. (2018). Use of *Spirulina platensis* in treatment of fish farming wastewater. *Revista Ciência Agronômica*, *49*, 599-606. https://doi.org/10.5935/1806-6690.20180068

Paquin, M., & Cosgrove, C. (2016). *The United Nations World Water Development Report 2016: Water and jobs*. UNESCO for UN-Water. Retrieved from https://unesdoc.unesco.org/ark:/48223/pf0000243938

Park, J. (2013). Environment and health: An overview of current trends at WHO and OECD. *Journal of Environmental Health Sciences*, *39*(4), 299-311. https://doi.org/10.5668/JEHS.2013.39.4.299

Prabhath, G. P. W. A., Shukla, S. P., Srivastava, P. P., Kumar, K., Sawant, P. B., Verma, A. K., ... dan Nuwansi, K. K. T. (2022). Downstream processing of biomass produced in aquaculture wastewater for valuable pigments from the cyanobacterium *Spirulina* (*Arthrospira*) *platensis*: a green and sustainable approach. *Aquaculture International*, 1-26. https://doi.org/10.1007/s10499-022-00949-w

Salunke, M. A., Wakure, B. S., dan Wakte, P. S. (2021). Neoteric Approaches for Extraction of Bioactives from Marine Macroflora. *International Journal of Research in Pharmaceutical Sciences*, *12*(4), 2507-2518. http://dx.doi.org/10.26452/ijrps.v12i4.4896

Soni, R. A., Sudhakar, K., dan Rana, R. S. (2019). Comparative study on the growth performance of *Spirulina platensis* on modifying culture media. *Energy Reports*, *5*, 327-336. https://doi.org/10.1016/j.egyr.2019.02.009

Sow, S. and Ranjan, S. (2021). Cultivation of *Spirulina*: An innovative approach to boost up agricultural productivity. *The Pharma Innovation*. *10*(3), 799-813. https://doi.org/10.22271/tpi.2021.v10.i3k.5889

Utomo, A. N. S., Julyantoro, P. G. S., dan Dewi, A. P. W. K. (2020). Pengaruh Penambahan Air Cucian Beras terhadap Laju Pertumbuhan *Spirulina* sp. *Current Trends in Aquatic Science*, *3*(1), 15-22. Retrieved from https://ojs.unud.ac.id/index.php/CTAS/article/view/51260

Vo, T., dan Tran, D. (2014). Carotene and antioxidant capacity of *Dunaliella salina* strains. *World Journal of Nutrition and Health*, *2*(2), 21-23. Retrieved from http://www.sciepub.com/JNH/abstract/2307