Sriwijaya Journal of Environment
Article
ojs.pps.unsri.ac.id

Optimization of *Arthrospira platensis* Growth by Adding Different Carbon Sources on the Culture Media of Catfish Cultivation Waste and Technical Fertilizer

Nyayu Nurul Husna[1,2], Marini Wijayanti[2], Nuni Gofar[3*)]

[1]*Magister Program on Management of Environmental Study, Sriwijaya University, Palembang, Indonesia*
[2]*Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University, Palembang, Indonesia*
[3]*Department of Soil Science, Faculty of Agriculture, Sriwijaya University, Palembang, Indonesia*
[*)] *Corresponding Author: nigofar@unsri.ac.id*

Article history

Received	Received in revised form	Accepted	Available online
24 December 2021	22 February 2022	15 July 2022	30 August 2022

Abstract: Nutrients (C, N, and P) can affect the growth and biochemical content of *Arthrospira platensis*. Ethanol can be a potential carbon source compared to glucose and acetic acid. Glycerol is also potential as a carbon source for *Arthrospira platensis*. This research was carried out from June to July 2021 at the Aquaculture Laboratory and Experimental Pond Laboratory of the Aquaculture Study Program, Sriwijaya University. The research method used Completely Randomized Design (CRD) factorial with two factors consisting of the first factor with three treatments and the second factor with two treatments and three replications. The inoculant was cultured in liquid fertilizer technical F/2 media for culture stock as initial inoculum with a density of ± 1.0 g L^{-1}. Parameters observed were the density, the specific growth rate, and water quality. The results showed that M1S3 treatment had the highest density, reaching 4.95 g L^{-1} and the highest specific growth rate of 0,33% per day. ANOVA test result showed that the addition of carbon sources in the culture media of fertilizer and technical waste had a significant effect ($P<0.05$) on the growth rate of *Arthrospira platensis*. The water quality parameters of each treatment were suitable for *Arthrospira platensis* culture.
Keywords: growth, addition, Arthrospira platensis, different carbon sources, culture media

1. Introduction

Arthrospira platensis (*Spirulina*) is a cyano-bacteria that has been widely used in various industrial sectors, both food, and medicine. Various studies were carried out in cultivating *Arthrospira platensis* in modifying nutrient sources, carbon sources, lighting, temperature and providing nutrition. It increases the growth rate, biomass and chemical composition of *Arthrospira platensis*. Nutrients (carbon, nitrogen, phosphorus) can affect microalgae's growth and biochemical content, such as *Arthrospira platensis* [1].

Research on the modification of nutrient sources for *Arthrospira platensis* culture has been carried out, one of which is using catfish culture waste as a medium for *Arthrospira platensis* cultivation. This study shows that the density rate of *Arthrospira platensis* cultivated with catfish waste media is higher than technical media, so it is necessary to add a carbon source that can increase the fatty acid content of *Arthrospira platensis* [2]

Ethanol can be a potential carbon source compared to glucose and acetic acid in increasing the fatty acid content of *Arthrospira platensis* using the fed-batch method [3]. Besides ethanol, glycerol is also potential as a carbon source to increase the concentration of *Arthrospira platensis* fatty acids. Glycerol is a by-product of biodiesel from the transesterification reaction, in the form of a colorless, odorless, viscous liquid with a sweet taste, and alcohol compound with three hydroxyl groups [4].

Research on the chemical composition of glycerol states that crude glycerol contains 96% methanol [5]. Another study on lipid production using glycerol in *Schizochytrium limacinum* SR21 and *Cryptococcus curvatus* produced the highest cellular lipid, 73.3% [6].

This study aimed to analyze the effect of adding different carbon sources, were glycerol and ethanol in the cultivation of *Arthrospira platensis* cultured on fish culture waste media and technical fertilizers on the optimization of growth rate maximum density.

2. Material and Methods

2.1. Materials

The materials used in this study were, first, *Arthrospira platensis* which was used in this study previously cultured in liquid technical fertilizer F/2 media for culture stock as initial inoculum with a density of ± 1.0 g L^{-1}. Second, the culture media from catfish farming waste already two weeks and technical fertilizers. Third, the carbon source consists of ethanol and glycerol. Furthermore, the research was carried out from June to August 2021 at the Aquaculture Laboratory and Experimental Pond Laboratory, Aquaculture Study Program, Sriwijaya University, Indonesia.

2.2. Methods

2.2.1. Tools Preparation

The equipment used in this study was cleaned first by washing it with soap, then drying it and finally spraying it with 70% alcohol. It aims to minimize contaminants that can inhibit the productivity of *Arthrospira platensis*. The equipment used in this study was cleaned first by washing with soap and then drying and finally spraying with 70% alcohol to minimize contaminants that can inhibit the productivity of *Arthrospira platensis.*

2.2.2. Preparation of growing media for Arthrospira platensis

The culture media used were catfish culture waste and technical fertilizer. The culture media from catfish culture waste was first filtered using a 30-40 micron Asahi cloth to minimize waste water from dirt and insoluble feed residue. After filtering, the media water is cooked to a boil to kill microorganisms. Then cooled and given baking soda so that the pH level of the water becomes 8 - 8.5 and salt to a salinity of 30 ± 1 ppt; the last is left for 12 hours. The catfish culture waste comes from an intensive catfish rearing pond measuring 1m x 1m x 1m. The water was put into the 500 liter pond and 100-150 catfish seeds are stocked/m; the seed size was 5gr/head (SNI 6484.3:2014). Maintenance time for 14 days by feeding 5-6 mm floating pellets (30-32% protein, 4% fat, 5% fiber, 13% ash, and 10% moisture content) was given three times a day at satiation without any water change.

2.2.3. Culture and Harvesting of Arthrospira platensis

Arthrospira platensis was previously cultured in liquid fertilizer technical media F/2 for culture stock as initial inoculum with a density of ± 1.0 g L^{-1}. The stock of *Arthrospira platensis* was put into 10 liters of aerated catfish culture wastewater and lighting using a 36 watt TL lamp (1500 - 1700 lux) for 24 hours/day. Harvesting of *Arthrospira platensis* was carried out in an exponential phase to a stationary phase. Harvesting was done by filtering using a 30-40 micron Asahi cloth [7]. *Athrospira platensis* was then weighed on wet weight and then put into a porcelain exchange and then dried in an oven at 40°C for 18 hours [8]. And then mashed using a mortar.

2.2.4. Experimental variable and analytical procedures

The research method used Completely Randomized Design (CRD) factorial with 2 factors consisting of the first factor with three treatments and the second factor with two treatments and three replications. The first factor is the treatment of carbon sources (S), were SC = control, SG = glycerol, and SE = ethanol. The second factor is the treatment of culture media (M), were WM = catfish culture waste media, FM = technical fertilizer media.

The data obtained were analyzed by analysis of variance (ANOVA) at the 5% level using the SPSS 24 program to determine the effect of the treatment to being tried. If the test results are significantly different, further tests were carried out that are adjusted to the magnitude of the of diversity coefficient. [9].

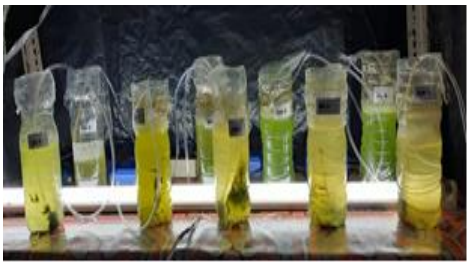

Figure 1. Culture Media using Catfish Culture Waste

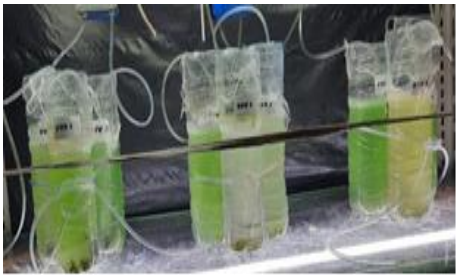

Figure 2. Culture Media using Technical Fertilizer

2.3. Data Analysis

The density of *Arthrospira platensis* was measured using the wet and dry weight methods. Calculation of the specific growth rate of Arthrospira platensis was done using the formula [10]. Checking the water quality on the culture media was carried out once a day during the culture period.

3. Results and Discussion

3.1. Density

The research observations carried out for 12 days of the culture process showed that adding different carbon sources (glycerol and ethanol) in the culture media of cultivation waste and technical fertilizers, the average density of *Arthrospira platensis* was shown in Table 1. Based on the data from Table 1, it is known that the highest achievement of the density of *Arthrospira platensis* culture for each treatment occurred at the same time were the eight- day with the maximum density value of each treatment.

Table 1. Density Data for *Arthrospira platensis* (g L^{-1})

Factor S	Factor M		Main Effect of Carbon Source (S)
	WM	FM	
SC	3,0600[a]	3,0967[a]	3,0783[a]
SG	3,2267[ab]	3,2800[abc]	3,2533[a]
SE	3,5400[c]	3,4800[bc]	3,5100[b]

Notes: Numbers followed by the same letter in the same column mean that they are not significantly different at the 5% test level (Duncan)

The results of the analysis using the ANOVA test showed that the single factor of carbon source treatment (S) had a significant effect on the growth of *Arthrospira platensis* with a significance value less than 0.05 (P<0.05). Then Duncan's further test was carried out for the carbon source treatment factor (S). Treatment SC (control) was not significantly different from treatment SG (glycerol), but treatment SE (ethanol) was significantly higher than for the other treatments. The addition of carbon (S) sources as one of the nutrients can be a solution to accelerate the growth of microalgae [12].

The single factor treatment of culture media (M) was not significantly different (P> 0.05). It means that the culture media (M) of aquaculture waste media and technical fertilizers media do not effect on the growth of *Arthrospira platensis*. In addition, it can also be seen that the use of aquaculture waste as a culture medium for *Arthrospira platensis* produces an effect that is not much different from the use of technical fertilizers as a culture medium. Even the use of aquaculture waste as an alternative culture medium has advantages over technical fertilizers. Because besides being able to reduce pollution, microalgae can grow better on aquaculture waste media [11].

The addition of growth nutrients into microalgae culture media is considered the most influential aspect of the biomass produced by microalgae cultivation [13]. Ethanol contains lipid elements (C, H, O) that have lower bonds than glycerol, where these elements have the primary reserve function as an energy source [12].

Based on the results of the calculation of the specific growth rate of *Arthrospira platensis* in each different treatment, as shown in Figure 2, the specific growth rate of WMSC treatment reached 0.19 % per day. WMSG treatment reached 0.20 % per day, WMSE treatment reached 0.33 % per day, FMSC treatment reached 0.16 % per day, FMSG treatment reached 0.20 % per day, FMSE treatment reached 0.22 % per day.

The addition of ethanol (SE) as a carbon source resulted in a better growth rate than the addition of glycerol (SG), as shown in Table 1, this was indicated by the high cell density of *Arthrospira platensis* reaching 4.95 g L^{-1} in the WMSE treatment and 4.23 g L^{-1} in FMSE treatment. Based on Figure 4, the growth pattern of *Arthrospira platensis* follows the normal growth pattern, were through the lag phase (adaptation phase), exponential phase (log phase), stationary phase (decreased log phase), and death phase [14].

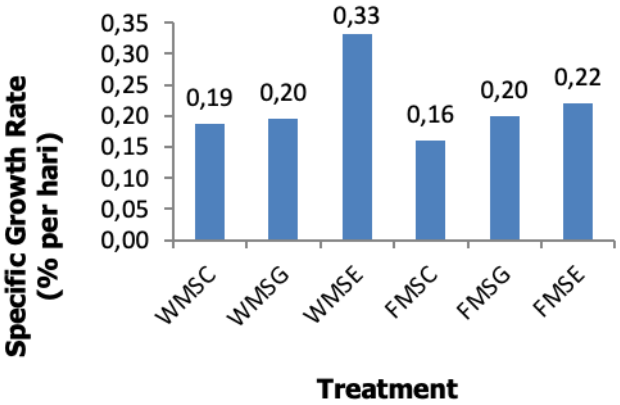

Figure 3. Specific Growth Rate Chart of *Arthrospira platensis*

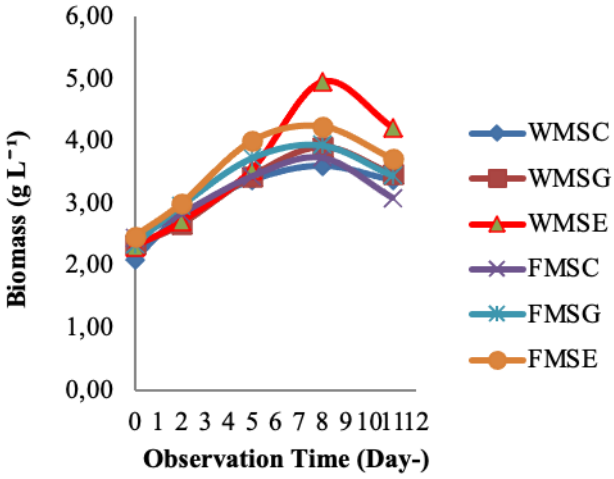

Figure 4. Growth Rate Curve of *Arthrospira platensis*

The exponential phase is indicated by a significant and comparable increase in the growth graph from day 2 to peak population density on day 8. In this phase, there is a very fast growth marked by an increase in the daily population density. In this phase, it is assumed that WMSE and FMSEtreatment media contain the nutrients needed by *Arthrospira platensis* to breed. Nutrient factors are important factors that need to be considered in the growth of microalgae. The nutrients needed by microalgae consist of macronutrients and micronutrients [15].

After reaching the peak of growth on the eighth

day, there was no additional density on the ninth day because the growth rate was balanced with the death rate (stationary phase) due to the limited nutrient content in the culture media. In this phase, the rate of reproduction or cell division is the same as the rate of death in the sense that the addition and reduction of plankton are relatively the same so that the density of plankton tends to remain constant [16].

The death phase in each treatment was characterized by population growth that continued to decrease with time of culture and the death rate was higher than the growth rate until it finally reached the death phase on day 12. In this phase of death, the rate of death is faster than the rate of reproduction and the number of cells decreases geometrically due to the depletion of nutrients in the medium and energy reserves in the cells. The speed of death is influenced by nutrient conditions, environment and types of microalgae [17].

3.2. Water Quality

The growth of *Arthrospira platensis* (*Spirulina*) is not only influenced by the nutrient content in the culture media that is given the addition of a carbon source but is also influenced by environmental factors. Environmental factors that support the growth of *Arthrospira platensis* are temperature, pH, dissolved oxygen (DO), and salinity.

Table 2. Water Quality of *Arthrospira platensis* Culture Media

Parametere	Unit	Result
Temperature	ºC	33 - 34
pH		8.2 - 8.8
Dissolved oxygen (DO)	mg L^{-1}	4.9 – 5.9
Salinity	ppt	20

Based on Table 2, the observations on each treatment using culture media for cultivation waste and technical fertilizers with the addition of different carbon sources and control treatments indicate that the treatment temperature at the time of the study was still suitable for the growth *Arthrospira platensis*.

Temperature is a factor that determines the growth of microalgae. Generally, under laboratory conditions, changes in the temperature of the water media are influenced by room temperature and light intensity [18].

During the study the temperature of the culture media of *Arthrospira platensis* (*Spirulina*) ranged from 33-34 ºC. It was appropriate because Spirulina sp. belongs to mesophilic microalgae, which can grow at a temperature of 20 – 40 ºC with an optimum growth temperature of 25 – 33 ºC [19].

The increase in the pH value in the treated water media is caused by the decomposition of proteins and other nitrogen compounds [20]. The pH range value obtained from the measurement results during the study was in the range from 8.2 to 8.8. The range was suitable for culturing *Spirulina* sp. which is known that the optimal pH for the growth of *Spirulina* sp. ranged from 7 – 11 [21]. It is following the measurements on the research culture media to be said that the pH of the culture media is optimal.

Dissolved oxygen (DO) in culture media is needed by microalgae. for the respiration process. In this study, the source of oxygen in the culture media came from the provision of aeration and the photosynthetic process of *Arthrospira platensis* (*Spirulina*). The DO value at the time of culturing in each treatment ranged from 4.9 – 5.9 mg L^{-1}. This value was suitable for the growth of *Arthrospira platensis* (*Spirulina*). DO levels of 3.0 – 5.0 ppm are less productive, while 5–7 ppm are high productivity and above seven ppm are very high in productivity. DO in culture media that has a value of > 5 mg L^{-1} is good for phytoplankton growth [22][23].

The salinity range during the study was 20 ppt, which means that the salinity of the culture media from the beginning of cultivation to harvesting was stable and still in a good salinity range for the growth of *Arthrospira platensis* (*Spirulina*). Salinity affects organisms in maintaining osmosis with their environment. Spirulina sp is euryhaline with a salinity range between 15–30 ppt [24].

4. Conclusion

The addition of different carbon sources had a positive effect on increasing the density and growth rate of *Arthrospira platensis* (Spirulina). The addition of the highest carbon source of ethanol occurred in the culture media of aquaculture waste (WMSE) with a density of 4.95 g L^{-1} and a specific growth rate of 0.33% per day. The use of aquaculture waste culture media resulted in better growth than technical fertilizers.

Acknowledgement

We want to thank the Institute for Research and Community Services, Sriwijaya University, which funded this study through Hibah Kompetitif Stage II 2021 Nomor: 0022/UN9/SK.LP2M.PT/2021. Also thanks to Aquaculture Laboratory and Experimental Pond Laboratory of the Aquaculture Study Program, Sriwijaya University.

References

[1] A. Juneja, R. M. Ceballos, and G. S. Murthy, "Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review," *Energies*, vol. 6, no. 9, pp. 4607–4638, 2013.

[2] D. B. D. Raja Guk-Guk, "Profiles of Fatty Acid And Beta Carotene of Spirulina platensis Cultured in Technical Media and Catfish Culture

Wastewater (Profil Asam Lemak dan Beta Karoten Spirulina platensis yang dikultur di Media Teknis dan Air Limbah Budidaya Ikan Lele)," Sriwijaya University, 2020.

[3] M. T. Golmakani, K. Rezaei, S. Mazidi, and S. H. Razavi, "γ-Linolenic acid production by Arthrospira platensis using different carbon sources," *Eur. J. Lipid Sci. Technol.*, vol. 114, no. 3, pp. 306–314, 2012.

[4] M. Pagliaro and M. Ross, *The Future of Glycerol*, 2nd Editio., vol. 2008, no. 6. The Royal Society of Chemistry, 2008.

[5] C. F. Hansen et al., "A chemical analysis of samples of crude glycerol from the production of biodiesel in Australia, and the effects of feeding crude glycerol to growing-finishing pigs on performance, plasma metabolites and meat quality at slaughter," Anim. Prod. Sci., vol. 49, no. 2, pp. 154–161, 2009.

[6] Y. Liang, N. Sarkany, Y. Cui, and J. W. Blackburn, "Batch stage study of lipid production from crude glycerol derived from yellow grease or animal fats through microalgal fermentation," *Bioresour. Technol.*, vol. 101, no. 17, pp. 6745–6750, 2010.

[7] A. Hidayani, "Effect of different ph and salinity of catfish rearing wastewater for spirulina platensis growth and phycocyanin rendement," Sriwijaya University, 2019.

[8] S. Afriani, Uju, and I. Setyaningsih, "Chemical Composition of Spirulina platensis Cultivated in Photobioreactors with Different Photoperiods (Komposisi kimia spirulina plantesis yang dikultivasi dalam fotobioreaktor dengan fotoperiode berbeda)," *J. Pengolah. Has. Perikan. Indones.*, vol. 21, no. 3, pp. 471–479, 2018.

[9] K. A. Hanafiah, *Rancangan percobaan : Teori dan aplikasi*, 6th ed. Jakarta, Indonesia: Raja Grafindo Persada, 2001

[10] A. Vonshak, *Spirulina Platensis Arthrospira: Physiology, Cell-Biology And Biotechnology*. CRC Press, 1997.

[11] S. C. Wuang, M. C. Khin, P. Q. D. Chua, and Y. D. Luo, "Use of Spirulina biomass produced from treatment of aquaculture wastewater as agricultural fertilizers," *Algal Res.*, vol. 15, pp. 59–64, 2016.

[12] Nurmayana, "Effect of Different Carbon Sources on The Growth of Spirulina sp. at The Laboratory Scale (Pengaruh sumber karbon yang berbeda terhadap pertumbuhan Spirulina sp. pada skala laboratorium)," Mataram University, 2019.

[13] N. K. E. Juniantari, A. A. M. D. Anggreni, and I. B. W. Gunam, "Effect of Media Type on Growth (Pengaruh Jenis Media terhadap Pertumbuhan Nannochloropsis sp.)," *J. Rekayasa dan Manaj. Agroindustri*, vol. 3, no. 2, pp. 1–9, 2015.

[14] F. Astiani, I. Dewiyanti, and S. Mellisa, "Effect of Different Culture Media on Growth Rate and Biomass of Spirulina sp. (Pengaruh Media Kultur Yang Berbeda Terhadap Laju Pertumbuhan Dan Biomassa Spirulina sp.)," *J. Ilm. Mhs. Kelaut. dan Perikan. Unsyiah*, vol. 1, no. 3, pp. 441–447, 2016.

[15] M. Kawaroe, T. Prartono, A. Rachmat, D. W. Sari, and D. Augustine, "Specific Growth Rate and Fatty Acid Content in Microalgae Spirulina platensis, Isochrysis sp., and Porphyridium cruentum (Laju Pertumbuhan Spesifik dan Kandungan Asam Lemak pada Mikroalga Spirulina platensis, Isochrysis sp. dan Porphyridium cruentum)," *Ilmu Kelaut.*, vol. 17, no. 3, pp. 125–131, 2012.

[16] F. Y. A. Sari, I. M. A. Suryajaya, and Hadiyanto, "Cultivation of Microalgae Spirulina platensis in Pome Media with Variations in Pome Concentration and Nutrient Amount Composition (Kultivasi Mikroalga Spirulina platensis dalam Media Pome dengan Variasi Konsentrasi Pome dan Komposisi Jumlah Nutrien)," *J. Teknol. Kim. dan Ind.*, vol. 1, no. 1, pp. 487–494, 2012.

[17] M. Muliani, E. Ayuzar, and M. C. Amri, "The effect of vermi compost fermented with different doses in Spirulina sp culture (Pengaruh pemberian pupuk kascing (bekas cacing) yang difermentasi dengan dosis yang berbeda dalam kultur Spirulina sp.)," *Acta Aquat. Aquat. Sci. J.*, vol. 5, no. 1, pp. 30–35, 2018.

[18] S. Maryam and G. Diansyah, "The Effect of Phytoplankton Feeding (Tetraselmis sp., Porphyridium sp., and Chaetoceros sp.) towards Growth Rate of Zooplankton Diaphanosoma sp. On Laboratory Scale (Pengaruh Pemberian Pakan Fitoplankton (Tetraselmis sp., Porphyridium sp. dan Chaetoceros ," *Maspari*, vol. 7, no. 2, pp. 41–50, 2015.

[19] N. D. A. Wulandari, "Penggunaan Media Alternatif Pada Produksi Spirulina fusiformis," IPB, 2011.

[20] D. R. Amanatin and T. Nurhidayati, "Effect of Combination Concentration of Bean sprout Extract (MET) with Urea Fertilizer on Protein Levels of Spirulina sp. (Pengaruh Kombinasi Konsentrasi Media Ekstrak Tauge (MET) dengan Pupuk Urea terhadap Kadar Protein Spirulina sp .)," *J. Sains dan Seni Pomits*, vol. 2, no. 2, pp. E182–E185, 2013.

[21] O. Ciferri, "Spirulina, the edible microorganism," *Microbiol. Rev.*, vol. 47, no. 4, pp. 551–578, 1983.

[22] J. M. Fox, *Intensive Algal Culture Techniques In: CRC Handbook of Marine Culture Series.*, Crustacean. Boca Rotan. Florida: CRC Press Inc., 1983.

[23] D. E. Satriaji, M. Zainuri, and I. Widowati, "Study of growth and N, P content of microalgae chlorella vulgaris cultivated in different culture

media and light intensity," *J. Teknol.*, vol. 78, no. 4–2, pp. 27–31, 2016.

[24] N. B. P. Utomo, Winarti, and A. Erlina, "Growth of Spirulina platensis Cultured with Inorganic Fertilizer (Urea, TSP and ZA) and Chicken Manure (Pertumbuhan Spirulina platensis yang dikultur dengan Pupuk Inorganik (Urea, TSP dan ZA) dan Kotoran Ayam)," *Akuakultur Indones.*, vol. 4, no. 1, pp. 63–67, 2005.