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Research article

Effect of Different Purities of Seed Cells and Culture Media on the Growth Pattern and Protein Content of *Spirulina Platensis*

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Abstract

Spriulian platensis is a type of microalgae commonly used as natural fish feed. The purity of cell seedlings represents an internal factor, while the culture media acts as an external factor that can limit microalgae growth. This research investigates how varying purities of cell seedlings and culture media affect the growth patterns and protein content of *Spriulian platensis*, as well as to identify the optimal purity of cell seedlings and culture media that best promote growth patterns and protein content. The research method employed was an experimental design using a completely randomized factorial design with four treatment combinations and three replicates. The results showed that factor A (purity of seed cells) significantly affected growth patterns, including the lag phase duration, specific growth rate, and maximum density, but did not influence the final density *Spriulian platensis*. Factor B (culture media) had no significant effect on any of the variables. The combination of axenic seedlings with Pro Analis media was identified as the best treatment, producing a lag phase duration of (-4.17 ± 0.03 cells/day), a growth rate of (0.33 ± 0.01 cells/day), a maximum density of (6.43 ± 0.02 log cells/ml), and a final density of (5.78 ± 0.03 log cells/ml). The protein content across all treatments remained consistent at 58-60%. The study concludes that the purity of cell seedlings is a critical factor in determining the growth pattern of *Spirulina platensis*, particularly affecting the lag phase, growth rate, and maximum density.

Keywords: Culture media, growth pattern, protein, *S. platensis*, seedling purity.

1. Introduction

Spirulina platensis is one of the potential microalgae in fisheries that is used as natural feed in fish [1,2,3], shrimp, and shellfish hatcheries as well as used for ornamental fish coloring because *Spriulian platensis* contains 60-71% protein, 8% fat, 16% carbohydrate, 1.6% chlorophyll-a, 18% phycocyanin, 17% betacarotene, 20-30% vitamins and linoleic acid [4]. *Spriulian platensis* is called blue-green-algae because it has a high content of carotenoid color pigments (*Zeaxanthin*), and has good digestibility [5]. The utilization of *Spriulian platensis* in several fields has resulted in its increasingly high demand.

The growth pattern and nutrient content of *Spriulian platensis* are influenced by the purity of the seed cells and environmental factors such as water quality and culture media. Growth rate can be influenced by the purity of the cell seeds, so seeds that are free from contaminants are needed [6]. Axenic cell seedlings, which only have one type of microalgae in liquid medium, do not have other microorganism contaminants [7]. *Spriulian platensis* culture requires proper nutrition for optimal growth and nutritional content [8,9]. One of the media that can be used in microalgae culture is Zarrouk media because this

media contains nutrients, namely carbon, nitrogen and phosphorus that can support the growth of *Spriulian platensis*. Zarrouk media contains higher dissolved bicarbonate compared to walne media, which is utilized as a carbon source for the photosynthesis process. Culture media can be composed of Pro Analyst and technical chemicals, Pro Analyst chemicals have a very high purity level (>99.5%) which is commonly used for laboratories while technical chemicals have lower purity and are used for production processes.

Seedlings resulting from cell washing can shorten the lag phase time and extend the stationary phase. The presence of competitors in *Spriulian platensis* culture in the form of other types of microalgae and bacteria, there will be competition in obtaining food, so that cell division will be disrupted and the nutrient content in cultured microalgae will be lower. The use of different culture media has no significant effect on microalgae growth [10]. Each microalgae has different seed purity and best culture media for growth, so research is needed to produce good growth patterns and protein content.

2. Materials and methods

2.1. Description of the research sites

This research was conducted at the Aquaculture Natural Feed Laboratory, Faculty of Fisheries and Marine Science, Diponegoro University and PT Algae Biotechnology Indonesia, Semarang in September-November 2023.

2.2. Preparation of tools and materials

Tools in the form of glassware and culture media that will be used are sterilized first using an autoclave at 121℃ for 15 minutes with a pressure of 1 atm. The materials used in this research are xenic *Spriulian platensis* seeds obtained from PT Algae Biotechnology Indonesia, Semarang City, Central Java and axenic *Spriulian platensis* obtained from cell washing in the Natural Feed Laboratory of the Department of Aquaculture, FPIK UNDIP. The culture media used were Zarrouk Pro Analyst and technical media.

2.3. Experimental design

This research was conducted using laboratory experimental method and the experimental design used was Factorial Randomized Complete Design (factorial RAL). Factorial RAL is a complete randomized design consisting of two or more independent variables. The research consisted of 4 treatment combinations and 3 replicates with the following treatment combinations:

A_1B_1 treatment: axenic seedlings, Pro Analyst media;

A_1B_2 treatment: axenic seedlings, technical media;

A_2B_1 treatment: xenic seedlings, Pro Analyst media; and

A_2B_2 treatment: xenic seedlings, technical media.

The treatment of the use of cell seeds and culture media above refers to the research of [11], that the washing of cell seeds affects the growth pattern of *Spriulian platensis*. This is in accordance with the research of [12], which states that cell washing can increase protein content in the maximum density phase.

2.4. Media composition

Zarrouk media used for microalgae culture with a composition of 16.8 g NaHCO3, 2.5 g NaNO3, 0.5 g K2HPO4, 1 g K2SO4, 1 g NaCl, 0.2 g MgSO4.7H2O, 0. 04 g CaCl2, 0.01 g FeSO4.7H2O, 0.08 g Na2EDTA and A5 micronutrients with a composition of 0.0177 g CuSO4.5H2O, 1.81 g MnCl2.4H2O, 0.22 g ZnSO4.7H2O and 2.86 g H3BO3 [13]. The composition was used for 1 liter of culture media. The culture media used used thecomposition of pro analis and technical media whit same composition. Preparation of culture media for *Spriulian platensis* begins with mixing the culture media ingredients in 1 liter of distilled water, then sterilized using an autoclave at 121℃ for 15 minutes with a pressure of 1 atm.

2.5. *Spriulian platensis* inoculation

Spriulian platensis culture was carried out in a 100 ml erlenmeyer. The sterilized Zarrouk culture medium was put into the erlenmeyer as much as 60 ml. The next step was to put *Spriulian* platensis inoculant with a density of 5×104 cells/ml into the erlenmeyer. Cell seedlings were added as much as 10-20% of the water volume [14].

2.6. Data Analysis

a. Lag phase time

The calculation of the lag phase time is by calculating the linear

regression during the exponential phase [15], with the following formula:

$$Y = Ak + B$$

where:

Y = logarithm of cell density (log cells/ml)

A = lag phase time (days)

B = constant

The duration of lag phase time (A) was then calculated with Y = initial culture density (5×10^4 cells/ml).

b. Specific Growth Rate (SGR)

The specific growth rate of *Spriulian platensis* was calculated from population abundance data on day 0 to the peak of the population with the formula [16]:

$$K = (\text{Log } (Wt - W0))/\Delta t$$

where:

K = specific growth constant

Wt = density at the end of exponential phase (log cells/ml)

W0 = density in the early exponential phase (log cells/ml)

Δt = difference between the days of the late exponentiall and early exponential phase (days)

c. Maximum density of Spriulian platensis

The daily density of *Spriulian platensis* cells was calculated using the formula of [15] which uses 400 haemocytometer boxes with a size of 1 mm^2 with a depth of 0.1 mm. The formula used is as follows:

Volume of 400 haemocytometer boxes = 1 mm2 × 0.1 mm

= 0.1 mm^3

= 0,0001 ml

The volume of 400 haemocytometer boxes is 0.0001 ml, so the density formula used is:

$$\text{Cell density } (P) = \frac{\text{number of cells in 400 haemocytometer squares}}{\text{volume haemocytometer}}$$

P = cell/ml

P = N x 104 cell/ml

Where:

P = cell density (cells/ml)

N = number of cells counted in 400 haemocytometer box

The maximum density of *Spriulian platensis* cells used the highest cell density value during the research.

d. Protein Content of Spriulian platensis

Analysis of protein content in *Spriulian platensis* using the Kjedhall semimicro method SNI, 1992. Protein content analysis was carried out by taking samples from the culture results in the form of *Spriulian platensis* flour. The samples were tested at the Fishery Product Technology Laboratory, Faculty of Fisheries and Marine Science, Universitas Diponegoro.

e. Water Quality

Water quality measurements were taken three times, on the first day, seventh day and fourteenth day at 10:00 am. Water quality variables measured were temperature, salinity and pH using a thermometer, refractometer and pH meter.

2.7 Statistical analysis

The results of the data obtained after the research include growth pattern data which includes lag phase time, specific growth rate, maximum density and final density. The results that have been obtained are then analyzed by statistical tests and presented in graphical form. Furthermore, statistical analysis is carried out with normality, homogeneity and additivity tests. Data that are normal, homogeneous, additive are further tested, namely analysis of variance (ANOVA). Data that have been analyzed using ANOVA, if found to have a significant effect ($P < 0.05$), Data on protein content and water quality were analyzed descriptively with reference to relevant references.

3. Results and discussion

Based on the results of research on differences in seed purity and culture media on the growth pattern of *Spriulian platensis* protein content, the growth pattern graph is presented in Figure 1.

3.1. Growth pattern of *Spriulian platensis*

Based on the growth pattern graph, it can be seen that the abundance of *Spriulian platensis* is the result of observations during the 15-day culture period.

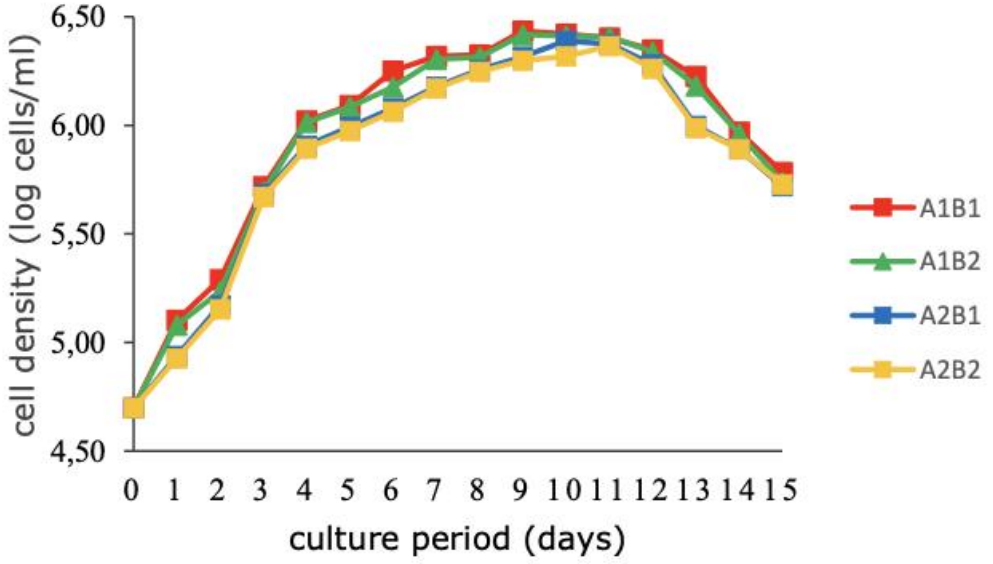

Figure 1. Growth pattern of Spriulian platensis during the research. A_1B_1 with axenic seedlings, Pro Analyst media; A_1B_2 with axenic seedlings, technical media; A_2B_1 with xenic seedlings, Pro Analyst media; and A_2B_2 with xenic seedlings, technical media.

The initial cell density of *Spriulian platensis* in all treatments was the same, which was 5×10^4 cells/ml. A_1B_1 treatment increased exponentially until day 4 and the highest cell density peak occurred on day 9, then a decrease in cell density occurred on day 12 until the end of the culture period. The A_1B_2 treatment experienced the same exponential phase and stationary phase as the A_1B_1 treatment. The stationary phase in the A_2B_1 treatment occurred for 8 days with maximum density occurring on day 10, cell density decreased on day 12 until the end of the culture period. The stationary phase in the A_2B_2 treatment occurred on day 6 to day 12 with maximum density on day 11. Growth pattern data observed in this research include lag phase time, specific growth rate, length of stationary phase, maximum density and final density (Figure 1).

The data are presented in Table 1. Based on Table 1, the best lag phase time value in the A_2B_2 treatment (xenic cell seedlings, technical culture media) with a value of -4.61 days, the highest specific growth rate value in the A_1B_1 treatment (axenic cell seedlings, Pro Analis media) and A_1B_2 (axenic cell seedlings, technical media) with a value of 0.33 cells/day. The highest maximum density value in treatments A_1B_1 (axenic cell seedlings, Pro Analis media) and A_1B_2 (axenic cell seedlings, technical media) with a value of 6.43 log cells/ml, and the highest final density value in treatment A_1B_1 (axenic cell seedlings, Pro Analis media) with a value of 5.78 log cells/ml. Based on the results of the analysis of variance (ANOVA) test in Table 2, it shows that Based on the results of the analysis of variance ANOVA test, the lag phase time of *Spriulian platensis* presented in Table 4.2 shows that there is no interaction between the purity of the cell seed and the culture media because ($P>0.05$), so there is no relationship between the two factors. The difference in cell seed purity has a significant effect ($P<0.05$) on the lag phase time, but the difference in culture media has no significant effect ($P>0.05$) on the lag phase time.

3.2. Protein content

Based on the results of the research, a T test was conducted to determine the difference in protein content of dried *Spriulian platensis* flour. The results obtained showed that the different combinations of cell seed purity and culture media were not significantly different from the protein content of *Spriulian platensis*. Histogram of protein content is presented in Figure 2.

The protein content of dried *Spriulian platensis* flour obtained consecutive results, namely the A_1B_1 treatment

(axenic media, Pro Analyst media) of 60.02%, A_1B_2 treatment (axenic cell seeds, technical media) of 58.05%, A_2B_1 treatment (xenic cell seeds, Pro Analyst media) of 59.68%, and A_2B_2 treatment (xenic cell seeds, technical media) of 60.40% (Fugure 2).

3.3. Water quality

Based on the results of research on differences in seed purity and culture media on growth patterns and protein content of *Spriulian platensis*, water quality data are presented in Table 3.

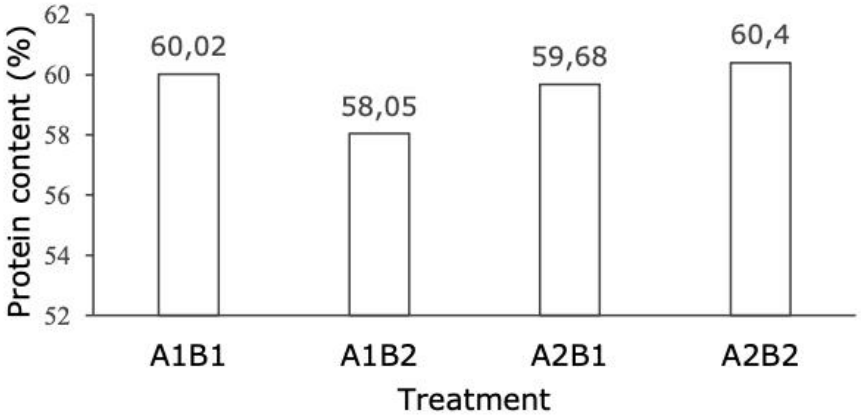

Figure 2. Protein content of *Spriulian platensis* in the stationary phase. Note: A_1B_1 with axenic seedlings, Pro Analyst media; A_1B_2 with axenic seedlings, technical media; A_2B_1 with xenic seedlings, Pro Analyst media; and A_2B_2 with xenic seedlings, technical media.

Table 1. Growth patterns of *Spriulian platensis* including lag phase time, specific growth rate, maximum density, and final density.

Growth pattern				
Treatment	**Lag phase time (days)**	**Specific growth rate (cells/days)**	**Maximum density (log cells/ml)**	**Final density (log cells/ml)**
A_1B_1	-4.17 ± 0.03^a	0.33 ± 0.01^a	6.43 ± 0.02^a	5.78 ± 0.03^a
A_1B_2	-4.19 ± 0.03^a	0.33 ± 0.00^a	6.43 ± 0.00^a	5.74 ± 0.03^a
A_2B_1	-4.56 ± 0.11^b	0.30 ± 0.00^b	6.39 ± 0.02^b	5.72 ± 0.02^a
A_2B_2	-4.61 ± 0.10^b	0.30 ± 0.01^b	6.36 ± 0.02^b	5.73 ± 0.03^a

Note: A_1B_1 with axenic seedlings, Pro Analyst media; A_1B_2 with axenic seedlings, technical media; A_2B_1 with xenic seedlings, Pro Analyst media; and A_2B_2 with xenic seedlings, technical media.

Table 2. Results of ANOVA Time Lag Phase *Spriulian platensis*

Source of Diversity	Degrees of Freedom	Sum of Squares	Mean	F count	F Table (0,05)
A	1	0.50	0.50	83.84*	5.32
B	1	0.00	0.00	0.70	5.32
AB	1	0.00	0.00	0.13	5.32
Galat	8	0.05	0.01		
Total	11	0.55			

Note: (*) indicates data has a significant effect ($P<0.05$). A_1B_1 with axenic seedlings, Pro Analyst media; A_1B_2 with axenic seedlings, technical media; A_2B_1 with xenic seedlings, Pro Analyst media; and A_2B_2 with xenic seedlings, technical media.

Table 3. Water Quality Parameters of Each Treatment

Treatment	Water Quality Parameters			
	Temperature (℃)	pH	Salinity (ppt)	Light intensity (lux)
A_1B_1	22.7-25.6	9.5	11	2890-2950
A_1B_2	22.5-25.3	8.9	19	2890-2950
A_2B_1	22.5-25.8	9.5	11	2890-2950
A_2B_2	22.5-25.6	8.9	19	2890-2950
Optimum range	20-30	7-11	0-35	500 –350000

Note: A_1B_1 with axenic seedlings, Pro Analyst media; A_1B_2 with axenic seedlings, technical media; A_2B_1 with xenic seedlings, Pro Analyst media; and A_2B_2 with xenic seedlings, technical media.

4. Discussion

The growth of *Spriulian platensis* is characterized by increasing cell abundance by microalgae by cell division. An increase in the number of cells indicates that microalgae cells are able to survive and perform cell division [17]. Based on the results of the research, the growth of *Spriulian platensis* in all treatments increased. *Spriulian platensis* growth with the highest density occurred in the axenic seed treatment and pro-analyst culture media with a cell density of 6.43 log cells/ml. This result is lower than the results of research by [18] that the maximum density occurred in the treatment of washing three times, namely 7.75 log cells/ml. Washing of cell seedlings aims to obtain contaminant-free *Spriulian platensis* cell seedlings. Culture media with high purity can increase the growth phase. This is in accordance with the results of research by [19], which states that the results of microalgae culture with Pro Analis media have a longer growth phase compared to technical media. The growth pattern of *Spriulian platensis* observed in this research includes lag phase time, specific growth rate, maximum density and final density of the research.

The time of the lag phase is the phase where *Spirulina platensis* adjusts to the new environment. According to [20], in the lag phase the cell size will increase and experience cell metabolism but not yet dividing.

There is no interaction in the combination of differences in cell seed purity and culture media, so there is no relationship between the two factors. Differences in the purity of *Spriulian platensis* cell seedlings have different effects on lag phase time. Axenic cell seedlings require longer time for adaptation than xenic cell seedlings. This is not in accordance with the results of [18], which states that *Spriulian platensis* which received cell washing treatment has a faster adaptation time, so that microalgae can utilize nutrients in the culture medium optimally. The difference in culture media did not have a different effect on lag phase time. This is thought to be because the composition of the culture media used is not different from the previous media, so it does not require a long time for adaptation. The lag phase time in all treatments was less than one day. This is thought to occur because the culture media and environmental conditions of the cultured microalgae are the same as the previous media. The composition of Zarrouk media can support the growth of *Spriulian platensis*, and contains complete macronutrients and micronutrients. This media contains high $NaHCO_3$, which functions to increase CO_2 so that the photosynthesis process occurs faster. The lag phase time shows the length of the microalgae adaptation period to the new media. The lower the lag phase value, indicating that the cell adaptation time to the culture media is faster so that the growth rate value is higher. The difference in the length of the lag phase shows the length of time for microalgae adaptation to the new media [21].

The specific growth rate value indicates that microalgae enter the exponential phase, where there has been an increase in cell

size and number due to the process of cell division. The exponential phase is characterized by cell division and is characterized by an increase in growth rate so that the population density is getting higher [12].

The results showed that there was no interaction in the combination of differences in seedling cell purity and culture media so that there was no relationship between the two factors. This is thought to occur because differences in the purity of cell seeds and culture media do not affect the absorption of nutrients by *Spriulian platensis*. Differences in cell seed purity had a significant effect ($P<0.05$) on specific growth rate. This is thought to be because the absorption of xenic cell seedlings is less than optimal due to the presence of contaminants. The presence of contaminants or competitors in microalgae cultures causes competition for nutrients, so that cell division and growth are disrupted [12]. The specific growth rate value in the treatment without washing is quite low due to the presence of contaminants that are not identified, causing less than the maximum growth rate and cell density of *S. platesis* is not optimal. Contamination of bacteria or other microorganisms can cause the amount of cell production and nutritional value to decrease [12]. Differences in the purity of culture media had no significant effect ($P>0.05$) on the specific growth rate of *Spriulian platensis*. This is thought to be because the purity of the Pro Analis media composition and the technical media used are not much different, so they have the same growth rate. The growth rate value is influenced by the culture media used. Carbon, nitrate, phosphate and micronutrients contained in the culture media affect the growth rate so that the right dose can increase the growth rate. Differences in culture media have no significant effect on growth patterns [22].

Maximum density indicates that growth has entered the stationary phase. The stationary phase occurs where the number of cells in microalgae is relatively the same because nutrients begin to decrease [23]. There is no interaction in the combination of differences in purity of seedling cells and culture media so that there is no relationship between the two factors. This is thought to be because in different conditions of cell seed purity and culture media, *Spriulian platensis* can absorb nutrients in the culture media optimally. The results showed that differences in the purity of cell seedlings had a significant effect ($P<0.05$) on the maximum density of *Spriulian platensis* seedlings that are axenic have a higher maximum density, because cell washing can shorten the lag phase time, increase the growth rate and get a high maximum density. This is in accordance with the results of research by [12], which states that this is because microalgae cells have been cleaned of contaminants and bacteria so that competitors in obtaining food are reduced. Axenic cell seedlings can extend the stationary phase because they are free from contaminants so that there are no competitors in nutrient absorption. Cell washing treatment affects growth patterns, especially in the stationary phase [17]. Different culture media had no significant effect ($P>0.05$) on maximum density. Different purity of Pro Analyst and technical media composition did not affect the maximum density. The use of Pro Analyst culture media has a higher value of specific growth rate and maximum density compared to technical media. This is in accordance with the results of research by [20], which states that Pro Analyst media has a longer growth phase compared to technical media. This maximum density is influenced by the culture media. High carbon, nitrate, and phosphate content in culture media can increase the maximum density of microalgae.

The final density occurs because the nutrients contained in the culture media have been reduced and are not optimal so that they cannot meet nutritional needs. The death phase occurs in all treatments after the peak cell density. *Spriulian platensis* reaches the peak density then the growth of *Spriulian platensis* cells will stop, where the need for nutrients at this point will decrease due to the absence of additional nutrients from fertilizers [14]. The results showed that there was no interaction in the combination of differences in cell seed purity and culture media so that there was no relationship between the two factors. Differences in cell seed purity and culture media did not affect the final density of the research. This is thought to be because on the last day of the research, *Spriulian platensis* had not yet entered the death phase but the cell density dropped. The

decrease in cell density occurred because the nutrients contained in the culture media had decreased, so it could not meet the nutrient requirements needed by *Spriulian platensis* for growth. This phase is the last phase in the microalgae growth phase, where the nutrients in the culture medium have run out so that they cannot be utilized by microalgae in the growth process [24].

Based on the results of the research, it was found that differences in seed purity and culture media did not affect the protein content of *Spriulian platensis*. Protein content in dry conditions with a value of 58-60%. This protein content is in accordance with the results of research by [25], which states that the protein content in *Spriulian platensis* in wet conditions reaches 58.3%, while in dry conditions it contains 45-75%. Protein content is influenced by nitrate in culture media because nitrate can increase protein content. Differences in the purity of cell seeds and culture media did not affect the protein content allegedly because the results of cell washing did not increase the protein content. This result is different from the results of research by [12], which states that cell washing can increase protein content in the maximum density phase. The higher protein content in xenic cell seedlings is thought to occur due to the presence of other types of microalgae which cause the protein content to increase. Another thing that causes differences in protein content between treatments is the possibility of competition for food, causing cell division and growth to be disrupted [11]. The different use of culture media had no effect on protein content. This is thought to be because differences in the purity of the culture media composition do not affect protein content. Differences in culture media have no significant effect on the growth pattern and nutrient content of *Spriulian platensis* [26].

Based on water quality (Tabel 3) measurements during the research, a pH value of 8.9-9.5 was obtained. The optimal pH range for *Spriulian platensis* growth is 7-9. However, there are several types of *Spriulian platensis* that can survive in an environment with a pH close to 7 or above 11. *Spriulian platensis* grows in an alkaline environment [27]. The pH value can affect nutrient availability and physiology of *Spriulian platensis*. pH conditions that exceed the threshold can cause an increase in dissolved carbon dioxide [28]. The temperature measurement results during the research were in the range of 23℃-26℃. This is in accordance with the opinion of [29], which states that the optimal temperature range for the growth of *Spriulian platensis* is between 20-30°C. Generally, in laboratory conditions, changes in water temperature are influenced by room temperature and light intensity. The salinity of culture media for Pro Analyst media was 11 ppt while that of technical media was 19 ppt. This is thought to be because the composition of the technical media has a higher NaCl content. High salinity produces osmotic pressure and will inhibit the absorption of nutrients so that growth is inhibited [30]. According to [31;32]. the salinity content for *Spriulian platensis* growth ranges from 0-35 ppt. During the research, the light intensity obtained was 2890-2950, this result was sufficient for the growth and photosynthesis of microalgae. Light acts as a light source used for photosynthesis. The light intensity required for algae photosynthesis is 500-5000 lux, while the optimal light intensity for *Spriulian platensis* is 2000-3000 lux. *Spriulian platensis* can grow well at a light intensity of 500-350,000 lux [33,34,35].

5. Conclusion

The conclusions of this research, factor A (purity of seed cells) had a significant effect on growth patterns including lag phase time, specific growth rate and maximum density, but did not affect the final density of *Spriulian platensis*. Factor B (culture media) had no effect on all variables. Differences in cell seed purity and culture media were not significantly different on protein content; and The best treatment in the research was the A1B1 treatment which produced the best growth pattern with the value of lag phase time (-4.17 ± 0.03a days), specific growth rate (0.33 ± 0.00a cells/day), maximum density (6.43 ± 0.02a log cells/ml) and final density (5.78 ± 0.03a log cells/ml), while the protein content was the same with a value of 58-60%.

Authors Contribution

Diana chilmawati as conceptualized the study, designed the experimental framework, conducted the laboratory experiments, performed data analysis, and wrote the manuscript. Aldhira

Martaningrum as, assisted in designing the experiment, provided technical support during the cultivation process, and contributed to data interpretation. Lestari Lakhsmi Widowati as provided expertise in statistical analysis, assisted with the interpretation of results, and reviewed the manuscript for critical content. Pranata Candra Perdana Putra as supervised the research, and provided guidance throughout the study, as well as contributed to manuscript editing and final approval.

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Conflicts of Interest

There are no conflicts of interest reported by the writers.

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Data Availability statement

The data presented in this study are available on request from the corresponding author.

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