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Abundance and Chlorophyll-a Content of *Spirulina* sp in Given Different Light Intensity Media's

Kelimpahan dan Kandungan Klorofil-a Spirulina sp dalam Media yang diberi Intensitas Cahaya yang Berbeda

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Abstract

Spirulina sp is one of the organisms used because it has twice the chlorophyll content of the alfalfa plant, the chlorophyll of which has previously been explored. Chlorophyll-a is a photosynthetic pigment, and light is very influential in photosynthesis in microalgae cultivation because light is a crucial part of the photosynthetic pigment as a source of energy for microalgae life. The purpose of this research is to get the best light intensity. This research started in September 2022 at the Aquaculture Environmental Quality Laboratory, Faculty of Fisheries and Marine, Universitas Riau. This study used a one-factor, Completely Randomized Design (CRD) method with five treatments and three replications. The treatment in this study was the light intensity consisting of P0: without giving light, P1: 2.974 lux, P2: 4.274 lux, P3: 5.575 lux, P4: 6.877 lux. The results of this study indicate that light intensity affects the locking of *Spirulina* sp. The best treatment was a light intensity of 4.274 lux with a cell density of 327.176,2 ind/mL with a chlorophyll-a content of 0.4 µg/mL with a range of water quality parameters that were optimal for the growth of *Spirulina* sp temperature value 28-31, pH 7-8.5, DO 5-7.5.

Keywords: Intensity, Abundance, Growth, *Spirulina* sp, Lux.

Abstrak

Spirulina sp merupakan salah satu organisme yang dimanfaatkan karena memiliki kandungan klorofil dua kali lebih tinggi dari tumbuhan alfalfa yang lebih dahulu di eksplorasi klorofilnya. Klorofil-a merupakan pigmen fotosintesis dan cahaya sangat berpengaruh dalam proses fotosintesis budidaya mikroalga, karena cahaya adalah bagian yang sangat penting dalam pigmen fotosinsetik sebagai penyedia energi bagi kehidupan mikroalga. Tujuan penelitian ini adalah untuk mendapat-kan intensitas cahaya terbaik. Penelitian ini dilaksanakan pada bulan September 2022 di Laboratorium Mutu Lingkungan Budidaya Fakultas Perikanan dan Kelautan Universitas Riau. Penelitian ini menggunakan metode Rancangan Acak Lengkap (RAL) satu faktor dengan lima perlakuan dan tiga ulangan. Perlakuan dalam penelitian ini adalah intensitas cahaya yang terdiri dari P0: tanpa cahaya, P1: 2.974 lux, P2: 4.274 lux, P3: 5.575 lux, P4: 6.877 lux. Hasil penelitian ini menunjukkan bahwa intensitas cahaya berpengaruh terhadap kelimpahan dan kandungan klorofil-a *Spirulina* sp. Hasil intensitas cahaya terbaik yakni pada perlakuan intensitas cahaya 4.274 lux dengan kelimpahan sel 327.176,2 ind/mL dengan kandungan klorofil-a 0,4 µg/mL dengan kisaran parameter kualitas air yang optimal bagi pertumbuhan *Spirulina* sp nilai suhu 28-31, pH 7-8,5, DO 5-7,5

Kata kunci: Intensitas, Kelimpahan, Pertumbuhan, *Spirulina* sp, Lux.

1. Introduction

Cultivation is an activity in great demand by people in Indonesia, and it continues to experience outstanding improvement and has expanded to many countries. However, cultivation activities have remained within natural food components. The density rate of fish and shrimp during the larval period can also increase by providing natural food (Anggraeni et al., 2013). This is what causes natural feed to be in great demand, with the advantage of easily digested feed for fish larvae during the growth phase. *Spirulina* sp is one of the natural feeds developed in the cultivation industry.

Spirulina sp is a type of bluish-green algae, phytoplankton, widely found in nature in fresh and brackish water. In culturing *Spirulina* sp, an appropriate and accessible alternative was found, using organic fertilizer as an alternative that is easy to obtain, reduces chemical fertilizers, and is economically valuable. Following the opinion of Kalsum et al. (2011), rice washing water contains nutrients needed for plant growth, including Vitamins B1, B2, nitrogen, and phosphorus. This is also reinforced by the opinion (Astiani et al., 2016) that rice-washing water is one of the appropriate and easy-to-find alternatives. Furthermore, according to research by Nur et al. (2020), rice washing water contains phosphorus and nitrogen, the most important elements needed for microalgae growth. Nitrogen functions as a form of chlorophyll-a, and phosphorus functions as a cellular metabolism needed for the growth and reproduction of microalgae. Based on Kurniawan et al. (2010), *Spirulina* sp is one of the organisms used because it has a chlorophyll content twice as high as alfalfa plants, whose chlorophyll was previously explored. This microalgae has a high nutritional content, namely: protein content of 55-70%, carbohydrates 15-25%, essential fatty acids 18%, and the rest are vitamins, minerals, and pigments, namely chlorophyll, carotene, xanthophyll, and phycocyanin (Prasanna et al. al., 2010). It was reported by Herawati & Hutabarat (2014) that one of the goals of algae culture is to obtain the highest cell abundance with optimal nutritional content.

Chlorophyll-a is a photosynthetic pigment, and light is very influential in the photosynthesis process of microalgae cultivation because light is a vital part of photosynthetic pigments as an energy provider for microalgae life. According to Hadiyanto et al. (2012), the activity of the photosynthetic process of microalgae will increase with an increase in the intensity of optimum light with a light range of 3500-5000 lux. If there is a lack of light in cultivating microalgae cultures during photosynthetic activity, it will disrupt subsequent cell biosynthesis. Based on the author's explanation, it is interesting for the author to raise a title about the abundance and chlorophyll-a content of *Spirulina* sp in media given different light intensities.

2. Material and Method

2.1. Time and Place

This research was carried out for approximately 14 days in September 2022 to determine the abundance and content of chlorophyll-a in *Spirulina* sp at the Aquaculture Environment Quality Laboratory, Faculty of Fisheries and Marine, Universitas Riau, Pekanbaru.

2.2. Methods

This research uses an experimental method using a 1 factor Completely Randomized Design (CRD) with five treatments and three replications. The treatments in this research are P0: No light, P1: 16-watt lamp light (2,974 lux), P2: 23-watt lamp light (4,274 lux), P3: 30-watt lamp light (5,575 lux), P4: 37-watt lamp light watts (6,877 lux).

2.3. Procedures

2.3.1. Container Preparation

The containers used in this research were 15 bottles of mineral water with a volume of 5 L as culture containers. Research containers are provided with lighting, and there are also containers without light. The lamps used are 16-watt LED lamps, 23-watt LED lamps, 30-watt LED lamps, and 37-watt LED lamps, each with three lamps. Next, 15 used cardboard boxes and black plastic will be prepared to cover the open parts of the cardboard and the gaps to protect the Container. The purpose of the cardboard and black plastic boxes in the treatments is as a partition so that the containers in each treatment are not exposed to light bias from each treatment.

2.3.2. Spirulina sp Culture

Spirulina sp seeds used in this research came from Sukabumi and were ordered online. According to Budiardi & Santosa (2010), the water that will be used for culturing *Spirulina* sp sanitizes first by boiling until boiling. After cooking, the water is cooled for one day so it is ready to be used. Put 2 L of water into a 5 L bottle, then spread the *Spirulina* sp seeds into the bottle along with fermented rice, washing water as much as 0.5 ml/L. The initial abundance used in culture was 196,178.34 cells/mL. Then, partitions were made between the containers in each treatment to ensure that the containers in each treatment were not exposed to light bias from other treatment containers.

This initial inoculant calculation was carried out by taking samples of *Spirulina* sp as much as 0.5 mL/L, which was then counted under a microscope using a Sedgewick Rafter counting tool. The amount of fertilizer given was 0.5 ml/L in each treatment container, so the total fertilizer required for this research was 15 mL, after which it was given aeration during the culture process, namely for 14 days. In the control container (P0), 0.5 ml/L of fermented rice washing water was added without light. In comparison, in treatment container 1 (P1), 0.5 ml/L of fermented rice washing water was added at an intensity of 2,974 lux under 16-watt light, treatment 2 (P2) added, 0.5 ml/L fermented rice washing water at an intensity of 4,274 lux under 23 watts lamp light, treatment 3 (P3) added 0.5 ml/L fermented rice washing water at an intensity of 5,575 lux under 30 watts lamp light, and treatment 4 (P4) added 0.5 ml/L fermented rice washing water at an intensity of 6,877 lux under 37-watt lamp light.

2.4. Parameter Measured

The parameters measured in this research are nitrate, phosphate, pH, dissolved oxygen, chlorophyll-a content, and temperature, as well as measuring light intensity. Water Quality Parameters were measured at the beginning of the study (day 1), mid-study (day 7), and end of the study (day 14).

2.6. Data Analysis

All data obtained during the research, including abundance (ind/mL) and chlorophyll-a content (µg/mL), were presented in the form of tables and graphs and analyzed according to the Completely Randomized Design (CRD) model. An analysis of variance (ANOVA) was carried out to determine the effect between treatments using the F statistical test and the SPSS version 24 application. If $p<0.05$, the Student Newman-Keuls test was conducted to determine the difference between treatments. If $p<0.05$ in the treatment, it shows that the abundance and chlorophyll-a content of *Spirulina* sp in media given POC in fermented rice washing water and different light intensities have a significant effect.

3. Result and Discussion

3.1 The Abundance of Spirulina sp

In the growth phase of *Spirulina* sp, there are four stages: the lag phase, exponential phase, stationary phase, and death phase. This is presented in the graph in Figure 1.

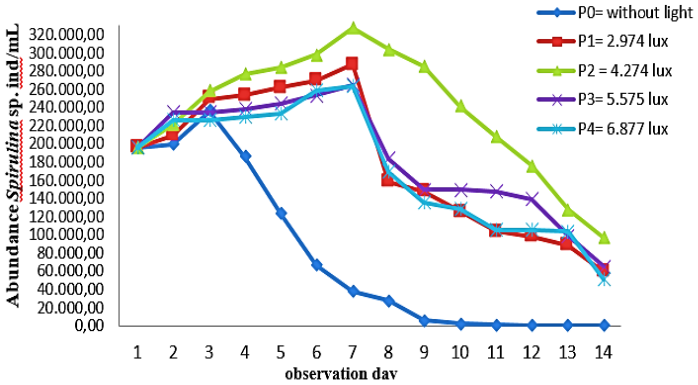

Figure 1. The effect of different light intensities on the abundance of *Spirulina* sp

P0 is the growth of *Spirulina* sp, which experienced significantly different growth from other treatments. Day 1 is called the lag phase or adaptation phase. However, on the second and third days, followed by the fourth day until the ninth day, the microalgae gave a decreasing growth response where there was no more microalgae growth, or the growth speed became zero until it approached the death phase. This is thought to be because, at P0, there is no light, so at P0, there is no good growth, and cell growth only lasts for a very short time, so microalgae growth experiences very premature growth. Furthermore, the microalgae showed a death phase from the 10th to the 14th day.

Furthermore, treatments P1, P3, and P4 experienced similar growth. The lag phase in treatments P1, P3, and P4 occurred on days 1 to 3; according to Putri & Setyati (2019), the first, second, and third days are a lag phase for *Spirulina* sp, which is adapting to its new environment, so that cell growth has not occurred significantly because *Spirulina* sp cells are still undergoing adaptation to growing. Even though growth is still relatively small, growth increases slowly. Furthermore, on days 4 to 7 in treatments P1, P3, and P4, an exponential phase occurs in which microalgae experience rapid growth. The exponential phase is one of the phases of the second growth after the phase of adaptation to its environment. According to Hadiyanto et al. (2012), if microalgae are in a suitable environment, the rate of cell growth and cell metabolism will increase; with the increase in cell growth rate, more biomass will be produced. Next, From day 8 to day 12, in treatments P1, P3, and P4, the microalgae began to decline until they approached the death phase, usually called the stationary phase. This is supported by

Armanda's statement (2013) that in this stationary phase, the growth of *Spirulina* sp tends to be static, meaning that cell division and cell death are balanced. Furthermore, on days 13 and 14 in treatments P1, P3, and P4, the microalgae experienced a death phase where the cells could not grow and develop because they did not receive a nutrient supply.

Furthermore, during P2 treatment from day 1 to day 3, the microalgae began to adapt to their environment, and their growth was still relatively small. Furthermore, from the fourth to the seventh day, the microalgae experienced very good growth compared to P0, P1, P3, and P4, which peaked after the fourth to the seventh day. This is because the microalgae are in a suitable environment and have adapted well to their environment. According to Hadiyanto et al. (2012), if microalgae are in a suitable environment, the rate of cell growth and cell metabolism will increase. This indicates that *Spirulina* sp can absorb and utilize nutrients for growth. Next, entering the 8th day until the 11th day, and the 12th day, the microalgae slowly begin to decline until they approach the death phase. This happens because the nutrients contained in the culture media are not optimal and have been reduced to meet the nutritional needs of *Spirulina* sp. Furthermore, on days 13 and 14, in treatments P1, P3, and P4, the microalgae experienced a death phase. In the death phase, it will decrease due to insufficient nutrient availability, reduced water quality parameters, and the accumulation of metabolites (NO_2^- and NH_4^+), which causes suboptimal growth so that the phytoplankton are unable to grow and develop (Nababan et al., 2021).

According to Rusyani (2001), the decline in microalgae population or cell number is due to the limited nutrient content in the culture media. At the start of culture, the nutrient content in the media is still high, so microalgae can utilize it to carry out the growth process. Cell growth will stop after the number of cells increases to a peak population. At this point, the need for nutrients decreases because nutrients are not added to fermented rice washing water. The following is a graph of *Spirulina* sp's average growth in abundance, presented in the histogram graph in Figure 2.

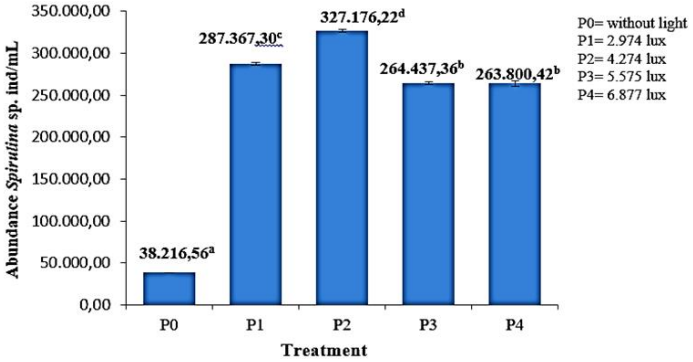

Figure 2. Average abundance histogram *Spirulina* sp

Based on Figure 2, the abundance of *Spirulina* sp cells is known. In each treatment in the study, the best and highest treatment was found to occur in P2 at a light intensity of 4,274 lux in the 23-watt lamp light; an abundance of 327,176.22 ind cells/mL was obtained, which happened in the exponential phase, namely day 7. According to Hadiyanto et al. (2012), if microalgae are in a suitable environment, the rate of cell growth and cell metabolism will increase. As the cell growth rate rises, more biomass will be produced. However, light intensity's role also greatly determines microalgae growth, namely as the main limiting factor in primary productivity.

Furthermore, the lowest treatment was obtained at P0 without providing light, with an abundance of 38,216.56 ind cells/mL. The low number of cells produced at P0 is due to the absence of light intensity, resulting in a slow rate of photosynthesis and cell growth. To see the best growth of microalgae abundance in the P2 treatment with a value of 327,176.2 cells/mL/day, it can be seen from the ANOVA test results (ANOVA), which show that light with different intensities has a significant effect ($p<0,05$) on the abundance of *Spirulina* sp.

3.2. Specific Growth Rate (SGR) Spirulina sp

Furthermore, the Specific Growth Rate in the research carried out during the study can be seen in Table 1.

Table 1. Average specific growth rate abundance of *Spirulina* sp

Growth Phase	Treatment (cell/mL/day) ± std. deviate				
	P0	P1	P2	P3	P4
Lag	0.1406±0.002^a	0.2398±0.003^c	0.2733±0.009^d	0.1788±0.008^b	0.1856±0.001^b
Eksponensial	0.0215±0.006^a	0.3976±0.15^b	0.7273±0.27^c	0.4136±0.032^b	0.2722±0.049^b
Stasioner	0.0000±0.00^a	0.0585±0.008^a	0.5532±0.018^b	0.0569±0.049^a	0.0010±0.0017^a
Death	0.0000±0.00^a	0.0636±0.004^c	0.166±0.022^e	0.0430±0.002^b	0.0931±0.010^d

Note: Superscript letters that are different on the same line are significantly different between treatments ($p<0.05$).

In Table 1. the lag phase has the highest SGR value, namely at P2= 0.2733±0.009^d cells/mL/day, followed by P1= 0.2398±0.003^c cells/mL/day, P4= 0.1856±0.001^b cells/mL/day, P3= 0.1788±0.008^b cells/mL/day, and P0=

0.1406±0.002[a] cells/mL/day. The specific growth rate can change daily depending on the increase or decrease in *Spirulina* sp cells. Furthermore, in the exponential phase, there was a faster increase with the largest SGR value, namely at P2= 0.7273±0.27[c] cells/mL/day, followed by P3= 0.4136±0.032[b] cells/mL/day, P1= 0.0636±0.004[c] cells/mL/day, P4= 0.2722±0.049[b] cells/mL/day, and P0= 0.0215±0.006[a] cell/mL/day. The increasing SGR value indicates the growth of *Spirulina* sp cells. Grow and develop well. The high SGR value occurred due to a high increase in cell density, and the nutrients contained in P2 were more optimal than those in other treatments. This also happened in the stationary phase, where the best treatment occurred in P2 with the highest SGR value, 0.5532±0.018[b] cells/mL/day.

Next, entering the death phase, the SGR value began to decrease, but still, the best treatment occurred in P2 with an SGR value of 0.166 ± 0.022[e] cells/mL/day. This is thought to be because the microalgae do not receive the nutrient requirements, and environmental conditions are no longer optimal (Kawaroe et al., 2012). The decrease in population growth of *Spirulina* sp, which occurs in culture, is thought to be due to the increasingly low nutrient content in the culture water medium. SGR indicates that the higher SGR value means cell abundance increases, and the cell doubling time occurs more quickly. SGR is directly proportional to cell growth because an optimal cell growth rate will also produce an optimal SGR value. The following is the specific growth rate for each treatment, which has different growth patterns (Figure 3).

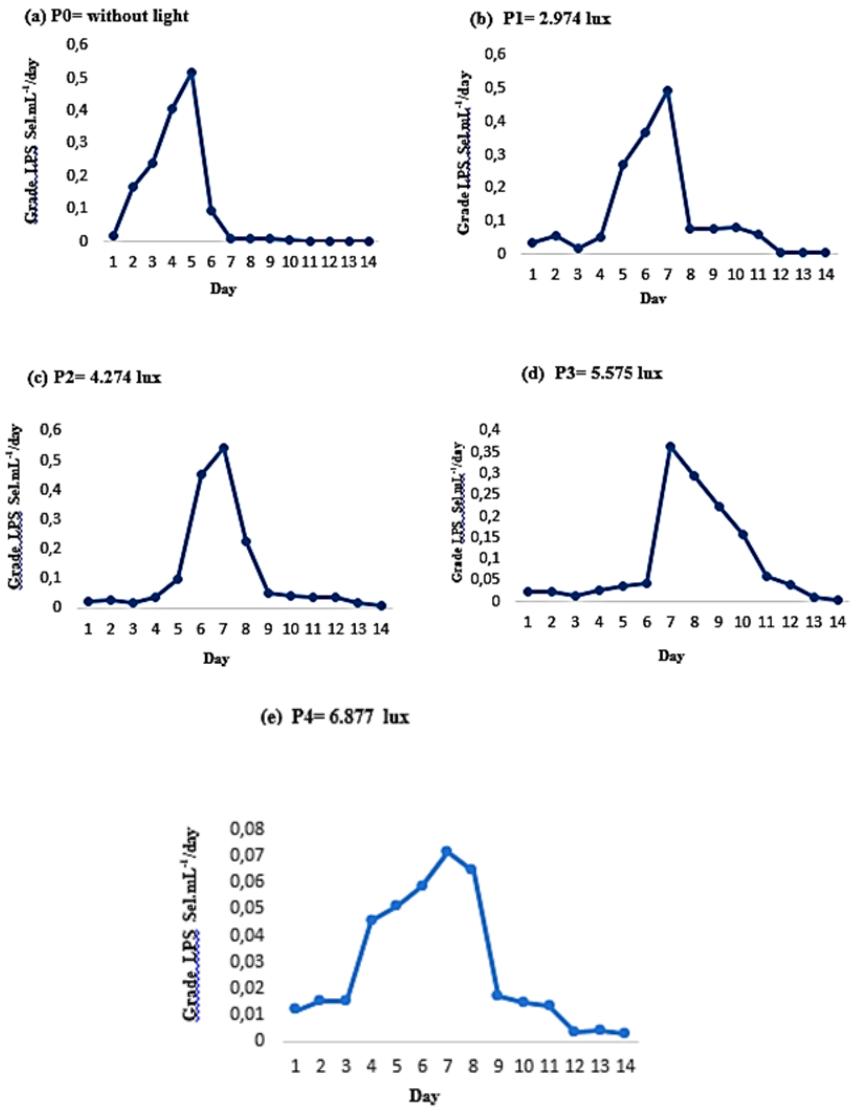

Figure 3. Specific growth rate *Spirulina* sp

Light intensity is important in microalgae culture because light is an energy source for photosynthesis. Figure 3 shows that the growth response of microalgae has varied growth in each treatment for each day. From the various light intensities, genuine differences can be seen. One of these differences can be influenced by the provision of different light intensities, which influences each treatment so that each treatment experiences a different phase on other days. Furthermore, with various light intensities used, microalgae show different growth rates. Higher light

intensity will produce more heat, increasing the temperature and causing a decrease in growth rate. High light intensity will speed up cell division but can cause nutrients to run out more quickly.

3.3. Survival Relationship between Abundance and Chlorophyll-a Content

The relationship between chlorophyll-a values and the cell abundance of *Spirulina* sp is interconnected. If cell abundance increases, the value of chlorophyll-a content will also increase. If cell density decreases, the value of chlorophyll-a content also decreases. This follows the opinion of Octhreeani et al. (2014) that the number of phytoplankton cells determines the high and low chlorophyll content of phytoplankton with parts of the cell wall containing chlorophyll.

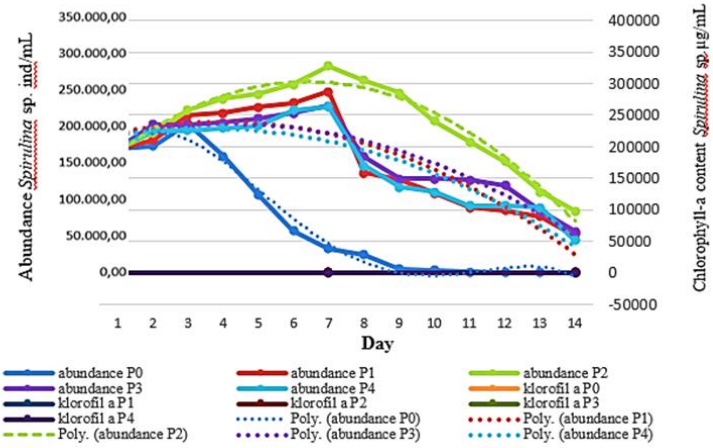

Figure 4. The relationship between abundance and chlorophyll-a content *Spirulina* sp

Figure 4 shows that the relationship between chlorophyll-a values and the abundance of *Spirulina* sp is interconnected and in tandem with each other. If cell abundance increases, the value of chlorophyll-a content also increases. If cell abundance decreases, the value of chlorophyll-a content also decreases. Chlorophyll-a is one of the parameters that determine primary productivity in waters (Simatupang et al., 2023). Furthermore, Octhreeani et al. (2014) reported that phytoplankton's high or low chlorophyll content is determined by the number of phytoplankton cells containing parts of the cell wall. The chlorophyll content in a body of water will increase or decrease as phytoplankton abundance increases or decreases. The role of chlorophyll-a in microalgae is that the chlorophyll-a pigment in microalgae utilizes the light it absorbs as energy in photosynthesis. Furthermore, chlorophyll-a also has a role in protecting chloroplasts from excess light.

Table 2. Calculation of Chlorophyll-a *Spirulina* sp

Day	Content chlorophyll-a				
	P0	P1	P2	P3	P4
Day 1	0.07 ± 0.002^{a}	0.12 ± 0.004^{b}	0.14 ± 0.009^{b}	0.09 ± 0.006^{a}	0.13 ± 0.13^{b}
Day 7	0.14 ± 0.004^{a}	0.20 ± 0.03^{c}	0.40 ± 0.10^{d}	0.21 ± 0.015^{c}	0.16 ± 0.04^{b}
Day 14	0.07 ± 0.005^{a}	0.11 ± 0.005^{c}	0.14 ± 0.006^{d}	0.12 ± 0.004^{c}	0.09 ± 0.005^{b}

Note: different superscript letters on the same row indicate significant differences between treatments ($P<0.05$).

Based on Table 2. the measurement of the chlorophyll-a content of *Spirulina* sp. has different values between treatments, with values that are still minimal because they are still in the lag phase. The values of chlorophyll-a content of *Spirulina* sp on day 1 for P0 and P3 were not significantly different, while values for P1, P2, and P4 were also not significantly different. The subsequent measurement was carried out on the seventh day, the peak phase of the abundance growth rate in microalgae. Based on the measurement results obtained, the chlorophyll-a content of *Spirulina* sp experienced an increase in the peak growth rate of *Spirulina* sp cells. The highest chlorophyll-a content value occurred in P2, with a 0.40±0.100b µg/mL value.

Parslow et al. (2008) classify the chlorophyll-a content in waters based on the trophic status of the seas; namely, the range of 0-0.02 µg/mL is classified as oligotrophic, 0.02-0.05 µg/mL is classified as meso-oligotrophic, 0.05- 0.20 µg/mL is classified as mesotrophic, 0.20-0.50 µg/mL is classified as eutrophic, and >0.50 is classified as hyper-eutrophic. On day 1, measurements of chlorophyll-a content showed that P0= $0.07\pm0.002_{a}$ µg/mL, P1= 0.12 ± 0.004^{b} µg/mL, P2= 0.14 ± 0.009^{b} µg/mL, P3= 0.09 ± 0.006^{a} µg/ mL, and P4= 0.13 ± 0.13^{b} µg/mL. Each treatment is classified as mesotrophic; that is, the level of fertility is moderate. On the seventh day, P0 = 0.14 ± 0.004^{a} µg/mL, P3 0.21 ± 0.015^{c} µg/mL, and P4 0.16 ± 0.043^{b} µg/mL are classified as mesotrophic with moderate fertility levels. Furthermore, P1= 0.20 ± 0.03^{c} µg/mL and P2= 0.40 ± 0.100^{d} µg/mL are classified as eutrophic; that is, they are classified as high fertility levels.

Furthermore, on the 14th day the chlorophyll-a content values on the 14th day were P0= 0.07±0.005[a] µg/mL, P1= 0.11±0.005[c] µg/mL, P2= 0.14±0.006[d] µg/mL, P3 = 0.12 ±0.004[c] µg/mL and P4= 0.09±0.005[b] µg/mL are classified as mesotrophic levels (medium fertility). The results obtained on the 14th day, the chlorophyll-a content values in each treatment all decreased because the culture of *Spirulina* sp has experienced death, so the chlorophyll-a content also reduced according to the cell density of *Spirulina* sp. This happens because the availability of nutrients and light affects the formation of chlorophyll in *Spirulina* sp (Nur et al., 2020). Meanwhile, the decrease in chlorophyll levels is thought to be due to the decline in nutrition in all treatments, thus affecting chlorophyll-a formation. The calculation of the results of the analysis of variance (ANOVA) test was tested using the SPSS version 24 application, showing that the different light intensities given fermented rice washing water were then followed by the Newman Keuls Study, which showed that the treatment used was significantly different from the treatment.

3.4. Light Intensity

The effect of light intensity on the growth of the microalga *Spirulina* sp. In this study, the growth results varied but were similar. However, the best growth results were proven in treatment P2 at a light intensity of 4,274 lux. Light intensity is suspected to be related to the abundance of *Spirulina* sp. The following graph shows the relationship between light intensity and the abundance of *Spirulina* sp.

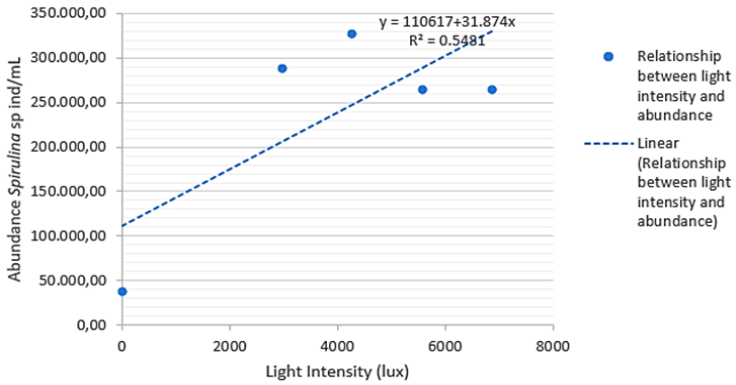

Figure 5. The relationship between light intensity and the abundance of *Spirulina* sp

Figure 5 shows that the relationship between light intensity and the abundance of *Spirulina* sp is interconnected. Different light intensities have different effects on the growth of microalgae. According to Nielsen (2017), if photoautotrophic organisms experience a lack of light, it will reduce the rate of photosynthesis. Decreased photosynthesis will also impact the biomass produced, microalgae's abundance, and specific growth rate. Light can increase the ATP produced in the photosynthesis process; the increase in ATP will trigger a faster metabolic rate and affect carotenoid metabolism in algal cells (Febriani et al., 2020). This aligns with the relationship between light intensity and microalgae abundance produced in the correlation coefficient graph, which shows an influence of R2 = 0.5481.

Hadiyanto et al. (2012) Reported that the activity of the photosynthetic process of microalgae will increase with an increase in the intensity of optimum light with a light range of 3500-5000 lux. Suppose the light intensity is in the optimal range required by the microalgae. In that case, the resulting abundance value will be practical because the light intensity can absorb the nutrients received. However, if the amount of light intensity required is not in the optimal range, it will affect the growth of the microalgae. At a light intensity of 4,274 lux in treatment P2, it is the highest treatment because cells divide more than in other treatments, and the size obtained in treatment P2, the light provided can support the growth rate of *Spirulina* sp.

3.5. Water Quality

An important factor that significantly influences the growth of *Spirulina* sp during culture is water quality, which includes physical conditions, temperature, and light intensity. In contrast, chemical conditions include temperature, pH, dissolved oxygen (DO), nitrate, and phosphate levels. Water quality is the most important part of the growth of the microalga *Spirulina* sp because water quality factors can provide optimal growth in an optimal environment. The parameters measured during the 14-day research process were temperature, pH, DO, chlorophyll-a, and nitrate and phosphate, measured three times during the research. The results of measuring water quality parameters are presented in Table 3.

Table 3 shows the range of water quality values for *Spirulina* sp culture. During the 14 days of research, it was found that the growth of the microalgae *Spirulina* sp's good results can be influenced by environmental factors that support the growth process of the microalgae *Spirulina* sp are the factors temperature, pH, and DO. In the research carried out, the temperature measurement results were 28-31^{0}C. Thus, the temperature measurement results are still at optimal intervals and can be tolerated by the microalgae *Spirulina* sp.

Table 3. Water quality value of *Spirulina* sp culture

Treatment	Temperature (ºC)	Optimum	pH		DO (mg/L)	Optimum
P0	28		7-8		5-6,5	
P1	28-30		7-8,5		5-6,5	
P2	30	*20-30	7-8	**5-9,5	4,8-7,3	***4-7
P3	30-31		7-8,3		4,8-7,3	
P4	31		7-8,3		4,7-7,5	

Sources: *(Buwono & Nurhasan, 2018); **(Putri & Setyani, 2019); ***(Muliani et al., 2018)

This is supported by the opinion (Buwono & Nurhasan, 2018) that *Spirulina* sp has a temperature range of 20-30^{0}C. Temperature can also affect cell growth and division. Cell division that occurs when the temperature increases results in a decrease in the solubility of CO_2 so that the pH of the water becomes alkaline, which will then affect the availability of nutrients. The temperature changes during the research were not much different for each treatment. Temperature plays an important role in the culture process as an organism's metabolic process.

The research results on pH measurements prove optimal growth of the microalgae *Spirulina* sp, which is around 7-8.5, so that cells can still grow and develop well. This is reinforced by the opinion (Putri & Setyani, 2019) that a pH range of 5-9.5 is suitable for the growth of *Spirulina* sp in the waters. Environmental conditions such as pH are a factor that has an important role in the growth of the microalgae *Spirulina* sp, so with optimal pH conditions, it can be maintained that cells can grow and develop well.

Furthermore, when measuring dissolved oxygen (DO) in this study, the DO values obtained were around 4.7-7.5 mg/L. P4 experienced the highest DO value in this study at 4.7-7.5 mg/L, which occurred on days 7 to 9 and gradually decreased in the phase leading up to the death phase. The percentage of dissolved oxygen (DO) measurement results obtained is still relatively high. Dissolved O_2 levels of 3.0–5.0 mg/L are less productive, and above 7 ppm, the productivity is very high. Increased dissolved oxygen content in the *Spirulina* sp culture media. Due to the large oxygen supply from photosynthesis and aeration processes during the research.

Nitrate is an important element that helps the growth process in microalgae, including *Spirulina* sp. The nitrate and phosphate content was measured three times during the study: on day 1, day 7, and day 14. More complete results of nitrate measurements can be seen in Table 4.

Table 4. Nitrate Content Measurement

Treatment	Day 1	Day 7	Day 14	Optimum value
P0	0,90	0,96	0,87	3,9-15,5 mg/L (Mackentum, 1969)
P1	0,58	0,89	0,75	
P2	1,07	0,91	0,81	
P3	0,66	0,77	0,76	
P4	0,73	0,74	0,70	

According to Mackentum (1969), the optimal growth of phytoplankton requires a nitrate content of 3.9 - 15.5 mg/L. Based on Table 4, day one nitrate measurements at P0, P1, P2, P3, and P4 have different values. The highest nitrate measurement results from day one were obtained, namely P2= 1.07 mg/L, followed by P0= 0.90 mg/L, P4= 0.73 mg/L, P3= 0.66 mg /L and P1= 0.58 mg/L. Furthermore, for nitrate measurements on day 7, the highest nitrate concentration was at P0= 0.96 mg/L, followed by P2= 0.91 mg/L, P1 = 0.89 mg/L, P3= 0.77 mg/L L, and P4= 0.74 mg/L. Furthermore, entering the 14th day of measurement, the nitrate concentration content decreased slowly, namely for the nitrate value at P0= 0.87 mg/L, followed by P2= 0.81 mg/L, P3= 0.76 mg/L, P1= 0.75 mg/L, and P4= 0.70 mg/L. The decrease in nitrate concentration is likely because nitrate has been utilized by Spirulina sp. According to Sukmawan et al. (2014), a decrease in nitrate content is caused by phytoplankton's use of nitrogen as nitrate for nutritional needs. Optimal and sound growth of phytoplankton will be in line with the presence of chlorophyll-a concentration. If phytoplankton growth is good, the chlorophyll-a concentration value will also be good. This nitrate nutrient is one of the main requirements for the metabolism and growth of microalgae and is used by microalgae in photosynthesis (Meirinawati & Fitriya, 2018).

Phosphate is one of the main elements needed for the growth and increase of microalgae biomass. So, phosphate is necessary to transfer energy from outside to inside organism cells. Furthermore, to find out more complete results of measurements on nitrate, see Table 5.

Table 5. Phosphate content measurement

Treatment	Day 1	Day 7	Day 14	Optimum Value
P0	0,51	0,99	0,83	*0,27-5,51 mg/L
P1	0,63	0,72	0,70	
P2	0,67	0,82	0,55	
P3	0,65	0,96	0,65	
P4	0,45	0,72	0,58	

Based on Table 5, day one phosphate measurements at P0, P1, P2, P3, and P4 have different values. The highest phosphate measurement results occurred at P2= 0.67 mg/L, followed by P3= 0.65 mg/L, P1= 63 mg/L, P0= 0.51 mg/mL, and P4= 0, 45 mg/L. According to Tungka et al. (2016), fertile phosphate can lead to a nutrient enrichment process. If the microalgae are fertile in these conditions, they will more easily utilize phosphate for their metabolism. Furthermore, phosphate measurements on day 7 occurred at P0= 0.99 mg/L, P3= 0.96 mg/L, P2= 0.82 mg/L, P1 and P4= 0.72 mg/L. Furthermore, entering the 14th day of measurement, the phosphate concentration content decreased slowly, namely for the phosphate value, namely at P0= 0.83 mg/L, followed by P1= 0.70 mg/L, P3= 0.65 mg/L, P4= 0.58 mg/L, and P2= 0.55 mg/L.

According to Boroh et al. (2019), the optimal phosphate content for phytoplankton growth is between 0.27 and 5.51 mg/mL. The decrease in phosphate concentration is thought to be due to *Spirulina* sp utilizing phosphate. If phosphate levels are low, they will become a limiting factor, whereas if they are high, they will inhibit cell growth and activity.

4. Conclusions

Different light intensities significantly affected the abundance and chlorophyll-a content of *Spirulina* sp ($p<0.05$) in fermented rice washing water on the abundance and content of chlorophyll-a. The best light intensity influences the abundance and chlorophyll-a content of *Spirulina* sp. The best light intensity was proven in treatment P2, namely 4,274 lux in the 23-watt lamp light, which gave an abundance of 327,176.2 ind cells/mL, which had the best chlorophyll-a content value in P2 of 0.4 µg/mL on day 7 of the exponential phase. The suggestion in this research is that further research needs to be carried out with different light intensities on other types of phytoplankton, which can be helpful in aquaculture.

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