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Effectiveness Of Direct And Indirect Sunlight Irradiation In The Cultivation Of Chlorella Pyrenoidosa

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Abstract—This study discusses the effectiveness of direct and indirect sunlight exposure in the cultivation of *Chlorella pyrenoidosa*. This microalga plays a role in various aspects of human life, both as a food source, an alternative energy source, and a pharmaceutical ingredient. Additionally, its relatively high protein content serves as food for aquatic microorganisms. Direct sunlight exposure can increase the density and growth rate of *C. pyrenoidosa*. This study employs an experimental approach using an independent t-test design, incorporating two treatments and six replications: Treatment A, which involves direct sunlight exposure, and Treatment B, which involves indirect sunlight exposure. The research results show that direct and indirect sunlight exposure significantly affects the growth rate of the *C. pyrenoidosa* population ($P < 0.05$). Direct sunlight exposure (Treatment A) yielded the best results with a density of 1838.94±116.27 ind/mL and a growth rate of 18.02±1.35 ind/mL/day.

Keywords – Sunlight Exposure, *Chlorella pyrenoidosa*, Density, Growth.

I. INTRODUCTION

Microalgae play an important role in various aspects of human life, both as a source of food, alternative energy, and pharmaceutical materials. In the field of energy, microalgae offer a promising solution as a source of biofuel and bioethanol, which can reduce dependence on fossil fuels ([1], [2], [3]). In the pharmaceutical and nutraceutical industries, microalgae contain various bioactive compounds that exhibit anti-inflammatory and anti-cancer activities, making them attractive candidates for the development of drugs and health supplements ([4], [5]). Shiels et al. [4] suggest that we can use the lipids from microalgae to reduce the risk of heart disease and inflammation. Biotechnology advancements can further utilize microalgae to produce high-value products that can meet the ever-growing demands of the global market ([6], [7]). In the context of nutrition, microalgae such as Chlorella and Spirulina are known as high sources of protein, omega-3 fatty acids, and antioxidants, which contribute to a healthy and sustainable diet ([8], [9]). Compared to conventional food sources, microalgae can provide a more sustainable alternative, particularly when it comes to producing essential fatty acids typically found in fish [9].

In addition to playing an important role in various aspects of human life, microalgae also function as primary producers in aquatic ecosystems, significantly contributing to primary productivity and water quality. As photosynthetic organisms, microalgae convert solar energy into biomass, which becomes a food source for various aquatic organisms, including zooplankton and fish. *C. pyrenoidosa*, one type of microalgae, offers significant benefits in the fields of aquaculture, the environment, and health. In the context of aquaculture, this microalga serves as a natural feed rich in nutrients for various species of fish and shrimp. The use of Chlorella in cultivation systems can enhance feed quality and support the growth of fish larvae, ultimately increasing harvest yields [10]. This microalga is rich in protein, vitamins, and minerals, making it an excellent source of nutrition. Research shows that *C. pyrenoidosa* contains up to 44% protein, making it one of the best sources of plant-based protein [11]. Additionally, this microalga also contains essential and non-essential amino acids that are important for growth and health [1]. The high chlorophyll content in *C. pyrenoidosa* also contributes to health benefits [11]. Additionally, *C. pyrenoidosa* is also capable of improving the quality of pond water by reducing levels of ammonia and nitrates that are harmful to aquatic life. The nutrients contained in this microalga

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are crucial during the larval phase, where the nutritional needs are higher compared to adult fish, because larvae require nutrients not only for activity and maintenance but also for optimal growth [13]. Thus, the use of *C. pyrenoidosa* as a natural feed can contribute to the increased success of fish farming.

The cultivation techniques of *C. pyrenoidosa* involve various methods designed to maximize the growth and biomass production of this microalga. Factors affecting the growth of *C. pyrenoidosa* include light, nutrients, temperature, and pH. Light is an important element in photosynthesis, where the intensity and quality of light can affect the growth rate of microalgae. Research by [14] shows that the use of LED lights with varying wavelengths can enhance the growth and lipid accumulation of *C. pyrenoidosa*, with the best results obtained at specific wavelength combinations. This shows that not only the intensity of light is important, but also the quality of light received by microalgae can affect the production of biomass and lipids.

In addition, temperature also plays an important role in the photosynthesis process, where excessively high temperatures can disrupt the balance between light absorption and energy utilization [15]. Nutrients, especially nitrogen and carbon, are another key factor in the cultivation of *C. pyrenoidosa*. Various sources of organic carbon can affect the growth and biochemical composition of *C. pyrenoidosa*, where the use of the appropriate carbon source can enhance production yields [16]. Additionally, highlight the importance of selecting the right nutrients in cultivation systems, which can affect the production of lipids and proteins in microalgae. Thus, effective nutrient management is crucial to achieving optimal productivity in microalgae cultivation [11].

The cultivation techniques of *C. pyrenoidosa* involve various methods designed to maximize the growth and biomass production of this microalga. Factors affecting the growth of *C. pyrenoidosa* include light, nutrients, temperature, and pH. Light is an important element in photosynthesis, where the intensity and quality of light can affect the growth rate of microalgae. Refers to [14] shows that using LED lights with different wavelengths can help *C. pyrenoidosa* grow and store more lipids, with certain wavelength combinations giving the best results. This demonstrates that the production of biomass and lipids by microalgae is not solely dependent on light intensity but also on the quality of light they receive.

In addition, temperature also plays an important role in the photosynthesis process, where excessively high temperatures can disrupt the balance between light absorption and energy utilization [15]. Nutrients, especially nitrogen and carbon, are another key factor in the cultivation of *C. pyrenoidosa*. Various sources of organic carbon can affect the growth and biochemical composition of *C. pyrenoidosa*; the use of the appropriate carbon source can enhance production yields [16].

Few studies specifically compare the effects of direct and indirect light on the growth of *C. pyrenoidosa*, despite the numerous studies conducted to understand the light requirements of microalgae. Light is a key factor in photosynthesis, and the intensity and quality of light can affect the efficiency of photosynthesis and the growth of microalgae. The pigment composition in microalgae, which consists of about 80% chlorophyll, plays an important role in photosynthesis efficiency [17]. However, this research does not directly compare the effects of direct and indirect light on the growth of *C. pyrenoidosa*, leaving room for further exploration on how these two types of illumination affect the growth of *C. pyrenoidosa*.

Another more relevant study is about the effects of various lighting conditions on the growth of microalgae, including *C. pyrenoidosa*. They found that microalgae exposed to direct light showed better growth compared to those exposed to indirect light, although this study focused more on toxicity effects rather than lighting comparison [18]. This research indicates that, despite many studies on the light requirements of microalgae, there is still a lack of understanding of how direct and indirect lighting can specifically affect the growth and productivity of *C. pyrenoidosa*.

Thus, further research is needed that explicitly examines the effectiveness of direct and indirect sunlight exposure in the cultivation of *C. pyrenoidosa* to optimize cultivation techniques and enhance the production yield of this microalga in supporting cultivation efforts.

II. MATERIALS AND METHODOLOGY

The microalgae species used in this study was *C. pyrenoidosa*. This strain was obtained from the Freshwater Aquaculture Centre of Sungai Gelam, Jambi. Cultures of *C. pyrenoidosa* were maintained in 2.5 L of freshwater that had been sterilised and fertilised. The

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microalgae were cultivated for 16 days in the wet laboratory of the Faculty of Fisheries and Marine Science, Universitas Bung Hatta, Padang. The method used for this research is experimental method with Independent t-test design of test variables with 2 treatments and 6 replicates. The treatments used in this study are referred to as Treatment A The use of direct sunlight and Treatment B The use of indirect sunlight. The initial density of microalgae used in this study was 256 cells mL^{-1}. The growth of microalgae was observed until day 16. The density of C. pyrenoidosa was calculated using an equation that refers to [19]:

$$\textbf{Density} = \textbf{N x } 10^4 \textbf{ cells mL}^{-1}$$

Where: N - number of cells obtained; 10^4 - haemocytometer constant.

Meanwhile, to measure the specific growth rate of *C. pyrenoidosa*, it is calculated using an equation that refers to [20].

$$GR = \frac{Wt - Wo}{t_2 - t_1}$$

GR = Growth rate (ind/mL/day), Wo = Average growth at the beginning of the study (ind/mL), Wt = Growth on day t (ind/mL/day), t = Duration of maintenance (day)

To measure the intensity of sunlight, refer to [21] using a lux meter. Water quality parameters were observed every 4 days during the maintenance period, including temperature, DO, pH, ammonia, nitrate, nitrite, and phosphate.

III. Result and Discussion

Based on the research results, it is evident that the influence of direct and indirect sunlight exposure significantly affects the growth rate of the *C. pyrenoidosa* population ($P < 0.05$). Table 1 presents the research results on the density and growth rate of the *C. pyrenoidosa* population.

Table 1. Population Density and Growth Rate of *C. pyrenoidosa*

Treatment	Density (Ind/mL)	Growth Rate (ind/mL/day)
A	1838.94±116.27	18.02±1.35
B	1024.12±7.36	16.66±0.34

Based on Table 1, it can be seen that the effect of direct and indirect sunlight exposure has a significant impact on the population density of *C. pyrenoidosa* ($P < 0.05$). The research results indicate that treatment A had the highest density, measuring between 1838.94±116.27 ind/mL, with treatment B following closely behind. Meanwhile, the highest growth rate was found in treatment A (18.02±1.35) ind/mL/day, followed by treatment B.

The intensity of direct sunlight greatly influences the growth of *C. pyrenoidosa*, a green microalga known for its bioproduction potential. The main energy source in photosynthesis, light, is crucial to *C. pyrenoidosa* growth. Microalgae use light to convert carbon dioxide and water into glucose and oxygen, which is a vital process for their growth and reproduction [14]. The fundamental difference between direct and indirect illumination has significant implications for the efficiency of photosynthesis and the growth of *C. pyrenoidosa*. Direct irradiation pertains to the direct impact of light on the surface of microalgae, whereas indirect irradiation involves the reflection or scattering of light before it reaches the microalgae. According to [22], direct irradiation can increase the efficiency of photosynthesis because microalgae receive high-intensity light directly, allowing the photosynthesis process to occur more quickly. On the other hand, indirect irradiation can decrease the received light intensity, leading to a decrease in the rate of photosynthesis and microalgae growth.

In this study, results showed that *C. pyrenoidosa* cultured under direct sunlight experienced a significant increase in density, reaching 1.7 times that of those cultured under indirect light. This is in line with the finding that higher light intensity can increase the rate of photosynthesis, which contributes to better growth. The high growth rate of *C. pyrenoidosa* in direct sunlight intensity is in accordance with the opinion of [23] which states that this microalga can achieve optimal growth rates when exposed to direct sunlight, which allows the photosynthetic process to take place more efficiently.

Indirect sunlight, although still providing some benefits, is not able to support the growth of *C. pyrenoidosa* as efficiently as direct sunlight. According to [24] found that less optimal lighting conditions, like indirect sunlight, can inhibit the accumulation of lipids and microalgal biomass. The results obtained in this study show that, although *C. pyrenoidosa* can still survive and grow under indirect light, its growth rate is much slower, and the biomass yield obtained is only 40% of that produced under direct light. This shows that even though *C. pyrenoidosa* has the ability to adapt, higher light intensity remains a key factor in achieving optimal growth results.

The study found that *C. pyrenoidosa* grew at a good rate. In this study, the light intensity obtained from the measurement of direct sunlight intensity was 3197.5±871.87 lux, while the light intensity under indirect sunlight was 1618.7±324.16 lux. Research conducted by [17] showed that the optimal light intensity for microalgae growth is in the range of 50 to 150 µE m^{-2} s^{-1}, where, at this intensity, the growth rate and biomass accumulation reach their peak. However, at higher intensities, such as 300 µE m^{-2} s^{-1}, growth can be inhibited due to changes in carbon allocation and decreased photosynthetic efficiency. This suggests that while light plays a crucial role in photosynthesis, too much light can lead to stress, which can negatively impact microalgae and cause them to enter the death phase. We suspect that the highest population growth of *C. pyrenoidosa* in treatment A is due to differences in the available sunlight intensity. Each species of microalgae has different tolerances to variations in light intensity [25].

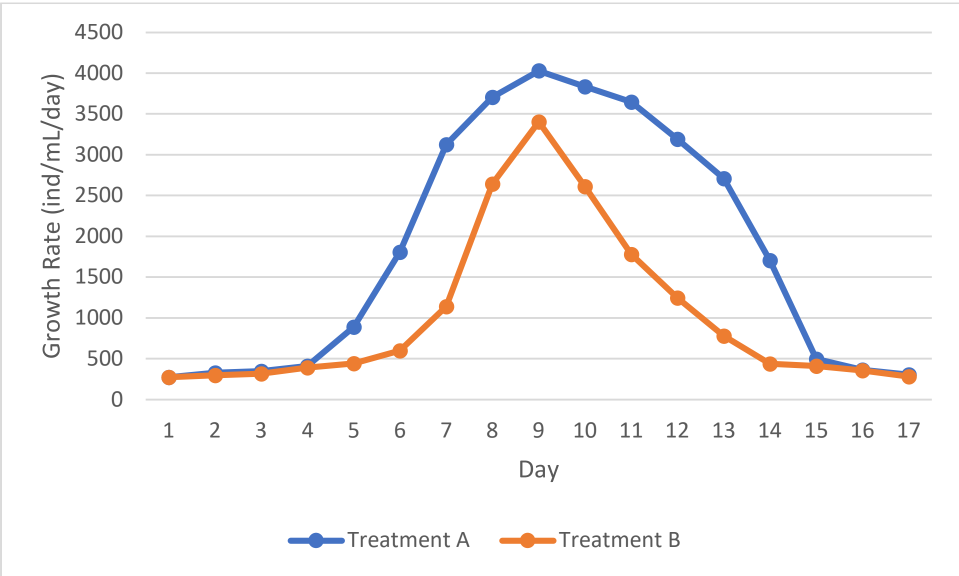

Fig. 1. Growth rate of *C. pyrenoidosa*

Based on Figure 1, The results of observing the growth of *C. pyrenoidosa* on a semi-mass scale can show that the density of *C. pyrenoidosa* on the first day is still small. This is due to the process of adaptation to the cultivation environment which is called the resting phase. This phase is characterised by the size of *C. pyrenoidosa* increasing physiologically and the process of synthesising new proteins in the microalgae, but not yet dividing into new individuals [26]. The growth of *C. pyrenoidosa* increases from day 4 to 8 which is called the logarithmic phase (log). The logarithmic (log) phase shows that density increases tend to be drastic, the growth rate is optimal, and the nutrient content is still high. In the peak growth phase, *C. pyrenoidosa* shows good adaptability to a variety of energy sources. Under mixotrophic conditions, where microalgae can utilise organic carbon sources such as glucose, growth can increase significantly [27]. In addition, the availability of nutrients such as nitrogen and phosphorus also plays an important role in supporting the optimal growth of these microalgae [28]. Thus, the combination of the right light intensity and the availability of adequate energy sources is essential to reach the peak growth phase.

Each microalgae species has a different tolerance to variations in light intensity [29]. After population growth reaches a peak on day 10, after that there is no more addition of individuals because the growth rate is balanced with the death rate (stationary phase). Each treatment showed a different decrease in population density. The ongoing decline in population density is thought to be caused by the availability of food in the media that is no longer sufficient for the needs of *C. pyrenoidosa*. Pyrenoidosa. The density of *C. pyrenoidosa* decreased which is generally called the stationary phase on days 10 and 14 due to the reduced amount of nutrients in the cultivation medium along with the increasing population of microalgae.

The results of water quality observations during the study were carried out once every 4 days. During the study, aeration was used to increase oxygen supply. In addition, aeration is intended to cause turbulence and cyculation of the culture medium, which is important for nutrient distribution to prevent precipitation. The results of the measurement of water quality parameters are as follows:

Table 2. Water quality measurement results

Treatment	Temperature	DO	pH	Ammonia	Nitrate	Nitrite	Phosphate
A	28.73±0.13	7.83±0.05	7.58±0.17	0.80±0.34	0.86±0.10	0.08±0.03	0.20±0.06
B	28.55±0.13	7.70±0.08	7.45±0.13	0.85±0.33	0.88±0.06	0.10±0.03	0.21±0.07

Based on Table 2, The temperature range in both treatments A and B ranged from 28.4 - 28.9ºC. Temperature plays a crucial role in the growth of *C. pyrenoidosa*, where the optimal temperature range can increase the photosynthetic rate and biomass productivity of this microalgae. Research shows that the ideal temperature for *C. pyrenoidosa* growth ranges from 25°C to 30°C [30]. At this temperature, photosynthetic activity produced by microalgae can reach its peak, thereby increasing the efficiency of energy conversion from light to biomass. For example, an increase in temperature can improve electron transfer in the photosynthetic process, which contributes to an increase in microalgae productivity [31]. In addition, proper temperature regulation can optimise microalgae growth conditions, which is important for applications in aquaculture and water treatment [32].

Dissolved oxygen (DO) is an important parameter that greatly affects the metabolic processes of these microalgae. The ideal DO range for *C. pyrenoidosa* growth ranges from 5 to 10 mg/L [24]. In this range, photosynthetic activity and respiration of microalgae take place efficiently, which contributes to an increase in biomass. Conversely, if the DO concentration is below 3 mg/L, microalgal growth may be inhibited, and anaerobic conditions may occur, which negatively impacts productivity [30]. The DO range in this study was 7.7 -7.9 mg/L so the DO range in the study was quite ideal.

pH serves to influence the rate of photosynthesis and metabolism of microalgae. The optimal pH for *C. pyrenoidosa* growth ranges from 6.5 to 8.5 [31]. The pH range in this study was 7.3 - 7.8. In this range, microalgae can perform photosynthesis efficiently, which contributes to an increase in biomass. Conversely, pH that is too low or too high can cause stress to microalgae, inhibit growth, and even cause cell death [32]. Changes in pH can affect the ability of *C. pyrenoidosa* to remove nitrate and phosphate from sewage, indicating the importance of pH management in microalgae cultivation applications [31].

The Ammonia range in this study was 0.41-1.15 mg/L. Ammonia is one of the important nutrients that play a role in the growth of *C. pyrenoidosa*, where this compound serves as an essential nitrogen source for microalgae. The ideal ammonia concentration for *C. pyrenoidosa* growth ranges from 0.5 to 2.0 mg/L [33]. In this range, microalgae can utilise ammonia efficiently for protein and biomass synthesis. Conversely, ammonia concentrations that are too high can be toxic and inhibit growth, even causing cell death [34]. In addition, the addition of ammonia in the culture medium can increase the cell density of Chlorella sp., indicating the importance of managing ammonia concentration in microalgae cultivation [35].

Nitrate functions in protein synthesis and microalgal biomass. The ideal nitrate concentration for *C. pyrenoidosa* growth ranges from 10 to 50 mg/L [36]. In the study, the range of nitrate was 0.06 - 0.93 mg/L. In this range, microalgae can utilise nitrate efficiently for photosynthesis and cell growth. Nitrate concentrations that are too high can cause toxic accumulation that negatively affects microalgae growth and water quality [37]. Research by [38] revealed that the addition of nitrate in culture media can increase the productivity of Chlorella sp., thus showing the importance of managing nitrate concentrations in microalgae cultivation.

The range of Nitrite in this study was 0.03 - 0.12 mg/L. This range is quite ideal where the ideal range for *C. pyrenoidosa* growth ranges from 0.1 to 1.0 mg/L [39]. In this range, microalgae can utilise nitrite efficiently for protein synthesis and cell growth. However, too high a concentration of nitrite can be toxic and inhibit microalgal growth, even causing cell death [40]. In addition, the addition of nitrite in culture media can increase the density of Chlorella sp. cells [34]. The last water quality parameter is Phosphate, where the range of Phosphate in this study is 0.06 - 0.25 mg/L. and is the ideal kisaaran. This is in accordance with the opinion of [35] that the ideal concentration of phosphate for the growth of *C. pyrenoidosa* ranges from 1 to 5 mg/L. From all observed water quality parameters, the water quality parameters in the study were in ideal conditions for the growth of *C. pyrenoidosa*.

IV. CONCLUSION

The results showed that direct and indirect solar irradiation can effectively increase the density and growth rate of *C. pyrenoidosa* on a mass scale. Direct sunlight (Treatment A) gave the best results with a density of 1838.94±116.27 ind/mL and a growth rate of (18.02±1.35) ind/mL/day.

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