Eduvest – Journal of Universal Studies
Volume 6, Number 3, March, 2026
p- ISSN 2775-3735- e-ISSN 2775-3727

# The Effect of Co2 Concentration and Light Intensity on the Productivity of Scenedesmus sp. In Culture Media

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***Keywords:***
*Scenedesmus sp., Productivity, CO2, Light Intensity, Photobioreactor*

## ABSTRACT

The microalga Scenedesmus sp. is a biological resource with high potential for biomass production and carbon dioxide (CO2) fixation. The productivity of microalgae is highly dependent on the availability of two primary resources: light as the energy source and CO2 as the carbon source. This study aimed to evaluate and determine the optimal combination of CO2 concentration and light intensity that is most effective in maximizing the growth and productivity of Scenedesmus sp. The research utilized a batch cultivation system over a 10-day period in 16 Photobioreactor (FBR) units. A 4×4 factorial experimental design was applied, varying four levels of CO2 concentration (2%, 2.5%, 3%, 3.5%) and four levels of light intensity (3500, 4000, 4500, 5000 Lux). Growth was monitored based on cell density (cells/mL), counted using the Haemacytometer method every two days, and the specific growth rate (μ) was calculated. The results indicated that the growth pattern exhibited a Lag phase (Day 0−2) and an Exponential phase (Day 2−8). The treatment combination of 3% CO2 concentration and 4500 Lux light intensity (FBR 11) consistently yielded the highest cell density, peaking at 1.41×106 cells/mL on Day 8, which also reflected the maximum specific growth rate. Other conditions showed inhibitory effects: the 5000 Lux light intensity caused photoinhibition or damage to the photosynthetic apparatus, while the highest CO2 concentration of 3.5% led to a decline in cell density due to a suspected drop in the medium pH. It is concluded that the optimal condition of 3% CO2 and 4500 Lux is the most effective condition for enhancing the productivity of Scenedesmus sp. in the FBR system.

## INTRODUCTION

Global climate change is one of the main issues facing the world today, especially due to increased $CO_2$ emissions from human activities such as fossil burning, deforestation, and industry that causes global warming and extreme climate change. According to a report by the Intergovernmental Panel on Climate Change (IPCC), atmospheric $CO_2$ levels have increased significantly beyond levels that have occurred in the last thousand years, triggering various negative impacts on the environment and human life (IPPC, 2021).

One of the most prominent efforts is biocarbon fixation because it is more environmentally friendly, cost-effective, and supports sustainable development. Microalgae play an important role in ecosystems because they are able to absorb large amounts of carbon through photosynthesis, even more than 50% of the total carbon captured naturally worldwide. Microalgae are an effective choice because they grow quickly, have a short life cycle, and require only simple nutrients to thrive (Yao et al., 2024).

The presence of $CO_2$ can increase the productivity of microalgae by up to 2-5 times compared to normal conditions, especially when microalgae are cultivated in photobioreactors

(Sari et al., 2018). According to Yao et al. (2024), the optimal $CO_2$ concentration for microalgae growth is generally below 10%, as levels exceeding 10% can be toxic to most microalgae strains.

Some species of microalgae exhibit the ability to absorb $CO_2$ at higher concentrations than terrestrial plants. In the study of Sangtani et al. (2024), with a supply of 5% $CO_2$, Scenedesmus sp. grown in fertilizer-based media (BG-11) shows a significant increase in $CO_2$ absorption. Its ability to absorb carbon has been shown to be higher than that of Chlorella vulgaris and even superior to previous studies on Scenedesmus sp. in different conditions. This suggests that the combination of fertilizer media and $CO_2$ concentration can improve the efficiency of carbon sequestration by Scenedesmus sp..

According to Jin et al. (2024), the main factors influencing the growth of microalgae in this study include mixing and aeration, light, cell density, temperature, pH, and nutrient availability, which are optimized through the use of photobioreactors to create a stable and efficient culture environment.

Although microalgae have the potential to mitigate carbon emissions, there are still limited specific data on the effect of $CO_2$ concentration on absorption efficiency in Scenedesmus sp. Most studies focus more on other microalgae or general aspects of growth without delving into the relationship between $CO_2$ and other factors such as light intensity. Therefore, this study is important to understand the response of Scenedesmus sp. against $CO_2$ and light variations to optimize carbon absorption efficiency. This study was conducted to answer the problem of the effect of variations in $CO_2$ concentration and light intensity on the growth rate, cell density, and biomass productivity of Scenedesmus sp. microalgae biomass. in the culture medium, as well as determining the most optimal $CO_2$ concentration and light intensity to increase productivity.

The main objective of this study was to analyze the relationship between the variation of the two factors and the growth and productivity of microalgae biomass, as well as to identify the best conditions that support the efficiency of $CO_2$ uptake by Scenedesmus sp. The benefits of this research include three aspects, namely theoretical benefits that can enrich scientific knowledge about the productivity of microalgae and the factors that affect it, practical benefits that can be a reference for industry and governments in developing microalgae-based carbon sequestration technology as an environmentally friendly and economical solution, and environmental benefits in the form of real contributions to climate change mitigation through reducing $CO_2$ levels in the atmosphere on an ongoing basis.

## METHOD

The research utilized a batch cultivation system over a 10-day period in 16 Photobioreactor (FBR) units. A 4×4 factorial experimental design was applied, varying four levels of CO2 concentration (2%, 2.5%, 3%, 3.5%) and four levels of light intensity (3500, 4000, 4500, 5000 Lux). Growth was monitored based on cell density (cells/mL), counted using the Haemacytometer method every two days, and the specific growth rate (μ) was calculated.

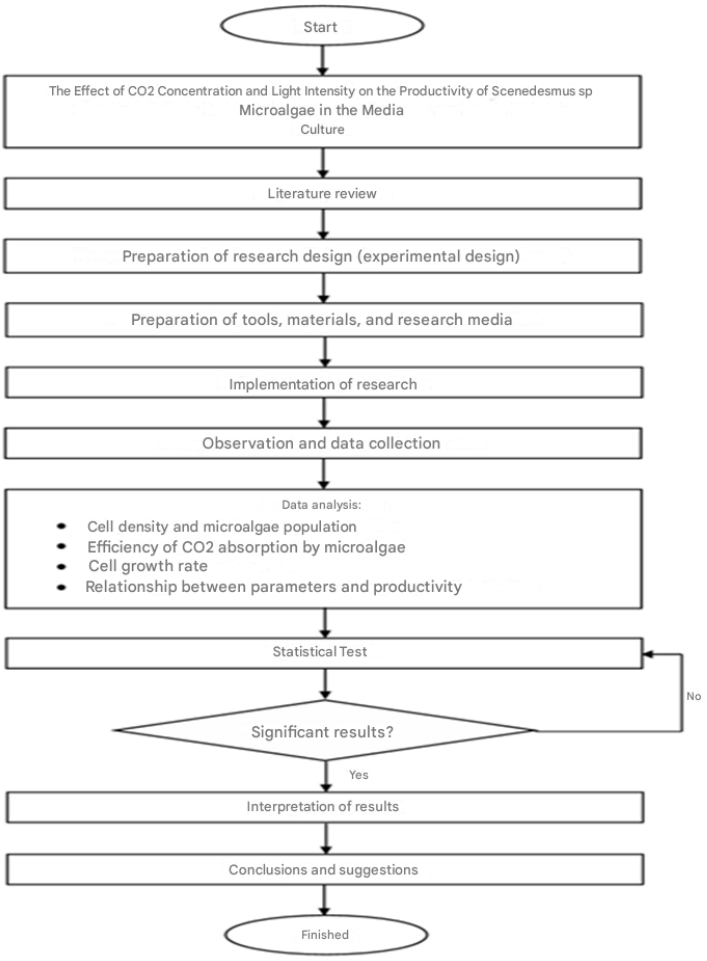


Figure 1. Research Flow Diagram
Source: Researcher Papers, 2025

This research framework is designed to analyze the effect of variations in $CO_2$ concentration and light intensity on the productivity of Scenedesmus sp. microalgae Scenedesmus sp. in the process of carbon dioxide absorption. The research was conducted using a photobioreactor system measuring 15 × 15 × 20 cm with a volume of 4 liters operated under controlled conditions. The photobioreactor is equipped with an aerator, flowmeter, $CO_2$ tube, regulator, pH meter, turbidimeter, microscope, thermometer, and TL Phillips Lifemax TLD 10W/54-765 lamp to ensure an optimal cultivation environment. Microalgae were cultured using Bold's Basal Medium (BBM) media with light intensity variations of 3500, 4000, 4500, and 5000 lux and $CO_2$ concentrations of 2%, 2.5%, 3%, and 3.5%. Cultures are maintained at 25–30°C, pH 7–8.5, and aerated continuously to maintain the homogeneity of the medium. Observations were made every 48 hours by measuring the cell density, turbidity, pH, and dry weight of microalgae. Observational data were analyzed to evaluate the relationship between $CO_2$ concentration and light intensity on biomass productivity and carbon dioxide absorption efficiency.

Data analysis used the Two-Way ANOVA method to determine the significant influence of these two independent variables on microalgae productivity. The normality test was carried out using the Kolmogorov-Smirnov method, while the homogeneity test used the Hartley-Pearson Fmax test to ensure that the data was distributed normally and homogeneously. If the

results of the analysis show a significant difference, a post-hoc test such as the Tukey test is carried out to find out the combination of treatments that gives the best results. The observed research parameters included specific growth rates, $CO_2$ absorption efficiency, and biomass productivity based on dry weight per day. The entire research process is scheduled to take place from February 2024 to October 2025, including the stages of preparing tools and materials, main tests, data collection, analysis, and finalizing the final report. This research is expected to contribute to the development of microalgae-based $CO_2$ bioseseseter technology to support sustainable carbon emission mitigation efforts.

## RESULT AND DISCUSSION

### Effect of CO2 Concentration and Light Intensity on Productivity based on Cell Density

Measurement of cell count using microscope is the most direct indicator of the growth rate of microalgae and biomass accumulation in culture. In this study, cell density was observed for 10 days in 16 variations of Photobioreactor (FBR) treatment with a combination of CO2 (2%–3.5%) and Light Intensity (3500–5000 lux) variables. The calculation of the number of cells (cells/mL) was carried out using a microscope and haemacytometer method.

**Table 1. Results of Analysis of the Effect of CO2 and Light Intensity on Cell Density**

| Concentration (CO2) | Intensity (Lux) | Number of Cells Day To - | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 |
| **2%** | 4000 | 6.90 x $10^5$ | 8.50 x $10^5$ | 6.60 x $10^5$ | 1.08 x $10^6$ | 1.14 x $10^6$ | 1.15 x $10^6$ |
| | 3500 | 6.04 x $10^5$ | 7.58 x $10^5$ | 9.40 x $10^5$ | 1.30 x $10^6$ | 1.36 x $10^6$ | 1.23 x $10^6$ |
| | 4500 | 6.70 x $10^5$ | 8.90 x $10^5$ | 1.17 x $10^6$ | 1.29 x $10^6$ | 1.33 x $10^6$ | 1.29 x $10^6$ |
| | 5000 | 6.54 x $10^5$ | 8.52 x $10^5$ | 1.08 x $10^6$ | 1.32 x $10^6$ | 1.33 x $10^6$ | 1.22 x $10^6$ |
| **2.5%** | 4000 | 7.10 x $10^5$ | 1.11 x $10^6$ | 1.02 x $10^6$ | 1.23 x $10^6$ | 1.35 x $10^6$ | 1.33 x $10^6$ |
| | 3500 | 6.30 x $10^5$ | 7.14 x $10^5$ | 9.30 x $10^5$ | 1.19 x $10^6$ | 1.25 x $10^6$ | 1.27 x $10^6$ |
| | 4500 | 5.98 x $10^5$ | 9.16 x $10^5$ | 1.18 x $10^6$ | 1.35 x $10^6$ | 1.34 x $10^6$ | 1.28 x $10^6$ |
| | 5000 | 6.38 x $10^5$ | 8.70 x $10^5$ | 1.07 x $10^6$ | 1.39 x $10^6$ | 1.26 x $10^6$ | 1.28 x $10^6$ |
| **3 %** | 4000 | 6.54 x $10^5$ | 1.08 x $10^6$ | 9.94 x $10^5$ | 1.37 x $10^6$ | 1.23 x $10^6$ | 1.39 x $10^6$ |
| | 3500 | 6.80 x $10^5$ | 7.54 x $10^5$ | 1.07 x $10^6$ | 1.24 x $10^6$ | 1.28 x $10^6$ | 1.25 x $10^6$ |
| | 4500 | 5.84 x $10^5$ | 9.46 x $10^5$ | 1.09 x $10^6$ | 1.41 x $10^6$ | 1.41 x $10^6$ | 1.33 x $10^6$ |
| | 5000 | 6.42 x $10^5$ | 9.74 x $10^5$ | 1.15 x $10^6$ | 1.31 x $10^6$ | 1.25 x $10^6$ | 1.27 x $10^6$ |
| **3.5%** | 4000 | 6.44 x $10^5$ | 7.64 x $10^5$ | 8.98 x $10^5$ | 1.14 x $10^6$ | 1.18 x $10^6$ | 1.23 x $10^6$ |
| | 3500 | 6.64 x $10^5$ | 8.36 x $10^5$ | 1.10 x $10^6$ | 1.24 x $10^6$ | 1.24 x $10^6$ | 1.22 x $10^6$ |
| | 4500 | 6.50 x $10^5$ | 8.64 x $10^5$ | 1.00 x $10^6$ | 1.32 x $10^6$ | 1.32 x $10^6$ | 1.33 x $10^6$ |
| | 5000 | 6.10 x $10^5$ | 8.60 x $10^5$ | 1.00 x $10^6$ | 1.24 x $10^6$ | 1.25 x $10^6$ | 1.26 x $10^6$ |

Source : Research Results

The data showed a significant increase in cell count in almost all treatments from Day 0 to Day 10. This increase reflects the success of the process of photosynthesis and cell division of microalgae, which culminate on Day 8 or Day 10, depending on the given environmental conditions.

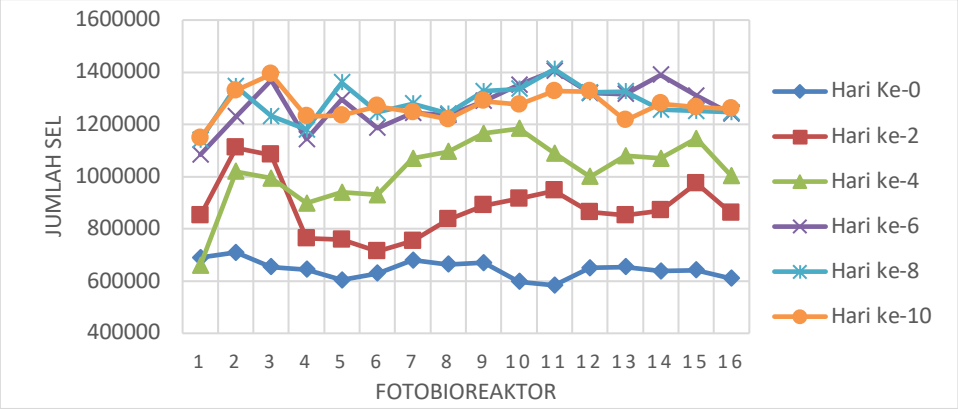


Figure 1. Dynamics of Microalgae Cell Density During Maintenance Period

The graph of the observation results shows the growth pattern in microalgae cultures, namely, the Adaptation Phase (Lag Phase) which occurs on day 0 to day 2. There is a relatively slow increase in the number of cells. In this phase, the cells adjust to the new media and gather energy and enzymes to initiate division. The length of this phase depends on the number and age of inoculants and the culture media used (Prasetyo et al., 2022).

Exponential Phase (Log Phase) which occurs on the 2nd to the 8th day. The majority of treatments show a very rapid spike in cell count, forming a steep growth curve. This is the period of maximum photosynthetic activity and cell division, since the resources (CO2, nutrients, and light) are still optimal and there are no dominant limiting factors. The protein content in cells is generally very high in the final exponential phase (Prasetyo et al., 2022).

Third, the Stationary Phase or Decline which occurs on the 10th day. Some treatments show a slight decline or stagnation, which indicates that the culture is beginning to enter a stationary or decline phase. This may be due to nutritional limitations, accumulation of toxic metabolite products, or limited light penetration due to high cell density (self-shading) (Hadi et al., 2023; Almutairi et al., 2024).

Light intensity is one of the factors in photosynthesis, which is the main mechanism of microalgae growth (Prasetyo et al., 2022; Almutairi et al., 2024). The graph shows that in general, cell density tends to increase as light intensity increases from 3500 Lux to 4500 Lux, after which there is a decrease at 5000 Lux.The intensity of 4500 Lux, especially at 3% (FBR 11), provides the highest cell growth yield.

The light intensity range of 2500 Lux - 5000 Lux is the optimal condition for various species of tropical microalgae, as it is able to provide sufficient energy without causing light inhibition (photoinhibition) (Almutairi et al., 2024; Zhan et al., 2023). Optimal light energy triggers a faster rate of photosynthesis, accelerating cell division and reproduction.

The cell density at 5000 Lux (FBR 13−16) tends to be lower than at 4500 Lux. This is thought to be due to the occurrence of photoinhibition, where excessive light energy damages the photosynthesis apparatus causing a decrease in the rate of photosynthesis and ultimately

inhibiting cell growth (Zhan et al., 2023). In addition, too high light intensity can increase the culture temperature excessively, which is also a growth limiting factor (Hadi et al., 2023).

Concentrations of 3% (specifically at 4500 Lux, FBR 11) result in the highest cell density. An increase in concentration from 2% to 3% generally gives a positive response because the abundant supply of carbon supports a high rate of photosynthesis. Some studies have also stated that the optimal CO2 concentration for most microalgae is in the range of 2%−10% (Zhang et al., 2025).

At the highest concentration (3.5%), there was a slight decrease in cell density compared to 3% at light intensity of 4500 Lux and 4000 Lux. High CO2 concentrations can cause a decrease in the pH of the culture medium, making it less optimal for the metabolic process of microalgae cells, and can even inhibit growth if it is outside the tolerance limits of the species (Politaeva et al., 2023). A drop in pH below the optimum limit can interfere with the activity of enzymes required in the cell's metabolic cycle.

The interaction between light intensity and CO2 concentration is crucial because they are both the main substrates in photosynthesis. The combination of 3% CO2 and 4500 Lux Light Intensity (FBR 11) proved to be the best conditions for cell growth, suggesting that at a concentration of 3%, microalgae are able to utilize light energy from 4500 Lux most efficiently without experiencing saturation or light inhibition (Politaeva et al., 2023; Zhan et al., 2023).

In general, a rapid exponential growth phase (log phase) is seen until Day 6 or Day 8, after which some treatments begin to enter the stationary phase or even show a downward trend (death phase) on Day 10 (e.g. FBR 5). The stationary phase occurs when the rate of cell growth is balanced with the rate of cell death, which is often caused by nutrient limitations or deteriorating environmental conditions (Almutairi et al., 2024).

Overall, these results confirm that there is an optimal concentration and intensity of light to achieve the highest cell density of microalgae, and that exceeding this point can trigger inhibitory phenomena that are detrimental to cells (Zhan et al., 2023). This study successfully identified that the conditions of 3% CO2 and 4500 Lux are the most effective treatments in increasing the density of microalgae cells.

**Effect of CO2 Concentration and Light Intensity on Productivity based on Turbidity (Turbidity)**

Turbidity is one of the parameters that reflects the level of turbidity of the culture, which is mostly caused by the density of growing microalgae cells. In this study, Turbidity (NTU) measurements were carried out for 16 days of observation with various combinations of CO2 Injection (2%, 2.5%, 3%, 3.5%) and Light Intensity (3500, 4000, 4500, 5000 lux) variables in the Photobioreactor (FBR). Data from turbidity observations showed an increase in NTU values as the cultivation days increased, which generally indicated an increase in cell density or biomass of microalgae in the medium. This relationship is in line with the principle that the more microalgae cells, the higher the level of turbidity of water due to the scattering of light caused by these cell particles (Prasetiya et al., 2021). The results of turbidity observation (NTU) in 16 Photobioreactor units (FBR) for 10 days are presented in Table 2.

**Table 2. Results of Analysis of the Effect of CO2 and Light Intensity on Turbidity (Turbidity)**

| CO2 concentration | Intensity (Lux) | Turbidity Day To - (NTU) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 |
| **2%** | 4000 | 13.8 | 17 | 13.2 | 21.7 | 22.8 | 23 |
| | 3500 | 12 | 15.2 | 18.8 | 25.9 | 27.2 | 24.7 |
| | 4500 | 13.4 | 17.8 | 23.3 | 25.8 | 26.5 | 25.9 |
| | 5000 | 13.1 | 17 | 21.6 | 26.3 | 26.5 | 24.3 |
| **2.5%** | 4000 | 14.2 | 22.2 | 20.4 | 24.6 | 27 | 26.6 |
| | 3500 | 12.6 | 14.3 | 18.6 | 23.7 | 24.9 | 25.4 |
| | 4500 | 12 | 18.3 | 23.7 | 27 | 26.7 | 25.5 |
| | 5000 | 12.8 | 17.4 | 21.4 | 27.8 | 25.1 | 25.6 |
| **3 %** | 4000 | 13.1 | 21.7 | 19.9 | 27.4 | 24.7 | 28 |
| | 3500 | 13.6 | 15.1 | 21.4 | 24.9 | 25.6 | 24.9 |
| | 4500 | 11.7 | 18.9 | 21.8 | 28.1 | 28.3 | 26.7 |
| | 5000 | 12.8 | 19.5 | 22.9 | 26.2 | 24.9 | 25.3 |
| **3.5%** | 4000 | 12.9 | 15.2 | 18 | 22.9 | 23.6 | 24.6 |
| | 3500 | 13.3 | 16.7 | 21.9 | 24.7 | 24.7 | 24.4 |
| | 4500 | 13 | 17.3 | 20 | 26.4 | 26.4 | 26.5 |
| | 5000 | 12.2 | 17.2 | 20 | 24.8 | 24.9 | 25.3 |

Source : Observation Results

The results of the observation showed that the turbidity value in all FBRs increased consistently from Day 0 until it reached its peak (stationary phase/peak of growth) around Day 8 to Day 10. The increase in NTU values is positively correlated with the increase in cell density observed in this study. This relationship occurs because microalgae cells, as suspended particles, reflect and absorb light from turbidimeters, the more cells, the higher their turbidity values are (Yusuf et al., 2021).

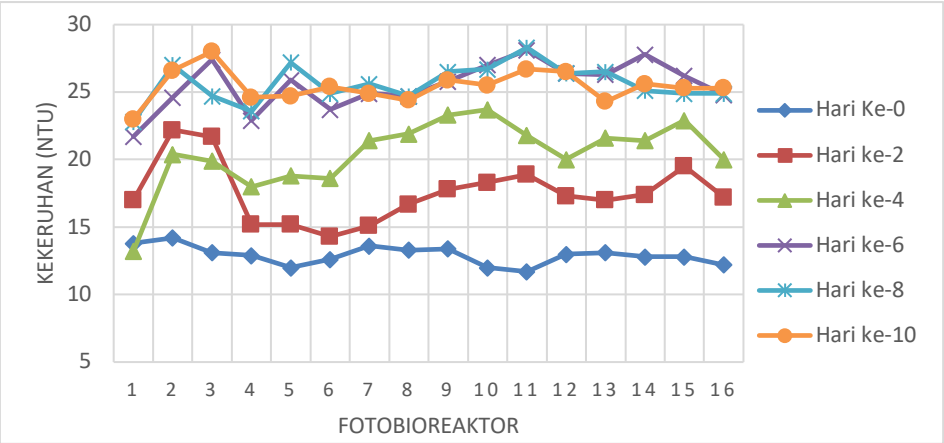


Figure 2. Turbidity Dynamics of Culture Media During the Observation Period

The highest turbidity value was recorded at FBR 11 (CO2 3%, 4500 Lux) with 28.3 NTU on Day 8, which was simultaneously also the highest cell density treatment (1.41 x 106 cells/mL). The intensity variations of 4000 Lux and 4500 Lux generally indicate the highest turbidity achievement, especially when combined with CO2 of 2.5% and 3% (FBR 2, FBR 3, FBR 10, FBR 11). Light intensity in this range (4000–4500 Lux) is often considered optimal for phototrophic microalgae species because it provides sufficient energy for the maximum

growth rate. Light at this range induces photosynthetic light reactions without causing significant stress (Setiawan et al., 2023). Research by Arsyad et al. (2020) also supports that medium light intensity provides better cell density compared to extreme conditions.

The turbidity at 3500 Lux (FBR 5–8) and 5000 Lux (FBR 13–16) treatments tends to be lower. At 3500 Lux, the limitation of light energy may be a light limiting factor, so that the rate of photosynthesis and cell growth is slower (Syarif et al., 2022). In contrast, at 5000 Lux, despite the abundance of energy, the decrease in turbidity and density of these cells is believed to be due to photoinhibition. Excessive light intensity can damage chlorophyll and photosynthetic reaction centers, inhibit cell division, and lead to lower biomass accumulation (Mubarok et al., 2023).

Treatments with a concentration of 3% CO2 consistently produced the highest turbidity values at most light intensities (FBR 3, FBR 7, FBR 11, FBR 15), indicating greater biomass production. The injected additional CO2 helps maintain the availability of dissolved carbon, accelerating carbon fixation through the enzyme RuBisCO, which has a great effect on cell growth (Yani et al., 2023).

A concentration of 3.5% results in a turbidity that is slightly lower than 3%. Very high CO2 concentrations can cause a drastic drop in the pH of the medium, creating an acidic environment and potentially inhibiting the metabolic activity of microalgae cells, even leading to cell death (Muktamar et al., 2020). As a result, the growth rate slows down and the accumulation of biomass (turbidity) is inhibited.

The best results were achieved at FBR 11 (3% CO2, 4500 Lux). This condition indicates an optimal synergy between an adequate supply of light energy and the high availability of carbon substrates. The achievement of maximum turbidity occurs between Day 8 and Day 10, which indicates that microalgae in this period have reached the stationary phase. In this phase, limiting factors such as nutrient depletion (e.g. nitrogen or phosphorus) or the accumulation of residual metabolites begin to compensate for the growth rate, even though light and CO2 conditions are optimal (Ningsih et al., 2022).

**Effect of CO2 Concentration and Light Intensity on Productivity by Dry Weight**

Dry weight measurement using gravimetric methods is the standard and most accurate way to determine the accumulation of microalgae biomass (Sari, Widiastuti, & Kusumaningrum, 2021). This dry weight reflects the total mass of harvested cells, which is a direct indicator of culture productivity. In this study, biomass measurements were carried out for 10 days on 16 variations of FBR treatment, The results of dry weight measurements from 16 units of Photobioreactor (FBR) for 10 days are presented in Table 3.

**Table 3. Results of Analysis of the Effect of CO2 Concentration and Light Intensity on Dry Weight**

| CO2 concentration | Intensity (LUX) | Dry Weight Day (gr/L) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 |
| **2%** | 4000 | 0.1550 | 0.4500 | 0.3500 | 0.5750 | 0.6050 | 0.6100 |
| | 3500 | 0.1350 | 0.4050 | 0.5000 | 0.6900 | 0.7250 | 0.6550 |
| | 4500 | 0.1500 | 0.4750 | 0.6200 | 0.6850 | 0.7050 | 0.6850 |
| | 5000 | 0.1450 | 0.4500 | 0.5750 | 0.7000 | 0.7050 | 0.6450 |
| **2.5%** | 4000 | 0.1600 | 0.5900 | 0.5400 | 0.6550 | 0.7150 | 0.7100 |
| | 3500 | 0.1410 | 0.3800 | 0.4950 | 0.6300 | 0.6600 | 0.6750 |
| | 4500 | 0.1335 | 0.4850 | 0.6300 | 0.7200 | 0.7100 | 0.6800 |
| | 5000 | 0.1425 | 0.4600 | 0.5700 | 0.7400 | 0.6650 | 0.6800 |
| **3 %** | 4000 | 0.1450 | 0.5750 | 0.5300 | 0.7300 | 0.6550 | 0.7400 |
| | 3500 | 0.1500 | 0.4000 | 0.5700 | 0.6600 | 0.6800 | 0.6600 |
| | 4500 | 0.1305 | 0.5000 | 0.5800 | 0.7500 | 0.7500 | 0.7050 |
| | 5000 | 0.1435 | 0.5200 | 0.6100 | 0.6950 | 0.6650 | 0.6700 |
| **3.5%** | 4000 | 0.1440 | 0.4050 | 0.4800 | 0.6100 | 0.6250 | 0.6550 |
| | 3500 | 0.1485 | 0.4450 | 0.5850 | 0.6550 | 0.6600 | 0.6500 |
| | 4500 | 0.1450 | 0.4600 | 0.5350 | 0.7000 | 0.7000 | 0.7050 |
| | 5000 | 0.1350 | 0.4550 | 0.5350 | 0.6600 | 0.6600 | 0.6650 |

Source : Research Results

The results of dry weight measurements showed that the highest biomass was achieved at the FBR 7 (3.0% CO2, 3500 Lux) and FBR 3 (3.0% CO2, 4000 Lux) treatments, reaching and (Day 8 and Day 10) respectively. These results are in slight contrast to the cell density data (Graph 4.1), where FBR 11 (3%, 4500 Lux) produces the largest number of cells. This difference shows that although it produces the most cells, it produces cells with a heavier size or internal biomass content per cell (Yusuf et al., 2021).

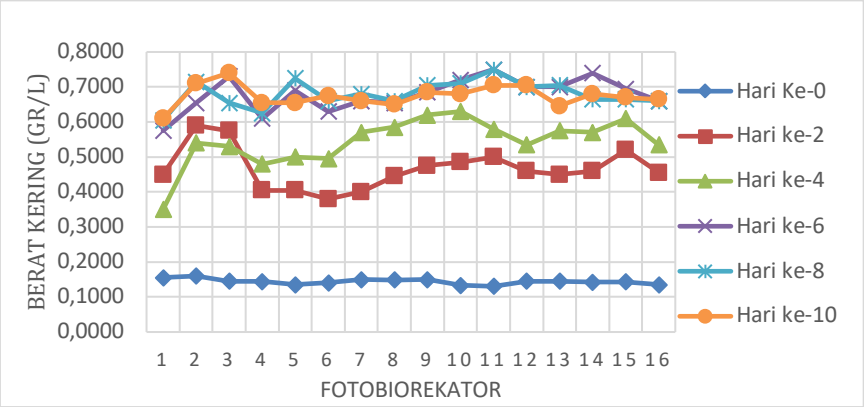


Figure 3. Dry weight dynamics of microalgae biomass during the observation period

The increase in biomass is directly proportional to the efficiency of fixation into organic carbon (Lee et al., 2022). The concentration of 3% CO2 predominantly results in the highest dry weight in almost all variations in light intensity (FBR 3, FBR 7, FBR 11, FBR 15). It confirms that a CO2 supply of 3% is an optimal point that provides an abundant carbon substrate for photosynthesis without causing significant pH stress. Studies by Yani et al. (2023) show that increased CO2 concentrations can increase carbon fixation, which in turn increases the accumulation of proteins, carbohydrates, and lipids within cells, all of which contribute to increased dry weight.

Dry weights at 3.5% CO2 (FBR 4, FBR 8, FBR 12, FBR 16) tend to be lower than 3%. The slightly higher concentration of CO2, this has likely led to a drop in the pH of the medium to a level that begins to inhibit key metabolic activity in the cell. This is in line with research that reports that although CO2 is necessary, excessive levels can lead to a decrease in biomass due to toxicity effects or disturbances of pH balance (Muktamar et al., 2020).

The highest dry weight was consistently achieved in the intensity groups of 4500 lux and 4000 lux. FBR 3 (3% CO2, 4000 lux) reached a high of 0.7400 g/L on Day 10, while FBR 11 (3% CO2, 4500 lux) reached 0.7500 g/L on Day 8. This shows that the intensity range of 4000–4500 lux is at a balanced point between sufficient energy availability and low risk of photoinhibition, thus maximizing the efficiency of photosynthesis and biomass accumulation (Maltsev et al., 2021; Sudhakar et al., 2011).

Although the intensity of 5000 lux (FBR 13–16) provides very high energy, its peak biomass value (approximately 0.6450–0.6800 g/L) is relatively lower than that of 4000 and 4500 lux. This phenomenon supports the hypothesis of photoinhibition that has been mentioned in the discussion of turbidity and cell count. Too strong light, especially in nutrient-restricted conditions, can divert energy to cell protection mechanisms, not to biomass growth (Gao et al., 2023; Barnes & Mann, 1999).

The intensity of 3500 lux (FBR 5–8) also indicates a lower biomass (about 0.6550–0.6750 g/L). Inadequate light energy limits the overall rate of photosynthesis, so that even as cells grow, the accumulation of dry mass becomes limited (Tasnim et al., 2023; Lavens & Sorgeloos, 1996).

**Specific Growth Rate Analysis (μ)**

Cell density measurement (number of cells/mL) is a direct method for monitoring the increase in biomass and determining the Specific Growth Rate (μ) of microalgae. The μ value is calculated during the exponential growth phase (Day 0 to Day 8, assumed as the peak point) using cell density data from Table 4 as follows:

**Table 4. Results of Specific Growth Rate Analysis**

| $CO_2$ Concentration | Intensity (Lux) | Number of Cells Day 0 | Number of Cells Day 8 | (μ)/ Hari |
|---|---|---|---|---|
| **2%** | 4000 | $6.90 \times 10^5$ | $1.14 \times 10^6$ | 0.0634 |
| | 3500 | $6.04 \times 10^5$ | $1.36 \times 10^6$ | 0.1011 |
| | 4500 | $6.70 \times 10^5$ | $1.33 \times 10^6$ | 0.0858 |
| | 5000 | $6.54 \times 10^5$ | $1.33 \times 10^6$ | 0.0881 |
| **2.5%** | 4000 | $7.10 \times 10^5$ | $1.35 \times 10^6$ | 0.0798 |
| | 3500 | $6.30 \times 10^5$ | $1.25 \times 10^6$ | 0.0853 |
| | 4500 | $5.98 \times 10^5$ | $1.34 \times 10^6$ | 0.1005 |
| | 5000 | $6.38 \times 10^5$ | $1.26 \times 10^6$ | 0.0844 |
| **3 %** | 4000 | $6.54 \times 10^5$ | $1.23 \times 10^6$ | 0.0792 |
| | 3500 | $6.80 \times 10^5$ | $1.28 \times 10^6$ | 0.0789 |
| | 4500 | $5.84 \times 10^5$ | $1.41 \times 10^6$ | 0.1105 |
| | 5000 | $6.42 \times 10^5$ | $1.25 \times 10^6$ | 0.0829 |
| **3.5%** | 4000 | $6.44 \times 10^5$ | $1.18 \times 10^6$ | 0.0759 |
| | 3500 | $6.64 \times 10^5$ | $1.24 \times 10^6$ | 0.0779 |
| | 4500 | $6.50 \times 10^5$ | $1.32 \times 10^6$ | 0.0877 |
| | 5000 | $6.10 \times 10^5$ | $1.25 \times 10^6$ | 0.0898 |

Source : Research Results

Specific Growth Rate (μ) is a parameter in bioprocesses because it reflects the speed at which cells perform division and biomass synthesis (Lee et al., 2022). The results of the calculation showed that the highest value was achieved at the FBR 11 treatment (3% CO2, 4500 Lux), which was 0.1105 per day, which also correlated with the maximum cell density on Day 8.

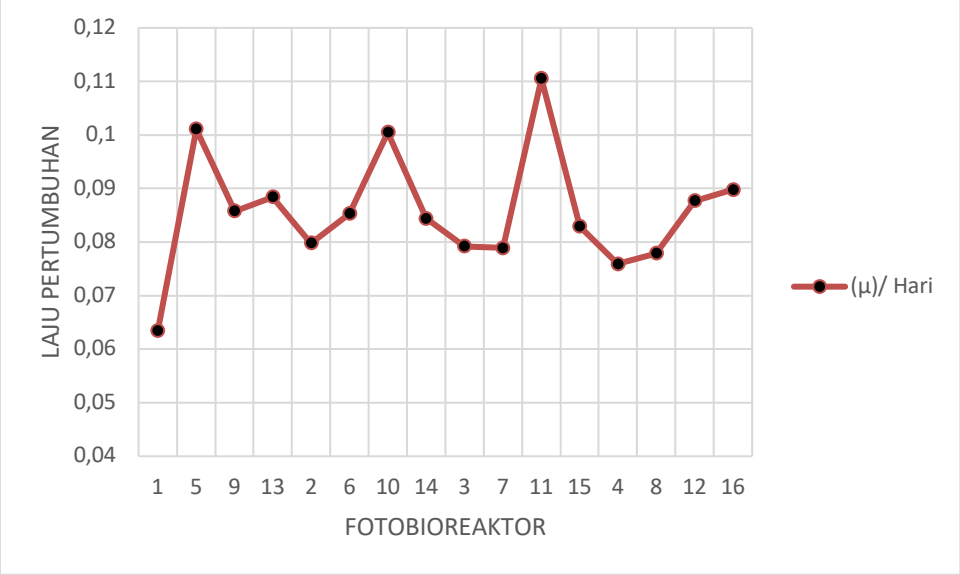


Figure 4. Cell Growth Rate Dynamics (μ)

Combine and create optimal synergistic conditions for rapid cell division. The intensity is estimated to be close to the light saturation point of the microalgae cell, providing enough energy to carry out the photosynthetic bright reaction at the highest rate. Simultaneously, the 3% CO2 supply ensures that the carbon fixation process in the Calvin cycle is not hampered by a shortage of substrates. This ideal balance maximizes the rate of cell division, resulting in the highest μ and peak cell density.

Although CO2 is 3% optimal, an increase in concentration to 3.5% (FBR 4, 8, 12, 16) actually results in lower μ values. This is seen in FBR 4 (CO2 3.5%, 4000 Lux) with only μ 0.0759 per day. Excessive CO2 concentrations are known to drastically lower the pH of the culture medium, creating stressful acidic conditions for the cell, which directly inhibits the activity of essential enzymes and slows down the rate of cell division, thereby lowering the μ.

The growth rate at 5000 Lux (FBR 13, 14, 15, 16) also did not reach the maximum value, indicating the presence of light inhibition (photoinhibition). Too high a light energy can damage the photosystem, divert the cell's energy from growth to damage repair, and effectively reduce the clean energy available for cell division, resulting in a less optimal growth rate.

Although FBR 11 shows the fastest growth rate (cell division), the Dry Weight data shows that the highest final biomass (0.7500 g/L and 0.7400 g/L) was achieved by FBR 7 (3% CO2, 3500 Lux) and FBR 3 (3% CO2, 4000 Lux). This difference underscores the difference between the rate of cell division and the accumulation of biomass per cell. In FBR 11 a high growth rate indicates that the cells divide very quickly, but the newly formed cells may be smaller or lighter (low dry matter content per cell). In FBR 3 and FBR 7 the cell growth rate is slightly lower, but the cells tend to allocate energy to a larger accumulation of macromolecules

(such as lipids or carbohydrates) before dividing, resulting in heavier cells and a higher total dry weight of the culture.

Therefore, for applications targeting internal metabolite production or total biomass (dry weight), FBR 3 or FBR 7 is superior. However, if the goal is to harvest cells at high frequencies based on cell density, FBR 11 is the most optimal treatment.

**Biomass Productivity Analysis (g/L/day)**

Biomass productivity (PB) is a measure to determine the industrial-scale potential of microalgae cultivation, as it reflects the rate of biomass accumulation per day. The data to be used are dry weight (g/L) data from Day 0 and the highest Dry Weight (Bmax). The results of biomass productivity calculations are presented in table 5 as follows:

**Table 5. Biomass Productivity Analysis Results**

| CO2 concentration | Intensity (Lux) | $B_0$ (g/L) | $B_{max}$ (g/L) | Day t ()$t_{max}$ | $PB_{max}$ |
|---|---|---|---|---|---|
| **2%** | 4000 | 0.1550 | 0.6100 | 10 | 0.0455 |
| | 3500 | 0.1350 | 0.7250 | 8 | 0.0738 |
| | 4500 | 0.1500 | 0.7050 | 8 | 0.0694 |
| | 5000 | 0.1450 | 0.7000 | 6 | 0.0925 |
| **2.5%** | 4000 | 0.1600 | 0.7150 | 8 | 0.0694 |
| | 3500 | 0.1410 | 0.6750 | 10 | 0.0534 |
| | 4500 | 0.1335 | 0.7200 | 6 | 0.0978 |
| | 5000 | 0.1425 | 0.7400 | 6 | 0.0996 |
| **3%** | 4000 | 0.1450 | 0.7400 | 10 | 0.0595 |
| | 3500 | 0.1500 | 0.6800 | 8 | 0.0663 |
| | 4500 | 0.1305 | 0.7500 | 8 | 0.0774 |
| | 5000 | 0.1435 | 0.6950 | 6 | 0.0920 |
| **3.5%** | 4000 | 0.1440 | 0.6550 | 10 | 0.0511 |
| | 3500 | 0.1485 | 0.6600 | 8 | 0.0639 |
| | 4500 | 0.1450 | 0.7000 | 8 | 0.0694 |
| | 5000 | 0.1350 | 0.6600 | 10 | 0.0525 |

Source : Research Results

Based on calculations from Day 0 to the achievement of maximum dry weight (Bmax), biomass productivity values vary widely, ranging from 0.0455 to 0.0996 g/L/day. The highest biomass productivity was found in FBR 14 (2.5% CO2, 5000 lux) weighing 0.0996 g/L/day obtained on Day 6, and FBR 10 (2.5% CO2, 4500 lux) weighing 0.0978 g/L/day was obtained on Day 6. These results show that the combination of CO2 of 2.5% with high light intensity (4500–5000 lux) results in the fastest efficiency of converting carbon and energy into biomass. High productivity indicates that FBR successfully keeps microalgae cells in an exponential growth phase for a longer period of time or at a faster rate (Prasetyo et al., 2022; Prasad et al., 2024)

The data showed a marked increase in productivity in the highest light intensity groups, especially at 4500 lux and 5000 lux, where most of the highest PB was achieved in a short period of time (Day 6). Higher light intensity (such as 4500 lux and 5000 lux) provides more photon energy that can be absorbed by chlorophyll, thereby increasing the rate of photosynthesis (Prasetyo et al., 2022). This is especially noticeable in the FBR 14 (5000 lux) and FBR 10 (4500 lux), which scored a productivity of almost 0.1 g/L/day.

Although 5000 lux (FBR 14) results in maximum productivity, this result is achieved at a concentration of 2.5% CO2. This may indicate that at 5000 lux, the cell needs slightly lower CO2 than 3% to prevent osmotic stress or photoinhibition exacerbated by very high CO2 concentrations. Some studies (such as in Chlorella sp.) suggest that increased light can increase the CO2 tolerance threshold, but the optimal limit must still be maintained to avoid saturation or damage (Prasad et al., 2024).

The CO2 concentration at 2.5% proved to be the best CO2 variable for maximizing productivity in this study, especially when combined with high light intensity. FBR 10 and FBR 14, both of which use 2.5% CO2, dominate the productivity rankings. This figure of 2.5% represents the best point capable of providing sufficient carbon for the high light-driven rate of photosynthesis, without causing a drastic drop in pH (Politaeva et al., 2023). CO2 concentrations in the range of 2%−10% are generally reported to be optimal for productivity (Politaeva et al., 2023).

Treatment of 3.5% CO2 shows significantly lower productivity. For example, FBR 16 (3.5% CO2, 5000 lux) reaches only 0.0525 g/L/day, well below FBR 14 (2.5% CO2, 5000 lux). This dramatic decline corroborates the conclusion that the CO2 concentration at 3.5% has exceeded the optimal tolerance limit of the microalgae species used, causing cellular stress and reducing cultivation efficiency (Prasad et al., 2024).

Biomass productivity analysis confirms the existence of a strong synergistic interaction between CO2 and light intensity. To achieve the highest productivity (0.0996 g/L/day), the culture requires a high energy supply (5000 lux) offset by an optimal supply of carbon (2.5% CO2). Although the highest Bmax is achieved at FBR 11 (0.7500 g/L), the highest PBmax is actually achieved by FBR 14. This shows that FBR 14 has superior daily efficiency in a short period of time (Day 0− Day 6), while FBR 11 shows better cultivation ability in maintaining growth to achieve the highest total biomass in a longer cultivation period (Day 0− Day 8). Therefore, the selection of optimal conditions depends largely on the harvest objectives: FBR 14 for fast harvesting, and FBR 11 for maximum total biomass.

**CO2 Absorption Efficiency Analysis**

The results of CO2 (ppm) observations after contact with culture (raw data) and CO2 absorption efficiency (in percent) are presented in Table 4.6 and Figure 6 as follows:

**Table 6. CO2 Measurement Results and CO2 Absorption Efficiency Analysis (ppm and %)**

| CO2 concentration | Intensity (Lux) | Lowest Waste (ppm) | Maximum Efficiency (%) | Achievement Day |
|---|---|---|---|---|
| **2%** | 4000 | 657 | 96.72 | 6 |
| | 3500 | 651 | 96.75 | 6 |
| | 4500 | 618 | 96.91 | 4 |
| | 5000 | 723 | 96.39 | 4 |
| **2.5%** | 4000 | 629 | 97.48 | 6 |
| | 3500 | 739 | 97.04 | 6 |
| | 4500 | 712 | 97.15 | 6 |
| | 5000 | 651 | 97.4 | 4 |
| **3%** | 4000 | 684 | 97.72 | 8 |
| | 3500 | 734 | 97.55 | 6 |
| | 4500 | 756 | 97.48 | 4 |
| | 5000 | 662 | 97.79 | 8 |
| **3.5%** | 4000 | 679 | 98.06 | 4 |
| | 3500 | 684 | 98.05 | 6 |
| | 4500 | 646 | 98.15 | 6 |
| | 5000 | 728 | 97.92 | 6 |

Source : Research Results

The efficiency of CO2 absorption is a critical parameter that indicates how well the microalgae are able to fix the carbon gas that is being delivered. The data show that overall, microalgae are highly efficient at fixing CO2, with an average efficiency of over 92% in almost all treatments. The highest efficiency value was recorded on FBR 12 (3.5%, 4500 Lux), reaching 98.15% on Day 6.

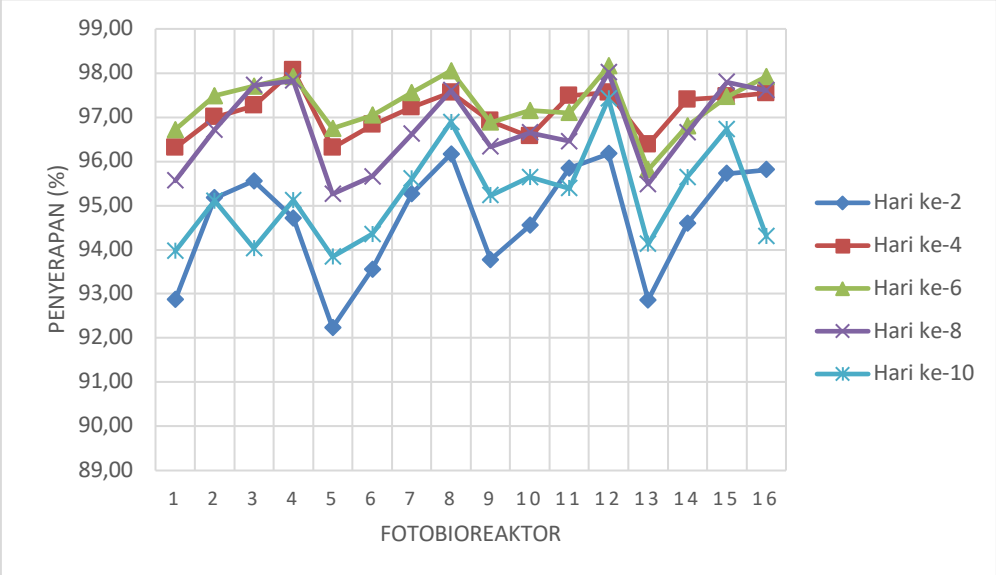


**Figure 5. Dynamics of Microalgae CO2 Absorption Efficiency During the Observation Period**

Improved CO2 absorption efficiency was seen on Day 4 to Day 8 in most FBRs. This period coincides with the exponential growth phase of cells, in which the cell population grows exponentially. The more cells there are, the larger the total surface area of the cell capable of absorbing and fixing CO2, thus increasing the efficiency of the system.

Disparity between Peak Efficiency and Peak Density: Although FBR 11 (CO2 3%, 4500 Lux) achieves the highest cell density, FBR 12 (CO2 3.5%, 4500 Lux) exhibits the highest CO2 absorption efficiency. This disparity suggests that at very high CO2 concentrations (3.5%), existing microalgae cells, even if they are slightly lower in number, may have activated a very aggressive Carbon Concentrating Mechanism (CCM) to take advantage of the abundant availability of CO2. This mechanism ensures the cell efficiently absorbs the available gases, which is reflected in the very high absorption percentage.

The 3.5% treatment resulted in consistently highest efficiency (FBR 4, 8, 12, 16). This is because the abundant CO2 encourages more intensive fixation reactions. However, as observed from previous cell density data, these very high ones also begin to exert negative effects (pH stress) that inhibit cell division and total biomass. This means that this high efficiency at 3.5% CO2 may come at the cost of a decrease in total cell productivity.

The graph shows a sharp drop in efficiency on Day 10 for almost all treatments. This decline occurs because the culture has entered a stationary phase where the rate of cell growth slows down due to nutrient depletion or metabolite buildup. Cells whose growth slows down will have decreased photosynthetic activity, and even if CO2 is injected, the culture's collective ability to absorb the gas is reduced. This can be seen from the increase in residual CO2 (ppm) on Day 10 (Graph 4.6) which shows more gas escaping absorption.

The highest efficiency at 4500 Lux (FBR 12) confirms that this intensity is very effective in supporting the overall photosynthesis process. Adequate energy (4500 Lux) ensures that ATP and NADPH (light reaction products) are available in sufficient quantities to run the Calvin cycle and fix CO2 efficiently.

Efficiency at 5000 Lux tends to be more volatile (e.g. FBR 13, 14, 15, 16). This very high intensity risks causing photosystem damage (photoinhibition). This damage disrupts the energy supply for CO2 fixation, so that although CO2 is abundant, the cell has difficulty processing it, resulting in an unstable absorption efficiency and tends to be lower than at 4000 or 4500 Lux.

In conclusion, the 3.5% and 4500 Lux (FBR 12) treatments showed the highest ability to maximize the percentage of CO2 absorption per volume of gas injected. However, in order to optimize biological output (biomass), absorption efficiency must be balanced with the rate of cell growth, of which 3% CO2 at an intensity of 4500 Lux is shown to be superior in producing the largest number of cells.

**Analysis of the Effect of CO2 Concentration and Light Intensity on Microalgae Productivity Using Two Way Anova**

Univariate Two Way Anova is an analytical tool to assess the level of significance of the influence of 2 qualitative independent variables on 1 bound variable on a quantitative scale (interval scale or ratio, i.e. measurement). The following are the results of Two Way Anova's analysis of concentration and intensity on microalgae productivity:

**Table 7. Statistical Analysis Results**

**Tests of Between-Subjects Effects**

Dependent Variable: Produktivitas_Sel

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 2.287E+11[a] | 7 | 3.267E+10 | 28.088 | .000 |
| Intercept | 7.142E+12 | 1 | 7.142E+12 | 6139.915 | .000 |
| KonsentrasiCO2 | 1.972E+11 | 3 | 6.572E+10 | 56.498 | .000 |
| Intensitas | 9506250000 | 1 | 9506250000 | 8.172 | .021 |
| KonsentrasiCO2 * Intensitas | 2.204E+10 | 3 | 7348250000 | 6.317 | .017 |
| Error | 9306000000 | 8 | 1163250000 | | |
| Total | 7.380E+12 | 16 | | | |
| Corrected Total | 2.380E+11 | 15 | | | |

a. R Squared = .961 (Adjusted R Squared = .927)

| **Source** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** |
|---|---|---|---|---|---|
| **Corrected Model** | 2.287E+11[a] | 7 | 3.267E+10$ | 28.088 | .000 |
| **Intercept (Titik Potong)** | 7.142E+12 | 1 | 7.142E+12$ | 6139.915 | .000 |
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| **Error** | 9306000000 | 8 | 1163250000 | | |
| **Total** | 7.380E+12 | 16 | | | |
| **Corrected Total** | 2.380E+11 | 15 | | | |

**R Squared = .961 (Adjusted R Squared = .927)**

Based on the results of the analysis of Two Way Anova with SPSS, it can be concluded that;

a) Corrected Model: The effect of all independent variables (CO2 concentration (%) and Light intensity (Lux)) together on the dependent variables Productivity (gr/day). If significant (sig.) < 0.05 (alpha) = significant. The table above shows the corrected Model Sig. 0.000 < 0.05 means a valid model and significant data.
b) Intercept: The value of the dependent variable changes without the need to be influenced by the existence of an independent variable, meaning that without the influence of an independent variable, the dependent variable can change its value. If significant (sig.) < 0.05 (alpha) = significant. The values shown from the table Sig. 0.000 < 0.05 mean Significant Intercept.
c) CO2 Concentration (%): The effect of CO2 Concentration (%) on Productivity (gr/day) in the model. If significant (sig.) < 0.05 (alpha) = significant. The table above shows that the value of Sig. 0.000 < 0.05 means that CO2 Concentration (%) has a significant effect on Productivity (gr/day).
d) Light intensity (Lux): The effect of light intensity (Lux) on Productivity (gr/day) in the model. If significant (sig.) < 0.05 (alpha) = significant. The table above shows the value of Sig. 0.021 < 0.05 means that light intensity (Lux) has a significant effect on Productivity (gr/day).

e) R Squared: The multiple determination value of all variables independent of the dependent. The table above shows the value of R Squared 0.961, which means that the size of the independent variable can affect the dependent variable by 96.1%.

So it can be concluded that there is an influence between concentration and light intensity on the productivity of Scenedesmus sp. microalgae Scenedesmus sp. This significant influence is in line with the biology literature on microalgae which states that microalgae are an essential source of carbon for the processes of photosynthesis and carbon fixation.

This study has shown that the injection of high concentrations into the culture medium can drastically increase the rate of growth and accumulation of biomass compared to the use of ordinary atmospheric air (Danish & Amini, 2022). In particular, microalgae such as Scenedesmus are known to have a high ability to utilize industrial exhaust gases, thereby increasing their productivity (Asriani et al., 2021).

Significant light intensity is also consistent with the principle that microalgae are phototrophic organisms that need light as an energy source for photosynthesis (Tewari et al., 2021). Increased light intensity, until it reaches the optimal point, is generally positively correlated with the rate of photosynthesis and cell growth, although excessive intensity can lead to photo-inhibition (Al-Haddad et al., 2022).

## CONCLUSION

Based on the results of the study on the effect of $CO_2$ injection variation (2%–3.5%) and light intensity (3500–5000 lux) on the cultivation of Scenedesmus sp. microalgae. Over 10 days, it can be concluded that variations in $CO_2$ concentration and light intensity have a significant effect on specific growth rate (μ), cell density, and biomass productivity of microalgae. The results of the statistical test showed that the two independent variables, both individually and interactively, had a significant influence on the productivity of microalgae (Sig. < 0.05). The highest cell density of 1.41 x $10^6$ cells/mL was obtained at FBR 11 treatment (3% $CO_2$ and 4500 lux), with the highest specific growth rate of 0.1105/day and peak biomass accumulation of 0.7500 g/L on day 8. Meanwhile, the fastest daily biomass productivity of 0.0996 g/L/day was achieved at the FBR 14 treatment (2.5% $CO_2$ and 5000 lux). Overall, the optimal combination of $CO_2$ concentration and light intensity that is most effective for increasing biomass growth and productivity is at 3% $CO_2$ and 4500 lux, as these conditions provide a maximum supply of photosynthetic energy without giving rise to photoinhibition. Higher $CO_2$ concentrations, such as 3.5%, actually reduce productivity due to disturbance of the pH balance of the medium, while the light intensity of 4500 lux proves to be an ideal point for maintaining the efficiency of photosynthesis and the growth of microalgae during the exponential phase.

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