

The Effect of Co2 Injection Duration on The Growth and Quality of Chaetoceros Muelleri at Intermediate Scale

Lalu Yusril Mazlan¹, Salnida Yuniarti Lumbessy^{1*}, Joko Sumarwan²

¹Aquaculture Study Program, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram, Jalan Pendidikan Mataram 83115, Nusa Tenggara Barat, Indonesia.

²Research Center for Marine and Land Bioindustry, National Research and Innovation Agency (BRIN), NTB, Indonesia

ABSTRACT

This study aimed to analyze the effect of variations in the duration and frequency of carbon dioxide (CO₂) gas injection on the productivity of the microalga *Chaetoceros muelleri* at an intermediate scale. This experimental study used a Completely Randomized Design (CRD) with five treatments using a CO₂ concentration of 50% v/v and three replications, namely P0 (Control/no CO₂ injection), P1 (6 seconds/1.2 hours), P2 (10 seconds/2 hours), P3 (20 seconds/4 hours), and P4 (40 seconds/8 hours). The parameters observed included daily cell density trends, chlorophyll content, dry biomass, final harvest weight, and water quality stability (pH, DO, temperature, and light intensity). Data were analyzed using ANOVA followed by Duncan's test. The results showed that CO₂ injection duration had a significant effect ($P < 0.05$) only on chlorophyll content and total harvest weight, but did not significantly affect water quality parameters, indicating that the culture medium remained stable. Microalgal cell density in all treatments reached its highest peak on Day 7, with the maximum population recorded in treatment P1 (6 seconds/1.2 hours) at 4,330,000 cells/mL, which also produced the highest chlorophyll content of 76.04 mg/g. In contrast, the highest final harvest weight was significantly obtained with the longer injection duration, namely treatment P4 (40 seconds/8 hours), at 5.42 g/L. It can be concluded that the short injection duration of 6 seconds/1.2 hours (P1) was optimal for stimulating the logarithmic growth phase and chlorophyll content, whereas the longer duration of 40 seconds/8 hours (P4) was more effective for maximizing final harvest weight.

KEYWORDS: Biomass, *C. muelleri*, Co2 injection, cell density.

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1. INTRODUCTION

Chaetoceros muelleri is one of the marine microalgae widely used in aquaculture as a natural feed source for fish and shrimp larvae because of its high nutritional content, including protein, lipids, and essential fatty acids, particularly eicosapentaenoic acid (EPA), as well as its cell size, which is suitable for the digestive system of larvae during the early stages. Meeting the natural feed requirements of aquaculture activities requires the production of *C. muelleri* biomass in large quantities and on a sustainable basis through cultivation, which involves growing and maintaining microalgae under controlled environmental conditions, including isolate selection, nutrient medium preparation, inoculation, regulation of physicochemical conditions, carbon supply, aeration, growth monitoring, and harvesting (Tan et al., 2020).

Microalgae cultivation can be carried out at small, intermediate, or mass scales, which differ in culture volume, purpose, and degree of environmental control. Intermediate-scale cultivation uses volumes ranging from tens to hundreds of liters as a transitional stage toward production with moderate environmental control, but it has limitations in maintaining uniformity of light, aeration, and CO₂ distribution throughout the culture volume compared with smaller and more stable laboratory-scale systems (Guieysse & Plouviez, 2024).

Marine microalgae have a high photosynthetic capacity because they can assimilate carbon dioxide (CO₂) as a carbon source for growth. However, CO₂ entering the medium through direct diffusion from air into water occurs very slowly and is insufficient to meet carbon fixation requirements in intensive culture systems. Therefore, direct CO₂ injection is required to increase the availability of dissolved carbon, accelerate the photosynthetic rate, and enhance biomass productivity (Sun et al., 2025). Although CO₂ supplementation has the potential to increase biomass, its effects depend strongly on the specific characteristics of each

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species, including carbon uptake mechanisms and pH tolerance, and therefore require targeted evaluation at the intermediate scale (Nagao et al., 2020).

Over the past 10 years, studies on CO₂ supplementation have largely compared variations in gas concentration without evaluating how gas delivery timing, including duration and frequency, affects the physiological response of cultures. Injection timing is important because CO₂ entering the medium not only increases carbon availability but also lowers the pH of the culture medium (Turnip et al., 2022). Proper regulation of the mode and frequency of CO₂ supply is essential to maintain a balance between increasing biomass productivity and maintaining stable chemical conditions in microalgal cultures (Guieysse & Plouviez, 2024). Based on this research gap, this study aimed to analyze the effects of variations in CO₂ injection duration and frequency and to determine the optimal injection schedule for obtaining the best growth and biomass quality of *C. muelleri* at an intermediate scale.

2. METHODOLOGY

2.1. Study Site and Time

This study was conducted in March 2026 at the Marine Aquaculture Research Center Laboratory, National Research and Innovation Agency (BRIN), Pemenang District, North Lombok.

2.2. Equipment and Materials

The equipment used included 14-L plastic containers, aerators, air stones, a CO₂ regulator and flow meter, a hemocytometer, microscope, spectrophotometer, pH meter, DO meter, lux meter, refrigerated centrifuge, freeze dryer, -80°C deep freezer, drying oven, desiccator, and analytical balance. The materials used included seawater with a salinity of 30 ppt filtered through a 1-μm filter, *C. muelleri* inoculum in the exponential phase, Walne fertilizer and silicate fertilizer, 90% acetone solution for chlorophyll-a extraction, chlorine for container and water sterilization, and sodium thiosulfate for chlorine neutralization.

2.3. Research Method

This study was an experimental study using a Completely Randomized Design (CRD) consisting of five CO₂ injection duration and frequency treatments with three replications, resulting in 15 experimental units. Each culture unit used a 14-L container placed in a room equipped with lighting and air conditioning for 24 hours to maintain stable light intensity and temperature. The culture medium was injected with CO₂ at a concentration of 50% v/v, and aeration was supplied at a flow rate of 1.1 L/min. The treatments consisted of intermittent CO₂ injection with different durations and intervals, while the total daily injection time was standardized at 120 seconds/day for all injection treatments (Table 1).

Table.1 Treatments used in the study

Code	Duration / Injection Interval		Frequency / Day	Total Injection Time / Day
P0 (Control)	-	-	-	0 menit (Aeration only)
P1	6 seconds	1.2 hours	20 times/day	120 seconds / day
P2	10 seconds	2.0 hours	12 times/day	120 seconds / day
P3	20 seconds	4.0 hours	6 times/day	120 seconds / day
P4	40 seconds	8.0 hours	3 times/day	120 seconds / day

2.4. Culture Container and Medium Preparation

Preparation was carried out by providing 15 plastic culture containers with a capacity of 14 L. The plastic containers were thoroughly washed with water and dishwashing liquid and treated with chlorine at 100–150 ppm. After being rinsed thoroughly with water, the containers were air-dried for 24 hours to ensure complete evaporation of residual chlorine. The culture medium was then prepared using sterile seawater. The seawater was first treated using an Ultraviolet Water Purification System and filtered through a 1-micron cartridge filter to remove large particles, residual detritus, and macroorganisms that could potentially interfere with the culture. After filtration, the water was sterilized using chlorine at a concentration of 5 mL/L as a basic disinfection treatment. The chlorinated water was then mixed with moderate aeration and left for approximately 24 hours to allow the sterilization process to proceed optimally. Chlorine neutralization was subsequently performed using sodium thiosulfate at a dose of 2.5 g/L

2.5. Fertilizer Preparation

The fertilizers used in this study were Walne fertilizer and silicate fertilizer because these types of fertilizer are commonly used in phytoplankton cultures. Walne fertilizer generally contains nitrogen (NO₃⁻) and phosphorus (PO₄³⁻), while silicate is an essential

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nutrient for frustule formation in diatoms. Walne fertilizer was added at a dose of 1 mL per liter of culture medium ($1 \text{ mL} \cdot \text{L}^{-1}$), while silicate fertilizer was added at a dose of 44.4 mL L^{-1} of culture medium to support diatom frustule formation. Thus, for each 10-L culture container, 10 mL of Walne fertilizer and silicate were added per container.

2.6. Inoculum Preparation

The *C. muelleri* inoculum was obtained directly from the Marine Aquaculture Research Center, National Research and Innovation Agency (BRIN). The stock culture was first observed under a microscope to ensure that it was free from contamination by protozoa, large bacteria, or other microalgae. Stock cell density was determined using a hemocytometer and then adjusted by dilution, following the formula below, so that the volume of inoculum added to the main culture containers produced a uniform initial density among experimental units of $2.5 \times 10^5 \text{ cells mL}^{-1}$. Dilution in the culture medium was determined using the formula according to Sopian et al. (2019): $V1 \times N1 = V2 \times N2$.

2.7. Monitoring

During the 10-day culture period, cell density was measured every 24 hours in the morning (approximately 09:00–12:00), while water quality parameters (pH, temperature, salinity, dissolved oxygen, and light intensity) were measured in the afternoon (approximately 16:00–17:00 WITA). Samples for dry biomass and chlorophyll-a analysis were collected during the exponential phase of the culture (Days 4–6), while final harvest weight was measured at the end of the culture period.

2.8 Experimental Parameters

The experimental parameters were biological and physicochemical indicators used to measure the response, productivity, and effectiveness of the CO₂ injection timing management treatments. The parameters observed in this study were as follows:

Cell Density (cells/mL)

Cell density was measured using the following formula according to Prasetyo et al. (2022):

$$\text{Cell Density} = \text{Average Number of Cells per Small Square} \times 10^4$$

Specific Growth Rate (SGR)

The specific growth rate was calculated using the following formula according to Turnip et al. (2022):

$$\text{SGR } (\mu) = (\ln N2 - \ln N1) / (t2 - t1)$$

Description:

SGR (μ): Specific Growth Rate

N1 : Initial cell number or density

N2 : Final cell number or density

t1 : Initial observation time

t2 : Final observation time

Doubling Time (DT)

Doubling time was calculated using the formula proposed by Mukhlis et al. (2017):

$$\text{DT} = \log(2) \times \Delta t / (\log Ct - \log C0)$$

Description:

DT : Doubling Time

Δt : Observation time interval

C0 : Initial cell density

Ct : Culture period (days)

Dry Biomass

Dry biomass was calculated using the following procedure and formula according to Khaw et al. (2021):

$$\text{Biomassa Kering} = [(W2 - W1) \times 1000] / V$$

Description:

W1 : Weight of the empty filter paper (g)

W2 : Weight of the filter paper + dry biomass (g)

V : Volume of filtered microalgal culture sample (mL)

Chlorophyll-a

The chlorophyll-a concentration of the microalgae was calculated according to Greipel et al. (2024) as follows:

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$$\text{Klorofil-a} = 13,7 \times (\text{OD665}) - 5,76 \times (\text{OD649})$$

Final Harvest Weight

Final harvest weight was obtained by harvesting and weighing the total wet *C. muelleri* biomass from each experimental unit at the end of the culture period using an analytical balance and was expressed in g/L.

DATA ANALYSIS

The data obtained were analyzed using one-way analysis of variance (ANOVA) at a 5% significance level ($\alpha = 0.05$) after meeting the assumptions of the Shapiro–Wilk normality test and Levene’s test for homogeneity of variance. If the ANOVA results showed a significant effect ($P < 0.05$), the analysis was followed by Duncan’s Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Daily Cell Density (cells/L)

Cell density is one of the growth parameters that can be used as a reference to determine whether microalgae are growing, in addition to biomass concentration. Observations over 10 days showed that the growth pattern of *C. muelleri* under all treatments with different CO₂ injection durations and frequencies followed a standard growth curve consisting of the adaptation phase (Day 0 to Day 1), exponential phase (Day 2 to Day 7), stationary phase, and death phase (Day 9 to Day 10). The control treatment (P0) reached its peak earlier on Day 6, with an average density of 3,320,000 cells/mL, whereas the other treatments maintained the exponential phase longer and reached their peaks simultaneously on Day 7. The 6-second injection duration treatment (P1) produced the highest cell density during the culture period, at 4,330,000 cells/mL, followed by the 20-second injection duration treatment (P3), the 10-second treatment (P2), and the 40-second treatment (P4), while the control treatment (P0) produced the lowest cell density at 2,090,000 cells/mL (Figure 1).

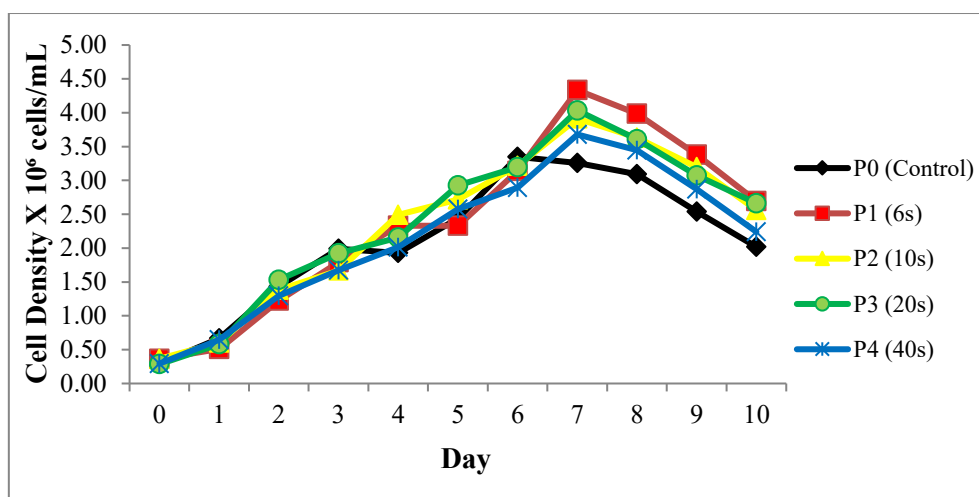


Figure 1. Cell Density of *C. muelleri* (cells/mL) under different CO₂ injection durations and frequencies during the 10-day culture period

Specific Growth Rate (SGR) and Doubling Time (DT)

Specific growth rate (SGR) and doubling time (DT) were evaluated during the exponential phase because this is the active growth phase of microalgae. Based on the calculated specific growth rates, the adaptation phase of *C. muelleri* in this study was very short. This was indicated by relatively high specific growth rates from the first and second days. On Day 1, the highest values were observed in the control treatment (P0) at 0.82/day and the 40-second injection treatment (P4) at 0.79/day, with corresponding doubling times (DT) of 0.85 and 0.88 days, respectively. On Day 2, the highest specific growth rates were recorded in the 6-second injection treatment (P1) at 0.89/day and the 20-second injection treatment (P3) at 0.94/day, with corresponding doubling times of 0.78 and 0.74 days, respectively. From Day 4 to Day 7, growth began to fluctuate, with the control treatment (P0) experiencing a marked decline and reaching a negative growth rate of $-0.02/\text{day}$. During the final stage of cultivation (Days 8–10), all treatments consistently showed declining growth rates and DT values, with negative SGR values. On Day 10, the lowest SGR values were observed in the 40-second injection treatment (P4) at $-0.25/\text{day}$ and the control treatment (P0) at $-0.23/\text{day}$. This indicates that all microalgal populations had passed their peak and uniformly entered the death phase at the end of the observation period (Table 2).

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Table 2. Specific Growth Rate (μ) and Doubling Time (DT)

	CO2 Injection Duration									
	P0 (Control)		P1 (6s)		P2 (10s)		P3 (20s)		P4 (40s)	
	SGR	DT	SGR	DT	SGR	DT	SGR	DT	SGR	DT
μ_1	0.82	0.85	0.32	2.14	0.51	1.37	0.71	0.97	0.79	0.88
μ_2	0.77	0.90	0.89	0.78	0.81	0.86	0.94	0.74	0.69	1.00
μ_3	0.32	2.17	0.38	1.83	0.21	3.36	0.26	2.72	0.26	2.68
μ_4	-0.02	-36.76	0.26	2.72	0.40	1.73	0.10	6.62	0.18	3.92
μ_5	0.23	2.96	-0.01	-75.28	0.08	8.67	0.29	2.37	0.23	2.99
μ_6	0.31	2.25	0.31	2.24	0.17	4.19	0.09	7.80	0.14	5.06
μ_7	-0.02	-37.57	0.32	2.14	0.19	3.56	0.23	3.04	0.23	3.01
μ_8	-0.05	-13.48	-0.08	-8.79	-0.07	-9.26	-0.10	-6.75	-0.05	-12.90
μ_9	-0.20	-3.51	-0.16	-4.23	-0.13	-5.35	-0.16	-4.44	-0.18	-3.78
μ_{10}	-0.23	-3.00	-0.23	-3.00	-0.21	-3.29	-0.14	-5.03	-0.25	-2.79

Dry Biomass (g/L)

The highest total dry biomass of *C. muelleri* (g/L) observed in this study is presented in Figure 2.

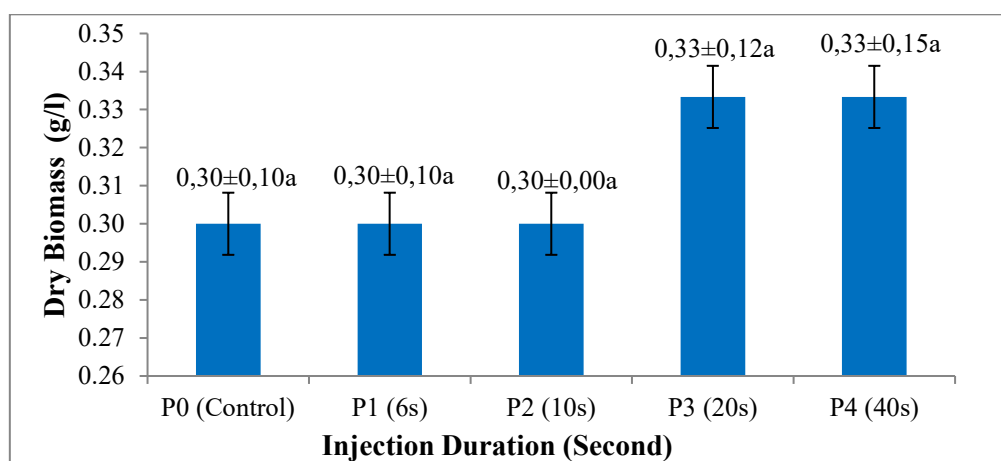


Figure 2. Dry Biomass of *C. muelleri* (g/L) under different CO2 injection durations: P0 (Control), P1 (6s), P2 (10s), P3 (20s), P4 (40s)

The highest dry biomass was observed in the 20-second injection duration treatment (P3) and the 40-second injection treatment (P4), with an average value of 0.33 g/L. Meanwhile, the lowest dry biomass was observed in the control treatment (P0), the 6-second injection duration treatment (P1), and the 10-second injection duration treatment (P2), at 0.30 g/L. The ANOVA results showed that different CO2 injection durations did not have a significant effect ($P > 0.05$) on dry biomass content.

Chlorophyll-a (mg/g)

The chlorophyll-a content of *C. muelleri* (mg/g) observed in this study is presented in Figure 3.

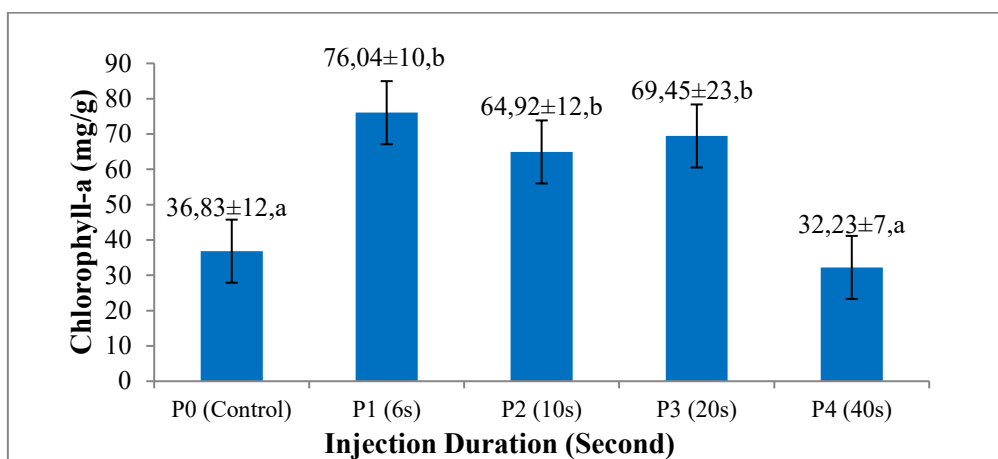


Figure 3. Chlorophyll-a of *C. muelleri* (mg/g) under different CO₂ injection durations: P0 (Control), P1 (6s), P2 (10s), P3 (20s), P4 (40s)

The highest chlorophyll-a content was observed in the 6-second injection duration treatment (P1), at 76.04 ± 10 mg/g, followed by the 20-second injection duration treatment (P3), at 69.45 ± 23 mg/g, and the 10-second injection duration treatment (P2), at 64.92 ± 12 mg/g. The lowest chlorophyll-a content was observed in the 40-second injection duration treatment (P4), at 32.23 ± 7 mg/g, and the control treatment (P0), at 36.83 ± 12 mg/g. The ANOVA results showed that different CO₂ injection durations and intervals had a significant effect ($P < 0.05$) on chlorophyll-a content. Duncan's post hoc test showed that the highest chlorophyll-a content was obtained in the 6-second injection treatment (P1) and was not significantly different from the 10-second (P2) and 20-second (P3) injection treatments; however, these three treatments differed significantly from the control (P0) and 40-second (P4) injection treatments.

Final Harvest Weight (g/L)

The final harvest weight of *C. muelleri* (g/L) observed in this study is presented in Figure 4.

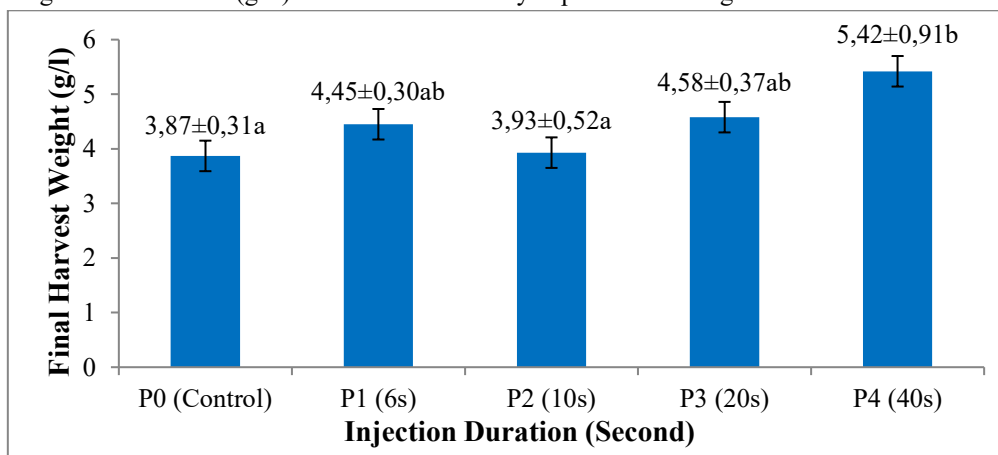


Figure 4. Final Harvest Weight of *C. muelleri* (g/L) under different CO₂ injection durations: P0 (Control), P1 (6s), P2 (10s), P3 (20s), P4 (40s)

The highest final harvest weight was observed in the 40-second injection duration treatment (P4), at 5.42 ± 0.9 g/L, followed by the 20-second injection duration treatment (P3), at 4.58 ± 0.3 g/L, the 6-second injection duration treatment (P1), at 4.45 ± 0.3 g/L, and the 10-second injection duration treatment (P2), at 3.93 ± 0.5 g/L. The lowest final harvest weight was observed in the control treatment (P0), at 3.87 ± 0.3 g/L. The ANOVA results showed that different CO₂ injection durations and intervals had a significant effect ($P < 0.05$) on the final harvest weight of *C. muelleri*. Duncan's post hoc test showed that the 40-second injection treatment (P4) produced the highest final harvest weight and was not significantly different from the 6-second (P1) and 20-second (P3) injection treatments, but differed significantly from the control (P0) and 10-second (P2) injection treatments.

Water Quality

Water quality measurements during the 10-day *C. muelleri* culture period are presented in Table 3, including temperature, pH, dissolved oxygen (DO), and light intensity, to describe the condition of the *C. muelleri* culture medium throughout the study.

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Table 3. Water Quality Measurements

Parameter	Observed Range	Optimal Value (BBPBAP Jepara, 2018)
Acidity (pH)	8.01 – 9.36	7.5 – 8.5
Dissolved oxygen (%)	29.30% – 76.10%	> 25%
Temperature (°C)	22.04°C – 24.30°C	25°C – 30°C
Light Intensity (Lux)	1015 – 3988 Lux	1000 – 4000 Lux

Overall, Table 3 shows that all water quality parameters remained within ranges that could support *C. muelleri* growth. However, the highest pH value exceeded the optimal range reported by BBPBAP Jepara (2018), which is 8.5. This condition was presumably caused by the uptake of dissolved CO₂ by microalgae during photosynthesis, which increased the pH of the medium. Nevertheless, the condition remained capable of supporting *C. muelleri* growth throughout the study.

DISCUSSION

The results showed that variations in CO₂ injection duration produced different responses in the growth of *C. muelleri*. In general, all treatments experienced adaptation, exponential, stationary, and decline phases. However, the 6-second CO₂ injection treatment (P1) produced the highest cell density of 4,330,000 cells/mL on Day 7, whereas the control (P0) reached only 3,320,000 cells/mL. This condition indicates that an appropriate supply of CO₂ can increase the availability of inorganic carbon for photosynthesis, thereby allowing cell division to proceed more efficiently. Nagao et al. (2020) reported that increased CO₂ availability can improve carbon fixation efficiency in diatoms, while Iwasaki et al. (2021) explained that *Chaetoceros* can efficiently utilize inorganic carbon as long as culture conditions remain within the optimal range. These results are also supported by Amaliandini et al. (2023), who stated that CO₂ supplementation can enhance microalgal growth by increasing photosynthetic activity, although the magnitude of the response depends on the species and CO₂ delivery method.

The increase in cell density was accompanied by a high specific growth rate (SGR) during the exponential phase. The 20-second injection duration treatment (P3) showed the highest SGR on Day 2, at 0.94/day, with the fastest doubling time of 0.74 days, while the 6-second injection duration treatment (P1) also showed high growth, with an SGR of 0.89/day. This indicates that an adequate carbon supply can accelerate cell division during the early culture stage. However, from Day 8 to Day 10, all treatments showed declining SGR values that became negative, indicating that the cultures had entered the death phase. Sen et al. (2020) reported that a decline in growth rate during the late culture phase is a normal response to nutrient depletion, increasing cell density, and the accumulation of metabolites that inhibit physiological activity. These results are consistent with Turnip et al. (2022), who showed that microalgal productivity decreases after the exponential phase because the capacity of cells to continue dividing becomes increasingly limited. Although no significant differences were observed among treatments, the SGR values tended to be higher in the CO₂ injection treatments, indicating that carbon supply still contributed to maintaining photosynthetic activity during the exponential phase. Turnip et al. (2022) reported that CO₂ supplementation can increase microalgal growth when supported by adequate light intensity and nutrient availability. Meanwhile, Xia et al. (2023) stated that the effectiveness of CO₂ supplementation is strongly influenced by the growth phase, so increased carbon availability does not always result in higher growth when cultures begin to enter the stationary phase.

The growth pattern subsequently affected biomass formation and chlorophyll-a content. Although dry biomass did not differ significantly among treatments, the 20-second injection duration treatment (P3) and 40-second injection duration treatment (P4) produced the highest dry biomass, at 0.33 g/L. This condition indicates that increased photosynthetic efficiency is not necessarily followed by a direct increase in biomass. At shorter injection durations, carbon fixed through photosynthesis was presumably used more for cell division and pigment synthesis, whereas at longer injection durations, part of the carbon may have been allocated to the formation of intracellular metabolic reserves. Wang et al. (2014) explained that carbon fixed through photosynthesis can be used for both growth and biomass accumulation, depending on the physiological condition of the microalgae. Meanwhile, Sun et al. (2025) stated that increased biomass productivity is determined more by carbon utilization efficiency than simply by increasing CO₂ concentration in the culture medium. The results of this study indicate that the available carbon under all treatments could still be utilized effectively for biomass formation. This is supported by Guieysse and Plouviez (2024), who stated that biomass accumulation is influenced not only by carbon availability but also by photosynthetic efficiency, nutrient availability, and environmental conditions during cultivation.

The highest chlorophyll-a content, however, was obtained in the 6-second injection duration treatment (P1), at 76.04 mg/g, and differed significantly from both the control (P0) and the 40-second injection duration treatment (P4). The high chlorophyll-a content indicates that photosynthesis proceeded more efficiently, allowing the resulting energy to support cellular metabolic activity. According to Amaliandini et al. (2023), increased chlorophyll-a content reflects the ability of microalgae to absorb light

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energy for photosynthesis. A similar finding was reported by Nagao et al. (2020), who found that CO₂ supplementation within an optimal range can enhance the formation of photosynthetic pigments. However, increasing CO₂ injection duration is not always accompanied by an increase in chlorophyll-a content. Iwasaki et al. (2021) explained that microalgae have an optimal limit for carbon utilization, so excessive CO₂ supply does not necessarily improve photosynthetic efficiency. Therefore, the high chlorophyll-a content observed in the 6-second injection duration treatment (P1) presumably indicates that the CO₂ supply under this treatment was the most suitable condition for supporting photosynthetic activity in *C. muelleri*.

This condition was further reflected in the final harvest weight, which showed significant differences among treatments. The 40-second treatment (P4) produced the highest final harvest weight, at 5.42 g/L, whereas the control (P0) reached only 3.87 g/L. These results indicate that although the highest cell density was obtained in the 6-second injection duration treatment (P1), a longer CO₂ injection duration was able to increase biomass accumulation by the end of the culture period. Wang et al. (2014) explained that increased biomass productivity occurs when carbon fixed through photosynthesis is more strongly allocated to the formation of organic compounds. These results are consistent with Guieysse and Plouviez (2024), who stated that biomass productivity is influenced by photosynthetic efficiency, carbon availability, and overall culture conditions, rather than cell growth rate alone.

In addition to the CO₂ injection treatment, the growth response of *C. muelleri* during the study was also supported by water quality conditions that remained within a tolerable range. The medium temperature ranged from 22.04–24.30°C, pH from 8.01–9.36, dissolved oxygen from 29.30–76.10%, and light intensity from 1,015–3,988 lux. These ranges were still capable of supporting photosynthetic activity and therefore did not constitute the main limiting factors during the study. Prasetyo et al. (2022) explained that stable water quality plays an important role in maintaining microalgal growth. In addition, Guieysse and Plouviez (2024) stated that successful cultivation is influenced by interactions among carbon, nutrients, light, and medium conditions. The increase in pH observed during cultivation was presumably associated with high photosynthetic activity that consumed dissolved CO₂, thereby reducing the concentration of free carbon dioxide in the medium. Iwasaki et al. (2021) similarly explained that increased photosynthetic activity is generally accompanied by an increase in culture-medium pH. Thus, the water quality conditions during the study were still capable of supporting *C. muelleri* growth, indicating that the observed responses were more strongly influenced by the CO₂ injection treatment.

CONCLUSION

CO₂ injection duration had a significant effect ($P < 0.05$) only on chlorophyll-a content and final harvest weight, but did not significantly alter water quality parameters, indicating that the culture medium remained stable. Microalgal cell density in all treatments reached its highest peak on day 7, with the maximum population recorded in treatment P1 (6 seconds/1.2 hours) at 4,330,000 cells/mL, which also produced the highest chlorophyll-a content of 76.04 mg/g. In contrast, the highest final harvest weight was significantly obtained with the longer injection duration, namely treatment P4 (40 seconds/8 hours), at 5.42 g/L. It can be concluded that the short injection duration of 6 seconds/1.2 hours (P1) was optimal for stimulating the logarithmic growth phase and chlorophyll-a content, whereas the longer duration of 40 seconds/8 hours (P4) was more effective for maximizing final harvest weight.

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