JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2023, 24(1), 289–298
https://doi.org/10.12911/22998993/156149
ISSN 2299–8993, License CC-BY 4.0

Received: 2022.10.19
Accepted: 2022.11.18
Published: 2022.12.01

The Effect of Different Additions of Carbon Dioxide on Growth, Lipids, Carotenoids and Chlorophyll-a of *Chaetoceros calcitrans*

Esza Rezky Amaliandini[1], Syarif Nashrullah[1], Anastasya Dewi Larasati[1], Fajar Taufan[1], Luthfiana Aprilianita Sari[2*], Nina Nurmalia Dewi[2], Sulastri Arsad[3,4], Nadirah Musa[5]

[1] Program Study of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Jalan Mulyorejo, Surabaya 60115 East Java, Indonesia.

[2] Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Jalan Mulyorejo, Surabaya 60115 East Java, Indonesia.

[3] Program Study of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Jl. Veteran Malang 65145, Indonesia

[4] Institute of Marine and Environmental Sciences, University of Szczecin, ul. Mickiewicza 16a, 70-383 Szczecin, Poland

[5] Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

* Corresponding author's e-mail: luthfianaas@fpk.unair.ac.id

ABSTRACT

Global warming gives phytoplankton a significant role in reducing carbon. *C. calcitras* is a phytoplankton which utilizes carbon dioxide for growth and the formation of secondary metabolites in order to survive. The purpose of this study was to determine the effect of differences in duration on growth, lipid content, carotenoids and chlorophyll-a in *C. calcitrans* and to determine the optimal duration. An experimental method with statistical analysis using ANOVA was used in this study. The treatments implemented were the addition of carbon dioxide for 0, 2, 4, 6, 8, 10, 12, and 14 minutes with a volume of carbon dioxide addition of 3 bps. The results showed that the addition of carbon dioxide had a significant effect ($P<0.05$) on the value of lipids, growth, carotenoids and chlorophyll-a. The highest density value was obtained in Treatment for 2 minutes to increase growth with the highest total value of 2,927,500 cells/ml. The best treatment to increase lipids was Treatment for 12 minutes with lipid content of 63.33% and lipid productivity of 1.82 mg/l/day. The best treatment to increase carotenoid content was Treatment for 4 minutes with carotenoid content of 2.20 g/mL and chlorophyll-a content of 1.4431 g/mL. Optimal treatment differences to obtain the highest value of each parameter differ from each other, depending on the synthesis and physiological processes of *C. calcitrans*.

Keywords: sustainable fisheries, marine, water quality, pollution, air, microalga, plankton.

INTRODUCTION

Phytoplankton plays a role in 50% of carbon fixation in the global photosynthesis process. This occurs because the amount of carbon dioxide that can be fixed by phytoplankton is close to the total carbon dioxide absorbed by land plants (Firdaus, 2019; Pratama et al., 2020). *C. calcitrans* is a photosynthetic phytoplankton that utilizes carbon dioxide in photosynthesis to form metabolism and cell growth (Mouahid et al., 2020). *C. calcitrans* is a phytoplankton that is widely used in the fishing industry as feed due to its high nutritional content. The nutritional content of *C. calcitrans* is fairly high, with a carbohydrate content of 12.8%, a fat comtent of 14.5%, and a protein content of 24.4% (Abraham, 2012).

In the field of aquaculture, *C. calcitrans* is a commonly cultivated species, because it can be used as a biocontrol of water quality and natural

feed. These microalgae are generally given to crustacean and mollusc larvae, because they contain lipids and specific fatty acid compositions (Wang et al., 2014). The pigment content in *C. calcitrans* consists of chlorophyll-a, chlorophyll-c, carotene, diatoxanthin (DT), and diadonixanthin (DD) (Balarumugan et al., 2013). In addition to nutrition, growth and density of *C. calcitrans* also greatly affects the utilization of these diatoms.

Photosynthetic phytoplankton can utilize air, sunlight, and carbon to produce their own food through the photosynthesis mechanism (Arifin, 2013; Rinawati et al., 2020). The main effect of the increase in carbon is the increasing rate of assimilation (rate of CO_2 binding to form carbohydrates). This condition affects microalgae, because the growth rate and metabolism of microalgae are influenced by the availability and ability of microalgae to absorb CO_2 in the environment (Mouahid et al., 2020; Obeid et al., 2022). Furthemore, Jawa et al. (2014) noted that a culture of *Chlorella vulgaris* exposed to carbon dioxide could increase the lipid content from 11.41% to 63.47% of dry weight. The research of Arbit et al. (2018) also showed that the addition of carbon dioxide was able to increase the carotenoid content in *Gracilaria changii* from the initial carotenoid content of 0.24 ± 0.51 g/g to 2.05 ± 1.32 g/g.

The success of the increase in lipids and carotenoids by giving carbon dioxide to several other microalgae species has been widely studied, but not many studies have been conducted on the effect of giving carbon dioxide on the growth and chlorophyll-a of *C. calcitrans*. Therefore, it is necessary to conduct further research on the effect of giving carbon dioxide on the growth, lipid content, carotenoids and chlorophyll-a of *C. calcitrans*.

MATERIALS AND METHODS

Tools and materials

The tools needed in the study were aquariums with a volume of 3 liters, pH pens, pH meters, DO meters, CO_2 tubes, aerators, an ultrasound cleaner, rotary evaporators, water aspirators, centrifuges, heaters, magnetic stirrers, spectrophotometers, cuvettes, a vortex and ovens. The materials needed in this study were *C. calcitrans* inoculants, 5 M HCL, sea water, walne, CO_2 gas, chlorine 20–30 mg/l, distilled water, label papers, absolute ethanol, diethyl ether n-hexane, ethanol, filter paper, NaOH 0.0227 N and phenolphthalein (PP).

Carbon dioxide addition

Carbon was added every day during culture with a volume of 3 bps (bubbles per second) (Nurmalitasari et al., 2014). The addition of carbon dioxide into the culture medium started on day 1, one day after day 0 which was the day of stocking *C. calcitrans* inoculants. The measurements of dissolved carbon were carried out every day during the culture period, and sampling was carried out 10 minutes after CO_2 injection was completed. The method used to measure the carbon dioxide levels was titration. A total of 50 mL of culture medium was dropped with phenolphthalein (PP) and then titrated with 0.0227 N sodium hydroxide (NaOH) solution until it turned pink.

C. calcitrans growth

The data on the growth of *C. calcitrans* were obtained by calculating the total density every day during the cycle. Supporting parameters observed were water quality consisting of temperature, pH, DO, salinity and dissolved carbon dioxide. According to Kwangdinata et al. (2013), the density of *C. calcitrans* can be calculated according to equation 1.

$$\text{Phytoplankton density}\left(\frac{\text{cells}}{\text{ml}}\right) = \frac{nA + nB + nC + nD}{4} \times 10^4 \quad (1)$$

Lipid extraction

Lipid extraction was conducted using the modified osmotic shock method (Nur, 2014). The first stage was biomass harvesting to obtain dry biomass. A total of 200 ml of sample water was transferred into a measuring cup and 0.5 M NaOH was added until the pH reached 10.5. The suspension was stirred with a magnetic stirrer at 1000 rpm for 10 minutes and then the speed was lowered to 250 rpm for 20 minutes. Next, the suspension was allowed to stand to create a concentrated biomass precipitate using filter paper number 041 with a vacuum pump, before being dried in an oven at a temperature of 70 °C for 5 hours. The weight of biomass obtained after heated was recorded as W1 (mg/l).

The second step was the extraction of lipids from the wet biomass of *C. calcitrans*. Firstly, 1 gram of broth was mixed with 5 ml of 5M HCl and then sonicated using an ultrasound cleaner for 20 minutes at a temperature of 35 °C. Following this, n-hexane was added to the solution in a ratio of 1:1 and stirred for 2 hours using a magnetic stirrer at 650 rpm. Next, re-sonication was carried out for 20 minutes, before the supernatant and residue were separated using filter paper. The supernatant was separated from n-hexane using a rotary evaporator at a temperature of 60 °C. Evaporated pure lipids were recorded as W2 (mg/l). Total lipids and lipid productivity were calculated using equation 2 (Nur, 2014).

$$L = \frac{W1}{W2} \times 100 \quad (2)$$

where: L – lipid content (%);
$W1$ – initial weight (g);
$W2$ – final weight (g).

Lipid productivity was calculated using equation 3 (Nur, 2014).

$$Y = \frac{L \times W2}{t} \quad (3)$$

where: Y – lipid productivity (mg/l/day);
L – lipid content (%),
$W2$ – final weight (g);
t – culture duration (day).

Carotenoid extraction

The UV-Vis spectrophotometric method was used to measure the carotenoid content of *C. calcitrans* in this study. According to Vo and Tran (2014), the measurements of carotenoid content begin by taking a sample of *C. calcitrans* culture and then centrifuging it at 3000 rpm for 10 minutes until two layers are formed, namely supernatant and precipitate. The precipitate obtained was then extracted with 3 mL of absolute ethanol and 1.5 mL of diethyl ether before 2 mL of distilled water and 4 mL of diethyl ether were added. The mixture was homogenized by vortex and centrifuged again at 3000 rpm for 10 minutes. The supernatant concentration was measured using a US-Vis spectrophotometer with a lambda of 450 nm. The results obtained are equivalent to micrograms (μg) per mL.

The carotenoid content was calculated using equation 4, referring to Menezes et al. (2022), Prieto et al. (2011).

$$\text{Carotenoid Concentration} \left(\mu\frac{g}{mL}\right) = = 25.2 \times A405 \quad (4)$$

where: A450 – absorption at a wavelength of 450 nm;
25.2 – carotenoid measurement constant value.

Chlorophyl-a extraction

The chlorophyll-a content of *C. calcitrans* was measured daily using the spectrophotometric method (Guo et al., 2014; Sari et al., 2019; Diana et al., 2021). The measurements began by taking 10 ml of microalgae sample, and then the sample was centrifuged at 6000 rpm for 10 minutes until two parts were formed (supernatant and precipitate). Afterwards, the supernatant was discarded before 10 ml of absolute methanol was added to the bottom sediment of the tub. The extract obtained was then homogenized with a vortex, and then the extract was incubated at 70 °C for 10 minutes. In the stage that followed, the extract was centrifuged again at 6000 rpm for 10 minutes, before the extract supernatant was taken. After being centrifuged in the next stage, 3 mL of the supernatant was taken to measure the absorption value using a spectrophotometer with a wavelength of 665 nm (Guo et al., 2014). The chlorophyll-a content was calculated using equation 5.

$$\text{Carotenoid Concentration} \left(\mu\frac{g}{mL}\right) = = 12.9447 \times A665 \quad (5)$$

where: 12.9447 – absorbance coefficient;
A665 – absorbance at wavelength of 665.

RESULTS AND DISCUSSION

Results

During the research period, the data were obtained in the form of daily density, lipid content and lipid productivity, daily carotenoid values and chlorophyll-a content of *C. calcitrans*. The results of average density of *C. calcitrans* can be seen in Table 1 and the graph of cell density in Figure 1.

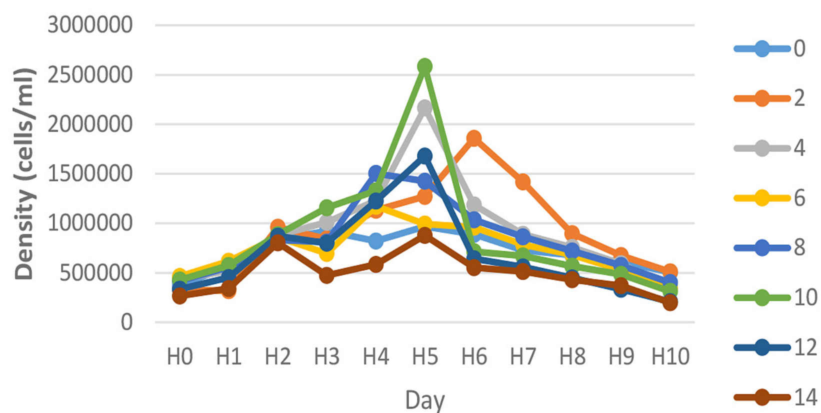

Figure 1. Graph of cell density of *C. calcitrans*

Table 1. Average density of *C. calcitrans* (cells/ml)

Treatment	Day
0	967500^{ab}
2	1270000^{d}
4	2165833^{c}
6	990833^{b}
8	1425000^{ab}
10	2582500^{ab}
12	1680000^{ab}
14	876667^{a}

Table 2. Data on average lipid content of *C. calcitrans*

Treatment	Lipid (%) ±SD
A	17.99^{b} ± 2.82
B	10.71^{a} ± 1.06
C	23.05^{c} ± 0.98
D	20.00^{bc} ± 1.75
E	28.90^{d} ± 1.98
F	41.20^{e} ± 3.51
G	63.33^{f} ± 3.38
H	60.58^{f} ± 2.16

All treatments other than control, namely the addition of carbon dioxide for 2, 4, 6, 8, 10, 12, and 14 minutes showed different amounts of density. The growth phase was passed at different times, some had the highest density on the 6th day, and some were on the 4th day. This indicates that the addition of carbon dioxide affects the growth of *C. calcitrans*.

As another parameter, lipids also showed an effect due to the addition of CO_2. The lipid content of *C. calcitrans* ranged from 10.71 to 63.33%. The data can be seen in Table 2, and the average level of lipid productivity is presented in Table 3.

Treatment B resulted in the lowest lipid productivity with a value of 0.07 mg/l/day. This occurred due to the low lipid content and measured biomass. The highest productivity value (1.82 mg/l/day) was much lower than that reported by Liu et al. (2021). However, the data that were not much different were shown by Adams and Bugbee (2014) who noted that *Chaetoceros gracilis*, given a stressor in the form of sodium chloride (NaCl) and silica, contained 73% lipids with a lipid productivity of 1.7 mg/l/day.

Table 3. Average lipid productivity level of *C. calcitrans*

Treatment	Lipid productivity (mg/l/day) ± SD
A	0.14 ± 0.06
B	0.07 ± 0.01
C	0.25 ± 0.04
D	0.11 ± 0.05
E	0.29 ± 0.12
F	0.63 ± 0.36
G	1.82 ± 0.46
H	1.53 ± 0.44

Table 4. Data on average content of carotenoids of *C. calcitrans* (μg/mL)

Treatment	Carotenoids (μg/mL) ± SD
A	1.00^{b} ± 0.05
B	1.42^{d} ± 0.05
C	2.20^{f} ± 0.04
D	1.83^{e} ± 0.06
E	1.10^{c} ± 0.01
F	1.14^{c} ± 0.01
G	0.95^{b} ± 0.07
H	0.84^{a} ± 0.03

Table 5. Daily carotenoid content of *C. calcitrans* during the culture period (µg/mL)

Treatment	Daily carotenoid content of *C. calcitrans* during the culture period (µg/L)									
	H0	H1	H2	H3	H4	H5	H6	H7	H8	H9
A	0.47	0.54	0.64	0.92	1.49	1.65	1.48	1.19	0.87	0.73
B	0.5	0.59	0.76	1.01	1.51	2.19	2.35	2.07	1.8	1.39
C	0.52	0.64	0.89	1.28	2.02	3.51	4.04	3.83	3.18	2.1
D	0.51	0.64	0.85	1.15	1.72	2.91	3.52	2.99	2.38	1.59
E	0.5	0.56	0.65	0.77	0.97	1.29	1.74	1.85	1.54	1.11
F	0.49	0.59	0.82	1.27	1.72	1.75	1.54	1.29	1.1	0.89
G	0.48	0.54	0.72	1.07	1.45	1.41	1.25	1.05	0.88	0.65
H	0.47	0.54	0.7	0.96	1.26	1.25	1.11	0.91	0.71	0.47

The average content of carotenoids in *C. calcitrans* during the culture period ranged from 0.84 g/mL – 2.20 g/mL. The data can be seen in Table 4.

Carotenoid production of *C. calcitrans* during the culture period experienced an increase and decrease in the carotenoid content based on the growth phase of *C. calcitrans* cells. Daily carotenoid data are presented in Table 5.

In the adaptation phase, there tended to be no increase in the carotenoid content, but an increase

Table 6. Data of daily chlorophyll-a content of *C. calcitrans* during the culture period

Treatment	Chlorophyll (µg/L) ± SD
A	0.6163[d] ± 0.10
B	1.1739[b] ± 0.04
C	1.4431[a] ± 0.10
D	0.9091[c] ± 0.11
E	0.8074[c] ± 0.03
F	0.7715[c] ± 0.04
G	0.5809[de] ± 0.13
H	0.4348[e] ± 0.08

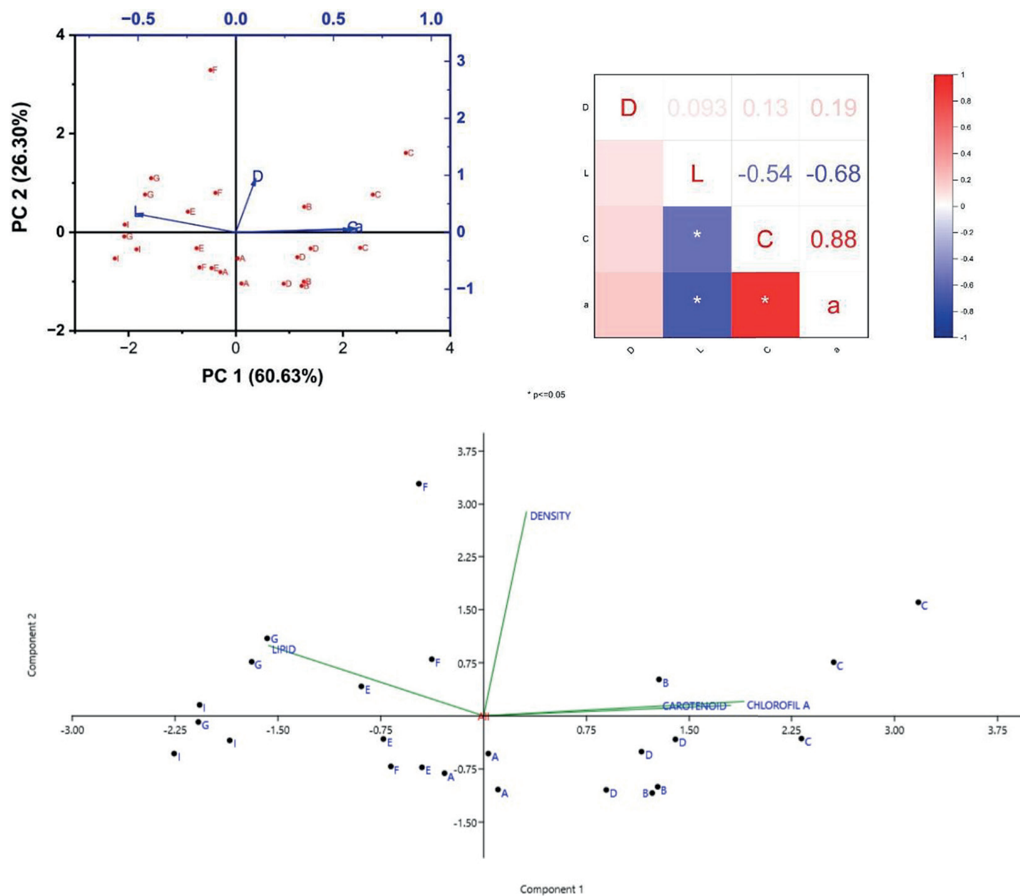

Figure 2. Principal component analysis (PCA) of cell density (D), Lipid (L), Carotenoid (Ca) and Chlorofil a (Ch) of *C. calcitrans*

Table 7. Water quality

Parameters	Score
Dissolved CO_2 (mg/l)	9.89–459.45
pH	7.47–9.11
Temperature (°C)	25–30.1
Salinity (ppt)	25–37
DO (mg/l)	1.86–4.08

occurred when entering the exponential phase. The highest carotenoid production occurred when *C. calcitrans* entered the stationary phase, but again experienced a decrease in the carotenoid production in the death phase.

The data on the daily chlorophyll-a content of *C. calcitrans* during the culture period are shown in Table 6. The highest average chlorophyll-a content of *C. calcitrans* was obtained in treatment C (1.4431 g/L), followed by treatment B (1.1739 g). /L), D (0.9091 g/L), E (0.8074 g/L), F (0.7715 g/L), A (0.6163 g/L), G (0.5809 g/L), and H (0.4348 g/L). Treatment A as a control produced an average chlorophyll-a content of 0.6163 g/L; this value was slightly higher than the value stated in the study conducted by Li et al. (2019) where chlorophyll-a production of *C. calcitrans* under normal conditions was 0.55 mg/L. The results of cell density (D), Lipid (L), Carotenoids (Ca), and Chlorophyll a (Ch) were then analyzed by Principal component analysis (PCA). It's an analytical technique used in statistics and also data science. It is to describe multivariate data. PCA results can be seen in Figure 2.

Growth, lipids, carotenoids and chlorophyll-a of *C. calcitrans* were also influenced by other water quality parameters. The water quality data during the research period can be seen in Table 7.

DISCUSSION

The addition of CO_2 during culture can increase the density of microalgae cells by up to 50%, and thus microalgae in waters, especially in the sea, have a high potential in reducing global warming due to carbon dioxide gas (Odunlami et al., 2022). According to Kawaroe et al. (2016), the high CO_2 content in the culture media still cannot guarantee the survival of microalgae due to the low growth of microalgae caused by levels of CO_2 concentration which are too high. This results in the death of the microalgae themselves, so it is necessary to do culture with the addition of CO_2 with a certain duration to prove the effectiveness of the duration of carbon dioxide addition in increasing cell density.

On the basis of the study results, all treatments other than control, namely the addition of carbon dioxide for 2, 4, 6, 8, 10, 12, and 14 minutes, showed different amounts of density. The growth phase was passed at different times, some of which had the highest density on the 6th day, and some were on the 4th day. This indicates that the addition of carbon dioxide affects the growth of *C. calcitrans*. The total density of *C. calcitrans* with the addition of carbon dioxide for a duration of 2 minutes had the highest number in the exponential phase accounting for 2,927,500 cells/ml, followed by a duration of 4 minutes which had a total of 1,907,667 cells/ml in the exponential phase, a duration of 6 minutes which had a total of 1,161,667 cells/ml in the exponential phase, control which had a total of 1,158,333 cells/ml in the exponential phase, a duration of 8 minutes which had a total of 1,033,333 cells/ml, a duration of 10 minutes which had a total of 957,500 cells/ml, a duration of 12 minutes which had a total of 843,500, and lastly a duration of 14 minutes which had a total of 777,667 cells/ml. This was due to too high concentration of carbon dioxide given for a long duration so that the density of *C. calcitrans* decreased, but conversely if carbon dioxide was given with a shorter duration, the total density of *C. calcitrans* could increase more than that in the control treatment.

The results of the *C. calcitrans* culture with the addition of carbon dioxide compared to the control treatment showed that the addition of CO_2 had a significant effect on the total density. This is in accordance with Jawa et al. (2014), who stated that CO_2 injection for 2 minutes had the highest total density compared to that in other behaviors. The lowest lipid content was obtained in treatment B with a value of 10.71%, which was thought to be caused by the presence of another stressor, namely salinity. According to Choi et al. (2012) high salinity (35 ppt) can reduce the lipid content of *C. calcitrans*. Treatment G produced the highest lipid among other treatments, which was 63.33%. This can occur because disolved carbon dioxide not only is used for photosynthesis, but also acts as a stressor that triggers lipid synthesis.

Under the conditions of optimum nutrition, light and carbon dioxide, microalgae tend to synthesize protein for growth and cell division (Jawa

et al., 2014; Dyachok et al., 2022). In addition, optimal light and carbon dioxide cause the rate of photosynthesis to increase and glucose production to increase as well. Glucose will undergo glycolysis and form pyruvic acid which is then converted to acetyl CoA, a lipid precursor (Hasanudin, 2012). In the A-D treatments, the carbon dioxide added to the media was still within the tolerance level of *C. calcitrans*, so that it could be well absorbed. The increase in the percentage of new lipid content increased in the E-G treatments and again experienced an insignificant decrease in the H treatment.

Carbon added to the culture media can also cause stress; if the volume of carbon dioxide exceeds the tolerance limit, the air quality decreases. However, stress conditions on microalgae can actually increase the lipid content. According to Hu et al. (2008), microalgae have the ability to produce triglycerides as the main component of lipids under stress conditions. Carbon acts as a stressor causing microalgae to change the biosynthetic pathway into the formation and accumulation of neutral lipids, especially in the form of triglycerides that can help microalgae to survive under unfavorable conditions (Nurmitasari et al., 2014). In this phase, microalgae will slow down their growth and cell division, so they do not spend a lot of energy when adapting (Menezes et al., 2022; Musa et al., 2020).

On the basis of the data obtained in this study, lipid productivity is not directly proportional to the percentage of lipid content. Liu et al. (2021) stated that microalgae with high lipids did not always have high lipid productivity. This is because lipid productivity is a product of biomass productivity and lipid content. These two things (biomass productivity and total lipid) are often inversely related when under stress conditions.

The highest carotenoid content was obtained in treatment C (4 minutes), which was 2.20±0.04 g/ml. The increase in the carotenoid content due to the addition of carbon dioxide in microalgae culture can increase the mechanism of inorganic carbon absorption in microalgae cells (Xia and Gao, 2005). The lowest carotenoid content was obtained in treatment H (14 minutes) with a carotenoid content of 0.84 ± 0.03 g/ml. The low content of carotenoids in the H treatment was caused by the high level of dissolved carbon dioxide in the H treatment, which was 9.98–459.45 mg/l. Dissolved carbon dioxide that is too high can change the cellular composition of microalgae, resulting in the degradation of carotenoids (Lee and Wang, 2014).

C. calcitrans utilizes carbon dioxide to carry out photosynthesis to produce glucose ($C_6H_{12}O_6$) which will be used in various biosynthetic pathways, such as primary metabolism, namely the preparation of biomass (carbohydrates, proteins, lipids); or secondary metabolism, one of which is the formation of pigments (Sri et al., 2019). Carbon dioxide, as an environmental stressor, triggers *C. calcitrans* to form carotenoids as a form of long-term self-defense. This could be the reason why the carotenoid content in treatments B – F increased from the one in treatment A as a control treatment. Treatments E and F did not experience significant differences, which is presumably because the dissolved carbon dioxide in treatments E and F was close to the tolerance limit of *C. calcitrans* in facing environmental stressors. Treatments G and H experienced a decrease in the content of carotenoids, because the dissolved carbon dioxide levels in treatments G and H exceeded the tolerance limit of microalgae. According to Lee and Wang (2014), the decrease in carotenoids can be caused by changes in the composition of microalgae cells.

The highest carotenoid formation in the stationary phase is in line with previous research (Singh et al., 2019) in the genus *Dunaliella* which showed that the formation of carotenoids in the stationary phase was higher than that in the exponential phase and other phases. According to Kusumaningrum and Zainuri (2013), an increase in the carotenoid content in the stationary phase was due to the fact that in the stationary phase, the nutrients in the culture media were depleted and microalgae were unable to perform cell division, so they produced carotenoids as their survival.

Treatments A and F entered the stationary phase on H-6 with the carotenoid content of 1.65 (μg/L) and 1.75 (μg/L). In treatments B, C, and D, the carotenoid content occurred at H-7 with respective carotenoid content of B: 2.35 (μg/L), C: 4.04 (μg/L), and D: 3.52 (μg/L), the carotenoid content in treatment E was obtained when entering H-8 accounting for 1.85 (μg /L), while in treatments G and H the carotenoid content was obtained when entering H-5 accounting for 1.45 (μg/L) and 1.26 (μg/L). The difference in time for each treatment to reach the stationary phase was influenced by the differences in the rate of cell growth, and the different time for decreasing the nutrient content in the media for each treatment (Zhu et al., 2017).

The addition of the duration of the CO_2 addition causes an increase in the concentration of dissolved carbon in the media. Inorganic carbon concentration affects cell growth, photosynthetic activity and the pigment content of microalgae (Azmi et al., 2020). In Treatment C with the addition of a duration of 4 minutes, CO_2 contained the highest chlorophyll-a with a value of 1.4431 (μg/L). This is reinforced by the statement made in a previous research that the addition of carbon dioxide causes a high supply of carbon dioxide needed in the photosynthesis process, so the chlorophyll-a content of microalgae increases as a natural response of microalgae due to increased photosynthetic activity (Cornwal et al., 2012; Dyachok et al., 2021).

The lowest chlorophyll-a content was obtained in treatment H with the addition of CO_2 duration of 12 minutes of and chlorophyll value of 0.4348 (μg/L). On the basis of the analysis of research data, the value of chlorophyll-a in Treatments G and H had a value of chlorophyll-a content under the value in the control treatment (A). Treatments G and H as a treatment with the addition of the highest duration of carbon dioxide cause the concentration of carbon dioxide to interfere with the physiological process of microalgae in producing chlorophyll. According to the research results of Khairy et al. (2014), the damage due to the addition of excess carbon dioxide can cause the damage to chloroplasts, disintegration of thylakoid membranes, and the occurrence of cell lyses when there is an increase in the concentration of carbon dioxide from 550 to 1,050 ppm in the culture of phytoplankton *Chaetoceros gracilis*.

Carbon dioxide measured during the study had a range of 9.98–459.45 mg/l. Most cultures have dissolved carbon dioxide that exceeds the tolerance limit of most aquatic organisms, which is 60 mg/l (Suyoso et al., 2022; Aryanto, 2017). The high dissolved carbon dioxide does not cause death, which is most likely because *C. calcitrans* is an autotrophic organism that absorbs and utilizes carbon dioxide for photosynthesis. The addition of carbon dioxide affects the pH of the media. The pH measured during the culture period was 7.47–9.11 and this value was not in accordance with the optimum pH for *C. calcitrans*, which was 8–10.2. Low pH was found in Treatments C-H, ranging from 7.47–7.59. However, this value is higher than that in the study of Jawa et al. (2014) which noted that the pH of the medium with the addition of carbon dioxide for 8 minutes was 5.1. Balarumugan et al. (2013) added that at a pH of 4.5–6.5 carbon dioxide which reacts with water will also form free carbon dioxide, because the water is saturated and cannot bind carbon dioxide anymore.

The temperature of the media during the study ranged from 25–30.1°C and was still within the optimum range for *C. calcitrans* (Prasetyo, 2017). Choi et al. (2012) stated that the lipids of *C. calcitrans* were higher at low temperatures, namely 20°C and 25°C. Salinity measured in each culture was in the range of 25–33 ppt, except in an culture with Treatment B which was recorded to have salinity up to 37 ppt, exceeding the optimum tolerance limit of *C. calcitrans*. The extreme difference in salinity affects the osmotic pressure of the cell (Arsad et al., 2022). Microalgae will form a cell wall to avoid the release of water from the cell. According to Duan et al. (2012), at this time the growth rate is low but the lipid content is higher. The measured DO value ranged from 1.86–4.08 mg/l and was not the optimum value for *C. calcitrans*. This is presumably because *C. calcitrans* is in an exponential phase which requires large amounts of nutrients and oxygen for cell growth and division.

CONCLUSIONS

The addition of carbon dioxide to the culture media significantly affected the growth, lipids, carotenoids and chlorophyll-a of *C. calcitrans*. The highest density was obtained in Treatment for 2 minutes with the value of 2,927,500 cells/ml, the highest lipid content was obtained in Treatment for 12 minutes at 63.33%, lipid productivity reached 1.82 mg/l/day, the highest carotenoid content was obtained in Treatment for 4 minutes reaching 2.20 g/mL and the chlorophyll-a content amounted to 1.4431 g/mL. The optimal duration for increasing each parameter differed from one another, depending on the synthesis and physiological processes of *C. calcitrans* itself.

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