



## Oxidation Stress of UV C Light on Growth, Carotenoids and Chlorophyll-a Content of *Chlorella vulgaris*

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### ABSTRACT

*C. vulgaris* is photoautotrophic, which refers to microalgae that require light as an energy source. One of the light sources used to affect pigments in microalgae is UV light. The advantage of UV light is that it can change gene composition, which results in mutant genes in biopigment mutations and causes a higher number of activities compared to the natural ones, thereby increasing the growth, carotenoids, and chlorophyll-a of microalgae. The aim of this research is to determine the effect of oxidation stress of UV light on growth, carotenoids and chlorophyll-a content of *Chlorella vulgaris*. The treatments were given based on differences in the power of the UV-C light from, 8W, 15W and 30W. This study used the UV-Vis Spectrophotometer method was used to calculate growth, carotenoids and chlorophyll-a. Data were analysed using ANOVA test, Duncan's Multiple Range Test, and Principal Component Analysis (PCA). The results showed a significant effect of UV-C light. The best treatment was found in the administration of 30 W UV light, with the results of growth was  $4.7746 \times 10^6$  cells/ml, content of carotenoids was 1.75 mg/ml and chlorophyll-a was 4.67 mg/ml of *C. vulgaris*. Biopigment can absorb radiation from UV-C light, which causes these microalgae to survive in environmental stress conditions. Absorbed light can affect the spectrum of pigments, thereby increasing growth, carotenoids, and chlorophyll-a.



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## 1. Introduction

Stress and pollution are triggers for the emergence of free radicals in the body that can damage cell membranes (Amjadi *et al.* 2021; Urrea-Victoria *et al.* 2022). The accumulation of free radicals in the body triggers metabolic disorders that cause various diseases (Tajvidi *et al.* 2021; Li *et al.* 2020). Modern society is starting to change their lifestyle by consuming free radical scavenging foods in the form of antioxidant foods in chlorophyll-a, carotenoids and lipids (Saranya *et al.* 2014; Serrà *et al.* 2020). These antioxidants are in the

form of active substances that are present in microalgae (Singh *et al.* 2019; Castro *et al.* 2021).

One of the microalgae which has the potential to be bio pigmented is *C. vulgaris*, which is dominated by green colour as it comes from the chlorophyll-a and chlorophyll-b pigments (Sharif *et al.* 2015). The pigment content present in *C. vulgaris* consists of chlorophyll-a, chlorophyll-b, chlorophyll-c, carotene, and xanthophyll (Ngesti *et al.* 2013). Chlorophyll-a and carotenoids are active bio pigment substances which are useful as a source of vitamin A, food additives, antibacterial agents, antioxidants and red blood cell boosters, and which are useful to replace damaged cells (Kusmiati *et al.* 2010). Chlorophyll-a and carotenoids are pigments produced by secondary metabolites in microalgae that can be

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synthesized by cells under oxidative stress from the environment (Chou *et al.* 2018; Samadi-Noshahr *et al.* 2021; Amjadi *et al.* 2021). One form of oxidative stress is in the form of UV light (Casazza *et al.* 2015; Stafsnes *et al.* 2010). Light activates transmembrane pathways in microalgae cells, thereby changing the location of gene expression in the nucleus and cytoplasm (Chen and Bridgeman 2017; Sharma and Sharma 2017; Serrà *et al.* 2020). The results of this gene arrangement are sent to the chloroplast and can lead to an increase in pigment production (Ramos *et al.* 2011).

One type of UV light is UV C which has a level of damage (Tekiner *et al.* 2019). The highlight of this research is applying UV C *C. vulgaris* culture on a laboratory scale. The purpose of this research is to determine the effect of oxidative stress by UV C light with different powers on the chlorophyll-a, and carotenoid content of microalgae. This research is expected to provide information on how much impact UV-C light has on microalgae as a result of secondary metabolism, which in this study refers to active substances to counteract free radicals. The urgency of this research is that it will become a reference to produce antioxidant materials from high-value marine sources in a sustainable manner.

## 2. Materials and Methods

### 2.1. Material

Media culture, DO Meter YSI 550A, pH Meter (ATC-SKU pH 2 Pro), T8 LED TL Lamp, Philips 8W Lamp, 15W UV-C Lamp, 30W UV-C Lamp, Lux Meter Lx 1330b, ATC Refractometer, LP20 Aerator, 20 XSP-12 Monocular Microscopes, 3000 RPM DSC-200T Centrifuge, centrifuge tube, Vortex Labtronics GM-A220, L7 Uv1700 UV-Vis Spectrophotometer and cuvette. *C. vulgaris* inoculant from Balai Situbondo, HCL 5%, Walne Fertilizer, Aquades Waterone, chlorine 20-30 ppm (Soghieklin), absolute ethanol, diethyl ether (Emsure), Na-thiosulfate.

### 2.2. Culture and Treatment *C. vulgaris*

The research began with the preparation and the rental of tools, materials and laboratories. The use of UV-C light at the same temperature in this research relied on previous research conducted by Pranagari *et al.* (2014). Moreover, the length of UV-C exposure time of 30 minutes relied on research conducted by Singh *et al.* (2019). The important thing to note in this research was the light intensity of UV light in different treatments.

Light intensity was measured using a lux meter. The intensity of a 15-watt UV lamp was 220 lux and a 30-watt UV lamp was 241 lux (Sarinaningsih 2018). The measurement of UV-C light was conducted using a lux meter with a spectrum of <280 nm. The intensity of UV light used was between 200-250 lux and the intensity of a TL lamp was 3000 lux.

The treatments used in this study were A (without UV C), B (UV C light of 8W for 30 minutes), C (UV C rays of 15W for 30 minutes), D (UV C rays of 30W for 30 minutes). The calculation of microalgae density, was carried out every day during the study. Microalgae density was calculated by using a 0.1 mm deep Neubauer haemocytometer and a microscope tool. Furthermore, carotenoid content was measured using the UV-Vis Spectrophotometry method (Vo and Tran 2014). Moreover, the chlorophyll-a content of microalgae was measured using the spectrophotometric method (Ritchie 2006).

### 2.3. Data Analysis

The data obtained were processed using tests of normality, homogeneity, Analysis of Variance (ANOVA), Duncan's Multiple Range Test and Principal Component Analysis (PCA). PCA is a dimension reduction method in machine learning. PCA will choose variables that are able to explain most of the variability of the data. PCA reduces dimensions by forming new variables called Principal Components. The PCA method is very useful when the existing data have a large number of variables and have a correlation between the variables.

## 3. Results

### 3.1. The Effect of UV C Light on the Growth of *C. vulgaris*

Based on the results of observing the density of *C. vulgaris* for 10 days of culture using the analysis of variance (ANOVA), it was found that UV-C light with different powers had a significantly different effect on the density of *C. vulgaris*. The statistical test of ANOVA was then followed by another testing method, namely Duncan's Multiple Range Test.

Based on the data, Treatment A was not significantly different from Treatments B and C, but it was significantly different from Treatment D. The results (Table 1) show that the highest peak density was found in Treatment D of  $4.7746 \pm 0.8616$  cells/ml. It was followed by Treatment C of  $3.3788 \pm 0.3628$

cells/ml, Treatment B of  $3.1228 \pm 0.2323$  cells/ml. and Treatment A with the lowest average of  $2.9262 \pm 0.1904$ .

During growth, microalgae pass through various growth phases, starting from the adaptation phase, the exponential phase, the deceleration phase, the stationary phase, to the death phase (Figure 1). The adaptation phase in Treatments A, B, C and D was observed in D0 and D1. The second phase was an exponential phase that started in D2 in all treatments, where cell growth and activity reached its maximum level due to double division from the first stocking. The third phase was the deceleration phase, characterized by an increase in cells but a decrease in density. The deceleration phase was observed in D5.

### 3.2. Production of Chlorophyll-a During Culture Periods

The chlorophyll-a value of *C. vulgaris* was calculated based on the average chlorophyll value during the culture period. Based on the results of the ANOVA test carried out, the results were significantly different ( $P < 0.05$ ). Differences and the specific effect of each treatment could be known by using Duncan's Multiple Range Test. Figure 2 shows the average data on the chlorophyll-a content of *C. vulgaris* which had been exposed to UV-C light for 30 minutes with the duration of the culture period for 10 days. In D9 of Treatment A (control) the lowest chlorophyll-a value was found, accounting for  $0.19 \pm 0.22$  mg/ml. Different superscript letters (a,b,c) in the same column

Table 1. Statistics of average cell density ( $\times 10^6$  cells/ml)  $\pm$  standard deviation of *C. vulgaris*

Length of incubation (day)	Treatment			
	A (Without UV C)	B (UV C light of 8W)	C (UV C light of 15W)	D (UV C light of 30W)
D0	2.0452 <sup>a</sup> $\pm$ 0.1928	2.1208 <sup>a</sup> $\pm$ 0.2080	2.0452 <sup>a</sup> $\pm$ 0.1928	2.4402 <sup>b</sup> $\pm$ 0.1906
D1	2.2772 <sup>a</sup> $\pm$ 0.2139	2.3836 <sup>ab</sup> $\pm$ 0.1930	2.2772 <sup>a</sup> $\pm$ 0.2139	2.5892 <sup>b</sup> $\pm$ 0.1678
D2	2.6546 <sup>a</sup> $\pm$ 0.1369	2.6928 <sup>a</sup> $\pm$ 0.1006	2.6546 <sup>a</sup> $\pm$ 0.1369	3.0692 <sup>b</sup> $\pm$ 0.2275
D3	2.7948 <sup>a</sup> $\pm$ 0.1098	2.7472 <sup>a</sup> $\pm$ 0.1574	2.7948 <sup>a</sup> $\pm$ 0.1098	3.5428 <sup>b</sup> $\pm$ 0.4665
D4	2.9262 <sup>a</sup> $\pm$ 0.1904	3.1228 <sup>a</sup> $\pm$ 0.2323	2.9262 <sup>a</sup> $\pm$ 0.1904	4.7746 <sup>b</sup> $\pm$ 0.8616
D5	2.7132 <sup>a</sup> $\pm$ 0.2352	2.9252 <sup>a</sup> $\pm$ 0.2544	2.7132 <sup>a</sup> $\pm$ 0.2352	4.6236 <sup>b</sup> $\pm$ 0.9259
D6	2.4508 <sup>a</sup> $\pm$ 0.2269	2.7542 <sup>a</sup> $\pm$ 0.2974	2.4508 <sup>a</sup> $\pm$ 0.2269	4.3836 <sup>b</sup> $\pm$ 0.5851
D7	2.2582 <sup>a</sup> $\pm$ 0.3233	2.5486 <sup>a</sup> $\pm$ 0.3525	2.2582 <sup>a</sup> $\pm$ 0.3233	3.5396 <sup>b</sup> $\pm$ 0.4837
D8	2.0372 <sup>a</sup> $\pm$ 0.1540	2.3622 <sup>a</sup> $\pm$ 0.3637	2.0372 <sup>a</sup> $\pm$ 0.1540	2.9256 <sup>b</sup> $\pm$ 0.3074
D9	1.9716 <sup>a</sup> $\pm$ 0.2747	2.1608 <sup>ab</sup> $\pm$ 0.2274	1.9716 <sup>a</sup> $\pm$ 0.2747	2.3926 <sup>b</sup> $\pm$ 0.4103

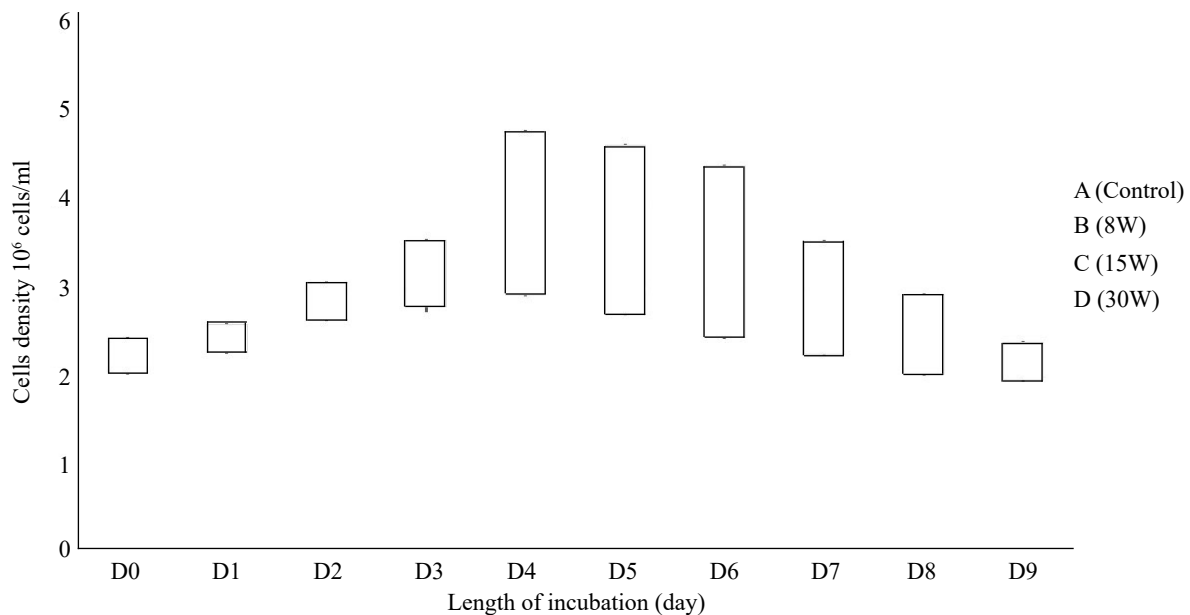


Figure 1. Graph of *C. vulgaris* cell density during the culture period ( $10^6$  cells/ml)

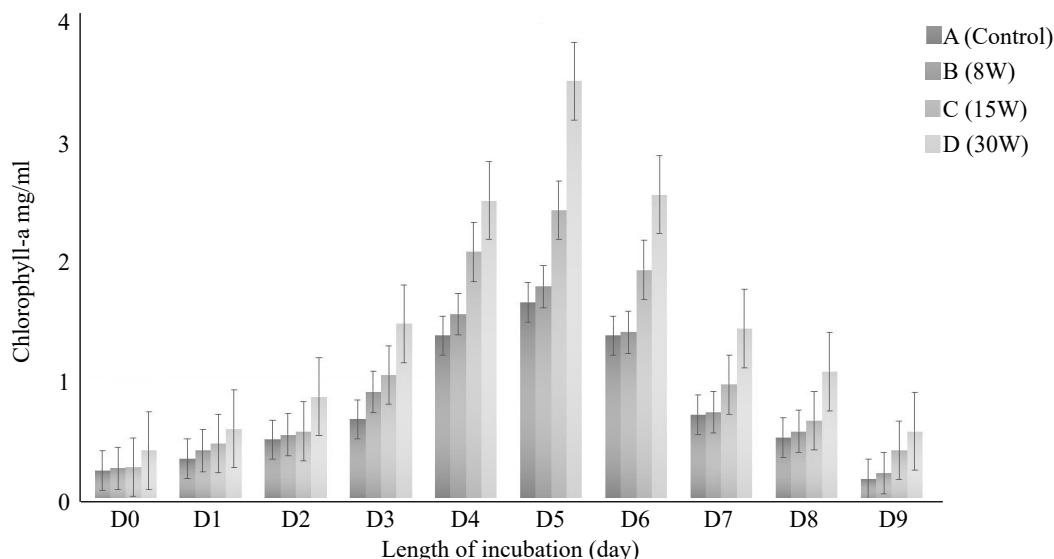


Figure 2. Graph of chlorophyll-a content of *C. vulgaris* (mg/ml)

Table 2. Data on average carotenoid content  $\pm$  standard deviation of *C. vulgaris*

Length of incubation (day)	Treatment			
	A (Without UV C)	B (UV C light of 8W)	C (UV C light of 15W)	D (UV C light of 30W)
D0	0.37±0.02 <sup>b</sup>	0.29±0.05 <sup>a</sup>	0.47±0.06 <sup>d</sup>	0.39±0.03 <sup>b</sup>
D1	0.61±0.08 <sup>a</sup>	0.56±0.09 <sup>a</sup>	0.82±0.05 <sup>b</sup>	0.64±0.07 <sup>a</sup>
D2	0.78±0.13 <sup>a</sup>	0.76±0.06 <sup>a</sup>	1.04±0.06 <sup>b</sup>	0.81±0.08 <sup>a</sup>
D3	0.90±0.11 <sup>ab</sup>	0.88±0.04 <sup>a</sup>	1.15±0.05 <sup>c</sup>	1.07±0.10 <sup>b</sup>
D4	1.03±0.09 <sup>a</sup>	1.02±0.10 <sup>a</sup>	1.33±0.09 <sup>b</sup>	1.46±0.17 <sup>b</sup>
D5	1.18±0.15 <sup>a</sup>	1.30±0.05 <sup>a</sup>	1.58±0.11 <sup>b</sup>	1.75±0.06 <sup>c</sup>
D6	0.99±0.06 <sup>a</sup>	1.11±0.08 <sup>b</sup>	1.30±0.09 <sup>c</sup>	1.56±0.08 <sup>d</sup>
D7	0.93±0.06 <sup>a</sup>	0.97±0.04 <sup>ab</sup>	1.05±0.04 <sup>b</sup>	1.32±0.11 <sup>c</sup>
D8	0.75±0.06 <sup>b</sup>	0.55±0.04 <sup>a</sup>	0.80±0.06 <sup>b</sup>	1.00±0.11 <sup>c</sup>
D9	0.50±0.06 <sup>b</sup>	0.39±0.06 <sup>a</sup>	0.50±0.06 <sup>b</sup>	0.80±0.10 <sup>c</sup>

indicate that there were significant differences in each treatment.

Measurement of the chlorophyll-a content of *C. vulgaris* was carried out every day during the culture period from D-0 to D-9 (Figure 2). There was an increase in chlorophyll-a content from D4 to D5, which was the growth phase when the abundance of cells experienced constant growth due to the balance of catabolism and cell metabolism (stationary phase). However, there was a decrease in the chlorophyll-a content during the treatment from D7 to D9.

### 3.3. Carotenoid Content

The average value of carotenoid content of *C. vulgaris* is shown in Table 2. Based on the one way statistical test of analysis of variance (ANOVA), the

results obtained were significantly different ( $P < 0.05$ ), and therefore a Duncan's Multiple Range Test was performed on the observation of the effect of UV-C rays for 30 minutes on the carotenoid content of *C. vulgaris*.

Based on the data above, it is known that the carotenoid content in D0 was between 0.29-0.47 mg/ml. The application of UV-C light with different powers affected the carotenoid content in each treatment during the culture period. The highest carotenoid content in each treatment occurred on D5. The highest carotenoid value on D5 of Treatment D was 1.75±0.06 mg/ml and of Treatments A, B and C was 1.18±0.15 mg/ml, 1.30±0.05 mg/ml, and 1.58±0.11 mg/ml respectively. From D6 to D9 in each treatment, the carotenoid content value decreased.

### 3.4. Principal Component Analysis

PCA results state that there is a strong correlation between density and carotenoids (Figure 3). This is because *C. vulgaris* contains carotenoids of 0.5-10% dry weight, which in UV-C treatment is a stressor for algae that can increase the content of pigments, one of which is carotenoids.

### 3.5. Water Quality

Water quality was measured after the culture was treated using UV-C light during the culture period. Water quality parameters that were measured included temperature, pH, DO, water salinity and light intensity. Water quality measurement data can be seen in Table 3.

Table 3 shows the water quality data obtained during the culture period of *C. vulgaris*. The lowest

pH value and the highest pH value were obtained in treatment B, accounting for 7.19 and 7.80 accordingly. The temperature values obtained ranged from 26.3-29.3°C, the water salinity values obtained ranged from 35-39 ppt, and the dissolved oxygen (DO) content obtained during the culture period ranged from 3.21-4.89 mg/ml. The light intensity in treatment A (control) was 2066 lux, while the light intensity in the treatment with UV-light for 30 minutes in Treatment B (8W) was 298 lux, in Treatment C (15W) was 680 lux and in Treatment D (30W) was 779 lux.

### 4. Discussion

The results of the ANOVA statistical test ( $P < 0.05$ ) showed that UV-C light given to *C. vulgaris* cultures had a significant effect on density, which was shown on D4.

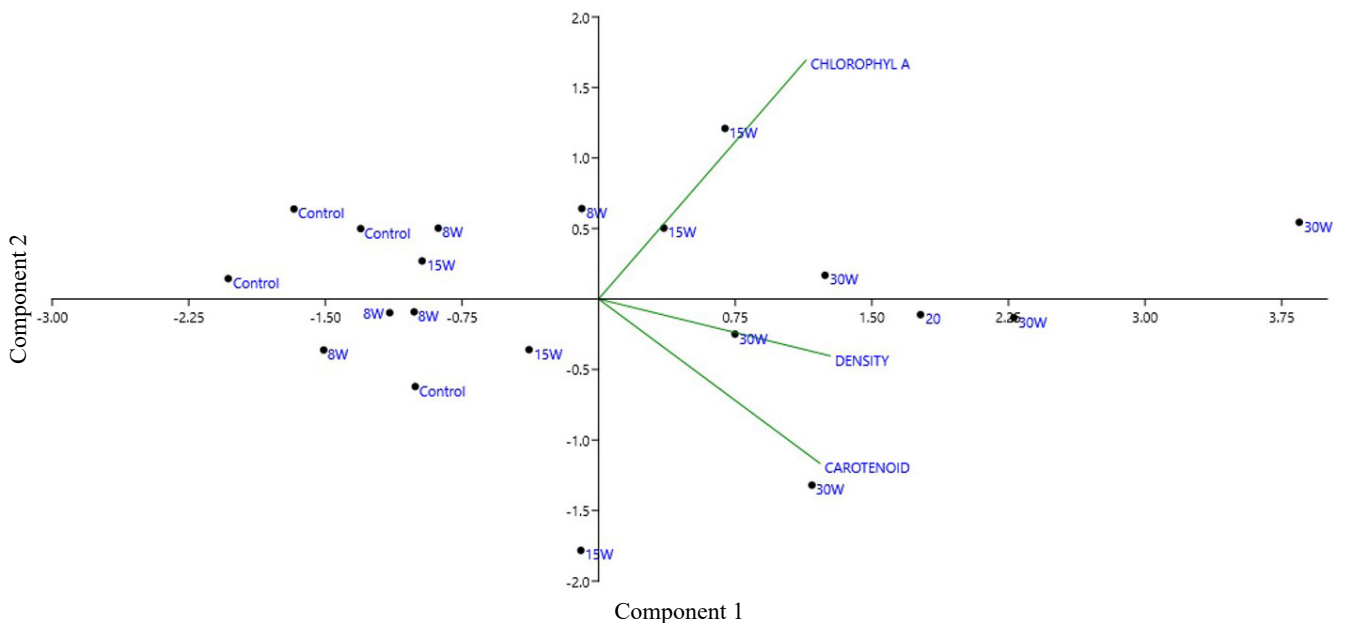


Figure 3. Principal component analysis density, carotenoids and chlorophyll-a content of *C. vulgaris*

Table 3. Water quality data

Treatment	Treatment				
	pH	Temperature (°C)	Salinity (ppt)	DO (mg/ml)	Light intensity (lux)
A (Without UV-C)	7.35-7.74	26.3-28.6	35-38	3.21-4.89	2066
B (UV-C light of 8W)	7.19-7.80	26.5-28.9	35-39	3.29-4.1	298
C (UV-C light of 15W)	7.21-7.76	26.3-28.9	35-39	3.25-4.68	680
D (UV-C light of 30W)	7.42-7.75	26.4-29.3	35-39	3.29-4.7	779
Optimum range	6.6-7.0 (Wang <i>et al.</i> 2010)	23-30°C (Pratama 2011)	30-35 (Kawaroe <i>et al.</i> 2016)	5-7 ppm (Facta <i>et al.</i> 2006)	500-5000 (Boroh <i>et al.</i> 2019)

UV-C light has energetic form of radiation that causes the metabolic activity of *C. vulgaris* to increase every day. D4 showed significantly different results because microalgae underwent double division from the first stocking so that cell activity reached a maximum level (Suantika and Hendrawandi 2009). According to Zul (2003), microalgae that are exposed to UV light can change the gene composition, resulting in more metabolic activity and an increase in the growth rate. The increase in the density of *C. vulgaris* in each treatment was highly influenced by the amount of light emitted. *C. vulgaris* experienced the peak growth on D4. In addition, *C. vulgaris* could grow up to 10 days as the amount of UV-C light used could still be tolerated by *C. vulgaris* and they utilized it for photosynthesis.

According to Wijoseno (2011), in the process of photosynthesis, light is absorbed by a green pigment called chlorophyll in the chloroplast, which will be converted into ATP and NADPH in the light reactions. Afterwards, ATP and NADPH are used to reduce CO<sub>2</sub> in the dark reactions into organic compounds. According to Campbell *et al.* (2002), basically photosynthesis is the process of making glucose and oxygen in organisms that have chlorophyll with the help of light energy. Glucose is used as a substrate for cellular respiration. In the process of glycolysis, glucose is broken down into pyruvate and then decarboxylated to produce acetyl Co-enzyme A which is later undergone the Krebs cycle to produce ATP. ATP can be used by microalgae for cell growth and cell development. According to Peri *et al.* (2009), light can increase the ATP produced in the process of photosynthesis, and the increase in ATP will trigger the growth of algal cells.

Treatment D (UV-C of 30 watts) had the highest effect on the density of *C. vulgaris* compared to other treatments on D4 and D5, accounting for  $4.7746 \times 10^6$  cells/ml and  $4.6236 \times 10^6$  cells/ml respectively. This could happen because Treatment D experienced environmental stress caused by UV-C light of 30W. UV-C light has short wavelengths, so the greater the radiation emitted, the greater the free radicals that cause environmental stress. According to Bouterfas *et al.* (2006), the growth of most microalgae increases in high light conditions. Carino & Vital (2021) added that the addition of UV-C light to *C. vulgaris* culture results in a high growth rate as it has more energetic form of radiation and has a significant effect on microalgae.

The lowest density in *C. vulgaris* culture was found in Treatment A (without UV-C). The peak density of *C. vulgaris* in the control treatment occurred on D4 with an

average of  $2.9262 \times 10^6$  cells/ml. The light intensity of a TL lamp was 2066 lux, and the energy produced by a TL lamp did not meet the light intensity requirements of *C. vulgaris*. Satriaji *et al.* (2016) stated that the optimal light intensity for the growth of *C. vulgaris* is between 3000-5000 lux. According to Suantika and Hendrawandi (2009), the availability of low light intensity causes cell growth to not be optimal, so cells are unable to grow and develop.

The research results regarding the application of UV-C light with different powers to the growth of *C. vulgaris* showed an effect on the density of microalgae which underwent various growth phases, namely the adaptation phase, the exponential phase, the deceleration phase, the stationary phase, and death phase (Fogg and Thake 1987). The growth of *C. vulgaris* is characterized by increasing cell size and increasing number of cells, which will directly affect plankton density (Isnansetyo and Kurniasty 1995).

Based on the results of the one-way ANOVA test, the difference in the average chlorophyll-a content in each treatment was affected by the difference in the power of UV-C light for 30 minutes during the culture period ( $P < 0.05$ ). Determination of the UV-C irradiation duration of 30 minutes during the culture period was based on the fact that this duration is the best duration for microalgae (Singh *et al.* 2019). The best power for giving UV-C light was treatment D (30W). This is in accordance with the statement of Dawes (1981) that proportional light intensity will provide an opportunity for *C. vulgaris* to carry out photosynthesis optimally. Microalgae show defense or protection mechanisms to adapt to increased exposure to UV-C radiation (Xue *et al.* 2005). Under the pressure from ultraviolet radiation, they sacrifice chloroplasts in order to protect cells and affect the enzymes involved in the chlorophyll synthesis pathway (Barufi *et al.* 2012). Peroxidase enzymes have functions related to the process of resistance to oxidative stress (Yanti 2011).

Based on the average value of chlorophyll-a content in this study, Treatment D of 30 Watt had the highest chlorophyll-a value with a value of 3.52 mg/ml, followed by Treatment C of 15 Watt (2.44 mg/ml), Treatment B of 8 Watt (1.8 mg/ml), and Treatment A as Control (1.67 mg/ml). The application of UV-C light gave a higher pigment yield compared to the control due to environmental stress factors. Chlorophyll is a secondary metabolite of microalgae that can be synthesized by cells when oxidative stress occurs from the environment. Chloroplast cells lack nutrients when exposed to intense UV-C rays, and algae produce high levels of chlorophyll pigment. This

indicates a survival mechanism for algae, so that the production of chlorophyll pigment serves as a shield to protect algae. Oxidative stress can lead to chlorophyll accumulation due to changes in secondary metabolite pathways in microalgae, one of which is strong light (Asker and Ohta, 1999; Stafsnes *et al.* 2010).

*C. vulgaris* is an autotrophic microalga which can form organic compounds with the help of energy from light. Light plays a role in the process of photosynthesis, where light energy is converted into chemical energy by chlorophyll activity. In general, photosynthesis increases as the light intensity increases to a certain optimum value. Above the optimum value, light is an inhibitor of photosynthesis, while light below the optimum value is a limiting light so that the presence of light determines the shape of the growth curve of microalgae carrying out photosynthesis (Edward 2010). According to Gunawan (2012), the required light intensity depends on the cultivation volume and microalgae density. The higher the cultivation density and volume, the higher the light intensity required. Samples treated with UV-C showed different photosynthetic activity with high light intensity, which significantly increased photosynthesis after UV-C treatment (Pfendler *et al.* 2018)

Environmental stress occurred in treatment D of 30W, which received the highest score compared to other treatments. This is in line with research conducted by Mona *et al.* (2016), stating that culture using UV-C light of 15W and 30W for 30 minutes obtained the highest chlorophyll-a value in the 30W UV-C treatment. This is further supported by the statement of Chairat *et al.* (2013) that the chlorophyll content was higher in samples given UV-C treatment than those that were not treated. Research conducted by Pfendler *et al.* (2018) suggesting that when *C. vulgaris* samples were exposed to UV-C light for 0.5 minutes, the chlorophyll-a content increased after 6-24 hours with the highest chlorophyll-a value of 2.5 mg/ml.

The lowest chlorophyll-a content was obtained in Treatment A as a control with a chlorophyll value of 0.19±0.22 mg/ml. The light intensity values of TL lamps ranged from 1957-2066 lux without the help of UV exposure to carry out photosynthesis, thus affecting the growth rate of microalgae. The small amount of chlorophyll-a pigment and low concentration in *C. vulgaris* resulted in light absorption that was less optimal due to light reception that is continuous and has high intensity (>1000 lux). According to Dewi and Winanto (2022) continuous lighting with high intensity also causes an imbalance in the ability of pigments to absorb light,

and under these conditions it will ultimately reduce the intensity of the photosynthesis process.

A good culture of *C. vulgaris* is influenced by the condition of the culture media when rearing. The results of water quality measurements during the study in the form of pH ranged from 7.19-7.80, water temperature quality ranged from 26.3-29.3°C, water salinity ranged from 35-39 ppt, dissolved oxygen (DO) ranged from 32.1-4.89 mg/L, and light intensity ranged from 298-2066 lux. The optimal range of water quality parameters of *C. vulgaris* included pH of 7-9 (Suthar and Verma 2018), temperature of 20-30°C (Effendi 2003), salinity of 30-35 ppt (Kawaroe *et al.* 2016), DO of 3.01-4.57 mg/L (Rahman *et al.* 2022), and UV-C light intensity of 500-5000 lux (Boroh *et al.* 2019). All supporting parameters were in accordance with the optimal range. Dissolved oxygen (DO) has a fairly low value but microalgae can still live. Oxygen is needed by *C. vulgaris* for respiration, and DO in water comes from photosynthesis and diffusion from the air. According to Garibaldi (2012), phytoplankton can live well at dissolved oxygen concentrations of more than 2 mg/L. The DO value in microalgae can be affected by temperature and light intensity in the culture media (Nurhayati *et al.* 2013).

ANOVA statistical test ( $P < 0.05$ ) on carotenoids of *C. vulgaris* exposed to UV-C rays at different powers for 30 minutes affected the average carotenoid content. The difference in carotenoid content results occurred due to the provision of UV-C rays with different light intensities for each treatment. Carotenoids can absorb radiation from UV-C light, which causes these microalgae to survive in environmental stress conditions. Borderie *et al.* (2014) stated that radiation with high intensity from UV-C light can cause high photosensitization of cell molecules.

Treatment D (UV-C 30W) produced the highest carotenoid content, accounting for 1.75±0.06 mg/ml, followed by Treatment C (UV-C 15W) of 1.58±0.11 mg/ml, Treatment B (UV -C 8W) of 1.30±0.05 mg/ml and Treatment A (control) of 1.18±0.15 mg/ml. This is similar to research conducted by Mona *et al.* (2016) that culture using 15W and 30W UV-C lamps for 30 minutes obtained the highest carotenoid values in the 30W UV-C treatment. According to Xue *et al.* (2005), exposure to radiation from UV-C rays will make microalgae form defences and protection to adapt to the environment. UV radiation can activate oxygen to become free radicals (Bold and Wynne 1985). Carotenoids can be synthesized by cells under oxidative stress from the environment. Light activates transmembrane pathways in the cells of *C. vulgaris*, thereby changing the location of gene

expression in the nucleus and cytoplasm. The results of this gene arrangement are sent to the chloroplast and can cause increased carotenoid production (Ramos *et al.* 2011).

The treatment with the lowest carotenoid content was found in treatment B (UV-C 8W) of  $1.30 \pm 0.05$  mg/ml. The application of UV-C light with high power can affect the carotenoid content in *C. vulgaris* as free radicals that cause oxidative stress in the environment are synthesized by carotenoids. According to Febriani *et al.* (2020) inappropriate environmental conditions, such as high light intensity, can cause an imbalance in the physiology of microalgae, and consequently microalgae will synthesize carotenoids and make the levels of carotenoids increase, thereby forming self-defence for microalgae. Light is absorbed by photosynthetic pigments in chloroplasts and is converted into chemical energy in the form of ATP and NADPH through the light reactions. Chemical energy is used to reduce carbon dioxide (CO<sub>2</sub>) into organic compounds during the dark reaction (Prihantini *et al.* 2005).

In conclusion, based on the results of research regarding determining the effect of oxidative stress by UV-C light with different powers on the chlorophyll a, and carotenoid content, the results showed a significant effect of UV-C light. The best treatment was found in the administration of 30W UV light, with the results of growth ( $4.7746 \times 10^6$  cells/ml), the content of carotenoids (1.75 mg/ml) and chlorophyll-a (4.67 mg/ml) of *C. vulgaris*.

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