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Research Article

Microalga *Chlorella sorokiniana* Response to Salinity: Effects on Cell Density, Size, and Pigment Accumulation

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Salinity

Abstract: Microalgae, such as those from the genus *Chlorella,* produce biochemical compositions such as lipids, protein, and pigment. This research investigated the effects of different salinity levels in a nutrient medium on the growth and pigment synthesis of *Chlorella sorokiniana*. Microalga *C. sorokiniana* was cultured and grown in 500 mL glass bottle with varying concentrations of sodium chloride (10,15, 20, and 25 g L^{-1} NaCl) in a BG-11 medium, starting at an initial cell density of 2.68 x 10^5 cell mL^{-1}. The cultures were maintained at 20 ± 1 °C, under continuous aeration, with a light intensity of 200 µmol photons m^{-2} s^{-1}, a 24 h light photoperiod, and pH 7.5 ± 0.2. The results revealed that the optimal salinity concentration for enhancing the cell density, and the specific growth was 10 g L^{-1}, demonstrating the highest cell density, exceeding the control group by 1.27-fold cell mL^{-1} at day 15 of the culture period. Additionally, the specific growth rate (SGR) was significantly higher in the 10 g L^{-1} of salinity concentration, achieving (0.05 ±0.14 day^{-1}) as early as day 6 of the culture period compared to the other experimental groups. Cell size also increased significantly with 20 g L^{-1} of salinity concentration (49.91 ± 2.39 µm). Regarding the pigment accumulation, total carotenoid levels and chlorophyll-a, the elevated salinity concentration of 20 g L^{-1} suppresses chlorophyll-a accumulation and exhibited a reduction in total carotenoid pigment accumulation. Thus, these findings suggest that lower salinity levels (10 g L^{-1} NaCl) can effectively enhance the growth of *Chlorella sorokiniana*, while higher salinity levels (20 g L^{-1} NaCl) tend to suppress pigment production, particularly chlorophyll-a and total carotenoids accumulation.

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1. Introduction

Microalgae are a diverse group of sunlight-powered organisms, ranging from single-celled species to more complex multicellular forms. They exhibit polyphyletic evolution across multiple biological kingdoms. It is estimated that 200 000 to 800 000 algal species exist worldwide, with

approximately 35,000 officially classified (Ebenezer et al., 2012; Guiry et al., 2014). These microorganisms flourish in a wide range of environments, including marine, freshwater ecosystems, and extreme conditions like hypersaline lakes and hot springs (Richmond, 2013; Singh and Saxena, 2015; Sarri and Elp, 2024). As primary producers, microalgae are essential to aquatic ecosystems, serving as the foundation of the food web and significantly contributing to global oxygen production (Richmond, 2004; Koç et al., 2024). Beyond their ecological importance, microalgae possess immense technological potential as an eco-friendly source of high-value bioactive compounds. They are known for producing high-value biomolecules, including pharmaceuticals, nutraceuticals, and biofuels (Chisti, 2007). Expanding the production and applications of microalgae is essential for enhancing their role in the global and European bioeconomy. However, achieving this requires overcoming technological and regulatory challenges, such as efficient water management, while maintaining optimal growth conditions for different microalgal strains. Additionally, integrating microalgae cultivation with its sustainability can be further enhanced through wastewater treatment and nutrient recovery systems (Wang et al., 2017; Verdelho Vieira et al., 2022).

Chlorella sp. is one of the most extensively researched and commercially utilized microalgae. This unicellular, eukaryotic, and non-motile freshwater green alga is widely used as a dietary supplement for humans due to its pharmacological health and numerous biological benefits. (Day et al., 2009; Jehlík et al., 2019). *Chlorella* consists of loosely structured, spherical cells ranging from 2 to 4 μm in diameter, classifying them as planktonic organisms (Converti et al., 2009; Ahmad et al., 2013 and 2014). Belonging to the Chlorophyta division within the Trebouxiophyceae family, *Chlorella* species thrive in both marine and freshwater environments (Wu et al., 2001; Lizzul et al., 2018). Driven by its diverse applications, the global *Chlorella* market is projected to grow steadily at an annual rate of 6.3% between 2021 and 2028, with an estimated valuation of USD 412.3 million (Abreu et al., 2023). Moreover, a specific species, *Chlorella sorokiniana*, has garnered significant attention for its high nutritional value and versatile applications. Its biomass is valued for its abundance of vitamins, carbohydrates, and proteins, positioning it as a promising resource for food and animal feed (Lucakova et al., 2022; Kumar et al., 2022). The rich vitamin content of *C. sorokiniana* makes it an ideal feed option for aquaculture systems, while its high protein levels make it serve as a promising substrate for single-cell protein production (Kumar and Das, 2012; Bratosin et al., 2021; Onyeaka et al., 2022). Additionally, *C. sorokiniana* has been reported to produce clean biohydrogen and energy, making it a potential contributor to renewable energy sources (Jiménez-Llanos et al., 2020). It is also widely employed in the production of high-value antioxidants, including α/β-carotene, lutein, α/β-tocopherol, and zeaxanthin (Matsukawa et al., 2000; Bianchini et al., 2024).

Carotenoids and chlorophylls are key components of microalgae, widely utilized across various industries. In the food sector, chlorophylls serve as natural colorants and nutraceuticals, offering multiple health benefits (Lourenço-Lopes et al., 2021; Martins et al., 2023). In pharmaceuticals and cosmetics, these pigments are valued for their anti-mutagenic, anti-inflammatory, and antibacterial properties (Ferruzi et al., 2006; Da Silva Ferreira and San'tAnna, 2017). Carotenoids, known for their antioxidant properties, are utilized as food supplements, natural dyes in human food products, and aquaculture feed colorants (Singh et al., 2019). Additionally, they offer a range of health benefits for both people and animals, acting as antibacterial, anti-inflammatory, and anti-carcinogenic agents in pharmaceutical and cosmetic formulations (Cezare-Gomes et al., 2019; Novoveská et al., 2019; Ogbonna et al., 2021). *Chlorella sorokiniana* is widely recognized for its ability to produce chlorophyll-a, carotenoids, and other essential pigments, which are crucial for photosynthesis and antioxidant defense (Banskota et al., 2024). Beyond their biological role, these pigments support health by helping lower the risk of cardiovascular diseases and certain cancers (Ampofo and Abbey, 2022). Furthermore, chlorophyll pigments are being explored as natural food colorants, providing a sustainable alternative to synthetic additives (Guiry et al., 2014). Although several studies have investigated the effects of salinity on microalgae, most have concentrated on general growth performance or biomass yield, with limited focus on pigment biosynthesis. In the case of *Chlorella sorokiniana*, research has primarily emphasized its nutritional value, antioxidant production, and applications in bioenergy and aquaculture. However, the combined influence of salinity on both growth parameters (such as cell division rate and cell size) and pigment production (chlorophyll-a and carotenoids) remains insufficiently explored. Addressing this gap is essential, as salinity is a critical factor in large-scale cultivation, influencing not only biomass productivity but also the accumulation of high-value bioactive compounds. This study

therefore aims to clarify how different salinity levels shape the growth and pigment profile of *C. sorokiniana*, contributing to the optimization of culture conditions for biotechnological applications. This study hypothesizes that moderate salinity levels enhance the growth of *C. sorokiniana*, whereas higher salinity suppresses pigment accumulation. It investigates the effects of different salinity levels on cell division rate, cell size, and pigment accumulation, such as chlorophyll-a and carotenoid. Understanding how salinity influences growth and biochemical composition is essential, as higher salt concentrations can inhibit cell growth and production. The results of this study will offer important insights into the relationship between salinity, growth performance, and pigment synthesis, contributing to the optimization of *C. sorokiniana* culture.

2. Material and Methods

2.1. Site of the study

This study was conducted over a period of 24 days of culture with sampling performed every three days at the Marine Integrated Laboratory, College of Fisheries, Mindanao State University Tawi-Tawi College of Technology in Sanga-Sanga, Bongao, Tawi-Tawi, Philippines.

2.2. Culture condition

Chlorella sorokiniana was cultured in improvised 500 mL glass bottles with BG-11 medium used as the nutrient source (Erbil et al., 2021; Sarri et al., 2024b). Sodium chloride (NaCl) was then introduced into the flasks at various concentrations, as described in Table 1. Each solution was autoclaved for 20 minutes at 121 °C to ensure sterility using the autoclave portable steam sterilizer (YX24LOJ). The experimental groups were inoculated at an initial density of 2.68 x 10^5 cell mL^{-1}. To ensure statistical robustness, each treatment was performed in triplicate, resulting in three independent replicates per salinity level. The cultures were maintained in a laboratory environment under artificial 24-hour lighting provided by fluorescent lamps. Aeration was facilitated by an air motor, and syringe filters with a 0.2 µm pore size were utilized to prevent contamination. The temperature was meticulously maintained at 20 ± 1 °C using an air conditioner to provide optimal growth conditions throughout the experiment. The pH of the culture medium was maintained to 7.5 ± 0.2 before inoculation and monitored daily to maintain optimal growth conditions. Cultures were maintained under continuous illumination using cool-white fluorescent lamps, providing an average photon flux density of 200 µmol photons m^{-2} s^{-1} at the surface of the culture flasks. A 24-hour photoperiod regime was applied to maximize photosynthetic activity and biomass yield, as continuous light has previously been reported to enhance growth in *Chlorella* species, including *C. sorokiniana* (Sonmez et al., 2023; Yılmaz Öztürk et al., 2024). While natural light–dark cycles are common, continuous illumination has been widely adopted in laboratory-scale cultivation to optimize productivity. Figure 1 illustrates the layout of the experimental design, showing the arrangement of replicate flasks for each treatment under randomized positioning to minimize environmental bias.

Figure 1. Layout of experimental design.

Table 1. Experimental treatments with salinity (NaCl) in a nutrient medium in *C. sorokiniana*

Experimental treatment	NaCl (g L^{-1})	BG-11 Medium (ml L^{-1})
Group 1 (Control)	0	10
Group 2	10	10
Group 3	15	10
Group 4	20	10
Group 5	25	10

2.3. Growth analysis

Microalgae *C. sorokiniana* samples were collected for counting and analysis every three days. A Neubauer hemocytometer was used to count cells under a light microscope, and regular visual checks for contamination were performed. The specific growth rate (μ) was calculated using the method described by Sanuddin et al.,2023.

$$\mu = \frac{\ln(M_2) - \ln(M_1)}{t_2 - t_1} \quad (1)$$

Where: M_2 is the cell number at the time (t_2).
M_1 is the initial cell number at a time (t_1).

2.4. Cell size measurement

Images were taken and uploaded to ImageJ software, which was created by the National Institutes of Health in the United States. This software was utilized to conduct measurements on individual cell clusters and a total of 10 randomly selected cells were measured.

2.5. Pigment analysis

Pigment analysis was conducted for the *C. sorokiniana* experimental groups following the methods described by Durmaz and Erbil (2020). From each experimental group, a 5 mL sample was collected, which then underwent centrifugation at 3,500 rpm for 10 minutes. After centrifugation, the supernatants were collected, and the water was removed. Next, 5 mL of methanol was added to the microalgae samples, followed by 30 seconds of vortexing. The samples were then centrifuged at 3500 rpm for 10 minutes. The obtained supernatant was analyzed using a spectrophotometer, measuring absorbance at 666 nm for chlorophyll-a and 475 nm for total carotenoids. Pigment concentrations were then determined using the equations below.

$$\text{Chlorophyll-a } (\mu g/ml) = 13.9\ A_{666} \quad (2)$$

$$\text{Total carotenoids } (\mu g/ml) = 4.5\ A_{475} \quad (3)$$

2.6. Statistical Analysis

The collected data on the growth and pigment accumulation of *C. sorokiniana* cultured in different concentrations of salinity were analyzed using IBM SPSS software version 20 at a significance level of $p<0.05$. The data were expressed as the mean ± standard error of the mean (SEM). Statistical significance was evaluated using One-way Analysis of Variance (ANOVA), while Levene's Test assessed the homogeneity of variance. Duncan's Post-Hoc Test was conducted to rank the means (Hairol et al., 2022; Sanuddin et al., 2023; Sarri et al., 2024c).

3. Results

3.1. Cell density

Below demonstrates the cell density of *C. sorokiniana* cultured at different concentrations of salinity in nutrient medium (Figure 2). The initial density of *C. sorokiniana* was started at 2.68 x 10^5 cells mL^{-1}, and the culture was done in triplicate. Based on the result of this study, the maximum cell density of groups 1, 2, 3, and 4 were 23 ± 1.73 x 10^6 cell mL^{-1}, 29.3 ± 3.08 x 10^6 cell mL^{-1}, 14.6 ± 1.18 x 10^6 cell mL^{-1}, 0.71 ± 0.18 x 10^6 cell mL^{-1}, respectively after 15 days of culture period. No growth was observed in group 5 starting from the 12th day of the culture period and onwards. Analysis of variance (ANOVA) revealed that group 2 was significantly higher ($p<0.05$) than the other experimental groups and the control group. Additionally, the mean cell density of group 1 (11.49 ±1.2 x 10^6 cell mL^{-1}) and group 2 (12.29 ± 0.88 x 10^6 cell mL^{-1}) significantly different ($p<0.05$) than the group 3 (6.94 ± 0.51 x 10^6 cell mL^{-1}), group 4 (0.74 ± 0.05 x 10^6 cell mL^{-1}), and group 5 (0.23 ± 0.06 x 10^6 cell mL^{-1}) (Figure 3).

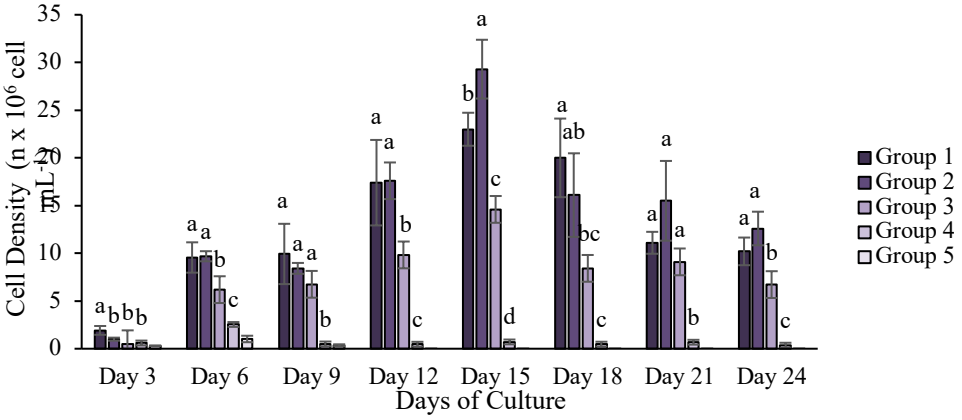

Figure 2. This graph shows the cell density (n x 10^6 cells mL^{-1}) of *C. sorokiniana* grown under different salinity levels (NaCl). The groups are as follows: Group 1 (0 g L^{-1} NaCl – control), Group 2 (10 g L^{-1} NaCl), Group 3 (15 g L^{-1} NaCl), Group 4 (20 g L^{-1} NaCl), and Group 5 (25 g L^{-1} NaCl). Bars that share the same letter are not significantly different (p > 0.05). Data are presented as mean ± SEM (Standard Error Mean).

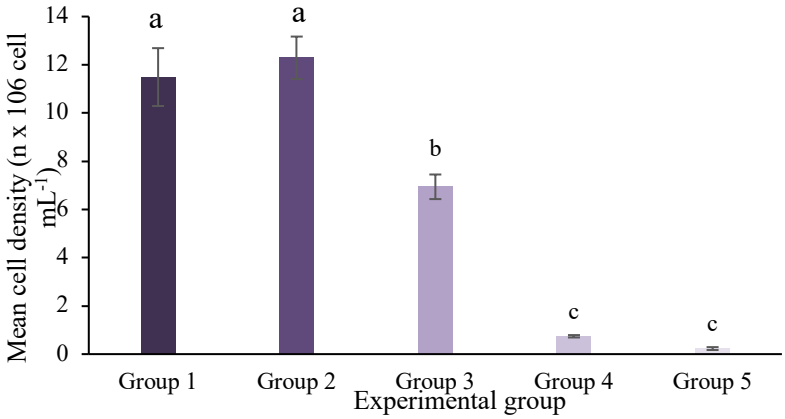

Figure 3. This graph shows the mean cell density (n x 10^6 cells mL^{-1}) of *C. sorokiniana* grown under different salinity levels (NaCl). The groups are as follows: Group 1 (0 g L^{-1} NaCl – control), Group 2 (10 g L^{-1} NaCl), Group 3 (15 g L^{-1} NaCl), Group 4 (20 g L^{-1} NaCl), and Group 5 (25 g L^{-1} NaCl). Bars that share the same letter are not significantly different (p > 0.05). Data are presented as mean ± SEM (Standard Error Mean).

3.2. Specific growth rate

The following demonstrates the specific growth rate (SGR, day^{-1}) of *C. sorokoniana* cultured at different concentrations of salinity in a nutrient medium (Figure 4). Based on the results of this study, the maximum SGR was achieved in group 2 (0.05 ±0.14 day $^{-1}$), which was significantly different (p<0.05) than the control group (0.03 ± 0.02 day $^{-1}$) as early as day 6 of culture period, however, it decreases at the succeeding culture period. Moreover, Figure 5 showed that the mean SGR of groups 1, 2, 3, and 4 were 0.15 ± 0.005 day $^{-1}$, 0.16± 0.006 day $^{-1}$, 0.13± 0.006 day $^{-1}$, and 0.01 ± 0.008 day $^{-1}$, respectively. ANOVA revealed that group 2 (0.16 ± 0.01 day $^{-1}$) was significantly higher (p<0.05) than group 3 (0.01 ± 0.01 day $^{-1}$).

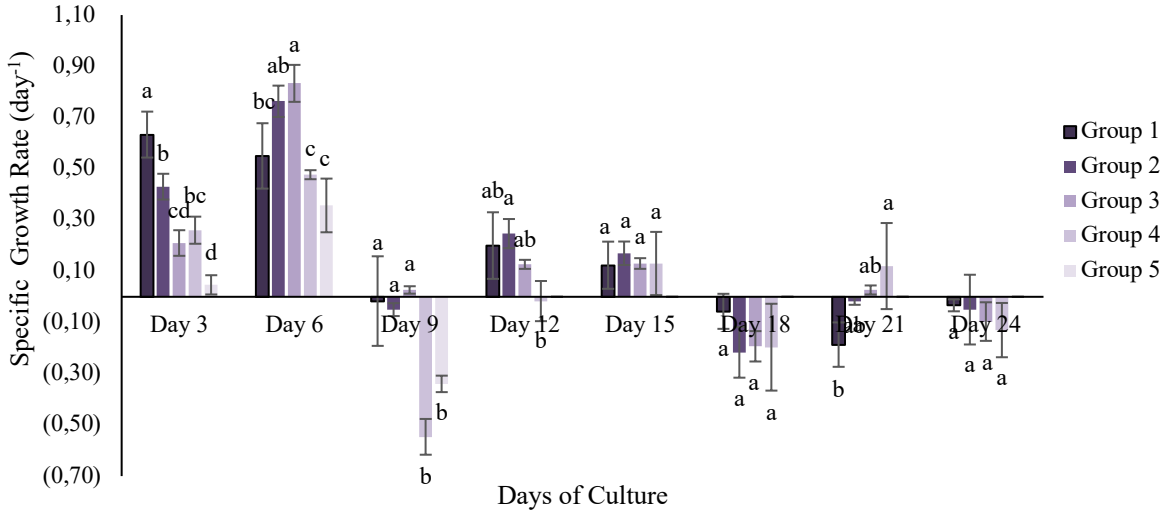

Figure 4. This graph shows the specific growth rate (SGR, day^{-1}) of *C. sorokiniana* grown under different salinity levels (NaCl). The groups are as follows: Group 1 (0 g L^{-1} NaCl – control), Group 2 (10 g L^{-1} NaCl), Group 3 (15 g L^{-1} NaCl), Group 4 (20 g L^{-1} NaCl), and Group 5 (25 g L^{-1} NaCl). Bars that share the same letter are not significantly different (p > 0.05). Data are presented as mean ± SEM (Standard Error Mean).

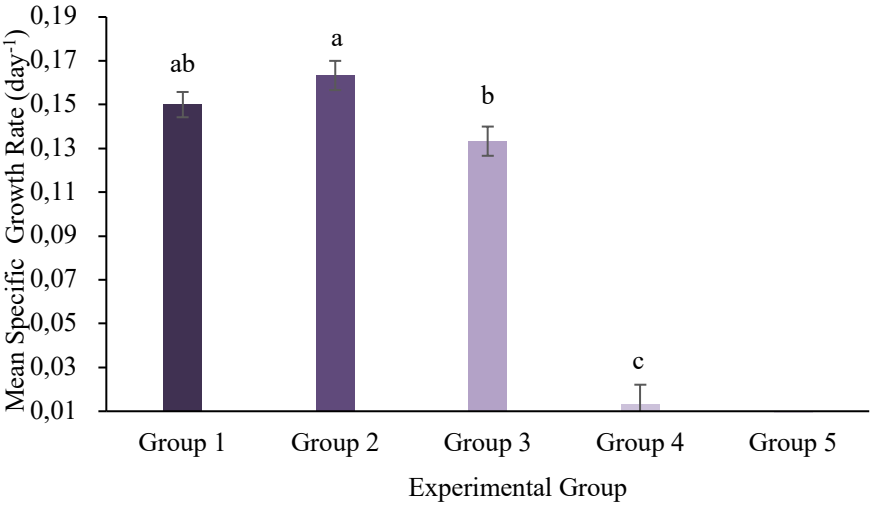

Figure 5. This graph shows the mean SGR (day^{-1}) of *C. sorokiniana* grown under different salinity levels (NaCl). The groups are as follows: Group 1 (0 g L^{-1} NaCl – control), Group 2 (10 g L^{-1} NaCl), Group 3 (15 g L^{-1} NaCl), Group 4 (20 g L^{-1} NaCl), and Group 5 (25 g L^{-1} NaCl). Bars that share the same letter are not significantly different (p > 0.05). Data are presented as mean ± SEM, with n = 15.

3.3. Cell size

Figure 6 revealed the cell size (µm) of *C. sorokiniana* cultured at different concentrations of Salinity in a nutrient medium. The ANOVA revealed that group 4 (49.91 ± 2.39 µm) was significantly different (p<0.05) from group 2 (14.68 ± 0.66 µm), group 3 (42.64 ± 2.05 µm), and the control group. This indicates that the addition of Salinity concentration in nutrient medium increases the cell size of *C. sorokiniana* culture.

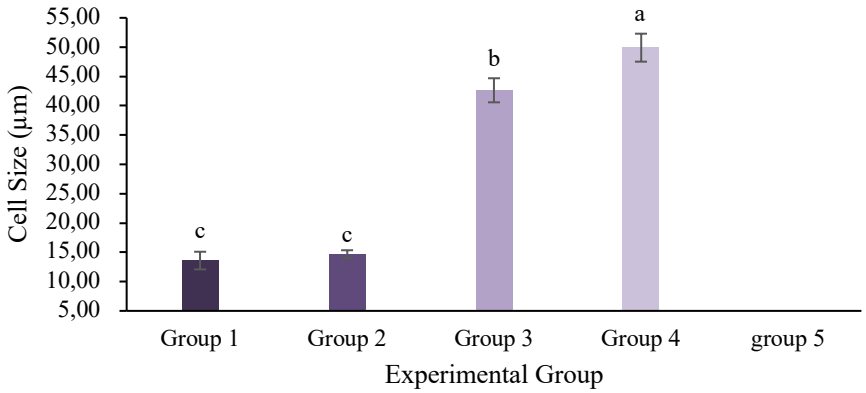

Figure 6. This graph shows the cell size (µm) of *C. sorokiniana* grown under different salinity levels (NaCl). The groups are as follows: Group 1 (0 g L^{-1} NaCl – control), Group 2 (10 g L^{-1} NaCl), Group 3 (15 g L^{-1} NaCl), Group 4 (20 g L^{-1} NaCl), and Group 5 (25 g L^{-1} NaCl). Bars that share the same letter are not significantly different (p > 0.05). Data are presented as mean ± SEM (Standard Error Mean).

3.4. Chlorophyll-a pigment

Figure 7 demonstrates the chlorophyll-a pigment accumulation of *C. sorokiniana* cultured at different concentrations of salinity in a nutrient medium. Based on the results of the study, the chlorophyll-a pigment accumulation in groups 1, 2, 3, and 4 were 13.38 ± 2.30 µg mL^{-1}, 13.17 ± 1.62 µg mL^{-1}, 12.73 ± 2.24 µg mL^{-1}, and 0.48 ± 0.04 µg mL^{-1}, respectively. ANOVA revealed that group 1 (13.38 ± 2.30 µg mL^{-1}), group 2 (13.17 ± 1.62 µg mL^{-1}), and group 3 (12.73 ± 2.24 µg mL^{-1}) were significantly different (p<0.05) from group 4 (0.48 ± 0.04 µg mL^{-1}). The figure indicates that increasing salinity concentrations enhanced chlorophyll-a pigment accumulation in *C. sorokiniana* cultures. Additionally, Figure 8 revealed that the cellular chlorophyll-a (pg. $cell^{-1}$) pigment accumulation in groups 1, 2, 3, and 4 were 1.31 ± 0.10 pg. $cell^{-1}$, 1.07 ± 0.15 pg. $cell^{-1}$, 1.96 ± 0.43 pg. $cell^{-1}$, 1.36 ± 0.21 pg. $cell^{-1}$, respectively. ANOVA revealed that group 3 was significantly different (p<0.05) from Group 2.

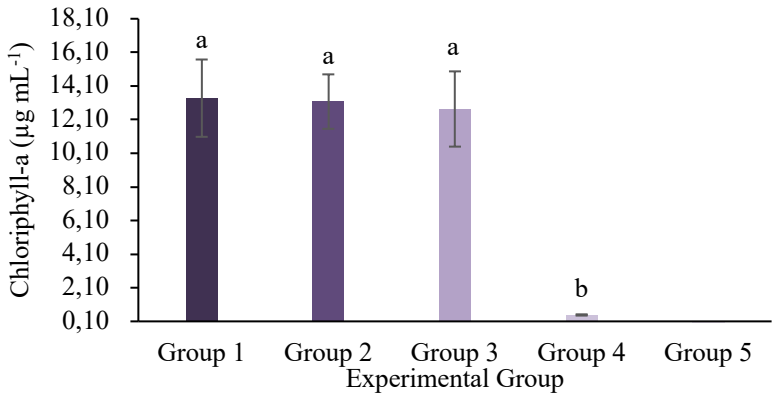

Figure 7. This graph shows the chlorophyll-a pigment accumulation of *C. sorokiniana* grown under different salinity levels (NaCl). The groups are as follows: Group 1 (0 g L^{-1} NaCl – control), Group 2 (10 g L^{-1} NaCl), Group 3 (15 g L^{-1} NaCl), Group 4 (20 g L^{-1} NaCl), and Group 5 (25 g L^{-1} NaCl). Bars that share the same letter are not significantly different (p > 0.05). Data are presented as mean ± SEM (Standard Error Mean).

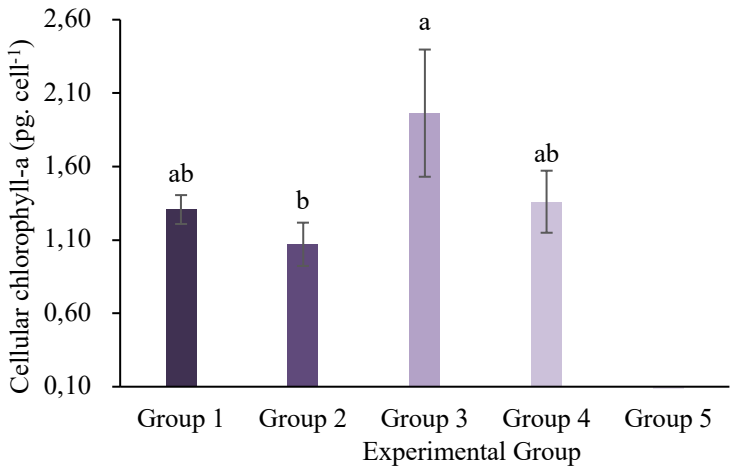

Figure 8. This graph shows the cellular chlorophyll-a (pg. cell^{-1}) of *C. sorokiniana* grown under different salinity levels (NaCl). The groups are as follows: Group 1 (0 g L^{-1} NaCl – control), Group 2 (10 g L^{-1} NaCl), Group 3 (15 g L^{-1} NaCl), Group 4 (20 g L^{-1} NaCl), and Group 5 (25 g L^{-1} NaCl). Bars that share the same letter are not significantly different ($p > 0.05$). Data are presented as mean ± SEM (Standard Error Mean).

3.5. Total carotenoid pigment

Figure 9 shows the total carotenoid pigment accumulation of *C. sorokiniana* cultured at different concentrations of salinity in a nutrient medium. Based on the results of this study, the total carotenoid pigment accumulation in groups 1, 2, 3, and 4 were 4.56 ± 0.73 µg mL^{-1}, 5.77 ± 0.50 µg mL^{-1}, 4.93 ± 0.87 µg mL^{-1}, and 0.09 ± 0.03 µg mL^{-1}, respectively. ANOVA revealed that group 4 was significantly lower (p<0.05) than group 1, group 2, and group 3. Moreover, the cellular total carotenoid pigment accumulation in groups 1, 2, 3, and 4 was 0.45 ± 0.03 pg. cell^{-1}, 0.48 ± 0.08 pg. cell^{-1}, 0.76 ± 0.18 pg. cell^{-1}, 0.24 ± 0.06 pg. cell^{-1}, respectively (Figure 10). ANOVA revealed that group 3 has a significant difference (p<0.05) from the control group.

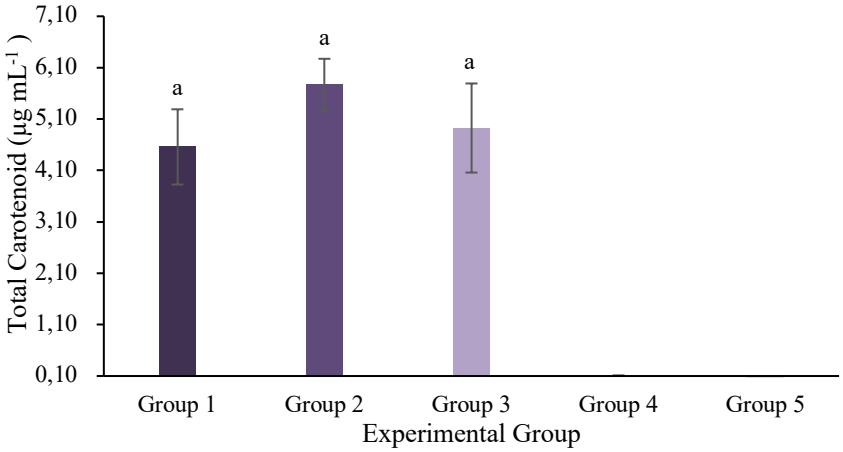

Figure 9. This graph shows the total carotenoid pigment accumulation of *C. sorokiniana* grown under different salinity levels (NaCl). The groups are as follows: Group 1 (0 g L^{-1} NaCl – control), Group 2 (10 g L^{-1} NaCl), Group 3 (15 g L^{-1} NaCl), Group 4 (20 g L^{-1} NaCl), and Group 5 (25 g L^{-1} NaCl). Bars that share the same letter are not significantly different ($p > 0.05$). Data are presented as mean ± SEM (Standard Error Mean).

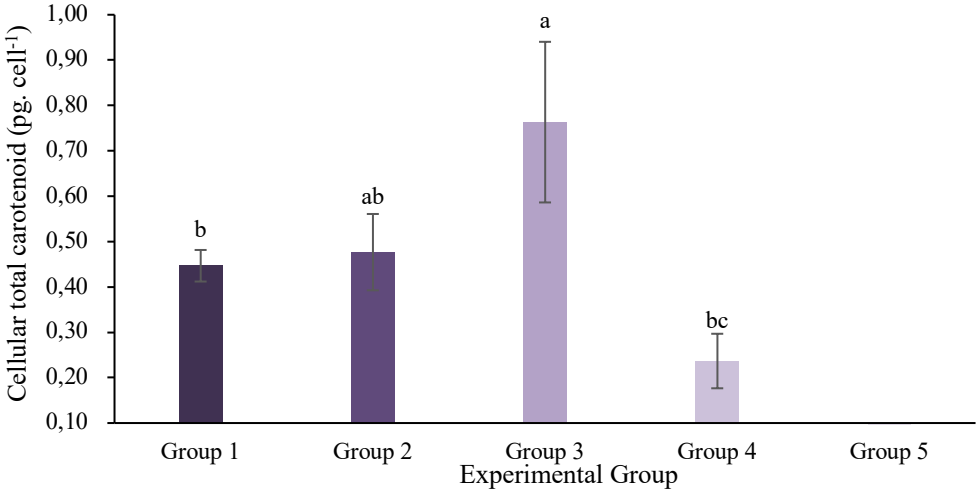

Figure 10. This graph shows the cellular total carotenoid *(*pg. cell^{-1}) of *C. sorokiniana* grown under different salinity levels (NaCl). The groups are as follows: Group 1 (0 g L^{-1} NaCl – control), Group 2 (10 g L^{-1} NaCl), Group 3 (15 g L^{-1} NaCl), Group 4 (20 g L^{-1} NaCl), and Group 5 (25 g L^{-1} NaCl). Bars that share the same letter are not significantly different ($p > 0.05$). Data are presented as mean ± SEM (Standard Error Mean).

4. Discussion

Cell density refers to the number of cells present within a given volume (Chadha et al., 2024; Sarri and Elp, 2024; Jalilul et al., 2025). The results of this study illustrated the influence of different salinity levels on the growth of *Chlorella sorokiniana* cultured in a nutrient medium. Maximum cell density was achieved after 15 days of cultivation, with 10 g L^{-1} salinity yielding the highest density compared to the control group. This indicates that moderate salinity enhances growth, likely by stimulating osmotic balance and ion homeostasis. However, higher salinity concentrations (15–20 g L^{-1}) significantly reduced cell density, and no growth was observed at 25 g L^{-1}, confirming that excessively high salinity surpasses the tolerance threshold of *C. sorokiniana*. Additionally, due to the highest concentration of salinity, particularly the 25 g L^{-1}, the cells exhibited no growth after 12 days of the culture period, emphasizing that salinity at this level exceeded the tolerable limit for *C. sorokiniana* culture. Prior research supports the results of this study, which showed an ideal salinity level for *Chlorella sorokinina* development, followed by inhibition at higher salinities. For instance, Gu et al. (2012) reported that *Nannochloropsis oculata* exhibited better growth in low-salinity conditions, while its growth rate slowed when cultivated in higher salinities. Additionally, *Chlorella capsulata* cultivated at a salinity level of 25 g L^{-1} achieved a maximum cell density of 3.1×10^6 cells mL^{-1} (Ebrahimi and Salarzadeh, 2016). Their study reported a lower cell density compared to the present study, where *Chlorella sorokiniana* cultured in a nutrient medium with a salinity level of 10 g L^{-1} reached a cell density of $9.3 \pm 3.08 \times 10^6$ cells mL^{-1}. This is consistent with the findings of the present investigation, which showed that 10 g L^{-1} of salinity concentration had the maximum cell density.

The specific growth rate (SGR) is a measure of how quickly a population of microorganisms or cells is increasing in size (Koch, 2005; Erbil et al., 2022; Sarri et al., 2025). The present study investigated the effect of salinity on the specific growth rate (SGR) of *Chlorella sorokinina* cultured in a nutrient medium. Cultures at 10 g L^{-1} showed the highest SGR during early growth (day 6), exceeding the control group. However, prolonged exposure reduced the growth rate, suggesting metabolic adjustments to salt stress, such as increased energy allocation toward ion regulation and antioxidant defenses rather than cell proliferation. A sharp decline in SGR at 20 g L^{-1} highlights the detrimental effect of osmotic imbalance, oxidative stress, and impaired nutrient uptake under extreme salinity (Church et al., 2017; Almutairi et al., 2021). Additionally, Pandit et al. (2014) looked at how *Chlorella vulgaris* was affected by salt stress and found comparable patterns. They discovered that growth was initially boosted by low to moderate salt levels.

Cell size refers to the physical dimensions of a cell. It can vary dramatically depending on the type of cell and organism (Dusenbery, 2011; Milo and Phillips, 2015). Cell size increased significantly under

higher salinity conditions, with 20 g L^{-1} cultures exhibiting the largest dimensions. This enlargement is consistent with adaptive responses involving vacuolation, water uptake, and accumulation of compatible solutes (e.g., glycerol, proline) that help maintain turgor pressure under osmotic stress. Mechanistically, cell swelling may also result from altered ion gradients across membranes, leading to changes in cytoplasmic osmolarity (Salama et al., 2013; Ji et al., 2018). In addition, the impact of salt stress on *Chlorella vulgaris* observed a significant increase in cell size at moderate salinity levels (Wang et al., 2016; Pandit et al., 2017). They attributed this phenomenon to osmotic adjustments, where cells actively increase their volume to counterbalance the external osmotic pressure and maintain internal turgor. These findings collectively suggest that the observed increase in cell size in *C. sorokiniana* may represent an adaptive mechanism to cope with osmotic stress. By enlarging their cell volume, the microalgae can potentially dilute the internal solute concentration, thereby mitigating the detrimental effects of high external salinity and maintaining cellular homeostasis (Singh et al., 2024; Zhang et al., 2024). Additionally, the impact of salinity on the growth of *Tetraselmis suecica* culture observed that low salinity levels did not significantly inhibit growth, of which they noted a reduction in cell size compared to optimal salinity conditions (Zhang et al., 2024). This finding suggests that while low salinity may not be detrimental, it might not be sufficient to support maximal growth and cellular proliferation.

Chlorophyll-a, a green pigment found in most photosynthetic organisms like plants, algae, and cyanobacteria, is vital for photosynthesis. It facilitates the conversion of light energy into chemical energy, sustaining the organisms' growth and metabolism (Allakhverdiev et al., 2016; Pareek et al., 2017). The effects of salinity concentration on chlorophyll-a pigment accumulation in *C. sorokiniana* culture demonstrated that chlorophyll-a accumulation (μg mL^{-1}) was consistent across experimental groups with no significant differences. This reduction at higher salinity levels could result from physiological stress, oxidative damage, or metabolic shifts that prioritize survival over pigment synthesis (Zhang et al., 2024). However, at 20 g L^{-1} salinity a statistically significant reduction was observed. This indicates that chlorophyll synthesis is stable under mild–moderate salt stress but becomes suppressed under severe stress, likely due to ROS-mediated degradation of chlorophyll molecules and/or downregulation of chlorophyll biosynthetic pathways (Ali et al., 2016; Kholssi et al., 2023). Additionally, according to Shetty et al. (2017), some microalgae may increase the amount of chlorophyll-a in each cell, hence improving their photosynthetic capability under mild salt stress. They also stated that to increase photosynthetic efficiency under less-than-ideal circumstances, this adaptation may entail the activation of genes related to chlorophyll production or the optimization of light-harvesting complexes.

Carotenoids are pigments that serve as secondary light absorbers in plants, aiding in the capture and conversion of light energy into chemical energy during photosynthesis (Zulfiqar et al., 2021; Kissae et al., 2025). The results demonstrated the effect of salinity concentration on the total carotenoid pigment accumulation in *C. sorokiniana* culture. The total carotenoid accumulation (μg mL^{-1}) was relatively stable in 10 g L^{-1} of salinity concentration, 15 g L^{-1} of salinity concentration, and the control group, with no significant differences among experimental groups. However, 20 g L^{-1} of salinity concentration exhibited a drastic and significant reduction compared to the other groups. The observed decrease in total carotenoid concentration at the highest salinity level at 20 g L^{-1} salinity concentration in *C. sorokiniana* culture aligns with findings from previous studies investigating the effects of salt stress on microalgal pigments. For example, a significant reduction in carotenoid content in *Chlorella vulgaris* under high salinity conditions (Kebeish et al., 2014; Annamalai et al., 2016; Ali et al., 2021). They attributed this decline to a combination of increased oxidative stress and the inhibition of carotenoid biosynthesis pathways. Similarly, León et al. (2004) observed a decrease in carotenoid levels in various microalgal species exposed to high salinity, suggesting that excessive salinity can disrupt the metabolic processes involved in carotenoid synthesis (Sun et al., 2018; Elloumi et al., 2020; Ren et al., 2021). These findings collectively indicated that the observed reduction in carotenoid content in *C. sorokiniana* at the highest salinity level may be a common physiological response to salt stress.

Carotenoids are pigments that serve as secondary light absorbers in plants, aiding in the capture and conversion of light energy into chemical energy during photosynthesis (Zulfiqar et al., 2021; Tunio, et al., 2022). The results demonstrated the effect of salinity concentration on the total carotenoid pigment accumulation in *C. sorokiniana* culture. Total carotenoid levels remained relatively stable in the control, 10 g L^{-1}, and 15 g L^{-1} groups, but a significant decline occurred at 20 g L^{-1}. This suggests carotenoids

may provide some protective role at moderate stress by scavenging ROS, but excessive salinity overwhelms their biosynthesis and stability. Previous research has shown that a significant reduction in carotenoid content in *Chlorella vulgaris* under high salinity conditions (Kebeish et al., 2014; Annamalai et al., 2016; Ali et al., 2021). They attributed this decline to a combination of increased oxidative stress and the inhibition of carotenoid biosynthesis pathways. Similarly, León et al. (2004) observed a decrease in carotenoid levels in various microalgal species exposed to high salinity, suggesting that excessive salinity can disrupt the metabolic processes involved in carotenoid synthesis (Sun et al., 2018; Elloumi et al., 2020; Ren et al., 2021). These findings collectively indicated that the observed reduction in carotenoid content in *C. sorokiniana* at the highest salinity level may be a common physiological response to salt stress. Collectively, these findings indicate that *C. sorokiniana* tolerates and even benefits from moderate salinity (up to ~10 g L^{-1}) but experiences severe physiological stress beyond this threshold. The enlargement of cell size under high salinity reflects adaptive osmotic adjustments, but such responses ultimately cannot compensate for the toxic effects of excessive salt exposure.

Conclusion

This study demonstrates that salinity exerts a significant influence on the growth dynamics and pigment production of *Chlorella sorokiniana*. Among the tested treatments, 10 g L^{-1} NaCl proved to be the optimal concentration, yielding the highest cell density and specific growth rate—exceeding the control by 1.27-fold at day 15 and showing faster growth kinetics as early as day 6. In contrast, higher salinity levels, particularly 20 g L^{-1} NaCl, significantly increased cell size but suppressed chlorophyll-a and total carotenoid accumulation, indicating a trade-off between biomass expansion and pigment synthesis under salt stress. These findings suggest that while moderate salinity can enhance the proliferation of *C. sorokiniana*, elevated salinity levels inhibit pigment accumulation, likely due to stress-induced physiological constraints. Overall, the study provides valuable insights for optimizing salinity conditions in controlled microalgal cultivation, balancing growth performance with pigment productivity.

Ethical Statement

Ethical approval is not required for this study because it did not involve human participants or experimental animals. The research focused solely on the cultivation and physiological assessment of microalgae under controlled laboratory conditions.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Author Contributions

Author 3 conceptualized the study. Authors 1 and 2 performed the experiments under the supervision of Authors 3 and 4. All authors participated in manuscript preparation and approved the final version.

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References

Abreu, A. P., Martins, R., & Nunes, J. (2023). Emerging applications of *Chlorella* sp. and spirulina (Arthrospira) sp. *Bioengineering*, *10*(8), 955.

Ahmad, A. L., Yasin, N. M., Derek, C. J. C., & Lim, J. K. (2013). Microfiltration of *Chlorella* sp.: Influence of material and membrane pore size. *Membrane Water Treatment*, *4*(2), 143-155.

Ahmad, A. L., Yasin, N. M., Derek, C. J. C., & Lim, J. K. (2014). Comparison of harvesting methods for microalgae *Chlorella* sp. and its potential use as a biodiesel feedstock. *Environmental Technology*, *35*(17), 2244-2253.

Ali, H. E. A., El-Fayoumy, E. A., Rasmy, W. E., Soliman, R. M., & Abdullah, M. A. (2021). Two-stage cultivation of Chlorella vulgaris using light and salt stress conditions for simultaneous production of lipid, carotenoids, and antioxidants. *Journal of Applied Phycology*, *33*, 227-239.

Allakhverdiev, S. I., Kreslavski, V. D., Zharmukhamedov, S. K., Voloshin, R. A., Korol'Kova, D. V., Tomo, T., & Shen, J. R. (2016). Chlorophylls d and f and their role in primary photosynthetic processes of cyanobacteria. *Biochemistry (Moscow)*, *81*, 201-212.

Almutairi, A. W., El-Sayed, A. E. K. B., & Reda, M. M. (2021). Evaluation of high salinity adaptation for lipid bio-accumulation in the green microalga Chlorella vulgaris. *Saudi Journal of Biological Sciences*, *28*(7), 3981-3988.

Ampofo, J., & Abbey, L. (2022). Microalgae: Bioactive composition, health benefits, safety and prospects as potential high-value ingredients for the functional food industry. *Foods*, *11*(12), 1744.

Annamalai, J., Shanmugam, J., & Nallamuthu, T. (2016). Salt stress enhancing the production of Phytochemicals in Chlorella vulgaris and Chlamydomonas reinhardtii. *J Algal Biomass Util*, *7*(1), 37-44.

Banskota, A. H., Stefanova, R., Hui, J. P., Bermarija, T., Stemmler, K., McGinn, P. J., & O'Leary, S. J. (2024). Comprehensive Analysis of Biomass from *Chlorella sorokiniana* Cultivated with Industrial Flue Gas as the Carbon Source. *Molecules*, *29*(14), 3368.

Bianchini, C. B., Ramos-Souza, C., Schappo, F. B., Farina, M., De Rosso, V. V., & Nunes, I. L. (2024). Ionic liquid and ultrasound as a fast and innovative combination for improved extraction of *Chlorella sorokiniana*-derived carotenoids. *Algal Research*, *82*, 103650.

Bratosin, B. C., Darjan, S., & Vodnar, D. C. (2021). Single cell protein: A potential substitute in human and animal nutrition. *Sustainability*, *13*(16), 9284.

Cezare-Gomes, E. A., Mejia-da-Silva, L. D. C., Pérez-Mora, L. S., Matsudo, M. C., Ferreira-Camargo, L. S., Singh, A. K., & de Carvalho, J. C. M. (2019). Potential of microalgae carotenoids for industrial application. *Applied biochemistry and Biotechnology*, *188*, 602-634.

Chadha, Y., Khurana, A., & Schmoller, K. M. (2024). Eukaryotic cell size regulation and its implications for cellular function and dysfunction. *Physiological Reviews*, *104*(4), 1679-1717.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, *25*(3), 294-306.

Church, J., Hwang, J. H., Kim, K. T., McLean, R., Oh, Y. K., Nam, B., ... & Lee, W. H. (2017). Effect of salt type and concentration on the growth and lipid content of *Chlorella vulgaris* in synthetic saline wastewater for biofuel production. *Bioresource Technology*, *243*, 147-153.

Converti, A., Casazza, A. A., Ortiz, E. Y., Perego, P., & Del Borghi, M. (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing: Process Intensification*, *48*(6), 1146-1151.

Da Silva Ferreira, V., & Sant'Anna, C. (2017). Impact of culture conditions on the chlorophyll content of microalgae for biotechnological applications. *World Journal of Microbiology and Biotechnology*, *33*(1), 20.

Day, A. G., Brinkmann, D., Franklin, S., Espina, K., Rudenko, G., Roberts, A., & Howse, K. S. (2009). Safety evaluation of a high-lipid algal biomass from *Chlorella* protothecoides. *Regulatory Toxicology and Pharmacology*, *55*(2), 166-180.

Durmaz, Y., & Erbil, G. Ç. (2020). Comparison of industrial-scale tubular photobioreactor to FRP (fiberglass reinforced plastic) panel photobioreactor on outdoor culture of *Nannochloropsis oculata* in the marine hatchery. *Ege Journal of Fisheries & Aquatic Sciences (EgeJFAS)/Su Ürünleri Dergisi*, *37*(3), 303-308.

Dusenbery, D. B. (2011). *Living at micro scale: the unexpected physics of being small*. Harvard University Press.

Ebenezer, V., Medlin, L. K., & Ki, J. S. (2012). Molecular detection, quantification, and diversity evaluation of microalgae. *Marine Biotechnology*, *14*, 129-142.

Ebrahimi, E., & Salarzadeh, A. (2016). The effect of temperature and salinity on the growth of *Skeletonema costatum* and *Chlorella capsulata* in vitro. *International Journal of Life Sciences*, *10*(1), 40-44.

Elloumi, W., Jebali, A., Maalej, A., Chamkha, M., & Sayadi, S. (2020). Effect of mild salinity stress on the growth, fatty acid and carotenoid compositions, and biological activities of the thermal freshwater microalgae Scenedesmus sp. *Biomolecules*, *10*(11), 1515.

Erbil, G. Ç., Durmaz, Y., & Mahmut, E. L. P. (2021). Indoor Growth Performance of *Chlorella* sp. Production at Tubular Photobioreactor. *Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi*, *7*(2), 90-95.

Erbil, G. Ç., Elp, M., & Durmaz, Y. (2022). Phycoerythrin accumulation of *Porphyridium cruentum* culture at indoor tubular photobioreactor. *Yuzuncu Yıl University Journal of Agricultural Sciences*, *32*(1), 81-88.

Ferruzzi, M. G., Böhm, V., Courtney, P. D., & Schwartz, S. J. (2002). Antioxidant and antimutagenic activity of dietary chlorophyll derivatives determined by radical scavenging and bacterial reverse mutagenesis assays. *Journal of Food Science*, *67*(7), 2589-2595.

Gu, N., Lin, Q., Li, G., Tan, Y., Huang, L. and Lin, J. 2012. Effect of salinity on growth, biochemical composition, and lipid productivity of *Nannochloropsis oculata* CS 179. *Engineering in Life Sciences, 12*(6), 631-637.

Guiry, M. D., Guiry, G. M., Morrison, L., Rindi, F., Miranda, S. V., Mathieson, A. C., ... & Garbary, D. J. (2014). AlgaeBase: an on-line resource for algae. *Cryptogamie, Algologie*, *35*(2), 105-115.

Hairol, M., Nian, C., Imlani, A., Tikmasan, J., & Sarri, J. (2022). Effects of Crab Shellmeal Inclusions to Fishmeal Replacement on the Survival, Growth, and Feed Utilization of Mangrove Crab *Scylla serrata* (Forsskal 1775). *Yuzuncu Yıl University Journal of Agricultural Sciences*, *32*(4), 714-726.

Jalilul, J. N. M., Jeva, M. A., Sarri, J. H., Robles, R. J. F., & Jamil, W. M. (2025). Evaluation of AMPEP as a natural biostimulant for enhancing biomass and pigment yield in *Chlorella sorokiniana*. *Jurnal Ilmiah Perikanan dan Kelautan, 17*(3):724-735

Jehlík, T., Kodrík, D., Krištůfek, V., Koubová, J., Sábová, M., Danihlík, J., ... & Čapková Frydrychová, R. (2019). Effects of *Chlorella* sp. on biological characteristics of the honeybee *Apis mellifera*. *Apidologie*, *50*, 564-577.

Ji, X., Cheng, J., Gong, D., Zhao, X., Qi, Y., Su, Y., & Ma, W. (2018). The effect of NaCl stress on photosynthetic efficiency and lipid production in freshwater microalga—*Scenedesmus obliquus* XJ002. *Science of The Total Environment*, *633*, 593-599.

Jiménez-Llanos, J., Ramírez-Carmona, M., Rendón-Castrillón, L., & Ocampo-López, C. (2020). Sustainable biohydrogen production by *Chlorella* sp. microalgae: A review. *International Journal of Hydrogen Energy*, *45*(15), 8310-8328.

Kebeish, R., El-Ayouty, Y., & Hussein, A. (2014). Effect of salinity on biochemical traits and photosynthesis-related gene transcription in Chlorella vulgaris. *Egyptian Journal of Botany*, *54*(2), 281-294.

Kholssi, R., Lougraimzi, H., & Moreno-Garrido, I. (2023). Influence of salinity and temperature on the growth, productivity, photosynthetic activity and intracellular ROS of two marine microalgae and cyanobacteria. *Marine Environmental Research*, *186*, 105932.

Kissae, A. N. H., Sarri, J. H., Amlani, M. Q., Jumsali, M. H., Jamil, W. M., Imbuk, E. S., Jalilul J. N. M., Jeva M. A., & Hairol, M. D. (2025). Incorporation of salinity and AMPEP concentration in nutrient medium on dry weight, cell density, growth response, and pigment accumulation of *Nannochloropsis* sp. culture. *Aquaculture, Aquarium, Conservation & Legislation*, *18*(3), 1593-1604.

Koç, N., Barbaros, S., Çelik, E., Uğuz, S., Şimşek, E., & Yaslıoglu, E. (2024). Removal of Nitrogen and Phosphorus from Liquid Dairy Manure using Microalgae. *Yuzuncu Yıl University Journal of Agricultural Sciences*, *34*(4), 571-583.

Koch, A. L. (2007). Growth measurement. *Methods for General and Molecular Microbiology*, 172-199.

Kumar, K., & Das, D. (2012). Growth characteristics of *Chlorella sorokiniana* in airlift and bubble column photobioreactors. *Bioresource Technology*, *116*, 307-313.

Kumar, R., Hegde, A. S., Sharma, K., Parmar, P., & Srivatsan, V. (2022). Microalgae as a sustainable source of edible proteins and bioactive peptides–Current trends and future prospects. *Food Research International*, *157*, 111338.

Lizzul, A. M., Lekuona-Amundarain, A., Purton, S., & Campos, L. C. (2018). Characterization of *Chlorella sorokiniana*, UTEX 1230. *Biology*, *7*(2), 25.

Lourenço-Lopes, C., Carreira-Casais, A., Fraga-Corral, M., Garcia-Oliveira, P., Soria, A., Jarboui, A., ... & Prieto, M. A. (2021). Carotenoids as natural colorful additives for the food industry. In *Natural Food Additives*. IntechOpen.

Lucakova, S., Branyikova, I., & Hayes, M. (2022). Microalgal proteins and bioactives for food, feed, and other applications. *Applied Sciences*, *12*(9), 4402.

Macıas-Sánchez, M.D., Mantell, C., Rodrıguez, M., De La Ossa, E.M., Lubián, L.M., & Montero, O. (2005). Supercritical fluid extraction of carotenoids and chlorophyll *a* from *Nannochloropsis gaditana*. *Journal of Food Engineering*, *66*(2), 245-251.

Martins, T., Barros, A. N., Rosa, E., & Antunes, L. (2023). Enhancing health benefits through chlorophylls and chlorophyll-rich agro-food: A comprehensive review. *Molecules*, *28*(14), 5344.

Matsukawa, R., Hotta, M., Masuda, Y., Chihara, M., & Karube, I. (2000). Antioxidants from carbon dioxide fixing *Chlorella sorokiniana*. *Journal of Applied Phycology*, *12*, 263-267.

Milo, R., & Phillips, R. (2015). *Cell biology by the numbers*. Garland Science.

Novoveská, L., Ross, M. E., Stanley, M. S., Pradelles, R., Wasiolek, V., & Sassi, J. F. (2019). Microalgal carotenoids: A review of production, current markets, regulations, and future direction. *Marine Drugs*, *17*(11), 640.

Ogbonna, J. C., Nweze, N. O., & Ogbonna, C. N. (2021). Effects of light on cell growth, chlorophyll, and carotenoid contents of *Chlorella sorokiniana* and *Ankistrodesmus falcatus* in poultry dropping medium. *J Appl Biol Biotechnol*, *9*, 157-163.

Onyeaka, H., Anumudu, C. K., Okpe, C., Okafor, A., Ihenetu, F., Miri, T., ... & Anyogu, A. (2022). Single cell protein for foods and feeds: A review of trends. *The Open Microbiology Journal*, *16*(1), e187428582206160.

Pandit, P. R., Fulekar, M. H., & Karuna, M. S. L. (2017). Effect of salinity stress on growth, lipid productivity, fatty acid composition, and biodiesel properties in *Acutodesmus obliquus* and *Chlorella vulgaris*. *Environmental Science and Pollution Research*, *24*, 13437-13451.

Pareek, S., Sagar, N. A., Sharma, S., Kumar, V., Agarwal, T., González-Aguilar, G. A., & Yahia, E. M. (2017). Chlorophylls: Chemistry and biological functions. *Fruit and Vegetable Phytochemicals: Chemistry and Human Health, 2nd Edition*, 269-284.

Ren, Y., Sun, H., Deng, J., Huang, J., & Chen, F. (2021). Carotenoid production from microalgae: biosynthesis, salinity responses and novel biotechnologies. *Marine Drugs*, *19*(12), 713.

Richmond, A. (2013). Biological principles of mass cultivation of photoautotrophic microalgae. *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*, 169-204.

Richmond, A. (Ed.). (2004). H*andbook of Microalgal Culture: Biotechnology and Applied Phycology* (Vol. 577). Oxford: Blackwell science.

Salama, E. S., Kim, H. C., Abou-Shanab, R. A., Ji, M. K., Oh, Y. K., Kim, S. H., & Jeon, B. H. (2013). Biomass, lipid content, and fatty acid composition of freshwater *Chlamydomonas mexicana* and *Scenedesmus obliquus* grown under salt stress. *Bioprocess and Biosystems Engineering*, *36*, 827-833.

Sanuddin, N. B., Hairol, M. D., Nian, C. T., Robles, R. J. F., Illud, H. A., Muyong, J. S., Ebbah, J. H., & Sarri, J. H. (2023). Impact of different nutrient enrichment concentrations on the growth of microalga *Nannochloropsis* sp. (Monodopsidaceae) Culture. *Acta Natura et Scientia, 4*(1), 87-93.

Sarri, J. H., & Elp, M. (2024). Optimization of iron, phosphate, and salinity in nutrient medium using response surface methodology for enhancing biochemical composition in *Chlorella* sp. culture. *Algal Research*, *84*, 103747.

Sarri, J. H., Erbil, G. Ç. & Elp, M. (2024b). Impact of Acadian Marine Plant Extract Powder (AMPEP) concentration in nutrient medium on the growth and lipid accumulation of *Chlorella* sp. culture. *Journal of Agricultural Sciences, 30*(4), 658-667.

Sarri, J. H., Erbil, G. Ç., Elp, M., & Kadak, A. E. (2024c). Acceptability of different concentrations of *Chlorella* sp. in Filipino Delicacy Puto as coloring agent. *Yuzuncu Yıl University Journal of Agricultural Sciences, 34*(1), 62-73.

Sarri, J. H., Ibno, D. C. V., Hassan, R. K., & Hairol, M. D. (2024a). Investigation of the effect of AMPEP concentration in nutrient medium on the cell density, growth response, and pigment accumulation of *Nannochloropsis* sp. culture. *AACL Bioflux, 17*(6), 2886-2898.

Sarri, J. H., Imlan, K. H., Ahajan, N. A., Hairol, M. D., Kissae, A. N. H., Yangson, N. A. T., Robles, R. J. F., & Talaid, E. M. (2025). Application of marine plant extract powder (AMPEP) as a nutrient supplement for optimizing *Nannochloropsis* sp. cultivation. *Israeli Journal of Aquaculture-Bamidgeh*, *77*(4), 23-35.

Shetty, P., Gitau, M. M., & Maróti, G. (2019). Salinity stress responses and adaptation mechanisms in eukaryotic green microalgae. *Cells*, *8*(12), 1657.

Singh, D. P., Khattar, J. S., Rajput, A., Chaudhary, R., & Singh, R. (2019). High production of carotenoids by the green microalga *Asterarcys quadricellulare* PUMCC 5.1. 1 under optimized culture conditions. *PloS One*, *14*(9), e0221930.

Singh, J., & Saxena, R. C. (2015). An introduction to microalgae: diversity and significance. In *Handbook of Marine Microalgae* (pp. 11-24). Academic Press.

Singh, R. P., Yadav, P., Sharma, H., Kumar, A., Hashem, A., Abd_Allah, E. F., & Gupta, R. K. (2024). Unlocking the adaptation mechanisms of the oleaginous microalga Scenedesmus sp. BHU1 under elevated salt stress: a physiochemical, lipidomics and transcriptomics approach. *Frontiers in Microbiology*, *15*, 1475410.

Sonmez, M. E., Altinsoy, B., Ozturk, B. Y., Gumus, N. E., & Eczacioglu, N. (2023). Deep learning-based classification of microalgae using light and scanning electron microscopy images. *Micron*, *172*, 103506.

Sun, X. M., Ren, L. J., Zhao, Q. Y., Ji, X. J., & Huang, H. (2018). Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation. *Biotechnology for Biofuels*, *11*, 1-16.

Tunio, A. A., Naqvi, S. H., Rehman, T., Bhutto, M. A., & Mugheri, M. H. (2022). Determination of antioxidant, antimicrobial properties with evaluation of biochemicals and phytochemicals present in Oscillatoria limosa of District Jamshoro, Pakistan. *Yuzuncu Yıl University Journal of Agricultural Sciences*, *32*(3), 538-547.

Verdelho Vieira, V., Cadoret, J. P., Acien, F. G., & Benemann, J. (2022). Clarification of most relevant concepts related to the microalgae production sector. *Processes*, *10*(1), 175.

Wang, T., Ge, H., Liu, T., Tian, X., Wang, Z., Guo, M., ... & Zhuang, Y. (2016). Salt stress induced lipid accumulation in heterotrophic culture cells of Chlorella protothecoides: mechanisms based on the multi-level analysis of oxidative response, key enzyme activity and biochemical alteration. *Journal of Biotechnology*, *228*, 18-27.

Wang, Y., Castillo-Keller, M., Eustance, E., & Sommerfeld, M. (2017). Early detection and quantification of zooplankton grazers in algal cultures by FlowCAM. *Algal Research*, *21*, 98-102.

Wu, H. L., Hseu, R. S., & Lin, L. P. (2001). Identification of *Chlorella* spp. isolates using ribosomal DNA sequences. *Botanical Bulletin of Academia Sinica*, *42*.

Yılmaz Öztürk, B., Dağlıoğlu, Y., Tezcan Ün, Ü., & Dağ, İ. (2024). Phycoremediation potential with ultrastructural and biochemical response of *Kirchneriella lunaris* to metribuzin. *International Journal of Environmental Science and Technology*, 22, 8195–8210.

Zhang, J., Yang, H., Sun, Y., Yan, B., Chen, W., & Fan, D. (2024). The potential use of microalgae for nutrient supply and health enhancement in isolated and confined environments. *Comprehensive Reviews in Food Science and Food Safety*, *23*(4), e13418.

Zou, N., and Richmond, A. (2000). Light-path length and population density in photoacclimation of *Nannochloropsis* sp. (Eustigmatophyceae). *Journal of Applied Phycology, 12*(3-5), 349-354. doi:https://doi.org/10.1023/a:1008151004317

Zulfiqar, S., Sharif, S., Saeed, M., & Tahir, A. (2021). Role of carotenoids in photosynthesis. *Carotenoids: Structure and Function in The Human Body*, 147-187.