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Optimizing Growth Conditions and Biomass Accumulation for *Chlorella vulgaris* of the Western Indian Ocean, Tanzania

Angelina MICHAEL[1*] and Kulwa MTAKI[2]

[1]*Department of Biology, College of Natural and Mathematical Sciences, University of Dodoma, P. O. Box 338, Dodoma, Tanzania.*
[2]*Mafia Island Marine Park, P.O. Box 74, Mafia, Tanzania.*
[*]*Corresponding author; E-mail: onkyangel@yahoo.com, ORCID: 0000-0003-3121-9630*
Co-Author's ORCID: 0000-0001-6947-5862.

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ABSTRACT

Microalgae hold significant potential for addressing global challenges such as climate change, food security, and environmental degradation. However, data on optimal culture conditions for growth and biochemical composition, particularly for microalgae from the Western Indian Ocean, are limited. This study evaluated the growth and biochemical composition of *Chlorella vulgaris* isolated from Tanzanian estuaries. Two experiments were conducted. In the first, *C. vulgaris* was cultured under high (5.5 ± 3.3 Klux) and low (2.9 ± 1.6 Klux) light intensities, with samples collected for biochemical analysis on days 7 and 14. The second experiment examined the effects of salinity (5, 10, 15, 20, and 33 g kg^{-1}) on growth under a light intensity of 2.9 ± 1.6 Klux. Optimal growth was observed at 15 g kg^{-1} salinity level. Higher light intensities increased crude protein and iron content while lower light favored lipid, carbohydrate, and phosphorus accumulation. Shorter culture periods (7 days) enhanced protein, lipid, and carbohydrate levels, whereas longer periods (14 days) increased ash and specific minerals like iron, potassium, and calcium. These findings provide strategies for optimizing *C. vulgaris* growth and composition for continually harnessing its potential for addressing global challenges.

Keywords: *Chlorella vulgaris,* Western Indian Ocean, Salinity and Light Intensity, Laboratory Conditions, Growth Performance, Biochemical Composition.

INTRODUCTION

Microalgae are increasingly recognized for their pivotal role in addressing key global issues like climate change, food security, and environmental degradation. Consequently, they are currently the focus for intensive research and industrial applications (Metsoviti et al., 2020). They have diverse applications in biofuel production, the synthesis of bioactive compounds, and the aquaculture industry (Rai

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et al., 2015; Khatoon et al., 2018; Metsoviti et al., 2020). Their protein-rich biomass offers promise as a solution to growing food security concerns, particularly in the context of aquaculture, where they serve as a natural and nutritionally rich food source for fish and shellfish larvae (Brown 2002; Sirakov et al., 2015). With increasing attention in aquaculture production due to the dwindling capture fisheries, microalgae culture would serve as an alternative to support the sustainable growth of aquatic species, providing a source of nutrition and aiding in the preservation of marine ecosystems. Similarly, in the pharmaceutical industries, microalgae are gaining recognition for their potential to produce bioactive compounds with various medicinal properties. Their ability to synthesize a wide range of secondary metabolites makes them valuable resources for drug discovery and development (Shimizu 2000; De Vera et al., 2018; Khavari et al., 2021).

Among the numerous microalgae genera, *Chlorella* stands out as a microorganism of immense potential for various applications. *Chlorella*, a single-celled green microalgae, possesses an exceptional capacity for photosynthesis and boasts remarkable nutritional value, often earning it the title of a "superfood" (Darwish et al., 2020; Fernández-Ríos et al., 2022). Extensive research have conducted on *C. vulgaris* in various geographical regions, covering the effects of culture media, light sources, and nutrient availability on its growth and biochemical composition (Gonçalves et al., 2014; Metsoviti et al., 2020; Mtaki et al., 2021; Mtaki et al., 2023). Similarly, studies have evaluated the suitable salinity for freshwater *C. vulgaris* microalgae and investigated strategies for enhancing biofuel production through the regulation of culture stresses (Pandit et al., 2017; Yun et al., 2019). However, limited information is available about the specific laboratory conditions that maximize biochemical composition and growth rates for *C. vulgaris*, particularly those originating within the context of the Western Indian Ocean region.

The optimization of laboratory conditions is crucial for scaling up microalgal production and enhancing its potential applications. The variations in environmental parameters such as salinity and light, influence the performance of microalgae either negatively or positively. Microalgae require optimal light intensity, quality, and wavelengths for photosynthesis and biochemical synthesis (Pandit et al., 2017). On the other hand, salinity variation influences microalgae growth performance, physiology, and biochemical processes (Pandit et al., 2017). Cultivation periods also may affect the composition of the specific microalgae. Therefore, this study aimed to investigate the optimal growth conditions and biomass accumulation of *Chlorella vulgaris* under varying salinity levels, cultivation durations, and light intensities for the species isolated from the estuaries of the Western Indian Ocean. The findings of this study will significantly contribute to the sustainable exploitation of *C. vulgaris* for various industrial, environmental, and nutritional applications, promoting economic development while supporting global efforts toward sustainability.

MATERIALS AND METHODS

Microalgae sampling and isolation

The collection of *C. vulgaris* was carried out in estuarine environments across three sites in the Western Indian Ocean namely, Ruvu Estuary (6°22'37"S, 38°51'56"E), Pangani Esturay (5°25'59"S, 38°58'53"E), and Donge Muanda, Zanzibar (5°55'03"S, 39°12'16"E). The sampling was conducted during the spring high tide in both the southeast and northeast monsoon periods to increase the chance of encountering the targeted species. At each site, water samples were collected from stations representing brackish and full-strength salinity conditions. A total of 100 litres of water were sampled per station and concentrated using a plankton net with a 20 µm mesh size. The concentrated crude samples were then transferred into glass bottles and immediately enriched with a few drops of a culture medium, Guillard's F/2

(Kang et al., 2011). The *C. vulgaris* was isolated using a combination of agar plating and serial dilution techniques. Agar plating involved mixing 5 g of plain agar with 100 ml of estuarine waters enriched with Guillard's F/2 culture medium. This technique facilitated the formation of microalgae colonies on the plate, which were then carefully transferred and inoculated into a 50 ml conical flask containing enriched estuarine waters. After several days of gentle aeration, 1 ml of the culture was transferred to a test tube to initiate the serial dilution process. Ten test tubes, each containing 9 ml of the culture medium, were prepared. After incubation for two days, 1 ml of the microalgae culture from one test tube was transferred to another test tube containing fresh culture medium. This transfer process was continuously repeated, accompanied by regular microscopic checks. To ensure aseptic conditions, all flasks and the culture media used in the isolation process were autoclaved for sterilization before use. The identification of the isolated species relied on morphological features observed under a compound light microscope. Based on the observed morphological characteristics, such as a lack of flagella, solitary, and a coccoid shape, the species was identified as *C. vulgaris.*

Experimental design

The successfully isolated species was cultured in a growth chamber in the laboratory at 28-29ºC for 14 days. The culture was illuminated with the artificial light provided by white LED lamps at a photoperiod of 12/12 hours for day and night. The light intensity was measured using a VXLM_636 Vertex light meter. Two different experiments were conducted to investigate the effects of salinity, light intensity, and cultivation period on the growth and biochemical composition of *C. vulgaris*. In both experiments, 100 ml of *C. vulgaris* culture was inoculated with 1900 ml of Guillard's F/2 culture medium in 2000 ml Erlenmeyer flasks. The cultures were provided with gentle aeration throughout the experiments. For experiment one, two light orientations were set to illuminate the culture at either the top or from a side of the flasks. The top light supplied an average intensity of 5.5 ± 3.3 Klux, considered high light intensity, while the side light supplied an intensity of 2.9 ± 1.6 Klux to the culture and was considered low light intensity. Under this experiment, *C. vulgaris* was grown at 25 g kg^{-1} salinity level, reflecting the estuarine environment conditions from which the species was originally collected. Biomass samples for biochemical composition analysis were harvested at two distinct time points i.e. day 7 and day 14. On day 7, a 200 ml subsample was collected from each flask of the main culture, while at the end of the experiment (day 14), the entire culture was harvested. The harvested sample was left to settle for about two hours and the supernatant was decanted and discarded. The concentrate was centrifuged (Model FC5706, Ohaus corporation, Germany) for 5 minutes at 4430 rpm to obtain the wet biomass. The wet biomass was spread on the pre-weighed glass filter and then placed in the oven dryer at 105°C for 24 h. The resulting dry biomass was then ground into a powder and stored in a cool place until further analysis of proximate composition and mineral contents. In experiment two, *C. vulgaris* was cultured at salinity levels of 5, 10, 15, 20, and 33 g kg^{-1} under side light illumination (2.9 ± 1.6 Klux). Salinity levels of 5 to 20 g kg^{-1} were prepared by diluting seawater with tap water, while the 33 g kg^{-1} salinity was achieved by adding sea salt to tap water.

Growth parameters of *C. vulgaris*

The *C. vulgaris* growth was determined in terns of cell density and specific growth rate (SGR). Cell density was determined through daily counting using a hemocytometer and the compound microscope (Olympus, BM 190, Japan). Specifically, one milliliter of a sample was taken from the main culture and placed on the hemocytometer at a magnification of 100X. The SGR of *C. vulgaris* were determined using Equation 1 below. C_f represents the final algae concentration, C_i is the initial algae concentration, and Δt is the difference between the final and initial times (in days).

$$\text{Specific Growth Rate (SGR)} = \text{In}(C_f/C_i)/(T_f - T_i) \quad (1)$$

Chemical composition of *C. vulgaris*

The determination of the biochemical composition, including proximate and mineral content, of *C. vulgaris* was performed using dry biomass. The proximate analysis assessed the percentages of crude protein, lipids, fiber, ash, and soluble carbohydrates. Mineral composition analysis focused on determining the composition of selected individual minerals in the dry biomass. All analyses were performed in triplicate following standard analytical methods.

Quantification of crude protein

Crude protein content in *C. vulgaris* biomass was determined using the semi-micro Kjeldahl digestion method and the indophenol blue colorimetric method (Emteryd 1989; Quarmby and Allen 1989). These methods were used to quantify the total nitrogen concentration in the biomass, which was then converted to protein content by multiplying the organic nitrogen concentration by a factor of 6.25.

Quantification of crude lipid

Lipid content was analyzed using a solvent extraction method based on the protocol by Bligh and Dyer (1959). The method involved the addition of a mixture of three organic solvents (chloroform, methanol, and water) in specific ratios to the polyethylene vial containing 2 g biomass of *C. vulgaris*. After the extraction, the filtrate was transferred into a graduated separating funnel whereas the upper alcoholic layer of methanol and water was discarded while the mixture of chloroform and lipid was retained. The chloroform was then evaporated under vacuum using a rotavapor (Heidolph, Germany) at a temperature of 40°C, and the lipid was oven-dried and weighed to get the percentage of its composition.

Quantification of crude fiber

The crude fiber content was determined gravimetrically after the acid-alkali hydrolysis (Allen 1989; Quarmby and Allen 1989). In this method, 1 g of *C. vulgaris*'s powder was added to boiling sulphuric acid (1.25% v/v) and then washed with boiling water. The extracted sample was added to 1.25% sodium hydroxide for further extraction and then washed with boiling water while suction pumping to separate it from the alkali. After oven drying the extracts at 105°C for 2 hours, the sample was weighed followed by calculation to obtain the composition of fibre in the biomass.

Quantification of carbohydrate

The total soluble carbohydrate content of *C. vulgaris* biomass was extracted using hot water and quantified using a colorimetric method with anthrone reagent, as described by Allen (1989). All other extraction and estimation procedures were performed according to the modifications by Michael et al. (2019).

Quantification of mineral content

Total mineral content of the biomass, termed ash was determined by combusting 1 g of the sample at 550ºC in the muffle furnace for 2 hours. The ash was then weighed and the percentage of composition was calculated. For the determination of the concentration of individual minerals in the dry biomass, the sample was oxidized using a mixture of acids (1 ml per-chloric acid and 5 ml nitric acid) followed by reading the concentration in the Atomic Absorption Spectrophotometer (AA240 Varian, USA). The analyzed minerals were the macro elements calcium (Ca), magnesium (Mg), potassium (K), and phosphorus (P), and two trace minerals namely iron (Fe) and the antioxidant selenium (Se).

Statistical analysis

All collected data were analyzed using R software (version 4.3.2). The normality and homogeneity of data were checked using the Shapiro-Wilk and Levene's tests, respectively. A two-sample t-test was used to analyze the variation in cell density and specific growth rate (SGR) of *C. vulgaris* grown using different light orientation. One-way analysis of variance (ANOVA) was used to analyze the variation in cell density and SGR of *C. vulgaris* grown at different salinity levels. Two-way ANOVA was used to analyze the proximate and mineral composition of *C. vulgaris* biomass. Tukey's post hoc tests were used to compare the mean pairwise between treatments. Results were presented as mean ± SE (standard error of the

mean) and the difference was considered significant when $p \leq 0.05$.

RESULTS

Microalgae growth performance

There was a variation in growth trend among microalgae cultured at different light sources, with those grown under the side light (2.9 ± 1.6 Klux) accumulating more cells than those cultured under top light (5.5 ± 3.3 Klux) (Figure 1). Although there was no significant difference in cell density of *C. vulgaris* cultured in the two light orientations ($p < 0.226$), the side light resulted in higher cell density (29.13 x 10^7 cell/ml) than the top light (18.85 x 10^7 cell/ml) (Figure 1). Similarly, the difference in specific growth rate (SGR) ($p = 0.484$) between the two light sources was not statistically significant (Table 1). In contrast, the salinity significantly impacted the SGR of *C. vulgaris* ($p < 0.001$). The *C. vulgaris* cultured at 15 g kg^{-1} salinity had significantly higher SGR than those cultured in other salinities ($p < 0.001$). The *C. vulgaris* cultivated in 5 and 10 g kg^{-1} salinity had statistically similar SGR, while that cultured in 20 g kg^{-1} salinity showed higher SGR than all except for 15 g kg^{-1} salinity ($p < 0.05$). The highest salinity level (33 g kg^{-1}) resulted in the poorest SGR (0.27 ± 0.12 d^{-1}) among all salinity levels (Table 1).

There was also variation in growth trend among *C. vulgaris* cultured at different salinity levels (Figure 2). The *C. vulgaris* grown at a salinity of 15 g kg^{-1} showed rapid growth, characterized by a short lag phase followed by a prolonged exponential growth phase (days 2-11). In contrast, *C. vulgaris* cultured at salinities of 20 and 33 g kg^{-1} exhibited poor growth, with a brief exponential phase and an early decline (day 5). Those cultured at salinities of 5 and 10 g kg^{-1} grew slowly, displaying short exponential phases and prolonged stationary phases. Furthermore, the variation in salinities had a significant effect on the accumulation of cell density in *C. vulgaris* ($p < 0.014$). The highest cell density (79.49 x 10^6 cell/ml) was observed at 15 g kg^{-1} salinity level, while the lowest cell density (14.43 x 10^6 cell/ml) was recorded in *C. vulgaris* cultured at 33 g kg^{-1} salinity. The cell density did not differ significantly between *C. vulgaris* cultured at 5 and 10 g kg^{-1} salinities ($p < 0.993$). However, the difference was significant between the salinity of 15 g kg^{-1} and all other salinities tested ($p < 0.001$).

Microalgae biomass composition

The proximate and mineral composition of *C. vulgaris* biomass are shown in Table 2. The biomass of C. *vulgaris* was recorded to have a large percentage of crude protein, more than 60% regardless of cultivation period, and light intensity. Both the light orientations and cultivation time and their interaction significantly affected the protein, carbohydrate, lipid, and ash content of *C. vulgaris* biomass ($p < 0.01$). The biomass of *C. vulgaris* harvested on day 7 had the highest crude protein and carbohydrate content under top light and side light, respectively. On the other hand, the microalgae biomass cultured for 14 days under top light had the lowest content of protein and carbohydrates. The lipid content was highest in *C. vulgaris* biomass grown for 14 days under side light, while the lowest value was recorded for microalgae biomass cultured for the same period under top light. The *C. vulgaris* cultured under top light for 14 days had the highest ash content compared to those cultured under other treatments. In addition, the fiber content of microalgae biomass was not affected by the cultivation days ($p = 0.984$). However, the crude fiber content was significantly affected by the light orientation and its interaction with cultivation days ($p < 0.05$). Although the fiber content was highest in *C. vulgaris* biomass cultured under side light for 14 days, it did not differ significantly from those cultured for 7 days under top light ($p > 0.05$).

The cultivation duration, light orientation, and their interaction significantly impacted the content of Fe, Se, K, P, Ca, and Mg in the biomass of *C. vulgaris* ($p < 0.01$). The biomass cultured for 14 days under top light exhibited significantly higher Fe and Se content ($p < 0.001$), although the latter was found in trace amounts. In contrast, there was no significant variation in Fe content observed

in *C. vulgaris* biomass cultured under side light, both on day 7 and 14 ($p < 0.017$). The K and P content was higher in *C. vulgaris* biomass cultured for 14 days under side light ($p < 0.001$). The P content was lowest in *C. vulgaris* biomass grown for 7 days under top light, while the K value was lowest in biomass cultured for 7 days under side light ($p < 0.001$). The biomass of *C. vulgaris* grown for 7 days under side light had the highest Ca content, while those cultured for 7 days under top light had the lowest value ($p < 0.001$). The Mg content of *C. vulgaris* biomass cultured for 7 days under top light was lower than all other treatments ($p < 0.001$).

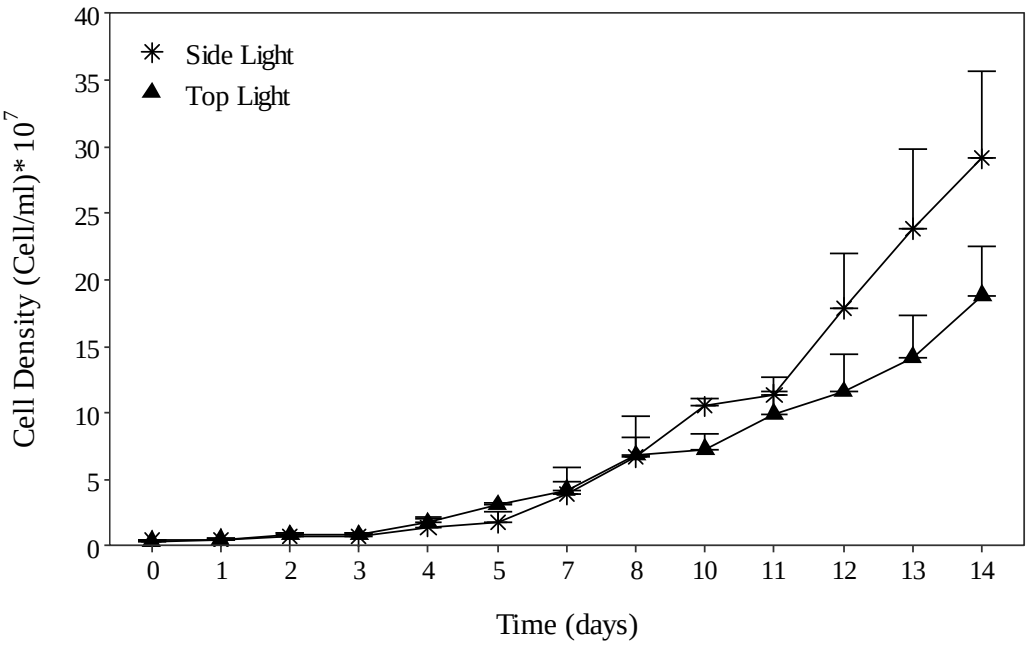

Figure 1: Cell density of *Chlorella vulgaris* grown at the top and light side orientations.

Table 1: The specific growth rate (SGR) of *Chlorella vulgaris* cultured at different salinities and light orientations.

Parameters	
Salinity (g kg^{-1})	**SGR (day^{-1})**
5	0.76 ± 0.07^{a}
10	0.90 ± 0.03^{a}
15	1.11 ± 0.00^{b}
20	0.96 ± 0.02^{c}
33	0.27 ± 0.12^{d}
Light orientation	
Side light	1.62 ± 0.11^{a}
Top light	1.3 ± 0.31^{a}

Means in the same column with different letters were significantly different ($p < 0.05$).

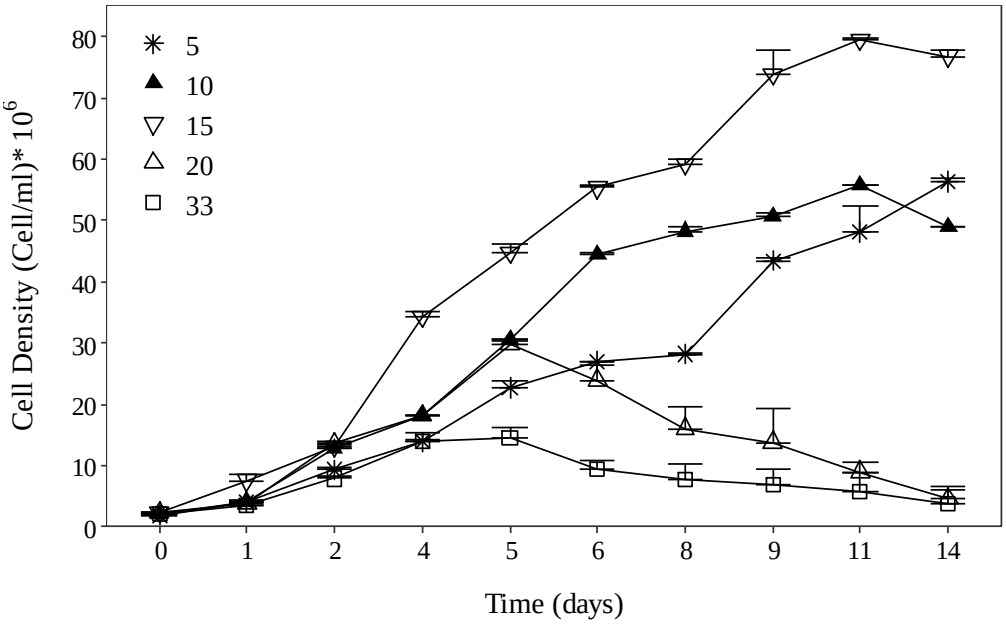

Figure 2: Cell density of *Chlorella vulgaris* cultured at different salinities.

Table 2: Biomass composition of *Chlorella vulgaris* harvested at different cultivation days under side and top light.

Biomass composition	**Cultivation days**			
	7		**14**	
Light	Side	Top	Side	Top
Proximate composition (%)				
Crude protein	61.65 ± 0.01^{a}	72.91 ± 0.44^{b}	68.34 ± 0.01^{c}	63.74 ± 0.01^{d}
Carbohydrate	8.67 ± 0.02^{a}	7.34 ± 0.03^{b}	7.25 ± 0.02^{b}	3.86 ± 0.00 ^{c}
Crude lipid	14.45 ± 0.06^{a}	11.29 ± 0.50^{b}	16.73 ± 0.00^{c}	3.76 ± 0.01^{d}
Crude fiber	1.31 ± 0.02^{ab}	1.44 ± 0.08^{bc}	1.57 ± 0.00^{c}	1.19 ± 0.01^{a}
Ash	9.78 ± 0.00^{a}	5.65 ± 0.01^{b}	12.01 ± 0.33^{c}	14.41 ± 0.14^{d}
Minerals composition (mg/100 g)				
Iron (Fe)	56.24 ± 0.01^{a}	61.70 ± 0.24^{b}	57.58 ± 0.40^{a}	76.25 ± 0.42^{c}
Selenium (Se)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± .0.01^{b}
Potassium (K)	1422.23 ± 0.86^{a}	1631.46 ± 0.70^{b}	1861.08 ± 0.57^{c}	176.17 ± 0.52^{d}
Phosphorus (P)	2857.80 ± 2.23^{a}	1873.88 ± 1.33^{b}	3119.20 ± 4.68^{c}	2181.71 ± 1.49^{d}
Calcium (Ca)	4963.58 ± 0.62^{a}	255.30 ± 1.17^{b}	728.27 ± 3.49^{c}	4476.39 ± 0.33^{d}
Magnesium (Mg)	403.99 ± 30.57^{a}	85.68 ± 1.46^{b}	368.72 ± 0.45^{a}	374.14 ± 1.21^{a}

Means in the same row with different letters were significantly different ($p < 0.05$).

DISCUSSION

The present study investigated the growth performance and biomass composition of *C. vulgaris* under different light intensities, salinity levels, and cultivation duration. The results revealed variations in growth trends, proximate composition, and mineral content in response to these factors. Light is an important factor in the growth of microalgae; insufficient or excessive light in terms of intensity and duration may lead to photooxidation and growth-limiting (Al-Qasmi et al., 2012; Gonçalves et al., 2014; Metsoviti et al., 2020). The observation that low light intensity resulted in slightly higher growth rates of *C. vulgaris* compared to high light intensity, although without a statistically significant difference, suggests that the light intensities used in this study fall within the optimal range for this microalga's growth. The higher growth rate and cell density observed in the low light intensity suggest its potential to provide more favorable conditions for the growth and proliferation of *C. vulgaris*. In contrast, a study by Gonçalves et al. (2014) documented an increased number of cells with higher light intensity for *C. vulgaris* sourced from the Culture Collection of Algae (United Kingdom). They reported a specific growth rate (SGR) of 1.190 ± 0.041 d^{-1} at a higher light intensity of 180 $\mu E\ m^{-2}\ s^{-1}$, which is lower than the growth rate (1.3 ± 0.31 d^{-1}) recorded in our study for high light intensity. However, the overall trend observed in our study, with higher density at low light intensities, is similar to the findings obtained by Gong et al. (2014). It is worth noting that the species used in our study appears to be highly replicative, as evidenced by its higher SGR compared to previous studies, such as in the freshwater environment of Tanzania (Mtaki et al., 2021) and China (Gong et al., 2014).

The salinity level significantly influenced the growth rate and cell density of *C. vulgaris*. Higher growth rates and cells were accumulated in lower salinity levels, with 15 g kg^{-1} salinity recording the highest growth rate. In contrast, higher salinity levels resulted in poor growth. This pattern is similar with the findings of Rai et al. (2015), who reported a decline in the growth of *Chlorella sp.* at salinity levels of 0.5 M. Similarly, Shah et al. (2003), observed that *Chlorella sp*. from Bangladesh achieved its maximum cell density at 14 g kg^{-1} salinity, which closely aligns with the optimal growth performance at 15 g kg^{-1} salinity observed in this study. Despite being sourced from an estuarine environment, the performance of *C. vulgaris* under laboratory conditions in a similar environment was not particularly high. This could be attributed to its tolerance limitations to salinity variations, as species often require highly specific environmental conditions to thrive (Padisák and Naselli-Flores, 2021).

Poor growth at elevated salinity levels might be associated with the accumulation of reactive oxygen species, as noted by Rai et al. (2015). Beyond growth inhibition, extreme salinity levels can result in mortality in both aquatic plants and animals when their tolerance thresholds are exceeded (Gueye et al., 2023). The observed variation in growth performance under different salinity levels signifies the adaptability of *C. vulgaris* to diverse environmental conditions. The SGR records reported in the current study even in the salinity levels, which showed low values are higher than those of previous studies for *C. vulgaris* in other parts of the globe (Yadavalli et al., 2020; Metsoviti et al., 2020), which recorded maximum values of 0.32 and 0.31-0.85 d^{-1}, respectively.

The *C. vulgaris* biomass accumulated a substantial amount of protein exceeding 60% regardless of the culture condition. This is particularly interesting as it surpasses those documented in previous studies on similar microalgae species, though of different origins (Tokuşoglu and üUnal 2003; Rai et al., 2015; Mtaki et al., 2021; Kandasamy et al., 2022; Mtaki et al., 2023). This higher crude protein content indicates that the biomass of *C. vulgaris* can be a valuable source of protein for various applications, including aquaculture and animal feed. However, this huge amount of protein might be associated among other factors, with the prolonged exposure of the microalgae in the dark environment, as the photoperiod was set as 12/12 day/night.

Microalage needs both light and dark regimes but the synthesis of essential biochemical compounds takes place in the dark (Al-Qasmi et al., 2012). Despite accumulating a substantial amount of protein, the biomass harvested on day 7 of the culture experiment exhibited a slightly higher protein content than that of day 14. This indicates that *C. vulgaris* accumulates much of its biochemical compounds during the steady state rather than the last time of cultures. Therefore, for maximizing the potential of *C. vulgaris* to obtain large protein, harvesting at the culture at 7 is the best option. On the other hand, the large composition recorded in the higher light intensity implies the optimal light amount for harnessing more protein.

Similarly, for carbohydrates, a significant accumulation was noted on day 7 compared to day 14. This contrasts with the findings by Solís-Salinas et al. (2021), who observed an increase in the carbohydrate content of cyanobacteria and green algae with retention time. Our study indicates that carbohydrate accumulation was favored with low light intensity conditions, as evidenced by the similar amounts accumulated on day 7 and day 14 under side light compared to top light.

Lipid content peaked under low light intensity provided by the side light, with both day 7 and day 14 biomass showing higher percentages compared to those under top light (higher light intensity). This contradicts previous research suggesting an increase in lipids with higher light intensity (He et al., 2015; Metsoviti et al., 2020). However, species-specific effects of light intensity are evident, as reported by Liu et al. (2012) for *Scenedesmus* spp which accumulated 41% of lipids at 400 μmol photons m−2 s−1 level. Furthermore, the observed increased lipid accumulation with an extending culture period is similar to Hu et al. (2019). This increased lipid accumulation at day 14 might be due to the maturity stage as the microalgae continues to grow, it accumulates more lipids, resulting in higher lipid content in the biomass. Differences in lipid compositions between studies might be associated with differences in *Chlorella* species and experimental setups, which altogether imply the importance of standardized protocols. Studies highlighted the salinity factor to be among the stresses that influence the production of lipids and that microalgae increase lipids when stressed by salinity (Yun et al., 2019). However, the culture conditions set for *C.vulgaris* in our study including the salinity level of 25, may play a key role in maximizing lipid composition as it was observed that lipid contents recorded were high not to a large extent of stress.

Significant amounts of minerals were recorded in the biomass of *C. vulgaris* except for selenium which was found to be almost negligible regardless of the culture condition. Longer cultivation periods were found to increase the content of iron, potassium, magnesium, phosphorus, and calcium, even selenium though it was very minute, suggesting that extended cultivation allows for greater mineral accumulation. Additionally, higher light intensity conditions resulted in higher mineral content compared to low light conditions. These findings emphasize the importance of optimizing cultivation conditions and light intensity for desired mineral profiles in *C. vulgaris* biomass. Notably, the observed mineral contents were generally larger than those reported in previous studies, such as Tokuşoglu and üUnal (2003), for the same species from different origins, except for iron and selenium. This discrepancy could reflect the influence of specific cultivation conditions, such as light intensity and duration, on nutrient assimilation. The observed laboratory conditions might reflect processes similar to those occurring in aquatic ecosystems, where environmental factors such as temperature, salinity, and pH play a critical role in shaping seasonal variations in mineral concentrations, particularly sodium, potassium, and calcium (Assemian-Niango et al., 2020).

Conclusion

The study found that the environmental conditions and cultivation days had a significant impact on the growth and biomass composition of *C. vulgaris*. High and low light

intensities had positive effects on growth, with different effects on biochemical composition. High light levels increased crude protein and iron content, while low light levels favored lipid, carbohydrate, and phosphorus accumulation. A salinity level of 15 g kg^{-1} yielded the highest cell density and growth rate. Cultivation time also influenced biomass composition, with certain compounds being abundant on day 7 and others, particularly minerals, reaching highest peak on day 14. Therefore, selecting the appropriate light intensity and cultivation period should align with specific production objectives. However, a salinity level of 15 g kg^{-1} is recommended for optimal growth performance. This information is crucial for optimizing the yield and composition of *C. vulgaris*, supporting sustainable cultivation practices and industrial applications.

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AUTHORS' CONTRIBUTIONS

AM contributed to the conceptualization, methodology design, data curation, funding acquisition, manuscript writing, and editing. KM contributed to data curation, analysis, writing, and manuscript editing.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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