



# Maximizing high-value biomass in *Scenedesmus* and *Chlorella*: Exploring light-driven macromolecular storage for nutraceutical and feed applications

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## ABSTRACT

The stringent safety requirements for using microalgal biomass in the pharmaceutical, food, and feed industries demand increased scientific research to identify optimal cultivation conditions under axenic environments. To achieve high biomass yields and macromolecular productivity, various cultivation parameters must be systematically evaluated. In particular, the strain-specific responses to these conditions underscore the importance of understanding how inorganic carbon supply and light irradiation influence biomass proliferation and macromolecular accumulation. To gain deeper insight into microalgae productivity, *Scenedesmus* sp. and *Chlorella* sp. were cultivated under axenic condition with a 2 % CO<sub>2</sub> supply. The effects of different light wavelengths (red, green and blue) and light intensities (ranging from 80 μE to 200 μE) were investigated. The highest biomass production for *Scenedesmus* sp. was observed under red, green, and blue light at an intensity of 200 μE, yielding 0.78 ± 0.12 g L<sup>-1</sup>, 0.94 ± 0.07 g L<sup>-1</sup>, and 1.00 ± 0.10 g L<sup>-1</sup>, respectively. Red, green, and blue light at 150 μE intensity increased lipid storage to 25.32 ± 1.50 %, 39.36 ± 1.81 %, and 33.91 ± 19.87 %, respectively. In contrast, the biomass of *Chlorella* remained stable under 100 μE of red, green, and blue light, producing 0.65 ± 0.04 g L<sup>-1</sup>, 0.66 ± 0.09 g L<sup>-1</sup>, and 0.66 ± 0.07 g L<sup>-1</sup>, respectively. Increasing light intensity did not significantly affect lipid storage, which remained at 6.87 %. Protein content was consistent in both microalgae strains across all experimental conditions, exceeding 15 %. Lipid characterization revealed a high concentration of oleic, palmitic, stearic, and vaccenic acids, highlighting the potential for integrating these microalgae strains into the pharmaceutical, nutraceutical and feed industries.

## 1. Introduction

The commercialization of microalgae biomass depends on achieving specific compositional characteristics and production costs that enable competitive pricing against non-microalgal products. Reducing cultivation costs is a crucial challenge across all industrial sectors, with research highlighting the impact of abiotic stress on biomass yield and macromolecular composition [1].

As photosynthetic organisms, microalgae are highly influenced by light irradiation, which directly affects their composition and productivity. However, other factors such as temperature fluctuations, nutrient limitations, and the presence of heavy metals also play significant roles in biomass growth and macromolecular accumulation [2]. Many studies have explored the potential of microalgae in phytoremediation, using waste or wastewater as a cultivation medium to produce biofuels at costs competitive with fossil fuels [3,4]. However, this approach is primarily

limited to the energy sector, as waste-based cultivation is unsuitable for nutraceutical and pharmaceutical applications. To integrate microalgae into food, feed, pharmaceutical, and cosmetic industries, cultivation must occur under axenic conditions with pure substrates, making cost reduction a key challenge.

Industrial microalgae cultivation typically employs photobioreactors with either natural or artificial light sources. While sunlight is commonly used in open pond systems, artificial lighting is preferred in closed tubular or panel photobioreactors [1]. The advantage of artificial lighting lies in its precise control over intensity, wavelength, and light-dark cycles, all of which significantly influence biomass proliferation and composition. However, the high energy costs of artificial lighting remain a major economic bottleneck for large-scale production [5]. The light sources applied, as fluorescent lamps, high-intensity discharge lamps and light-emitting diodes (LEDs), could influence the microalgae cultivation cost. Fluorescent lamps are commonly used,

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covering a large wavelength range, but LEDs influence on microalgae cultivation is currently evaluated, due to their longer lifetime, compactness, low heat emission and high electrical conversion efficiency.

To further optimize cultivation costs, CO<sub>2</sub> supplementation is essential. This enhances autotrophic metabolism, improves greenhouse gas sequestration, and boosts biomass productivity [5,6]. In microalgae, bicarbonate (HCO<sub>3</sub><sup>-</sup>) serves as the primary carbon source for CO<sub>2</sub> assimilation. Although CO<sub>2</sub> has low solubility in water, microalgae utilize the enzyme carbonic anhydrase to enhance its availability. Additionally, passive diffusion allows for further CO<sub>2</sub> uptake [7].

*Scenedesmus* is known for high lipid accumulation and robustness under varying light conditions, making it a promising candidate for biofuel and feed applications. *Chlorella vulgaris* is widely used in nutraceuticals and aquaculture due to its protein content and safety profile, with a strong cultivation track record in closed systems [8–15]. By applying abiotic stress under axenic conditions with CO<sub>2</sub> supplementation, it is possible to obtain microalgae biomass with compositions suitable for pharmaceutical and nutraceutical applications. The high economic value of these specialized biomasses helps offset the elevated production costs [13–15]. Notably, microalgae-derived lipids contain essential compounds such as  $\alpha$ -linolenic acid,  $\omega$ -3 fatty acids (including  $\alpha$ -linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid), and  $\omega$ -6 fatty acids (e.g.,  $\gamma$ -linolenic acid). These bioactive compounds contribute to human health by supporting nutritional needs and preventing conditions such as cerebral and ocular disorders, obesity, chronic inflammation, arteriosclerosis, and lung diseases while also promoting tissue regeneration [1,14].

Based on these insights, this study aimed to evaluate the effects of different abiotic factors on the biomass production of *Scenedesmus* sp. and *Chlorella vulgaris*. Specifically, the research focused on the impact of light irradiation and intensity stress under high CO<sub>2</sub> supplementation to enhance macromolecular storage, ultimately generating high-value biomass for nutraceutical and feed applications.

## 2. Material and methods

### 2.1. Microalgae strains

*Scenedesmus* sp. and *Chlorella vulgaris* Beij. were obtained by ACUF collection of University Federico II of Naples (Italy). Strains maintenance was carried out using Bold's Basal Medium (BBM), under axenic condition, at room temperature (25 °C) with continuous mechanical agitation (Heidolph UNIMAX 1010) at 150–160 rpm. Continuous white light irradiation was applied. BBM medium composition was 10 ml of NaNO<sub>3</sub> solution (25 g l<sup>-1</sup>), CaCl<sub>2</sub> · 2 H<sub>2</sub>O (2.5 g l<sup>-1</sup>), MgSO<sub>4</sub> · 7H<sub>2</sub>O (7.5 g l<sup>-1</sup>), K<sub>2</sub>HPO<sub>4</sub> · 3H<sub>2</sub>O (7.5 g l<sup>-1</sup>), KH<sub>2</sub>PO<sub>4</sub> (17.5 g l<sup>-1</sup>), NaCl (2.5 g l<sup>-1</sup>), 1 ml of vitamins mix solution (thiamine 0.1 g 100 ml H<sub>2</sub>O<sup>-1</sup>; biotin 25 · 10<sup>-6</sup> g 100 ml H<sub>2</sub>O<sup>-1</sup>; vitamin B<sub>12</sub> 15 · 10<sup>-6</sup> g 100 ml H<sub>2</sub>O<sup>-1</sup>), 6 ml of metal solution (FeCl<sub>3</sub> · 6H<sub>2</sub>O 0.097 g l<sup>-1</sup>; MnCl<sub>2</sub> · 4H<sub>2</sub>O 0.041 g l<sup>-1</sup>; ZnCl<sub>2</sub> 0.005 g l<sup>-1</sup>; CoCl<sub>2</sub> · 6H<sub>2</sub>O 0.002 g l<sup>-1</sup>; Na<sub>2</sub>MoO<sub>4</sub> · 2H<sub>2</sub>O 0.004 g l<sup>-1</sup>) [16].

### 2.2. Experimental set up

Microalgae cultivation experiments were conducted using a Multi-Cultivator MC 1000-OD MIX (Photon System Instruments), with continuous air bubbling, 0.8 L min<sup>-1</sup>, enriched with 2 % CO<sub>2</sub> provided by a Gas Mixing System (GMS 150). The experiment was conducted in batch, 8 days, in volume 80 mL. Biomass proliferation was monitored through OD measurements using the ONDA UV-30 SCAN system for spectrophotometric analysis at 680 nm and 720 nm. The initial OD of the inoculum at time 0 was 0.1, and the experiment lasted 8 days (192 h). LED irradiation was applied in four conditions: white light (WHITE, control), red light at 660 nm (RED), blue light at 470 nm (BLUE), and green light at 530 nm (GREEN). An initial comparison was performed

using a light intensity of 80  $\mu$ E across all conditions. Subsequent experiments tested RED, GREEN, and BLUE light irradiation intensities at 80, 100, 120, 150, and 200  $\mu$ E. A photoperiod of 24:0 (light:dark) was maintained. The total culture volume for each sample was 80 mL, the experiments and analysis were performed in duplicate for each experimental condition.

### 2.3. Biomass analysis

At the conclusion of the experiment, microalgal biomasses were collected through centrifugation (NEYA XS 16) at 4500 rpm for 20 min and lyophilized using a Thermo Scientific Heto Lyolab 3000 system. The dry weight of the biomass was determined post-lyophilization. Elementary analysis of nitrogen (N), carbon (C), hydrogen (H), and sulphur (S) was performed using 2 mg of lyophilized biomass with a Flash 2000 Organic Elemental Analyzer (Thermo Scientific). Total lipid storage (%) was quantified following the method described by Kapoor et al. [17]. Two millilitres of bi-distilled water were added to the lyophilized biomass, and the mixture was subjected to ultrasound treatment (Hielscher UP 200th) for 4 min. Subsequently, 1 ml of hexane was added, and the solutions were agitated at 4500 rpm for 10 min. After centrifugation (4500 rpm for 10 min), the hexane layer was recovered, evaporated using an Eppendorf concentrator (5310) at 30 °C for 30 min, and subjected to lipid characterization. Lipid transesterification was performed following the protocol by Hewavitharana et al. [18]. Dried lipids were treated with 1 ml of methanol:sulfuric acid (10 % v/v) and heated at 80 °C for 30 min. Hexane (1 ml) and water (1 ml) were added, and the hexane fraction containing fatty acid methyl esters (FAMES) was dried. For gas chromatography-mass spectrometry (GC-MS) analysis, the sample was re-suspended in 200  $\mu$ l of hexane. GC-MS analysis was conducted using an Agilent 7890B gas chromatograph equipped with a 25 m × 0.2 mm ID (0.5  $\mu$ m film thickness, coated with (5 %)-diphenyl-(95 %)-dimethylpolysiloxane). Injection was performed manually in split mode (1:50) with an injector temperature of 250 °C. Helium served as the carrier (1.2 mL min<sup>-1</sup>). The column temperature was programmed to increase from 130 °C (2 min) to 230 °C at a rate of 10 °C/min with a 3 min hold at 230 °C. The column effluent was introduced into the ion source of an Agilent 5977B GC/MSD. Ionization was achieved with a 70 eV electron beam at an emission current of 35  $\mu$ A. The detector voltage was set between 1500 and 2000 V, with an acceleration voltage applied after a solvent delay of 240 s. The ion source and transfer line temperatures were maintained at 230 °C and 260 °C, respectively.

Protein quantification was carried out using Organic Elemental Analyzer (FLASH2000, configuration CHNS, Thermo Fisher Scientific), using He as carrier and reference with flow 140 mL min<sup>-1</sup> and 100 mL min<sup>-1</sup> respectively. Oxygen flux, for the combustion, had flow 250 mL min<sup>-1</sup> for 3 s. Oven temperature was set 950 °C, when the oxygen was injected the temperature was 1800 °C the gasified sample was analysed using GC column (PTFE: 2 m 6 × 5 mm) with TCD detector (65 °C). 1–2 mg of dry matter (WLFW diluted 1:10 in BBM + V) was used as sample. Total time run was 720 s. K factor was applied as calibration method using 2,5-Bis(5-tert-butyl-2-benzo-oxazol-2-yl) (BBOT) as standard. Conversion factor applied to obtain protein concentration was 6.25 as reported by Boulos et al. [19].

All the analyses were performed in duplicate for each experimental condition.

### 2.4. Statistical analysis

Data obtained during the experiment were processed using average and standard deviation. On total lipid percentages, fatty acids percentages and total protein were applied the t-Student test using p value < 0.05 and p value < 0.01.

### 3. Result and discussion

#### 3.1. Biomass proliferation under white, red, green and blue light at 80 $\mu\text{E}$

The initial evaluation of light intensity (80  $\mu\text{E}$ ) revealed distinct physiological responses in *Scenedesmus* and *Chlorella*. These differences can be partially explained by light perception mechanisms involving photoreceptors such as phytochromes (red light), cryptochromes and phototropins (blue light), which modulate transcriptional responses affecting photosynthesis, cell division, and carbon metabolism. In *Chlorella*, photoreceptor-mediated regulation of light-harvesting complex genes has been observed under varying light wavelengths, influencing growth rates and stress responses [20]. Similarly, *Scenedesmus* exhibits light-specific gene expression changes through signalling pathways triggered by reactive oxygen species (ROS) and redox signals [21].

At the same light intensity, the proliferation of *Scenedesmus* was not affected by the different LED light applied. This was evident from comparable OD at 720 nm:  $1.30 \pm 0.00$ ,  $1.33 \pm 0.03$ ,  $1.29 \pm 0.07$ , and  $1.39 \pm 0.01$  under WHITE, RED, GREEN and BLUE LED lights, respectively. In contrast, *Chlorella* biomass proliferation showed enhanced growth under RED, GREEN, and BLUE lights compared to the control condition (white light). The OD values at 720 nm were  $1.1 \pm 0.1$ ,  $1.37 \pm 0.01$ ,  $1.22 \pm 0.03$ , and  $1.34 \pm 0.01$  under WHITE, RED, GREEN, and BLUE lights, respectively.

Dry weight analysis further highlighted a significant difference in biomass production between the control and experimental conditions, underscoring the varying effects of different light wavelengths on the two microalgae strain. For *Scenedesmus*, the dry weight was measured at  $0.19 \text{ g l}^{-1}$  under control conditions, and  $0.66 \pm 0.06 \text{ g l}^{-1}$ ,  $0.62 \pm 0.10 \text{ g l}^{-1}$ , and  $0.78 \pm 0.06 \text{ g l}^{-1}$  under RED, GREEN, and BLUE light irradiation, respectively. Similarly, for *Chlorella*, the dry weight was  $0.18 \text{ g l}^{-1}$  in the control condition and  $0.62 \pm 0.00 \text{ g l}^{-1}$ ,  $0.53 \pm 0.06 \text{ g l}^{-1}$ , and  $0.56 \pm 0.12 \text{ g l}^{-1}$  under RED, GREEN, and BLUE light irradiation, respectively.

Differences between OD and dry weight results led to different conclusions regarding the effects of experimental conditions on microalgae proliferation. This variation represents a potential bottleneck in scaling up microalgae biomass cultivation. While indirect measurement methods, such as OD, are cost effective and straightforward, they can be influenced by external factors during cultivation, resulting in inaccurate data. Integrating direct measurement methods, such as dry weight and cell counts, provided more reliable biomass estimations, enabling identification of optimal growth conditions. These findings are consistent with studies by Scagerl et al. [22] and Scarponi et al. [23], which noted the simplicity of indirect methods but also the inherent risks of under- or over-estimation.

Dry weight analysis in this study confirmed that RED, GREEN and BLUE light irradiation significantly enhanced biomass production in both *Scenedesmus* and *Chlorella*. However, comparing these results with literature was challenging due to variations in microalgae strains and light intensities used. For example, Leonardi et al. [24] reported biomass production of  $0.20 \text{ g l}^{-1}$  and  $0.32 \text{ g l}^{-1}$  for *Scenedesmus quadricauda* under red and blue light (36–44  $\mu\text{E}$ ), respectively. Meanwhile, Baidya et al. [25] observed biomass yields of  $0.04 \pm 0.00 \text{ g l}^{-1}$ ,  $0.04 \pm 0.00 \text{ g l}^{-1}$ ,  $0.05 \pm 0.00 \text{ g l}^{-1}$  and  $0.06 \text{ g l}^{-1}$  for *Chlorella ellipsoidea* under white, red, green, and blue light at 200  $\mu\text{E}$ , achieved respectively.

The differential responses of microalgae to light irradiation were partly attributed to changes in cell size. Cepak et al. [26] observed an increase in cell volume for *Scenedesmus obliquus* under 50  $\mu\text{E}$  irradiance at 30 °C. After 12 h of cultivation, cell volume exponentially increased under white, red, and blue light (170–190  $\mu\text{m}^3$ ), while green light had a lesser effect (150  $\mu\text{m}^3$ ). Similarly, Baidya et al. [25] reported that blue light irradiation significantly increased cell area in *C. ellipsoidea*, from 3.00 to 7.04  $\mu\text{m}^2$  under control conditions to 38.94  $\mu\text{m}^2$ .

This study's direct monitoring analysis demonstrated the positive

influence of RED, GREEN, and BLUE light irradiation on biomass production. Subsequent experiments examined the effects of these light wavelength at varying intensity - 80  $\mu\text{E}$ , 100  $\mu\text{E}$ , 120  $\mu\text{E}$ , 150  $\mu\text{E}$  and 200  $\mu\text{E}$  - to further refine optimal growth conditions.

#### 3.2. Influence of different light intensity on *Scenedesmus* and *Chlorella* proliferation

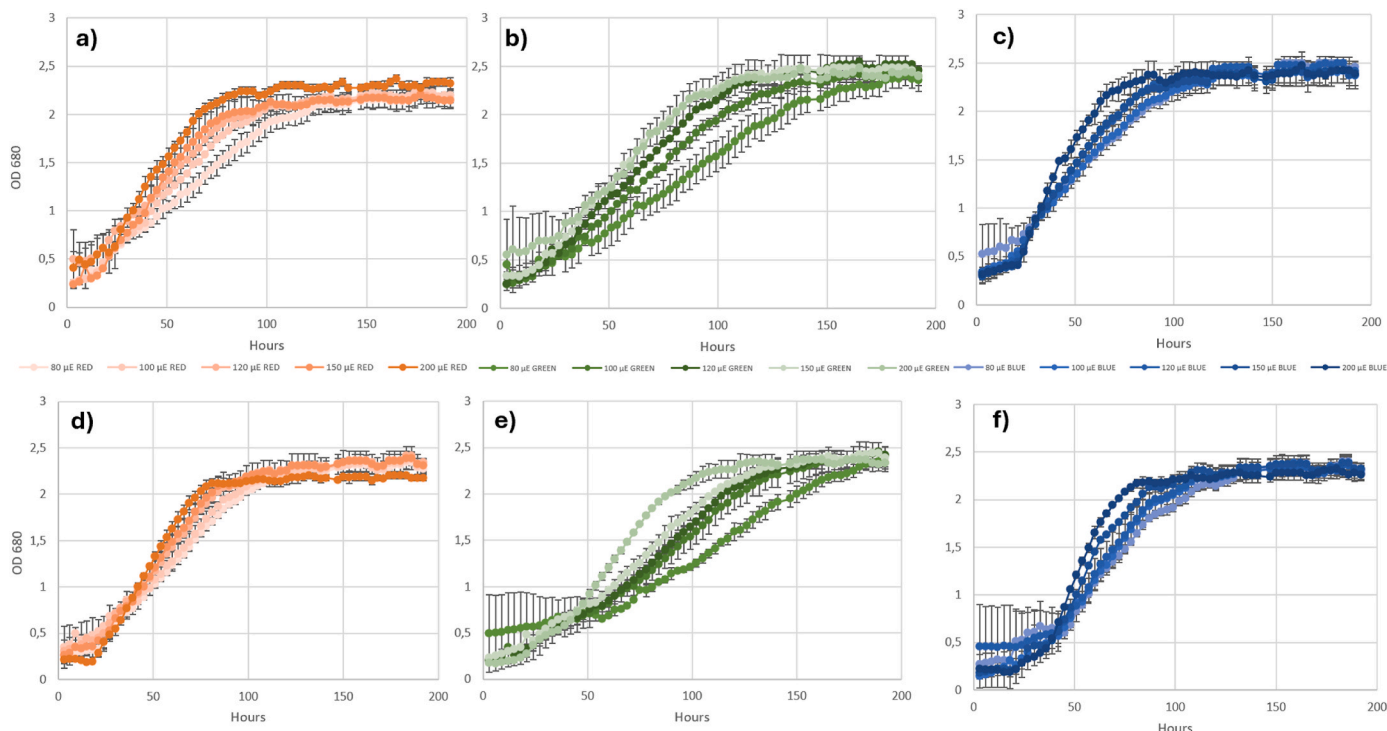
OD analysis (Fig. 1) revealed a reduction in the lag phase when a light intensity of 200  $\mu\text{E}$  was applied with RED, GREEN, and BLUE light for both microalgae strains. As summarized in Table 1, the effect of increasing light intensity on growth rate varied between *Scenedesmus* and *Chlorella*. For *Scenedesmus*, the highest growth rate under RED, GREEN, and BLUE lights occurred at specific intensities: RED light at  $\leq 150 \mu\text{E}$ , GREEN light at 100–120  $\mu\text{E}$ , and BLUE light at  $\geq 100 \mu\text{E}$ . Conversely, *Chlorella* exhibited no significant change in growth rates under varying RED and GREEN light intensities. However, under BLUE light, the highest growth rates were observed at 100  $\mu\text{E}$  and 150  $\mu\text{E}$ .

The 680/720 ratio (Table 1) remained stable throughout the tests, confirming the absence of bacterial or mold contamination. When comparing experimental data at 80  $\mu\text{E}$  with literature, discrepancies arose. For instance, Torres-Martínez et al. [27] reported lower growth rates for *Chlorella vulgaris* and *Scenedesmus acutus* under red and blue lights (200  $\mu\text{E}$ ), with values of  $0.20 \text{ d}^{-1}$ ,  $0.21 \text{ d}^{-1}$ ,  $0.20 \text{ d}^{-1}$ , and  $0.27 \text{ d}^{-1}$ , respectively. Similarly, Liu et al. [28] noted that while blue light (65  $\mu\text{E}$ ) enhanced cell density for *Chlorella* sp., the growth rates under red and blue lights ( $0.60 \pm 0.12 \text{ d}^{-1}$  and  $0.36 \pm 0.02 \text{ d}^{-1}$ , respectively) were higher than the control condition ( $0.27 \pm 0.02 \text{ d}^{-1}$ ).

##### 3.2.1. Biomass production and composition

Dry weight analysis (Fig. 2a) showed that biomass production for *Scenedesmus* increased with rising light intensity under all light irradiation conditions. In contrast, *Chlorella* biomass production was not significantly influenced by either light intensity or type. Comparison with literature data proved challenging. Difusa et al. [29] reported a biomass production of  $0.41 \pm 0.02 \text{ g l}^{-1}$  for *Scenedesmus* under 81  $\mu\text{E}$ , while Scharff et al. [30] observed higher values of  $2.70 \text{ g l}^{-1}$  and  $2.56 \text{ g l}^{-1}$  for *C. vulgaris* and *S. obliquus*, respectively, under white light. Khalili et al. [31] reported biomass production for *C. vulgaris* of  $0.04 \text{ g l}^{-1}$ ,  $1.2 \text{ g l}^{-1}$ , and  $0.6 \text{ g l}^{-1}$  under 50  $\mu\text{E}$ , 80  $\mu\text{E}$ , and 110  $\mu\text{E}$  red light, respectively, and  $0.3 \text{ g l}^{-1}$ ,  $1.0 \text{ g l}^{-1}$ , and  $0.2 \text{ g l}^{-1}$  under 50  $\mu\text{E}$ , 80  $\mu\text{E}$ , and 110  $\mu\text{E}$  blue light. Despite differences from the literature, the results here suggest that *Scenedesmus* strain may undergo secondary product modifications under specific conditions. Such as red and blue lights or unbalanced C/N ratio, as reported by Scarponi et al. [32] and Cepak et al. [26].

Light irradiation influenced macromolecular storage in both *Scenedesmus* and *Chlorella*, particularly lipid and protein content (Fig. 2b and c). For *Scenedesmus*, lipid storage increased under GREEN light, stabilizing between 35 % and 40 % across light intensity from 80  $\mu\text{E}$  to 150  $\mu\text{E}$ . Red, green, and blue light generally promoted lipid storage in *Scenedesmus*, whereas *Chlorella* showed no significant changes, with lipid content remaining below 10 %. These results were in line with studies by Baidya et al. [25] and Torres-Martínez et al. [27], reporting lipid storage at 30 % for *Scenedesmus* and 7–10 % for *Chlorella*. The observed variability in lipid accumulation under different wavelengths and intensities likely reflects an interplay between light-driven energy stress and oxidative metabolic regulation. Under blue and green light, higher excitation pressures on the photosystems may lead to excess electron flow, triggering oxidative stress that diverts carbon toward neutral lipid synthesis via upregulation of key genes such as DGAT and ACCase [33]. In *Scenedesmus*, this is further enhanced by chloroplast-to-nucleus retrograde signaling pathways that alter fatty acid biosynthesis to protect the photosynthetic apparatus. In contrast, the low lipid content in *Chlorella* may reflect efficient photoprotection mechanisms or metabolic channeling favoring protein or carbohydrate synthesis under similar



**Fig. 1.** OD at 680 nm analysis of *Scenedesmus* growth under 80 µE, 100 µE, 120 µE, 150 µE and 200 µE a) RED light, b) GREEN light and c) BLUE light; *Chlorella* growth under 80 µE, 100 µE, 120 µE, 150 µE and 200 µE d) RED light, e) GREEN light and f) BLUE light (ABS 680 nm). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 1**  
*Scenedesmus* and *Chlorella* growth rate ( $d^{-1}$ ) and 680nm/720 nm.

	µE	<i>Scenedesmus</i>		<i>Chlorella</i>	
		680/720	Growth rate ( $d^{-1}$ )	680/720	Growth rate ( $d^{-1}$ )
Red	80	1.62 ± 0.03	0.31 ± 0.01	1.64 ± 0.00	0.32 ± 0.03
	100	1.63 ± 0.06	0.31 ± 0.01	1.65 ± 0.06	0.29 ± 0.10
	120	1.63 ± 0.02	0.22 ± 0.09	1.67 ± 0.03	0.30 ± 0.06
	150	1.52 ± 0.02	0.32 ± 0.01	1.58 ± 0.03	0.31 ± 0.05
	200	1.63 ± 0.02	0.26 ± 0.06	1.60 ± 0.01	0.33 ± 0.00
Green	80	1.80 ± 0.03	0.24 ± 0.01	1.81 ± 0.02	0.25 ± 0.14
	100	1.75 ± 0.04	0.33 ± 0.01	1.74 ± 0.03	0.35 ± 0.00
	120	1.75 ± 0.03	0.32 ± 0.01	1.72 ± 0.01	0.35 ± 0.02
	150	1.64 ± 0.01	0.28 ± 0.02	1.62 ± 0.00	0.33 ± 0.02
	200	1.67 ± 0.06	0.23 ± 0.11	1.70 ± 0.01	0.37 ± 0.00
Blue	80	1.72 ± 0.01	0.23 ± 0.09	1.70 ± 0.01	0.31 ± 0.06
	100	1.71 ± 0.06	0.28 ± 0.02	1.70 ± 0.04	0.40 ± 0.02
	120	1.74 ± 0.00	0.28 ± 0.02	1.70 ± 0.02	0.27 ± 0.16
	150	1.61 ± 0.00	0.30 ± 0.02	1.61 ± 0.01	0.37 ± 0.03
	200	1.61 ± 0.01	0.29 ± 0.02	1.56 ± 0.02	0.33 ± 0.00

conditions.

Protein storage (Fig. 2c) was also affected by light type and intensity. For both strains, protein storage remained stable under RED light but decreased under GREEN and BLUE light as intensity increased. The highest protein content was observed at 80 µE under GREEN light, with values of 20 % and 23 % for *Scenedesmus* and *Chlorella*, respectively. Lower protein storage under red light has been reported by Baidaya et al. [25] and Scarponi et al. [34], as red light’s energy is insufficient to drive optimal synthesis compared to green and blue light, which promote  $^{14}C$  incorporation into protein-bound amino acids promoting the protein synthesis rate compared to red light [25,34].

The reduced macromolecular content under RED light align with other findings, as higher intensity red light can cause cell damage due to its lower energy compared to blue light [33,34]. Despite the discrepancies with literature data, this study highlights the significant role of light type and intensity in optimizing biomass production and macromolecular composition in *Scenedesmus* and *Chlorella* strains.

### 3.3. Lipid characterization

To explore the potential of *Chlorella* and *Scenedesmus* cultivation under specific light irradiation and intensity for use in food and feed applications, lipid characterization was performed to quantify the presence of fatty acids with marketable value. As shown in Fig. 3, light irradiation and intensity significantly influenced the lipid profiles of both microalgae strain, modifying the proportions of saturated and unsaturated fatty acids. Specifically, increased RED light intensity (>100 µE) enhanced the percentage of unsaturated fatty acids. Conversely, the *Scenedesmus* lipid profile varied with changes in GREEN and BLUE light intensities, with the highest unsaturated fatty acids percentage recorded at 100 µE (GREEN) and 150 µE (BLUE). The shift toward unsaturated fatty acids under certain light conditions may reflect redox imbalances within the chloroplast. Recent transcriptomic studies have shown that desaturase enzymes such as FAD2 and FAD7 are transcriptionally regulated under light-induced oxidative stress,

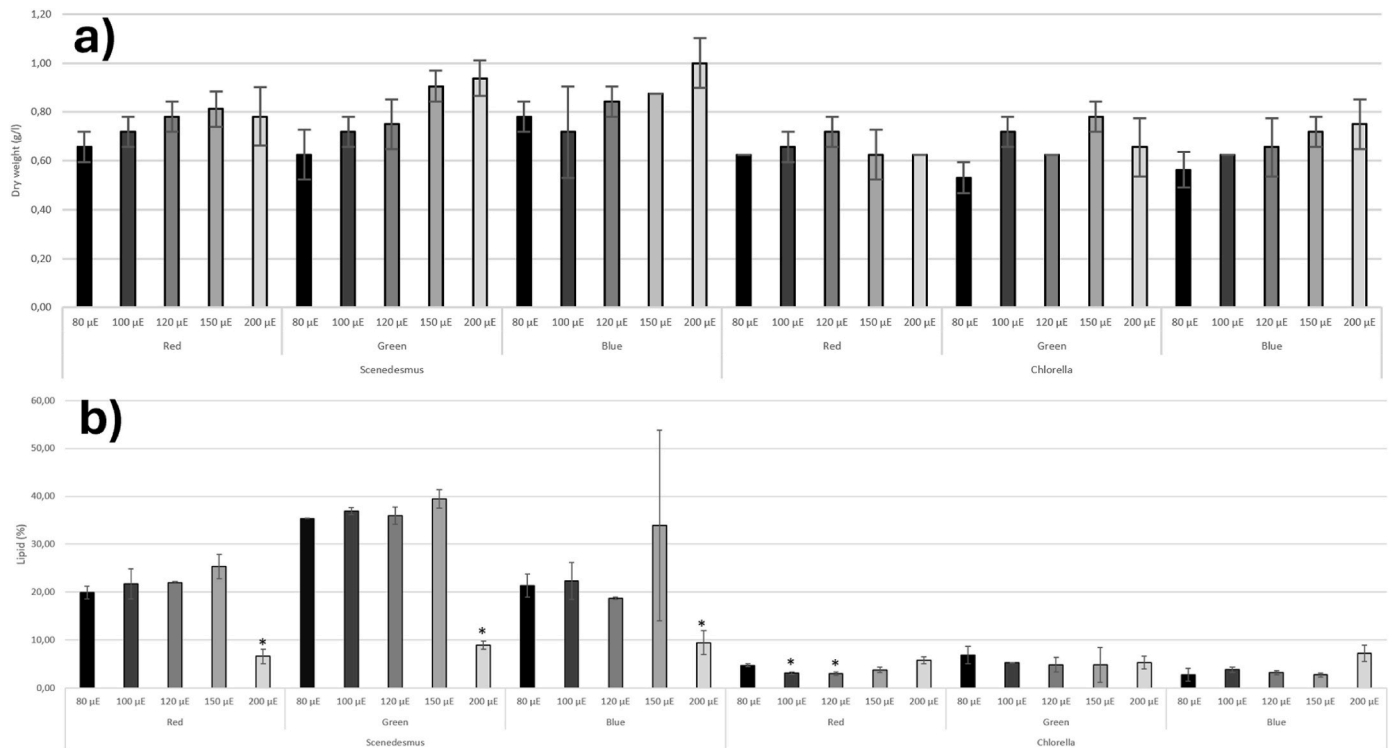


Fig. 2a. *Scenedesmus* and *Chlorella* a) dry biomass and b) lipid percentage under 80 μE, 100 μE, 120 μE, 150 μE and 200 μE of RED light, GREEN light and BLUE light. “\*” indicated the significant difference with control condition (80 μE)  $p < 0.05$ . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

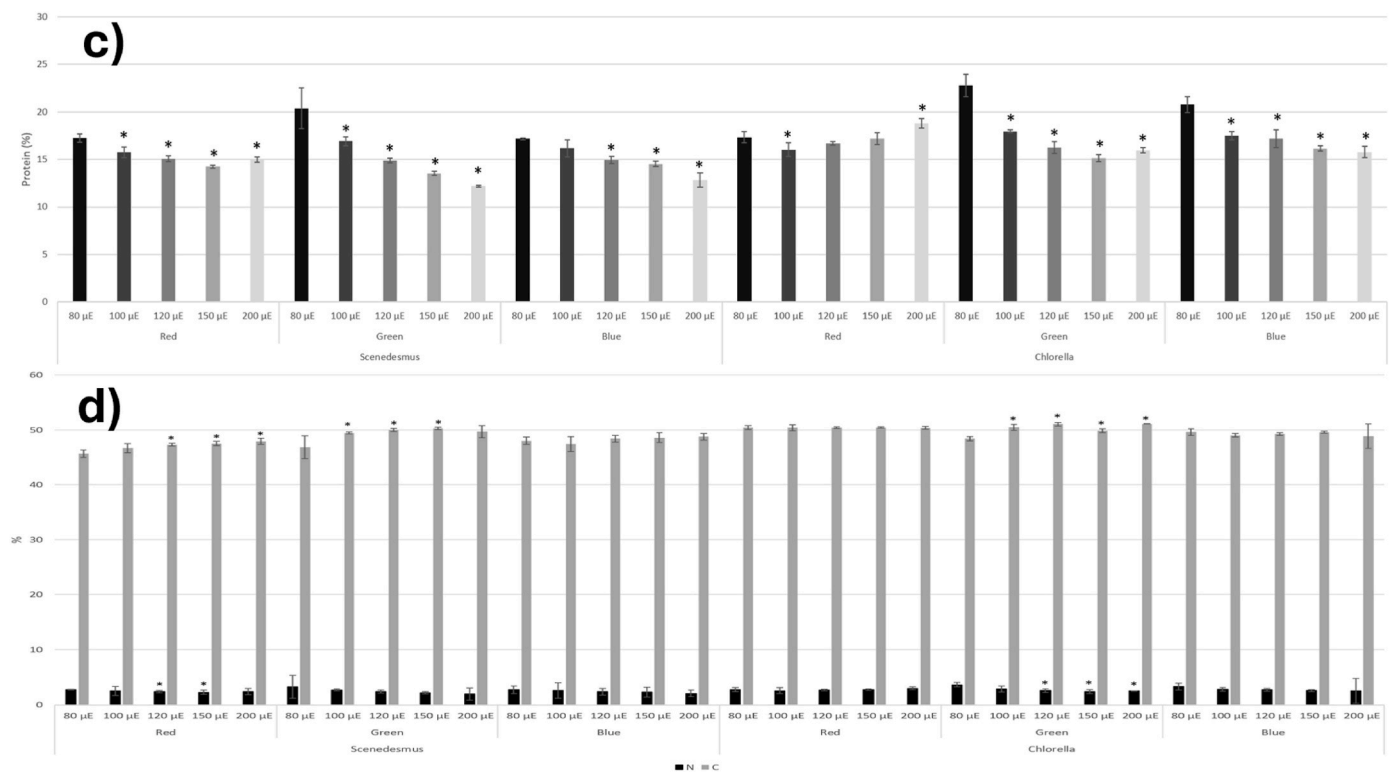
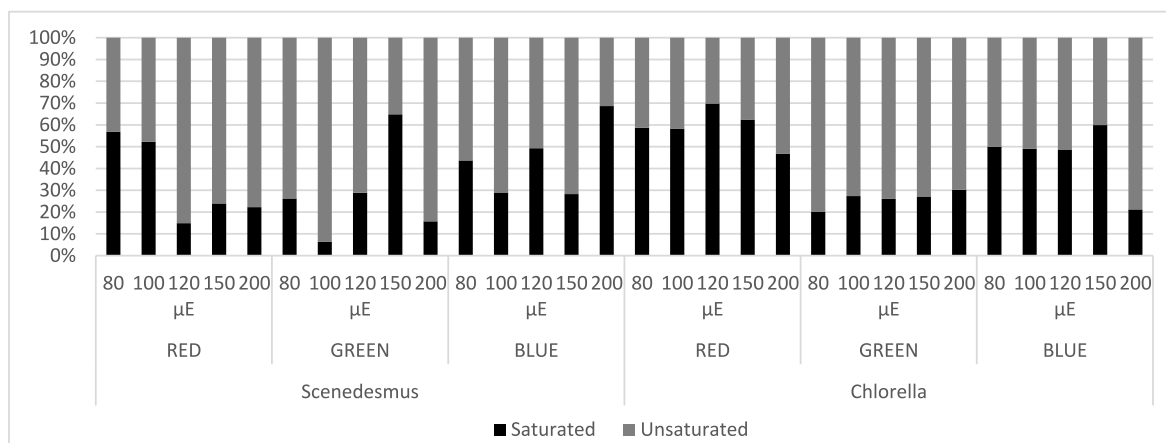


Fig. 2b. *Scenedesmus* and *Chlorella* c) protein and d) nitrogen and carbon percentages under 80 μE, 100 μE, 120 μE, 150 μE and 200 μE of RED light, GREEN light and BLUE light. “\*” indicated the significant difference with control condition (80 μE)  $p < 0.05$ . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 3.** *Scenedesmus* lipid characterization under 80 μE, 100 μE, 120 μE, 150 μE and 200 μE a) RED light, b) GREEN light and c) BLUE light; *Chlorella* lipid characterization under 80 μE, 100 μE, 120 μE, 150 μE and 200 μE d) RED light, e) GREEN light and f) BLUE light. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

contributing to changes in fatty acid saturation patterns [35]. Additionally, in *Chlorella*, light stress modulates plastid-localized fatty acid synthesis genes via the TOR and MAPK signaling pathways, integrating light quality with carbon allocation and stress mitigation [36,37]. In contrast, *Chlorella* showed a stable ratio of saturated to unsaturated fatty acids across all light conditions, with a notable increase in saturated fatty acids (30 %) under BLUE light at 200 μE. These findings align with existing literature, which reports stable saturated fatty acids percentage in *Chlorella* under blue (29.16 %) and green (25.14 %) irradiations respectively [34]. Interestingly, red light reduced saturated fatty acids (>3 %) in *Scenedesmus* in previous studies [38], but in this research, blue light significantly increased the saturated fatty acids content to 30 %. This increase may be linked to compensatory re-arrangement of thylakoid structure in response to low blue light absorption, boosting the synthesis of polyunsaturated and monounsaturated fatty acids. Such modifications render the biomass highly suitable for integration into food and feed sectors [39].

Tables 2 and 3 reveal an unconventional polyunsaturated fatty acid profile for both strains. Unlike typical profiles in *Chlorella* and *Scenedesmus* where linoleic and linolenic acids dominate [40,41], these were found in low abundance or entirely absent. Instead, the primary fatty acids detected included oleic, palmitic, stearic, and vaccenic acids. The analysis of *Chlorella* and *Scenedesmus* lipid profile revealed that the percentage of palmitic acid (C16:0) was higher than reported in the literature, ranging from 19.10 % to 22.30 %. Similarly, oleic acid (C18:1), the most abundant fatty acid identified, exceeded literature values, which typically range between 3 % and 17 %. In contrast, the stearic acid (C18:0) content was consistent with published data, falling within 2 %–16 % [27,35,42].

**Table 2**

Lipid relative abundance (%) in *Scenedesmus* lipid storage under RED, GREEN and BLUE lights at different light intensity (80 μE, 100 μE, 120 μE, 150 μE and 200 μE).

Relative abundance (%)	Scenedesmus														
	RED					GREEN					BLUE				
	80 μE	100 μE	120 μE	150 μE	200 μE	80 μE	100 μE	120 μE	150 μE	200 μE	80 μE	100 μE	120 μE	150 μE	200 μE
4,7,10,13-hexadecatetraenoate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.43	6.37	9.59	n.d.	7.10	16.91
Linolenic acid	15.73	n.d.	n.d.	n.d.	n.d.	18.33	37.22	n.d.	n.d.	n.d.	n.d.	n.d.	11.83	n.d.	n.d.
6,9,12-Octadecatrienoic acid	n.d.	n.d.	10.46	n.d.	23.20	n.d.	n.d.	n.d.	n.d.	17.88	0.66	14.39	n.d.	n.d.	14.45
Hypogeic acid	n.d.	0.79	39.92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Palmitic acid	28.61	27.52	n.d.	18.99	12.36	1.45	2.17	2.65	42.96	12.08	30.83	18.69	24.30	18.37	64.43
Oleic acid	27.04	46.71	24.30	74.22	54.56	31.51	42.92	62.97	35.15	57.86	49.25	47.08	35.83	55.31	n.d.
Stearic acid	27.82	24.42	13.51	4.96	4.12	16.30	3.25	26.20	21.89	3.30	12.88	10.26	22.03	9.86	4.21

A notable finding was the abundance of vaccenic acid in *Chlorella* lipid storage. Under GREEN irradiation at 120 μE, vaccenic acid was the predominant fatty acid, accounting for 53.9 % of the total lipid content in the microalgae biomass. Vaccenic acid has been extensively studied for its health benefit when integrated into the human diet, as well as its bioconversion into linoleic acid in the human body [43–45]. The Food and Agriculture Organization (FAO) recommends an omega-6 to omega-3 ratio (n6/n3) below 5 in the human diet to reduce risk of conditions such as cardiovascular disease, osteoporosis, autoimmune disorders, and cancer [46,47]. The lipid profiles identified in this study indicate potential applications in the food and feed industries, offering a promising source of biomass with positive effects on the nutraceutical and feeding stuffs sectors. However, achieving sustainable large scale production of microalgae biomass remains challenging due to the high labor costs and fixed capital expenditures associated with current methods. Results from this study suggest that wild type mesophilic strains of *Chlorella* and *Scenedesmus* could be integrated into industries requiring specific manufacturing processes and compliance with varying regulations and standards across different countries [48]. In the context of biorefinery processes, the removal of lipids for biofuels, pharmaceuticals, or nutraceutical applications could help, offset the high production costs of microalgae biomass. The remaining biomass could then be utilized as a protein-rich feed component, thereby enhancing its value and supporting sustainability in the food and feed sectors [49,50].

### 3.4. Economic implications of microalgal bioproducts from *Scenedesmus* and *Chlorella*

Recent investigations into the controlled cultivation of microalgae,

**Table 3**Lipid relative abundance (%) in *Scenedesmus* lipid storage under RED, GREEN and BLUE lights at different light intensity (80  $\mu$ E, 100  $\mu$ E, 120  $\mu$ E, 150  $\mu$ E and 200  $\mu$ E).

Relative abundance (%)	Chlorella														
	RED					GREEN					BLUE				
	80 $\mu$ E	100 $\mu$ E	120 $\mu$ E	150 $\mu$ E	200 $\mu$ E	80 $\mu$ E	100 $\mu$ E	120 $\mu$ E	150 $\mu$ E	200 $\mu$ E	80 $\mu$ E	100 $\mu$ E	120 $\mu$ E	150 $\mu$ E	200 $\mu$ E
6,9,12,15-Docosatetraenoic acid	n.d.	n.d.	n.d.	n.d.	22.75	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4,7,10,13-hexadecatetraenoate	n.d.	n.d.	n.d.	n.d.	n.d.	5.83	6.13	3.52	n.d.	n.d.	3	7.16	6.12	2.01	n.d.
Linolenic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.19	n.d.
Roughanic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.50	n.d.	12.92	n.d.	n.d.	7.56	6.89	n.d.	n.d.
9,12-Octadecadienoyl chloride	n.d.	n.d.	n.d.	n.d.	n.d.	8.41	n.d.	11.62	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hypogeic acid	n.d.	5.65	1.86	2.27	n.d.	n.d.	n.d.	n.d.	n.d.	26.70	3.87	n.d.	n.d.	n.d.	31.90
Palmitic acid	16.53	18.16	10.07	8.13	11.47	12.74	18.34	17.29	19.23	4.99	23.00	30.87	27.30	33.16	2.29
Oleic acid	n.d.	18.63	6.73	6.96	21.98	45.92	50.84	n.d.	49.51	30.03	27.49	26.87	31.64	28.01	34.57
Vaccenic acid	17.91	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	53.90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Stearic acid	14.78	15.57	9.80	7.21	27.73	2.36	6.64	6.98	6.61	19.63	11.40	9.27	15.07	19.41	15.65

particularly *Scenedesmus* and *Chlorella* species, have highlighted promising avenues for the production of high-value biochemical compounds. The results of this study underscore the potential economic advantages and current limitations associated with scaling such biotechnological systems.

One of the most significant findings is the ability of *Scenedesmus* to accumulate substantial lipid content, reaching up to approximately 40 % under optimized light and CO<sub>2</sub>-enriched conditions. The lipid profile is notably rich in oleic, palmitic, and vaccenic acids—fatty acids with well-documented applications in pharmaceuticals (e.g., anti-inflammatory and cardiovascular benefits), nutraceuticals (particularly omega-3/omega-6 balancing in dietary supplements), and animal feed, especially for aquaculture and poultry sectors. These specialized applications support a pricing strategy that justifies the higher production costs when compared to conventional crops or synthetic additives, thus offering entry into premium markets.

Beyond lipid extraction, the residual biomass retains a high protein content (>15 %), suggesting a dual-purpose utilization model suitable for biorefinery integration. This enables cascading value streams, where lipids are directed towards high-value products and the remaining biomass serves as a protein source for animal feed or as biofertilizers. Such an approach enhances resource efficiency and improves the economic viability of the system by maximizing output from a single biomass input.

The study employed cultivation under axenic, CO<sub>2</sub>-enriched, and light-controlled laboratory conditions. While these parameters increase operational complexity and costs, they are necessary for applications in highly regulated markets such as pharmaceuticals and food. These controlled conditions minimize contamination risk and enable compliance with stringent quality and safety standards. As a result, they support market access in high-barrier sectors but necessitate premium pricing strategies or cost mitigation through co-product valorization.

Importantly, the findings highlight the role of strain-specific optimization in microalgae cultivation. For instance, *Scenedesmus* was better suited for lipid production, while *Chlorella* displayed higher protein potential but low lipid accumulation and minimal responsiveness to light optimization. This suggests the feasibility of precision cultivation strategies tailored to specific end-use applications, improving efficiency and minimizing resource waste in high-margin industries.

An ancillary benefit of the cultivation method is its contribution to carbon management. The use of 2 % CO<sub>2</sub> in the growth medium presents an opportunity for CO<sub>2</sub> sequestration, which could be monetized through participation in carbon credit systems or by aligning with environmental, social, and governance (ESG) investment frameworks.

However, several economic limitations must be addressed before commercial scalability can be realized. Chief among them are the high

capital expenditure (CAPEX) and operational expenditure (OPEX) associated with artificial lighting (e.g., LEDs), sterile conditions, and specialized photobioreactor systems. These factors make the current approach economically viable only for niche applications unless cost-reducing innovations or substantial co-product value streams are realized.

Moreover, the study's findings are based on small-scale (80 mL) batch experiments, which do not capture the complexities of industrial-scale operations. The absence of pilot-scale validation raises questions about energy use, labor costs, and system durability in real-world conditions. Further work, including life cycle assessments (LCA) and cost-benefit analyses (CBA), is necessary to verify techno-economic feasibility at scale.

Another constraint lies in the lack of standardized benchmarks. Due to strain variability and condition-specific responses, it is difficult to compare biomass productivity across different studies, which complicates forecasting return on investment and standardizing production models for industry stakeholders.

In terms of biological performance, the limited lipid productivity and low sensitivity to light conditions exhibited by *Chlorella* pose a scalability challenge. Its utility may be confined to protein-centric applications unless further genetic or environmental optimizations are developed.

Finally, market entry for nutraceutical and pharmaceutical products derived from microalgae is hampered by stringent regulatory requirements and the need for extensive consumer safety validation. These non-technical barriers increase time-to-market and elevate legal and compliance costs, further complicating commercialization efforts.

In summary, while the production of high-value biocompounds from microalgae like *Scenedesmus* and *Chlorella* shows strong potential for economically viable applications in specialized markets, considerable challenges remain in terms of scalability, cost efficiency, and regulatory navigation. Continued interdisciplinary research and pilot-scale demonstrations will be crucial to bridging the gap between laboratory promise and industrial application.

#### 4. Conclusion

The results of this study highlighted the significant influence of light irradiation and intensity on microalgae culture. Blue and green light at 150  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> maximized lipid storage in *Scenedesmus*, exceeding 30 %, while biomass productivity remained stable across light conditions. Conversely, no significant changes in lipid storage were detected in *Chlorella*, with lipid content remaining below 6.87 %. Protein storage in both microalgae strains followed a similar trend, where higher light intensities reduced protein biosynthesis and accumulation; however,

protein content remained as high as 15 %. Additionally, stress-induced changes in pigment composition highlighted the role of wavelength-specific responses in optimizing microalgal performance. These findings suggest strong potential for tailoring light regimes in industrial-scale cultivation to enhance lipid yields. However, further studies are needed to assess economic viability and to explore the underlying metabolic pathways through omics-level analyses.

### CRedit authorship contribution statement

**P. Scarponi:** Writing – original draft, Supervision, Methodology, Data curation, Conceptualization. **F. Chiavolelli:** Formal analysis. **L. Arru:** Formal analysis. **L. Forti:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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### Data availability

Data will be made available on request.

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