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Insights into Microalgae Growth Performance Under Varying Blue LED Light Intensities Using the Monod Model Framework

Insights sobre o desempenho do crescimento de microalgas sob diferentes intensidades de luz LED azul usando a estrutura do modelo de Monod

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Abstract
Aim: *this study investigates the effects of varying blue LED light intensities on the growth performance and lipid production of the microalga Chlorella vulgaris using a Monod Model framework.* ***Methodology:*** *cultures were exposed to eight light intensities ranging from 14.7 to 370 µmol m^{-2} s^{-1} over 12 days.* ***Results:*** *results indicate that while higher intensities (120 and 370 µmol m^{-2} s^{-1}) increase growth coefficients (0.43-0.60 d^{-1}), optimal biomass accumulation occurred at a lower intensity of 55.5 µmol m^{-2} s^{-1} when growth is sustained uninhibited over a longer period. In contrast to recent suggestions of using light intensities close to 200 µmol m^{-2} s^{-1}, the findings suggest that efficient growth can be achieved with lower, less energy-consuming light intensities, as biomass production with blue LED light was approximately 50% higher than with white light. Although cellular lipid accumulation was higher under white light (27%), the increased biomass production from blue light compensates for the total lipid yield.* ***Conclusion:*** *overall, optimizing blue LED light intensity represents a promising strategy for enhancing microalgae productivity, reducing energy consumption, and mitigating the effects of potential inhibitors in cultivation systems.*
Keywords: Chlorella vulgaris; Microalgae biomass; Blue LED; Monod model.

Resumo
Objetivos: *este estudo investiga os efeitos de diferentes intensidades de luz LED azul no desempenho de crescimento e na produção de lipídios da microalga Chlorella vulgaris utilizando o modelo Monod.* **Metodologia:** *as culturas foram expostas a oito intensidades de luz, variando de 14,7 a 370 µmol m^{-2} s^{-1} ao longo de 12 dias.* **Resultados:** *os resultados indicam que, enquanto intensidades mais altas (120 e 370 µmol m^{-2} s^{-1}) aumentam os coeficientes de crescimento (0,43-0,60 d^{-1}), o acúmulo ideal de biomassa ocorreu em uma intensidade menor, de 55,5 µmol m^{-2} s^{-1}, quando o crescimento é sustentado sem inibição por um período mais longo. Em contraste com as sugestões recentes de uso de intensidades de luz próximas a 200 µmol m^{-2} s^{-1}, os resultados sugerem que o crescimento eficiente pode ser alcançado com intensidades de luz mais baixas e com menor consumo de energia, visto que a produção de biomassa com luz LED azul foi aproximadamente 50% maior do que com luz branca. Embora o acúmulo de lipídios celulares tenha sido maior sob luz branca (27%), o aumento na produção de biomassa com luz azul compensa o rendimento total de lipídios.* **Conclusão:** *de modo geral, a otimização da intensidade da luz LED azul representa uma estratégia promissora para aumentar a produtividade de microalgas, reduzir o consumo de energia e mitigar os efeitos de potenciais inibidores em sistemas de cultivo.*
Palavras-chave: Chlorella vulgaris; biomassa de microalgas; LED azul; modelo Monod.

INTRODUCTION

Light is a critical resource for phototrophic microalgae and directly influences their productivity[1]. Although inorganic nutrients can limit growth rates, factors such as light intensity, wavelength, and photoperiod significantly impact microalgae-based industrial yields[2]. While sunlight is cost-effective, its availability may be inconsistent due to seasonal variations, limiting continuous microalgae productivity in various regions[3].

Artificial light emerges as a viable option to support the microalgae-based industry. Renewable sources like wind farms can generate the power necessary

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for abundant LED light production[4,5]. Light-emitting diodes (LEDs) have become one of the most affordable and efficient light sources globally[6]. LEDs are known for lower heat emission[7], longer lifespan[8], reduced energy consumption, and the ability to produce high-intensity monochromatic light[8]. Optimised red or blue light intensity may enhance microalgae-based industrial yields[9,10].

Green microalgae contain chlorophyll-a and chlorophyll-b in a 3:1 ratio, exhibiting similar absorption peaks for blue and red light. Chlorella vulgaris has been shown to perform better under high-intensity blue light[11]. However, lower intensities of red light can lead to greater biomass production in C. vulgaris compared to blue light[9]. Blue light penetrates water more effectively and achieves a more uniform distribution within the culturing medium than red light, making it more advantageous for biomass production in open raceways[8]. This wavelength also activates specific photoreceptor proteins, such as phototropins (phot1 and phot2 genes) and phytochrome A (phyA gene), which directly regulate microalgal growth rates and significantly influence biomass production[12,13].

Despite the testing of C. vulgaris performance across various intensities of white, red, and blue light, systematic comparisons of growth performance across a broad range of light intensities are seldom conducted. This research seeks to address this gap by employing the Monod Model approach. Blue light has been shown to increase algal growth performance by 40%[14] and cellular lipid ratios by 23%[15]. Optimal light intensity for growing Chlorella vulgaris has been reported at 200 µmol m^{-2} s^{-1} with a 12:12 h photoperiod[16]. However, this cited study did not compare blue light intensities below 100 µmol m^{-2} s^{-1}. It is important to note that high light intensities (>100 µmol m^{-2} s^{-1}) can generate reactive oxygen species that may negatively impact growth performance[17,18].

The relationship between light and microalgal growth can be likened to the Monod model, which describes microbial growth kinetics in relation to substrate concentrations[19]. While the Monod model traditionally emphasises substrate utilisation by microorganisms, the growth of microalgae is an autotrophic process that depends on light intensity, alongside the availability of carbon dioxide and nutrients. Under controlled conditions, light emerges as the primary factor influencing growth performance. Thus, utilising a Monod model to predict the optimal light intensity for algal growth is a logical approach. The Monod growth parameters, typically used to determine critical substrate concentrations such as the affinity constant (ks) and the maximum growth rate coefficient (µmax), can be adapted to identify corresponding light intensities. Accordingly, this study seeks to evaluate the effects of varying intensities of blue LED light on the growth performance and lipid production of Chlorella vulgaris through the application of a Monod first-order approach.

METHODOLOGY

Microalgae strain

The Chlorella vulgaris strain used in this experiment was kindly provided by Iracema Nascimento Microalgae Bank, sustained by the Bioprospection Biotechnology Laboratory (LaBBiotec) from the Federal University of Bahia, Salvador, Bahia, Brazil. Algae were maintained in BG-11 medium[20], which contains 1.5g l^{-1} of NaNO3, 0.04g l^{-1} of K_2HPO_4, 0.075g l^{-1} of $MgSO_4$, 0.036g l^{-1} of $CaCl_2$, 0.02g l^{-1} of Na_2CO_3, 0.001g l^{-1} of EDTA, 0.006g l^{-1} of $C_6H_8O_7$ xFe^{3+} + yNH_3, 1 mL of trace metal solution (0.286g l^{-1} of H_3BO_3, 0.181g l^{-1} of MnCl, 0.022 g l^{-1} of $ZnSO_4$, 0.039g l^{-1} of Na_2MoO_4, 0.0079g l^{-1} of $CuSO_2$, 0.00494g l^{-1} of $Co(NO_3)_2$). The conditions are white LED light with an intensity of 100 µmol photons m^{-2} s^{-1} and a photoperiod of 12h:12h at 25°C (±1°C).

Growth conditions

For the experimental setup, Chlorella vulgaris was subjected to eight different blue LED light intensities ranging from 14.7 to 370 µmol m^{-2} s^{-1}. An orbital incubator shaker with temperature control (Tecnal, TE-424) was adapted with LED light sources distributed 50 cm above the culturing flasks. Light intensity was measured across the entire area of the incubator floor to ensure the correct distribution and number of LED sources, providing equal light intensities to the whole region. Measurements were also taken with the culturing flasks in place to detect potential shading. The selected light intensities were based on previous research, covering a wide range of conditions to assess their impact on algal growth and lipid production.

Algal cultures were grown in 1 L conical flasks containing 500 mL of BG-11 medium, inoculated with 10% (v/v) fresh algal biomass at the end of the exponential growth phase. Each treatment was performed in triplicate to ensure the reliability and reproducibility of results. The flasks were incubated under a 12-hour light/12-hour dark photoperiod using blue LED light (460 nm) at the specified intensities. Additionally, continuous agitation (100 rpm) and aeration with atmospheric air enriched with 2.5% CO_2 were maintained throughout the experiment to ensure optimal growth conditions.

Growth was monitored over a period of 12 days, with samples collected at regular intervals for analysis of biomass, lipids, pigment and nutrient contents.

Analytic methods

Aliquots were taken daily for assessing biomass, pH, chlorophyll a, b and carotenoids according to Conceição et al.[21] (2023). Pigments were evaluated using 5 mL of microalgae samples from each treatment. Cells were washed twice in deionised water using the centrifugation procedure (5000 × g for 10 min). The dried cells were resuspended in 5 mL of acetone in an ultrasonic bath

for one h. Free of cells supernatant was read at wavelengths 470, 645, and 662 nm, and the results were used for estimating total carotenoids, chlorophyll a, and b, respectively. Procedures for accessing final total biomass and its constituents' ratios were carried out according to Hynstova et al.[22] (2018).

Biomass productivity rates were calculated using a linear regression over the exponential phase of biomass accumulation. The growth rate coefficient (μ) was estimated by applying linear regression to the natural logarithm (LN) transformed biomass accumulation data during the exponential growth phase[21]. Lipid productivity rates were calculated according to the equation below:

$$\text{Lipid Productivity } (\text{mg } l^{-1}\ d^{-1}) = \text{ Pbiomass x } L_c$$

where Pbiomass is the biomass productivity rate and Lc is the percentage of cellular lipid content.

Samples were collected at the start and end of experiments for assessing phosphate P-PO_4 (Ascorbic Acid Method 4500-P E.) and nitrate N-NO_3 (Spectrophotometric Method 4500-NO3 B.)[23]. The removal efficiency of those inorganics was calculated according to the following equation:

$$\text{Removal Yields (\%)} = ((IC\text{-}FC)/IC) \times 100$$

where IC and FC represent the initial and final concentrations of phosphate and nitrate, respectively.

At the end of each experiment, microalgae biomass was recovered by centrifugation at 5000 rpm for 5 min and then freeze-dried for 12 h before storing. Total lipid content was estimated using the chloroform/methanol/buffer method[24]. Briefly, 50 mg of dry biomass was exposed to a mixture of chloroform:methanol: buffer (2:1:1) for 20 minutes under constant stirring. The mixture was filtered, the organic phase separated and evaporated to quantify the amount of lipids extracted[24].

Statistical analysis

All experiments were conducted in three independent replicates (n=3). Results are shown as the mean of replicates with their respective error bars (±). Results were compared using ANOVA ($p<0.05$) in GraphPad Prism 8.0.1.

RESULTS

Figure 1A shows that algal growth kinetics had a stable exponential phase when grown in synthetic medium under blue LED light intensities of 55.5 and 370 μmol m^{-2} s^{-1}. Interestingly, in the 55.5 μmol m^{-2} s^{-1} treatment, there was the longest exponential period in contrast to the 370 μmol m^{-2} s^{-1} treatment, which had the shortest exponential phase time. Overall production of chlorophyll in Figure 1B was significant under the same conditions. The kinetic performances and total biomass reported in Figure 1 were the base for the calculation shown in Figure 2.

Figure 1 – *Kinetics of Chlorella vulgaris biomass (A) and chlorophyll (B) grown in synthetic medium under blue LED light intensities from 14.7 to 370 μmol m^{-2} s^{-1}, with a 12:12h light/dark photoperiod and 25 ºC (±2 ºC). Error bars show the standard deviation (n=3).*

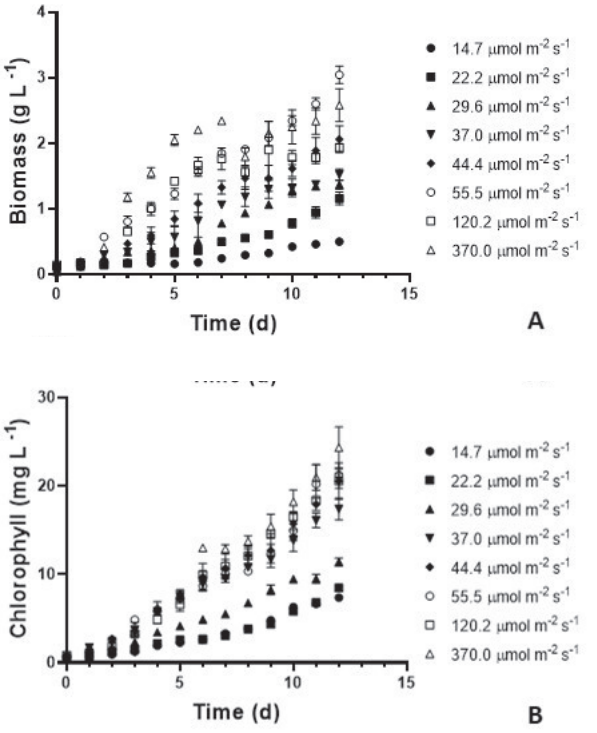

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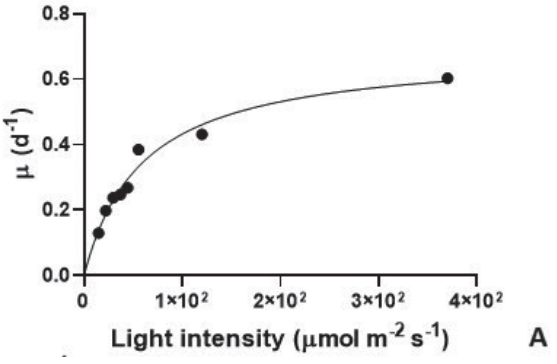
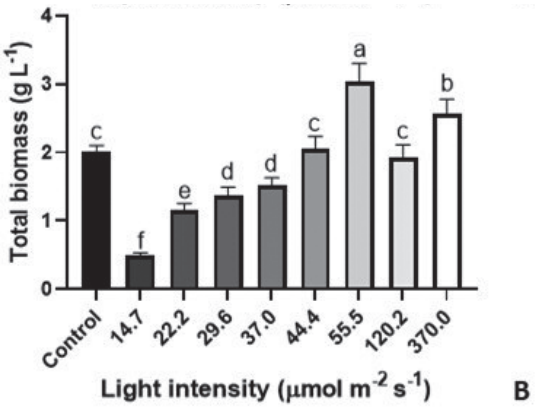
Figure 2 – *Specific growth coefficients (A) and total biomass production (B) of Chlorella vulgaris plotted against blue LED light intensities. The Lineweaver-Burk plot generated from data in panel A suggests a maximum growth coefficient (μ) of 0.68 d^{-1} and an affinity coefficient (comparable to the Monod k_s) of 61.8 μmol m^{-2} s^{-1}. The lowercase letters identify the statistical similarity and: a>b>c>d>e>f.*





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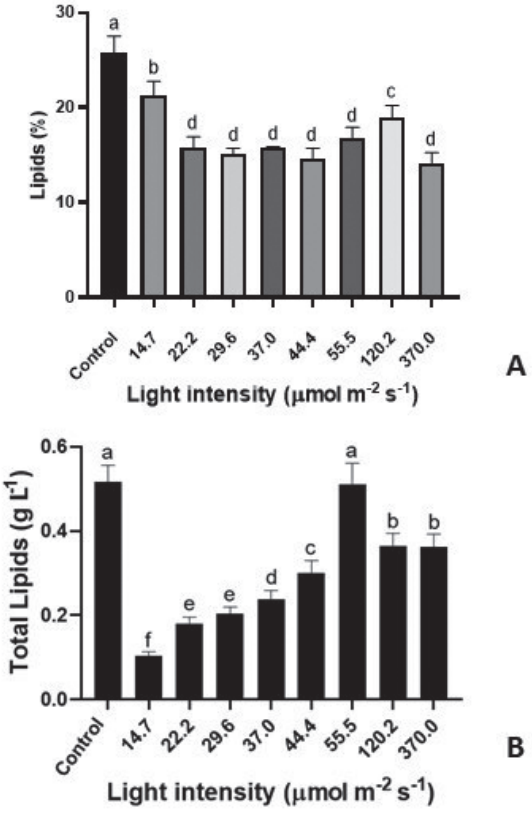
Lipid values ranged from 14.1 to 21.3% and total lipids ranged from 0.10 to 0.51 in all conditions exposed to blue LED light. Value is still below control using white LED light (Figure 3A and Figure 3B). However, in relation to total lipids at the end of cultivation, the treatment of 55.5 µmol m^{-2} s^{-1} of blue LED light is statistically similar to 55 µmol m^{-2} s^{-1} of white LED light, the control group of this work.

Figure 3 – *Percentage of lipids in the Chlorella vulgaris biomass (A) and total lipids (B) obtained under distinct blue LED light intensities, compared to the respective control with white light 55.5 µmol m^{-2} s^{-1}. The lowercase letters identify the statistical similarity and differences between the results: a>b>c>d>e>f.*



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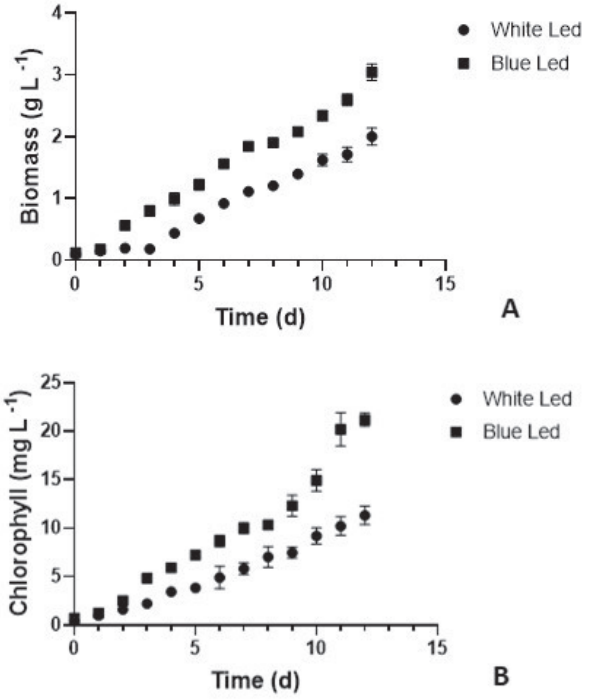
Comparing these two conditions in relation to growth kinetics under equal cultivation conditions, with the exception of the type of LED light used, it can be seen that the accumulation of biomass (Figure 4A) and chlorophyll (Figure 4B) is greater in blue LED light than in white LED light.

Figure 4 – *Results from an additional independent experiment showing the biomass growth curves of Chlorella vulgaris (A) and chlorophyll accumulation kinetics (B) under 55.5 µmol m^{-2} s^{-1} of blue and white LED light. Error bars show the standard deviation (n=3).*



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DISCUSSION

The results indicate that Chlorella vulgaris growth kinetics are significantly influenced by varying blue light intensities (Figure 1A). Highest intensities of 120 and 370 µmol m^{-2} s^{-1} exhibited two growth phases resembling a diauxic-like effect. The first phase lasted for the initial six days of the 12-day incubation period. This effect was not caused by a change in carbon source usage, as is typical in diauxic growth, but likely resulted from an inhibitory factor. High photosynthesis rates can substantially generate reactive oxygen species (ROS)[17]. Elevated light intensities may cause photooxidation of photosystem II (PS II), altering the reduced state of the primary quinone acceptor QA, which negatively impacts algal growth[25]. Although microalgae have mechanisms to neutralise ROS accumulation, these mechanisms are affected by intracellular ROS levels[18]. This inhibitory effect is less pronounced when

analysing cellular chlorophyll accumulation ratios (Figure 1B). For example, at 370 µmol m⁻² s⁻¹ of blue LED light, cells exhibited a chlorophyll accumulation ratio of 0.93% (Table 1). Safi et al.[26] (2014) reported that Chlorella sp. might accumulate chlorophyll-α between 1-2% under ideal conditions. Therefore, reduced biomass accumulation at high blue light intensities may not be directly linked to photosynthetic potential but rather associated with factors such as ROS accumulation affecting final energy yields. Elevated light flux, combined with high concentrations of dissolved oxygen resulting from intensified photosynthetic activity, can lead to photooxidative damage in microalgal cells[27,28]. Under such stress, often exacerbated by nutrient limitation or energy oversaturation, the electron transport chain becomes over-reduced, promoting the generation of reactive oxygen species (ROS)[29]. These conditions reduce photosynthetic efficiency and may lead to pigment bleaching, lipid peroxidation, and cellular damage[30]. In response, microalgae activate photoprotective mechanisms, including changes in biochemical composition, production of antioxidant compounds, and morphological adaptations such as increased cell size or enhanced oxygen tolerance, as part of an acclimation strategy to mitigate light-induced oxidative stress[31].

Microalgae growth rates may respond to light intensity in a manner similar to the Monod Model, commonly used to describe bacterial growth under varying carbon source concentrations. Therefore, plotting the growth coefficient against light intensity can help identify optimal performance conditions (Figure 2A). The growth coefficients consistently increased and did not decline within the range of 14.7 to 370 µmol m⁻² s⁻¹. Consequently, Monod parameters were calculated using the Lineweaver-Burk plot, indicating an affinity coefficient for light of 61.8 µmol m⁻² s⁻¹ and a maximum growth rate of 0.68 d⁻¹. According to the Monod model, the optimal growth coefficient might occur between 120 and 370 µmol m⁻² s⁻¹. However, Figure 2B reveals that the highest biomass accumulation was observed at 55.5 µmol m⁻² s⁻¹. This suggests that while higher light intensities may lead to increased growth rates, these rates are not sustained long enough to achieve maximum biomass concentration (Figure 1A). Given that the estimation for k_s was 61.8 µmol m⁻² s⁻¹, but the best growth performance was recorded at 55.5 µmol m⁻² s⁻¹, it may indicate that production peaks between 55.5 and 120 µmol m⁻² s⁻¹ rather than between 120 and 370 µmol m⁻² s⁻¹ (Figure 2A). The inhibitory effects at higher intensities likely disrupt the growth coefficients, becoming significant at midpoints in the growth curve. As a result, analysing only the highest growth coefficient does not fully capture the process; it is crucial to consider how long such high growth rates persist during cultivation.

Treatments with light intensities of 120 and 370 µmol m⁻² s⁻¹ supported algal growth rates of 0.43 and 0.60 d⁻¹ for 96 hours, respectively. In contrast, 55.5 µmol m⁻² s⁻¹ maintained a growth rate of 0.38 d⁻¹ for 168 hours, resulting in statistically higher biomass production (Figure 2B). Notably, a 75% shorter production period was observed with the higher intensities compared to the 55.5 µmol m⁻² s⁻¹ treatment. The hypothesis that rapid growth depletes media resources, thereby shortening the exponential phase, does not account for these results. Treatments at 55.5 µmol m⁻² s⁻¹ of blue or white light produced higher biomass and nutrient consumption ratios (N and P), indicating that nutrient availability was not limiting for the other treatments and supporting the notion that ROS generation may be a key factor affecting the performance at 120 and 370 µmol m⁻² s⁻¹ of blue LED treatment.

The algal biomass production rate at 55.5 µmol m⁻² s⁻¹ of blue light was 50% higher than the control, which utilized white LED light at the same light intensity. This demonstrates that microalgae performance is influenced not only by the total incidence of energy, but also by the specific wavelength used. Chlorophyll absorbs light primarily in the blue (430-470nm) and red (630-660nm) spectrum, promoting higher photosynthesis rates compared to other wavelengths[32-34]. Consequently, blue light directly impacts various physiological and biochemical processes in microalgae, including growth rates, cell division, and pigment synthesis[34].

Exploring light intensities between 100 to 300 µmol m⁻² s⁻¹, Atta et al.[16] (2013) found that C. vulgaris had higher growth and biomass accumulation at 200 µmol m⁻² s⁻¹, with around 2.7 g L⁻¹ biomass over 12 days. However, those authors did not compare growth under lower white or blue light intensities. This research shows that approximately 3 and 2 g L⁻¹ can be achieved using 55.5 µmol m⁻² s⁻¹ of blue or white light (12:12 photoperiod). Achieving significant biomass increases with less blue light energy than 200 µmol m⁻² s⁻¹ (Table 1). This is a very important observation, as it has the potential to reduce algae production system costs under LED light support.

The highest accumulation of cellular lipid (27%) was observed under 55.5 µmol m⁻² s⁻¹ of control white light (Figure 3A). The second and third highest under 14.7 and 120 µmol m⁻² s⁻¹ (23 and 20%, respectively). There was no significant difference between the averages of the other treatments (Figure 3A). This observation suggests that cellular lipid accumulation does not correlate with blue light intensities.

Total biomass impacts total lipid production most significantly. Highest total lipid levels were achieved when using either 55.5 µmol m⁻² s⁻¹ of blue or white light, respectively (Figure 3B). The difference in the percentage of cellular lipid ratios was compensated for by higher total biomass production. Although significant lipid was produced at 120 and 370 µmol m⁻² s⁻¹, they were 37% lower than at 55.5 µmol m⁻² s⁻¹ blue or white light. This finding has not been previously reported. Figure 4A, an independent test with 55.5 µmol m⁻² s⁻¹ white and blue LEDs, confirms that exponential growth starts earlier and lasts for longer under blue LED light (Figure 4B). Comparative studies have shown that blue LED light often enhances lipid accumulation in C. vulgaris compared to white light[16,35],

while white light tends to yield higher biomass productivity but similar lipid profiles[36].

Depending on the species and growth conditions, certain pigments may be more abundant, directly affecting the efficiency of light absorption across the visible spectrum[31]. Blue light, which is approximately 73% more energetic than red light, plays a significant role in regulating photosynthesis and metabolism[37]. While chlorophyll α absorbs mainly in the red (642 nm) and blue (372 nm) regions, accessory pigments such as chlorophyll ß and carotenoids extend light absorption across the full 400–700 nm range[38].

Microalgae of the genus Chlorella spp. have been widely studied under different LED spectra. Although red, blue, and white LEDs are commonly used, the physiological and photosynthetic responses remain inconsistent among species and studies[17]. Some reports show red LEDs promoting higher cell densities compared to blue light[9], while others demonstrate that blue LEDs enhance nutritional quality, leading to increased protein and lipid content, as well as larger cell sizes[16,39]. In particular, blue light has been shown to stimulate the production of nutrient-rich biomass suitable for aquaculture. Conversely, exposure to red light alone has been associated with lower biomass yield and even potential cellular damage, which may be alleviated by gradual blue light exposure[40,41]. These findings highlight the complex and species-specific nature of microalgal responses to light quality and emphasise the need for further investigation into the underlying cellular mechanisms[42].

The use of low-intensity LED lighting in microalgal cultivation has demonstrated the potential to stimulate biomass production while reducing operational costs. In this study, the highest algal productivities were achieved under intermediary light intensities, suggesting that energy input does not need to be maximised to optimise biomass yield. Economically, LEDs are at least 40% cheaper to install and operate over their lifetime compared to high-intensity discharge lamps, making them a more viable option for long-term applications[43]. Moreover, their ability to be programmed for specific light spectra and intensities allows for the fine-tuning of metabolic outputs, including the targeted production of high-value compounds[31]. While LEDs are generally more expensive than natural sunlight, they enable continuous illumination regardless of weather or geographic limitations, which can enhance productivity in indoor or controlled systems. Additionally, LEDs emit less waste heat, reducing the energy costs associated with temperature control. Integrating natural light with solar power and LED supplementation—especially at optimised, lower and intermediary intensities—can significantly improve energy efficiency and economic feasibility in large-scale photobioreactors[44].

The future of LED-based illumination in the commercial culture of phytoplankton is expected to hinge on case-specific demonstrations of its economic advantages. These advantages could include enhanced productivity and improved product quality achieved through a highly controlled, artificially lit indoor culture, compared to an equivalent outdoor production relying on natural light.

CONCLUSION

This study demonstrates that blue light intensity significantly influences the growth kinetics and total lipid production of Chlorella vulgaris. While high light intensities of 120 and 370 µmol m^{-2} s^{-1} initially promote rapid growth, they also result in some inhibition during later stages of culturing. In contrast, an intensity of 55.5 µmol m^{-2} s^{-1} consistently supports higher biomass and total lipid production over an extended period, outperforming both higher and lower intensities as well as the control using white light. Peak algal production may occur between the tested 55.5 and 120 µmol m^{-2} s^{-1}, possibly exceeding the estimated (k_s) value of 61.8 µmol m^{-2} s^{-1}. These findings underscore the importance of optimising light intensity through a framework such as the Monod Model, which could help identify the appropriate wavelengths to enhance photosynthetic efficiency while minimising stress. Future research should explore the effects of alternating white, blue, and red lights on cellular lipid accumulation further to improve the sustainability and efficiency of microalgae-based bioenergy systems.

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