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Effects of Different Light Spectrums on Growth, Chlorophyll and CO_2 Biofixation Capacity of Chlorella sp.

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ABSTRACT

Air pollution, increased traffic, and the extensive use of fossil fuels have led to elevated atmospheric CO_2 levels, undermining efforts to mitigate climate change. This study investigates the effect of different light spectra, on the growth performance and bioenergy potential of *Chlorella* sp. The strain was cultivated in BG11 medium under controlled conditions (25 ± 2 °C, 25 µmol/m²/s) for 15 days, exposed to monochromatic LED lights emitting blue, white, red, or green wavelengths. Growth parameters including optical density (OD_{600}), dry biomass (X), chlorophyll content (chl a+b), specific growth rate (µ), maximum biomass productivity (P_{max}), and CO_2 biofixation rate (FCO_2) were evaluated. The results demonstrated that red light significantly enhanced biomass accumulation (0.710 g/L), chlorophyll concentration (0.871 µg/mL), and CO_2 bio-fixation rate (0.0117 mg CO_2/d), outperforming other spectral conditions. Green light also supported notable growth, while blue light was the least effective. These findings contribute to optimizing light-driven cultivation strategies for sustainable microalgal biomass production and CO_2 mitigation in bioenergy applications.

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Keywords: *Bioenergy, Biomass, Chlorella sp., CO_2 biofixation, Light spectra.*

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1. Introduction

Anthropogenic activities, particularly the intensified use of fossil fuels and the surge in vehicular emissions, have markedly increased atmospheric carbon dioxide (CO_2) concentrations, fly ash and acerbating the global climate crisis [1]. Mitigating CO_2 emissions is a critical objective in achieving environmental sustainability, and biological CO_2 sequestration using microalgae has emerged as a promising strategy due to their high photosynthetic efficiency and rapid growth rates [2]. Microalgae have attracted growing interest as sustainable bioresources due to their rapid growth, high photosynthetic efficiency, and potential for producing valuable bioproducts such as biofuels, pigments, and pharmaceuticals [3].

Chlorella spp., a genus of unicellular green microalgae belonging to the Chlorophyta division, has been widely studied for its robust growth under various environmental conditions and its ability to sequester atmospheric CO_2 through photosynthesis [4,5]. Light quality, particularly spectral composition, is one of the most influential abiotic factors affecting photosynthetic activity, pigment synthesis, and biomass accumulation in microalgal cultures [6]. Light spectral composition plays a crucial role in regulating microalgal growth, photosynthetic efficiency, and pigment synthesis. Microalgae possess chlorophylls and accessory pigments that absorb light at specific wavelengths, with red and blue regions being most effective for photosynthesis due to their alignment with the absorption peaks of chlorophyll a and b [6,7]. Red light has been widely reported to enhance biomass accumulation and chlorophyll content in species such as *Chlorella vulgaris*, *Scenedesmus obliquus* and *Arthrospira platensis* by promoting efficient electron transport in Photosystem II [8,9]. White light is commonly used in microalgal biomass studies [10]. Overall, understanding the response of microalgae to different light wavelengths is essential for enhancing microalgal biomass production for bioenergy and bioproduct applications. Examining the effects of red, blue, orange and white light on growth, lipid synthesis etc. on *C. vulgaris*, the highest growth was found under red light and the highest lipid content under white light. It has been reported that white light increases the content of photosynthetic pigments. It has been reported that blue light gives the weakest results in terms of growth [11]. When the effects of red, blue and white lights were examined on *C. vulgaris*, *C. pyrenoidosa*, *S. quadricauda* and *S. obliquus* species, blue light was found to have higher concentrations of polyunsaturated fatty acids

(PUFA). In addition, it has been reported that blue light is effective in chlorophyll production and growth rate [12].

The present study aims to evaluate the growth performance, chlorophyll content, and CO_2 bio-fixation capacity of *Chlorella* sp. cultivated under different LED light spectra (blue, red, green, and white) by identifying the most favorable light condition for biomass production.

2. Materials and Methods

2.1 Microalgae and Culture Conditions

Chlorella sp., a eukaryotic green alga, was isolated from a freshwater source in Sorgun, Yozgat by Taştan et al. [4]. Figure 1 shows (a) the confocal microscopic image of Chlorella sp. and (b) the stock culture maintained in our strain collection at the Gazi University Center for Life Sciences Application and Research (Figure 1).

The strain was inoculated into 250 mL Erlenmeyer flasks containing 100 mL of BG-11 medium [13] and incubated under continuous illumination of 25 ± 2 °C and 25 µmol/m²·s (1750 lux). The microalgal cultures were maintained in a static incubator without continuous shaking; however, to prevent sedimentation and ensure more homogeneous light exposure and nutrient distribution, the cultures were manually shaken once per day. Although BG-11 is typically used for cyanobacterial cultivation, it is also commonly utilized for members of the Chlorophyta group [14,15]. The components of the BG-11 medium are summarized in Table 1. The BG-11 medium was prepared using standard stock solutions according to the formulation described by Rippka et al. [13], with macronutrients expressed as final concentrations and trace elements supplied via concentrated stock solutions.

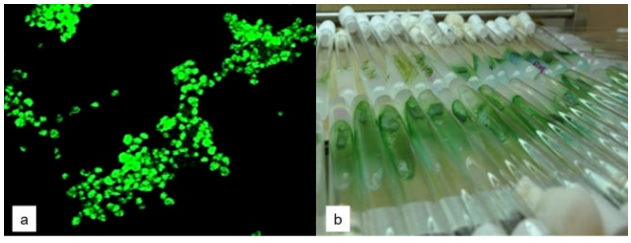


Figure 1. (a) *Chlorella* sp. confocal image (b) samples from the culture collection.

2.2 Incubation of *Chlorella* sp. Under Different Light Spectra

The *Chlorella* sp. cultures were incubated for 15 days at 25 ± 2 °C under four different light spectra: red, blue, green, and white. Light-isolated chambers were constructed using aluminum foil with separated shelves, each equipped with LED light sources emitting a specific spectrum, all adjusted to a uniform intensity of 1750 lux using a lux meter (Figure 2).

Each culture was grown in 250 mL Erlenmeyer flasks containing 100 mL BG-11 medium, incubated at 25 ± 2 °C under 25 µmol/m²s (1750 lux) light intensity. All experiments were carried out in triplicate. To ensure equal initial biomass, all flasks were inoculated with *Chlorella* sp. in the exponential phase to a final concentration of 0.1 g/L.

Table 1. Compositions of BG11 culture media

$NaNO_3$	**1.5 g/L**
Stock solutions	**g/L**
K_2HPO_4	0.04
$MgSO_4.7H_2O$	0.075
$CaCl_2.2H_2O$	0.03
Na_2CO_3	0.02
Citric acid	6.00
Ferric ammonium sulphate	6.00
Na_2 EDTA	1.0
A5 solution	**mL/L**
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.5H_2O$	0.390
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.6H_2O$	0.049

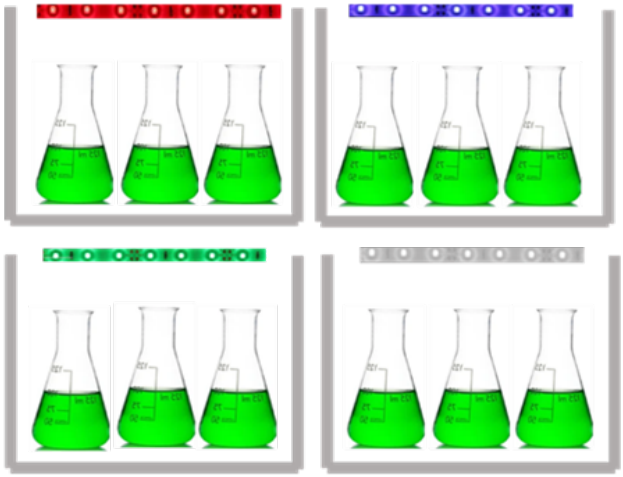


Figure 2. Schematic representation of incubation of *Chlorella* sp. in different light spectra.

2.3 Cell Growth Analyses

The cellular growth of *Chlorella* sp. was assessed by measuring optical density at 600 nm (OD_{600}), dry biomass (X), specific growth rate (µ), and maximum productivity (P_{max}). Optical Density (OD_{600}): Measured spectrophotometrically at 600 nm. Dry biomass determined by centrifuging the samples at 3421×g (5000 rpm) for 10 minutes, followed by overnight drying at 80 °C.

Specific Growth Rate (µ): Calculated using the following equation [16]:

$$\mu = \frac{lnX_2 - lnX_1}{t_2 - t_1} \quad (1)$$

where X_2 and X_1 are the dry biomass concentrations (g/L) at times t_2 and t_1, respectively.

Maximum Productivity (P_{max}) calculated by:

$$Pmax = \frac{X - X_0}{t - t_0} \quad (2)$$

where X and X_o are the dry biomass concentrations at times t and t_o.

CO_2 biofixation (F, g/g) was calculated according to the formula [17-19]:

$$F = aPxV \quad (3)$$

where a = 1.833 g CO_2, P_x is productivity, and V is the culture volume.

Chlorophyll analysis was conducted based on the method developed by Porra et al. [20]. Total chlorophyll (a+b) was calculated by summing individually determined chlorophyll a and b concentrations and expressed as micrograms per milliliter (µg/mL). All analyses were performed on day 15, which was the end of the incubation period.

2.4 Statistical Analyses

All recorded data were analyzed using Minitab® 14 and descriptive statistical analyses (mean ± standard error, S.E.). All experiments were performed in triplicate. Standard errors were calculated according to the equation described by Kenney and Keeping [21]:

$$SE = \sqrt{\sigma^2} \quad (4)$$

where SE is the standard error, and σ is the standard deviation of the measured variable.

3. Results and Discussion

3.1 Cultivation of *Chlorella* sp. Under Different Light Spectra

Chlorella sp. was incubated for 15 days in culture cabinets at 25 ± 2 °C and 25 µmol/m²s (1750 lx) of light intensity in 250 mL Erlenmeyer flasks containing 100 mL of BG11 medium, as illustrated in Figure 3.

Optical density measurements at 600 nm, taken on days 7 and 15 under blue, white, red, and green light conditions, are presented in Figure 4. Also figure 5 shows the biomass produced by *Chlorella* sp. at the end of the incubation under different light spectra. The highest biomass and chlorophyll concentrations were observed under red and green light, whereas the lowest values were recorded under blue light.

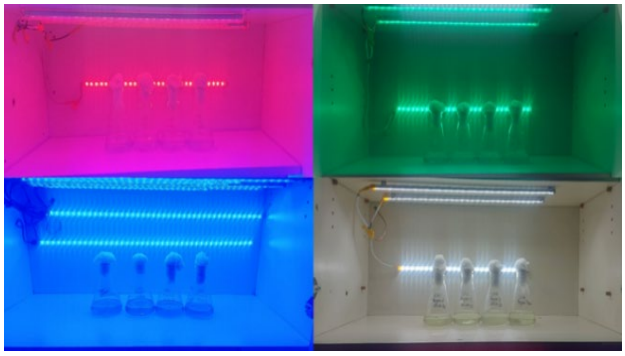


Figure 3. Specially designed incubation chambers equipped with green, white, red, and blue LED lights and constructed to be completely impermeable to external light.

The optical density (OD) values of *Chlorella* sp. cultured under different light conditions were measured on days 7 and 15, and the results are as follows: Under blue light, the OD value increased from 0.45 on day 7 to 1.02 on day 15. Under white light, the OD value rose from 0.875 to 1.36 over the same period. In cultures exposed to red light, the OD value increased significantly from 0.46 on day 7 to 1.62 on day 15. Under green light, the OD values were 0.43 and 1.42 on days 7 and 15, respectively. These results indicate a general increase in cell density over time under all light conditions. Notably, the most pronounced growth was observed under red light, followed by green, white, and blue light, respectively. This suggests that red light may be more favorable for the proliferation of *Chlorella* sp. compared to the other light wavelengths tested.

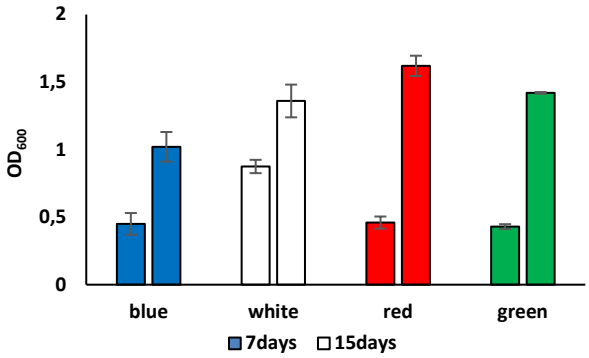


Figure 4. The effect of blue, white, red, and green light on the growth of *Chlorella* sp. (OD_{600}).



Figure 5. Images of *Chlorella* sp. cultivated under different light spectra, recorded on the 15th day.

3.2 Bioenergy Production

Dry biomass (X, g/L), chlorophyll concentrations chl (a+b, µg/mL), specific growth rates (µ, 1/d), maximum biomass productivity (P_{max}, g/L·d), and CO_2 biofixation rates (FCO_2, mg CO_2/d) of *Chlorella* sp. were summarized in Table 2. Among the tested conditions, the highest dry biomass concentration (0.710 ± 0.035 g/L) was achieved under red light, followed by green (0.623 ± 0.048 g/L), white (0.596 ± 0.035 g/L), and blue light (0.447 ± 0.06 g/L), indicating a favorable influence of red and green wavelengths on overall biomass accumulation.

Similarly, the total chlorophyll (a+b) concentration was found to be maximum under red light (0.871 ± 0.04

µg/mL), with green (0.763 ± 0.06 µg/mL) and white (0.731 ± 0.04 µg/mL) lights showing moderate pigment synthesis, and blue light resulting in the lowest chlorophyll content (0.548 ± 0.07 µg/mL). This suggests that chlorophyll biosynthesis in *Chlorella* sp. is enhanced under longer wavelength illumination.

Table 2. Dry weight (X) (g/L), chlorophyll concentrations chl (a+b) (µg/mL), specific growth rates (µ) (1/d), maximum biomass productivity (Pmax) (g/Ld) and CO_2 biofixation rates (FCO_2) ($mgCO_2$/d) of *Chlorella* sp.

	Blue	White	Red	Green
X (g/L)	0.4470 ± 0.0600	0.5960 ± 0.0350	0.7100 ± 0.0350	0.6230 ± 0.0480
Chl (a+b) (µg/mL)	0.5480 ± 0.0700	0.7310 ± 0.0400	0.8710 ± 0.0400	0.7630 ± 0.0600
µ (1/d)	0.1020 ± 0.0020	0.0550 ± 0.0030	0.1570 ± 0.0020	0.1500 ± 0.0070
P_{max} (g/Ld)	0.0310 ± 0.0050	0.0270 ± 0.0030	0.0630 ± 0.0030	0.0540 ± 0.0050
F_{CO2} ($mgCO_2$/d) (0.1 L)	0.0057 ± 0.0008	0.0049 ± 0.0005	0.0117 ± 0.0005	0.0099 ± 0.0010

The specific growth rate (µ), an indicator of cell division efficiency, peaked under red (0.157 ± 0.002 1/d) and green light (0.150 ± 0.007 1/d), followed by blue (0.102 ± 0.002 1/d), while white light produced the lowest growth rate (0.055 ± 0.003 1/d). In terms of maximum biomass productivity (P_{max}), *Chlorella* sp. again showed the highest value under red light (0.063 ± 0.003 g/L·d), with a slightly lower value under green light (0.054 ± 0.005 g/L·d). Blue and white light resulted in significantly lower productivities (0.031 ± 0.005 and 0.027 ± 0.003 g/L·d, respectively). Lastly, CO_2 biofixation rates, calculated for 100 mL cultures, correlated strongly with biomass productivity. The red-light spectrum supported the most efficient CO_2 fixation (0.0117 ± 0.0005 mg CO_2/d), followed by green (0.0099 ± 0.001 mg CO_2/d), blue (0.0057 ± 0.0008 mg CO_2/d), and white light (0.0049 ± 0.0005 mg CO_2/d).

4. Discussion

Several literature reviews have also emphasized the biomass-enhancing effect of red light in such microalgae species. For instance, red light, as well as combinations such as red-green or red-blue, have been reported as equally effective for promoting growth in species such as *Phaeodactylum tricornutum* and *Scenedesmus* sp. Specifically, red light enhanced biomass formation in *P. tricornutum*, while red and blue light combinations stimulated growth in *Scenedesmus* species, and red and yellow light promoted biomass in *Nannochloropsis* [9].

In another study effect of different light spectra was tested with different microalgae. *S. obliquus*, *C. vulgaris* and *A. platensis* were cultured in Bold's basal medium for 10 days under red, white, blue, green, and yellow LED lights. The results indicated that the highest biomass yield was obtained under red light, with S. obliquus exhibiting the most prominent performance, reaching a dry weight of 1.43 g/L. Similarly, *A. platensis* produced high biomass under red light (1.41 g/L) [8].

In a co-culture system of *C. vulgaris* and *Leptolyngbya*, conducted under a light intensity of 400 µmol/m²s, red light yielded the highest values for final biomass, growth rate, and productivity (913 mg/L, µ = 0.41/d) [22].

Red light has also shown efficacy in various *Haematococcus* strains. Notably, under low intensity red light (5 µmol photons/m²s), cell density was reported to be 24–77% higher compared to white light [23].

Overall, red light has been shown to significantly enhance biomass and growth rates in various green and siliceous microalgal species, particularly *S. obliquus*, *C. vulgaris*, *A. platensis*, and *Haematococcus* sp. [8].

This stimulatory effect is attributed to the absorption characteristics of chlorophyll pigments, which exhibit high absorption efficiency in the red spectrum (630–680 nm), thereby enhancing photosynthetic activity. Chlorophyll a has strong absorption peaks in the blue (~430 nm) and red (~665–666 nm) regions, whereas chlorophyll b absorbs maximally in the blue-green (~453 nm) and red (~642 nm) regions. Spectral analyses confirm that the red light region, particularly between 660–680 nm, is most effective for chlorophyll absorption. For example, red LEDs operating at these wavelengths have been shown to exert strong effects on chlorophyll a [7].

In heterotrophic cultures, *C. vulgaris* reached a biomass concentration of 0.79 g/L under white light by day 12. In mixotrophic conditions, the maximum specific growth rate under red light was reported as $\mu_{max} = 0.41\ d^{-1}$. Additionally, white light resulted in the highest biomass concentration (1.34 g/L), followed by blue (0.90 g/L), red (0.82 g/L), and yellow (0.76 g/L) light treatments [24].

Consistent with recent findings by Palikrousis et al. [25], higher light intensities significantly enhanced *Chlorella sorokiniana* biomass and altered biochemical composition, emphasizing the critical role of light parameters in microalgal cultivation. A recent review by Yu et al. [26] also highlights that optimization of light quality is crucial for advancing microalgae applications in sustainable bioenergy and environmental management. Studies on *Chlorella ellipsoidea* demonstrate that different LED exposure significantly increases chlorophyll and carotenoid contents, supporting our discussion on pigment responses to different light spectra [27].

Previous studies have demonstrated that the predominant FAME components synthesized vary depending on the microalgal species and light spectra. When the effects of light at different wavelengths on FAME composition in *Nannochloropsis* sp. and *Tetraselmis* sp. were investigated, it was reported that higher FAME production occurred under blue light compared to red, green, and white light. In *Tetraselmis* sp., blue light

exposure resulted in the synthesis of palmitic acid methyl ester at a rate of 53.7%, whereas this compound was observed at a rate of 16.49% in *Nannochloropsis* sp. Conversely, the most abundant FAME component in *Nannochloropsis* sp. under blue light was stearic acid methyl ester, with a proportion of 32.85%, while it was detected at 29.18% in *Tetraselmis* sp. [28]. In a similar study, it was reported that cultures grown under blue light exhibited the highest biomass conversion efficiency, the greatest lipid content, and the lowest carbohydrate content [29].

Conclusions

This study demonstrated that light spectrum significantly influences the growth performance, pigment synthesis, and CO_2 biofixation capacity of *Chlorella* sp. cultivated under controlled laboratory conditions. Among the tested wavelengths, red light most effectively enhanced biomass accumulation, chlorophyll (a+b) content, specific growth rate, and maximum biomass productivity, resulting in the highest CO_2 fixation rate. Green light also supported relatively high growth and carbon sequestration, while blue light was the least favorable for overall performance. These findings suggest that optimizing light quality, particularly through the application of red and green LED illumination, can enhance microalgal biomass production and improve the efficiency of CO_2 biofixation systems.

Conflict of Interest

There are no conflicts of interest in this work.

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Declaration of Generative AI

The authors confirm that no generative AI technologies were used in the preparation, writing, or data analysis of this manuscript.

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