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ORIGINAL RESEARCH

Effect of light on growth of green microalgae *Scenedesmus quadricauda*: influence of light intensity, light wavelength and photoperiods

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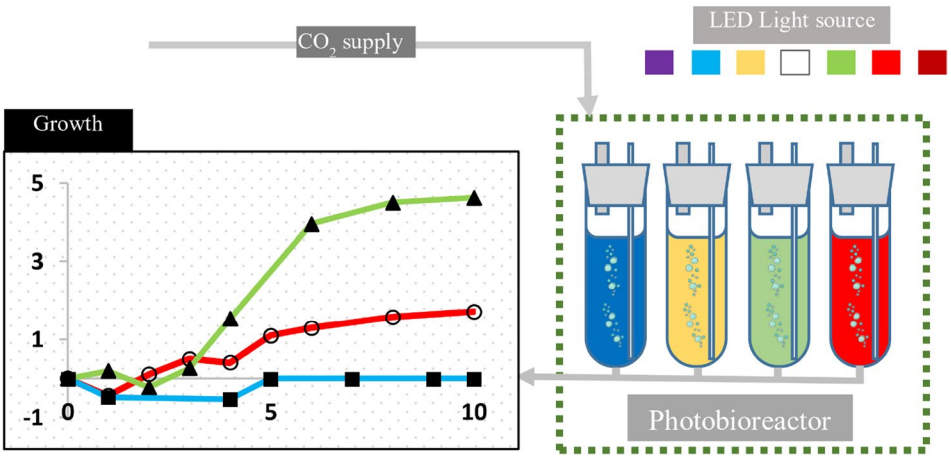
Abstract

Microalgae biomass is a potential source of biomass to be considered as a source of bioenergy, biochemicals and even food supply. The key to an economic and healthy algal cultivation is to optimize the growth conditions. The main objective of this research was to investigate the effects of light on the growth of green microalgae *Scenedesmus quadricauda*. This wild-type green microalgae *Scenedesmus quadricauda* obtained from the Porsuk River in the Eskisehir region of Turkey characterized by a high lipid content has been cultivated at different wavelengths, different light intensities and different photoperiod cycles. Our results show that for each light intensity, there is an optimal wavelength. The best performing light intensity was 500 µmol photons m^{-2} s^{-1} with a wavelength of 660 nm corresponding to specific growth rate of 0.621 d^{-1} and an average biomass productivity of 0.756 g/L following 10 days of growth. When the intensity of the light has been increased to 1000 µmol.m^{-2}.s^{-1} photons, growth is inhibited by showing signs of photodamage. At an intensity of 100 µmol.m^{-2}.s^{-1}, the growth was either very slow for some wavelengths or no growth for majority of them. This suggests that there is a minimal intensity threshold for growth being specific for each wavelength. Variable growth performances under different light/dark cycles imply necessity of the factor optimization. In this case, a photoperiod of 1: 1 h light:dark mode, with a moderate intensity of 500 µmol of photons m^{-2} s^{-1}, was the best for an efficient productivity.

Graphical abstract



Keywords Microalgae · Growth optimization · *Scenedesmus quadricauda* · Intensity of light · Photoperiod cycles · Light quality

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Introduction

Microalgae are considered as an interesting resource in biomass production since 1950 [1]. Microalgae are microscopic prokaryotic or eukaryotic photosynthetic organisms found in diverse aquatic habitats, marine and freshwater [2]. They are capable of converting light energy and carbon dioxide (CO_2) into a set of organic products or biomass [3]. These photosynthetic microorganisms have various biochemical characteristics for a wide range of applications in different fields. They produce numerous molecules with high added value (omega 3, beta carotene and antioxidant) used in the pharmaceuticals and cosmetics industries as well as environmental applications like the operations of removing pollution like heavy metals and CO_2 fixation [4]. Microalgae are of great interest for the large-scale production of algal biofuels like biodiesel, bio-oil and bio-hydrogen [3].

Nevertheless, progress is still needed to reduce the economic and environmental costs of cultivation processes and thus ensure the viability of the sector. In particular, better understanding on the effect of light on the microalgae productivity is needed. To obtain good productivity, it is therefore important to illuminate microalgae. In general, those wavelength range of light strongly absorbed by microalgae is necessary to obtain a high growth rate. However, it should be noted that the effect of the wavelength interacts with the intensity and photoperiod [5, 6]. In a range of intensity, the microalgae optimize its photosynthetic machinery to the light intensity it undergoes; this behavior is a consequence of a photoacclimation. However, the increase in intensity can cause a photodamage that manifests itself in a growth inhibition, called photoinhibition [7, 8]. In this sense, according to the literature, there are two levels of inhibition that is designated SPII and SPI; photodamage caused by absorbed light is balanced by repair. However, repair of PSII photodamage can be overcome by increases in light outside the photoacclimated condition, leading to severe PSII photodamage that slows the cell specific growth rate [9]. In addition, a very large increase in light intensity can lead to photodamage by PSI, which is semi-permanent in that it can take days or weeks to repair [9, 10]. On the other hand, the growth of algae is limited by light if the average of the light intensity received is less than that required by the saturation of photosynthesis. It should be noted that each type of microalgae has a minimum absorption, and saturating intensity in every specific wavelength [11]. The literature also reports that the metabolic activity of a microalgae may change depending on the wavelength applied [12] where using the *Scenedesmus sp* strain in a water treatment process showed that the growth rate of microalgae, the rate of nitrogen removal, the rate of phosphate removal differ according to the wavelength used (white, red or blue) or in a mixing ratio of different wavelengths. In the work of Yanan. XuIskander M.Ibrahim [13], it was reported that light influences the growth of microalgae, as well as the levels of certain constituents of cells.

The photoperiod cycle is as important as the intensity and quality of light. In this sense, many studies specify that the photoperiod condition has significant effects on the production of microalgae biomass as well as on the levels of certain valuable compounds in the cell; in addition, it has been shown that it has an effect on the adjustment of pollutant removal. As a result, photoperiod control has been proposed as a significant operating parameter in the production of microalgae and the treatment of wastewater by microalgae [14, 15]. In this context, our work in this paper is to understand the interactive effects of light intensity and quality on the growth of the strain *Scenedesmus quadricauda.* To the extent of our literature search, there are few research works invested in the culture of this strain [16, 17]. Our work is the first one that aims to highlight the best growth rate as well as productivity in a spectrum of eight wavelengths ranging from blue to red and therefore delineate the thresholds of saturation and the weakest absorption thresholds of the growth.

Materials and methods

Obtaining and isolating the strain *Scenedesmus quadricauda*

Green microalgae *Scenedesmus quadricauda* strain for this study was isolated from a slow flow point of the Porsuk River in the Eskisehir region of Turkey (Botanic Park 39.742747, 30.460088). The procedure of isolation and purification was described elsewhere [18]. The microalgae were cultured in BG11 medium in the artificial culture media for algae microorganisms which for one liter the composition is 1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4.7H_2O$, 0.036 g $CaCl_2.2H_2O$, 0.006 g citric acid, 0.001 g EDTA-2Na, 0.02 g Na_2CO_3 and 1 ml from trace metal solution stock with following composition in one liter: 2.86 g H_3BO_3, 1.81 g $MnCl_2.4H_2O$, 0.222 g $ZnSO_4.7H_2O$, 0.39 $NaMoO_4.2H_2O$, 0.079 g $CuSO_4.5H_2O$, 49.4 mg $Co(NO_3)_2 \bullet 6H_2O$ prepared according to [19].The pH of a medium was adjusted to 7.5 and temperature at 27 °C. All the chemicals were analytical reagent grade Fig. 1.

Preparation of pre-culture

The *Scenedesmus quadricauda* inoculum cultured in modified BG11 medium in.

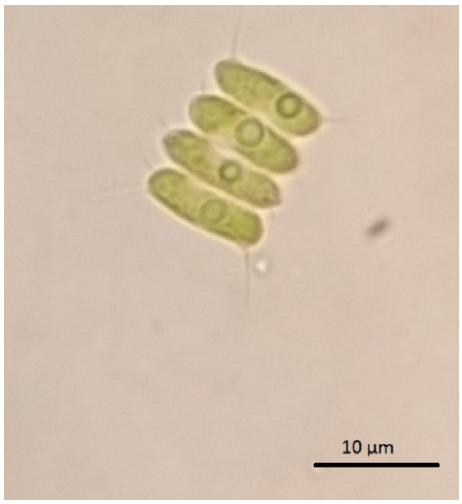

Fig. 1 Oil-immersion microscopic image at × 1000 of *Scenedesmus quadricauda*

Erlenmeyer flasks closed with a cotton stopper. These Erlenmeyer flasks maintained at 25 °C with a supply of air/CO_2 mixture containing 5% CO_2 with a flow rate of 2 vvm. The cultures were lit continuously with 3500 lx light intensity provided by 35 W fluorescent lamps.

Experimental culture device

To study the effect of light on the growth of microalgae, experiments were carried out in a photobioreactor (Multicultivator MC1000-OD, Photon Systems Instruments) which allows the algae to grow at controlled conditions of light, temperature and provides perfect mixing level in a semi-batch mode where gas was provided continuously. The cultivations per run were made simultaneously in eight separated identical cylindrical glass cells with diameter 2.5 cm and length 20 cm dimension. Active cell volume was 80 ml. The 8 cells were immersed in a water bath set at 27 °C. This temperature resembles the optimum growth range. Illumination was provided by LEDs placed equidistantly along the cell height providing homogeneous lighting. They generate incidental irradiations up to 1000 µmol.m^{-2}.s^{-1} independently adjustable for each cell. The provided lights quality is shown in Figs. 2 and 3.

Inoculation and photobioreactor start-up

As a rule, 75 ml of autoclaved BG11 and 5 ml of Scenedesmus quadricauda inoculum were loaded into each cell by syringes through 3-way luer-lock valves. The pH adjusted to 7.5, which is considered as the ideal pH for the growth of microalgae. Agitation inside the cells was achieved by aeration. A gaseous stream containing 5% carbon dioxide and air was provided using two gas mass flow controller (647B MKS Instruments, USA) and distributed evenly between the 8 cells of culture with a net flow rate of 2 vvm using pre-calibrated rotameters (mzb instruments, China). The gas stream was passed through 0.2 µm PTEF membrane filters (Fluoropore, Merck Millipore) which are mounted at the inlet of the gas to provide a sterile gas stream. The lost water due to evaporation was replenished with distilled water every day.

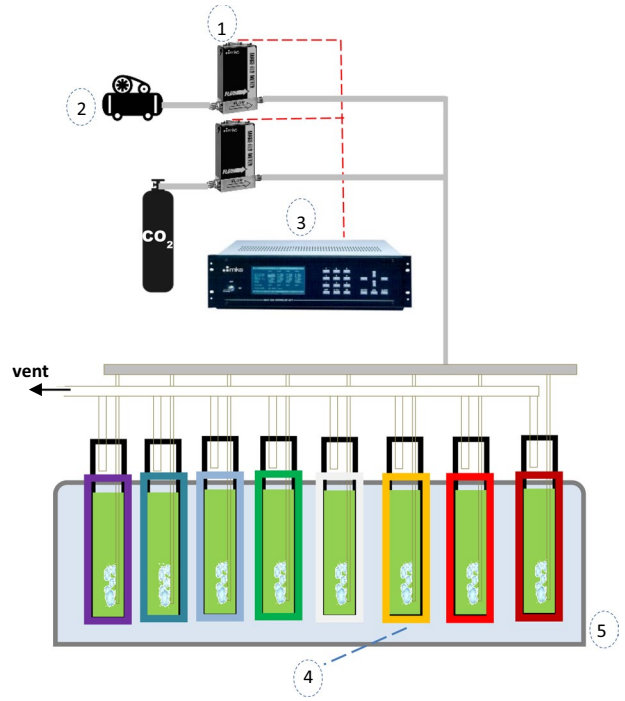

Fig. 2 Experimental setup.1**. 1:** Mass flow controller. **2:** Air compressor. **3:** Control unit **4:** Cultivation cells with different color LED's (1:8). **5:** Temperature controlled bath

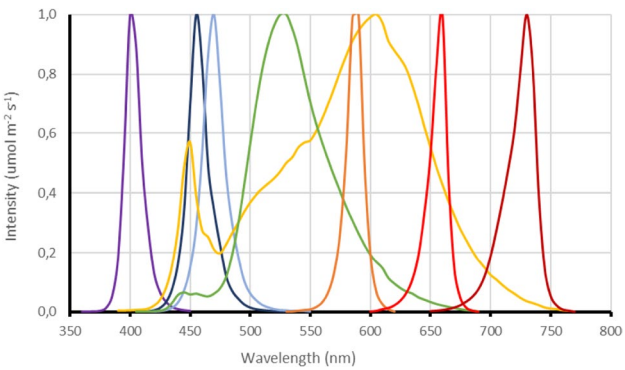

Fig. 3 The representative LED spectra and light quality used in multicultivator (provided by the manufacturer)

Determination of cell concentration

In order to follow the growth of the strain, the cell concentration was regularly determined by measuring the optical density (OD). The OD measured at 680 nm wavelength by means of a spectrophotometer (UV-1800 Shimadzu UV–Vis spectrophotometer) using a quartz cell. It was read in the OD range between 0.2 and 1.1 where concentration–OD_{680} curve was linear. Samples of 3 ml volume where taken using a syringe every two days. For the OD values higher than 1.1, dilution with distilled water was followed. BG11 with no microalgae was read and used as blank. A calibration curve was prepared to convert the optical density to cell concentration (g/l) on the basis of the dried biomass. A very well grown culture of microalgae was centrifuged and then divided into two parts with known volume. One part was left in oven at 90 °C to dryness until no meaningful change in dried mass weight was observed. The other part was gradually diluted, and OD was read and plotted. The linear regression provided correlation between concentration of biomass and optical density as Fig. 4:

$$\text{Conc.}\,(\text{g/l}) = 0.408 \times \text{OD}_{680}\left(\text{R}^2 = 0.997\right) \quad (1)$$

Calculation of the specific growth rate

The specific growth rate (**μ,** day^{-1}) was calculated as the slope of logarithmic growth phase according to:

$$\text{Ln}\left(\frac{C_t}{C_0}\right) = \mu t \quad (2)$$

where **μ** represents the specific growth rate (day^{-1}), $\boldsymbol{C}_0$ represents biomass concentration at the beginning of the culture in (gr/l) and $\boldsymbol{C}_t$ is the concentration of biomass at time t (gr/l).

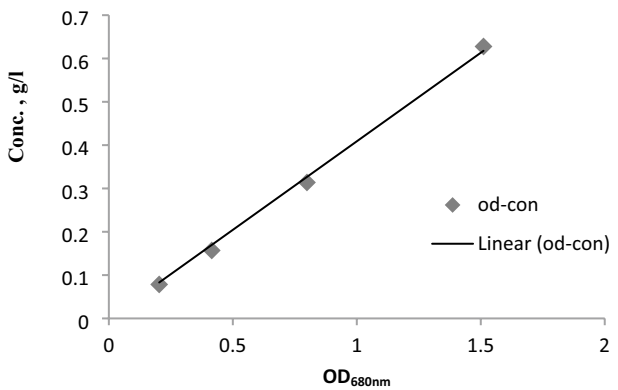

Fig. 4 Calibration curve for biomass determination

Results and discussion

Effect of the light intensity and light wavelength on the growth of *Scenedesmus quadricauda*

The cultures were exposed continuously (24/24 h) at different wavelengths in a range of 405 to 730 nm (Fig. 1). Three light intensities were tested being 100, 500 and 1000 µmol. m^{-2}. s^{-1}. The growth was monitored for nearly 10 days until the growth curve reaches the stationary phase or has entered the decline phase. The results obtained are shown in Fig. 5.

Light is the most important life parameter in the growth of microalgae. According to the results shown in Fig. 5, it is clear from the shape of the growth curve that the strain does not absorb light at different wavelengths to the same degree.

Figure 5a, corresponding to the wavelength λ = 405 nm, shows an absence of growth under the different intensity of light and also shows an inability of cells to adapt. This indicates that this wavelength 405 nm exceptionally cannot be tolerated by the strain Scenedesmus quadricauda.

With the different wavelengths, it was noted that the lag phase duration was different depending on the quality of light, but it does not constitute a significant factor of productivity. Indeed, the comparison between the different cases shows that the strain in the cases, where its lag phase was relatively long, was able to acclimate and resume growth which leads to a productivity very close to the cases where the lag phase was short. This is the case, for example, with growth under the intensity 500 µmol. $\text{m}^{-2}.\text{s}^{-1}$ for wavelengths corresponding 405 nm, 470 nm, 540 nm and 660 nm.

Under the intensity of 500 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$, it turns out that the growth with the wavelengths corresponding to the cells (470 nm, white light, 540 nm, 590 nm and 730 nm) represents a pace that can be governed by the Monod model with lag phases ranging from 1 to 4 days. As an exception, we noted that the growth under a wavelength of 660 nm is the only one that shows a start of growth without a lag phase. The growth rate obtained, compared with the other cells, was relatively high ($\mu = 0.816\ \text{d}^{-1}$) with a final concentration of 6.138 g/l obtained after 8 days of culture. Similarly, we note that the decline phase occurred suddenly, without a stationary phase. This phenomenon is explained by a strong excitation which reached an energy level which caused a damage or the cells were incapable of moderating its harmful effects. This is also called photoinhibition where the photosynthetic cycles inside cells is not able to quench the high rate of electron flow at primary photoreactions [20].

These results are comparable to the growth of Chlorella vulgaris at different wavelengths, which show

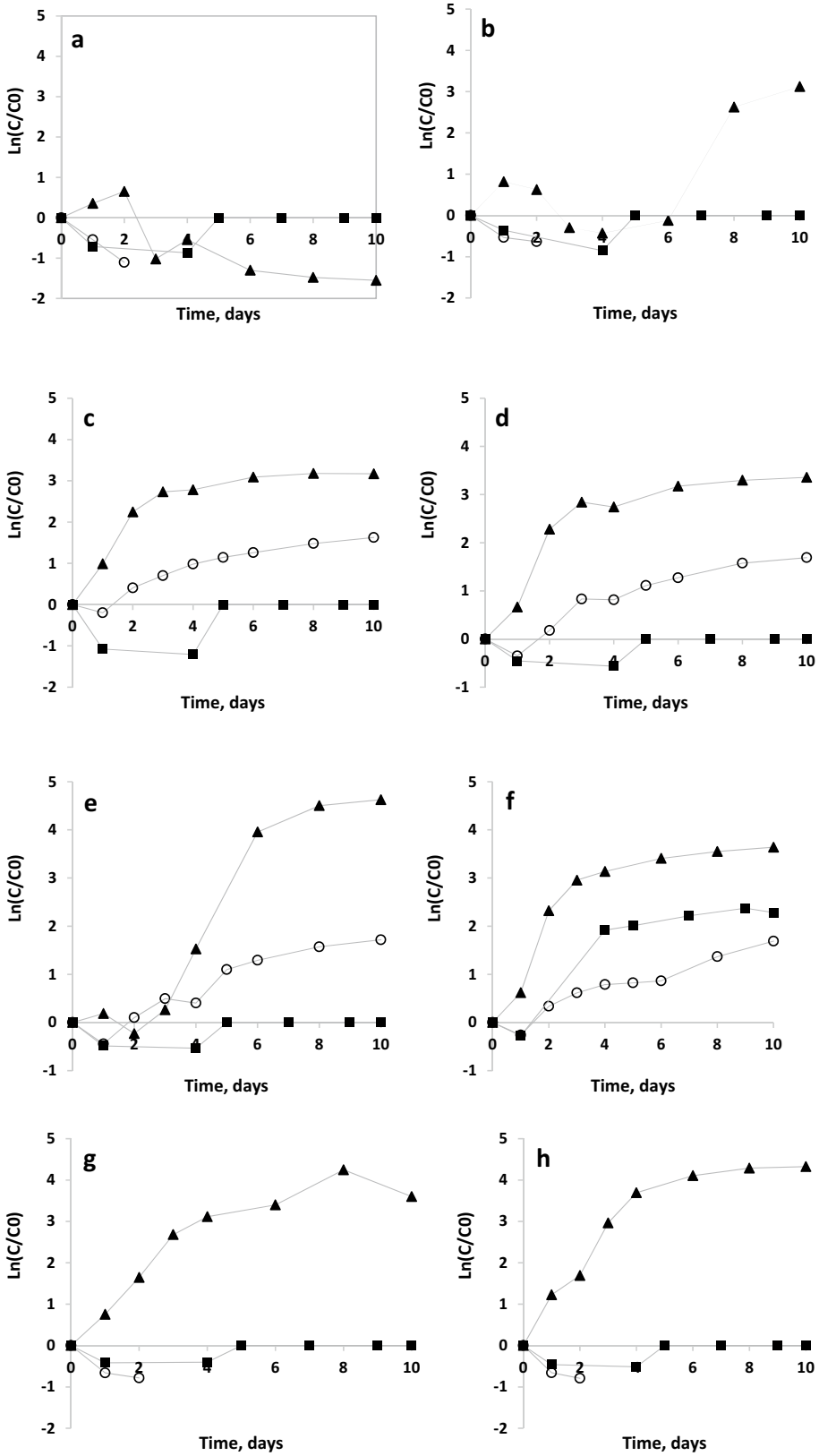

Fig. 5 Microalgae growth curves under continuous 24 h light at different wavelengths (**a**: 405 nm, **b**: 450 nm, **c**: 470 nm, **d**: white, **e**: 540 nm, **f**: 590 nm, **g**: 660 nm and **h**: 730 nm) and different light intensities (○:100; ▲: 500 and ■:1000 µmol.m^{-2}.s^{-1})

that the highest growth was in cultures lit by red light [21]. In contrast to our results, the case of the results of Mounica Asuthkar et al. [22] showed that pyrenoidosa has developed better under blue light than other types of light. From these results, it appears that the optimal wavelength is function of the microalgae strain. So the intensity 500 µmol.m^{-2}.s^{-1} generated the best growth at all wavelengths except 405 nm which with a 24 h lighting no growth was observed.

For the lowest intensity 100 µmol.m^{-2}.s^{-1}, the results show an absence of growth of the strain with the wavelengths of 405 nm, 450 nm, 660 nm and 730 nm; on the other hand, the growth was observed at cell wavelengths 470 nm, white light, 540 nm and 590 nm. However, it should be pointed out that the light intensity 100 µmol.m^{-2}.s^{-1} generated in these cells with less growth rate, compared with the growth intensity of 500 µmol.m^{-2}.s^{-1}. This is explained by the existence of a threshold of growth intensity which has not been reached in the cases with 405 nm, 450 nm, 660 nm and 730 nm wavelengths. On the other hand, this light intensity is considered in the growth range for the cases of 470 nm, white light, 540 nm and 590 nm. From these results, it emerges that the energy threshold required for growth also depends on photon energy, structural characteristics concerning the pigments of the cells.

In a general way with the highest intensity 1000 µmol m^{-2} s^{-1}, we noted a lack of growth. These results show that the intensity was very strong for the strain *Scenedesmus quadricauda* and led to an acute photodamage of PSII or a photodamage of PSI. On estimates in our case that it is a photodamage at the level of PSI. In this sense, the literature defines the photodamage system II as a reparable inhibition after a few hours [10], while the PSI is irreversible and is due to an imbalance between the balance of light responses and electron consumption in the chloroplast which is vital for plants and protected by several photosynthetic regulation mechanisms. Photosystem I (PSI) is particularly susceptible to photo-inhibition when these factors become unbalanced, which can occur at low temperatures or in bright light [23].

The growth obtained as an exception with 590 nm is consistent with the work of Tim and Mooij [24]. The results show that under culture conditions under 1000 µmol.m^{-2}.s^{-1}, the productivity and the absorption of light specific to biomass are inversely correlated. The highest productivity rate, measured under continuous illumination, was obtained using white, 540 nm and 590 nm lights corresponding to 1.014, 1,084 and 1,057 day^{-1} (Fig. 6a). These are close wavelengths at the middle range where a white color itself is a combination of other wavelengths. The quality of light as shown in Fig. 3 shows that white color actually covers

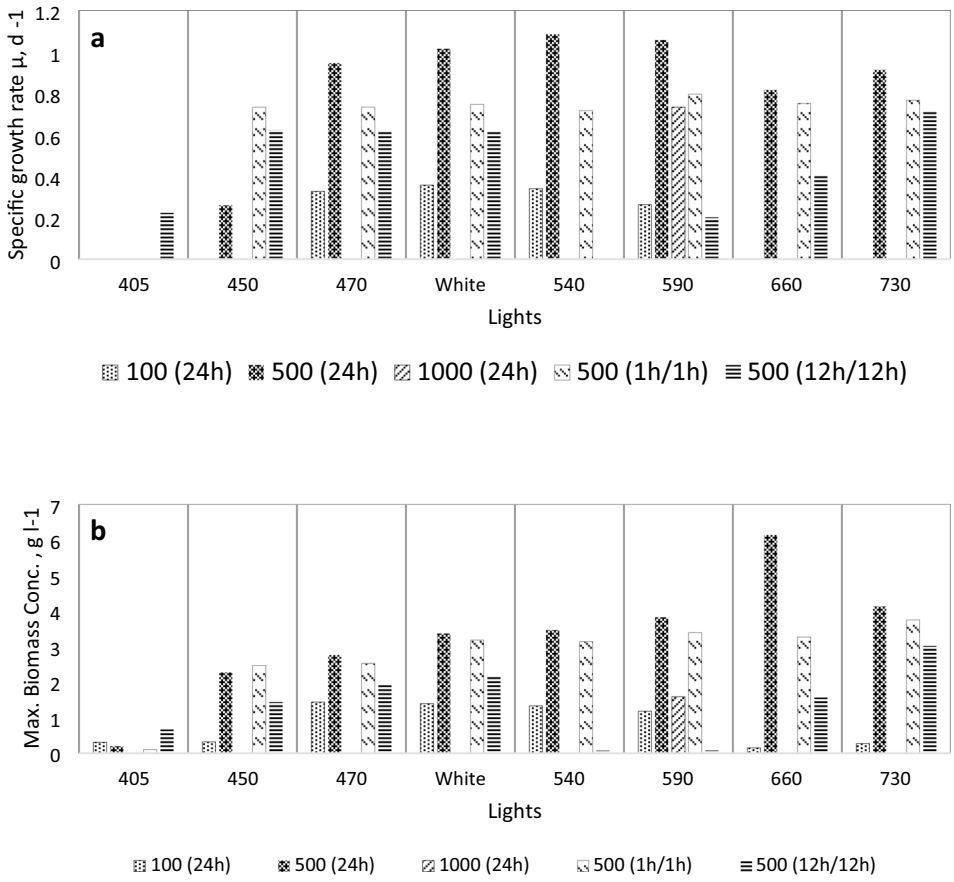

Fig. 6 Effect of the light intensity and light wavelength on max growth rate and biomass productivity. **a**: μ_{max}; **b**: biomass highest concentration

the 590 nm light and partly 540 nm color ranges very well. Therefore, it is expected for these lights to have similar outputs.

The non-growth in blue does not coincide with the general idea put forward in the literature [25] that chlorophyll **a** has maximum absorption for blue and red wavelengths, whereas little absorption occurs for green wavelength.

According to the results shown in Fig. 6b, the maximum biomass concentration was obtained at light intensity 500 $\mu mol.m^{-2}.s^{-1}$ under wavelength $\lambda = 660$ nm with values of 6.14 g/L and a specific growth rate of 0.816 day $^{-1}$. Whereas for intensities of 1000 and 100 $\mu mol\ m^{-2}\ s^{-1}$ the highest value of maximum biomass concentration and growth rate μ_{max} were (1.590 g/L and 0.732 d^{-1}) and (1.398 g/L and 0.356 d^{-1}), respectively. These results are related to the fact that the cultures which maintained grown at 500 $\mu mol.m^{-2}.s^{-1}$, resulted in an improvement in volumetric growth rate and concentrations.

These results are consistent with those of Gökçe Kendirlioğlu [21] reports that the number of cells depends largely on the wavelength of light and the highest cell growth rate in Chlorella vulgaris was observed for red light, while the lowest cell growth rate observed for blue light. Kim et al. [26] reported that the cell density of C. vulgaris grown in red light was 1.5 times of that in blue light after the first day of inoculation [13]. Wang et al. [25] analyzed the growth rate of *Spirulina platensis* under red, blue, yellow and white light and found that the highest growth rate occurred under a red light.

The effect of Photoperiod on the growth of *Scenedesmus quadricauda*

To explore the effect of photoperiod, eight cultures were inoculated and cultured under three different light: dark cycles including continuous 24 h light, 12 h:12 h and then 1 h:1 h, regimes under a luminous intensity of 500 $\mu mol.m^{-2}.s^{-1}$. Similar to the previous experiments, each cultivation cell corresponds to a specific wavelength in the range of 405 to 730 nm. The culture was monitored for about 12 to 17 days until the growth curve reached the stationary phase or entered the death phase. The cell concentration was determined regularly by measuring optical density (OD) at $\lambda = 680$ nm. Figure 7 shows the growth of microalgae under 8 different light color, i.e., wavelength, at three different photoperiods as mentioned earlier.

According to the results shown in Fig. 7 for all wavelengths, every dark: light cycles showed a positive growth of the strain except for the wavelength 405 nm where the absence of the growth under the cycles (1: 1) and (24: 0) was observed. A 12:12 mode under 590 nm light was also unsuccessful. A similar performance was observed for tested dark/light cycles of 470 nm, white and 540 nm light sources. The maximum concentration of biomass obtained was 6.138 g/l with 24.0 h continuous lighting. Considering the performance of 1:1 and 12:12 cycles which has the same lighting time per day, almost for all cases a 1:1 cycle showed considerably better performance. This can be seen from Fig. 6a, b, with an exception for 405 nm light, for the rest of light sources, 1:1 cycle stands at the second rank after 24 h lighting mode for maximum recorded biomass concentration and growth rate. This result is consistent with the findings of Wahidin and al. [27], who found that the photoperiod light cycle 24: 0 resulted in the highest growth rate of the Nannochloropsis sp. In addition, the effect of photoperiod has been reported as a key element of photosynthetic activity and growth rates of microalgae.

Energy efficiency in different lighting regimes

In order to determine the best cycle with respect to energy consumption, we have reported productivity at light duration p / t (light period), as are shown in Figure. To do so, a conversion factor of 1 $W.m^{-2} = 4.57\ \mu mol.m^{-2}.s^{-1}$ was used to convert light intensities according to [28]. The efficiency of light based on the produced biomass is presented in Fig. 8. The most efficient case with an efficiency factor of 10.56 $g.Mj^{-1}$ was observed when a red (730 nm) light at 500 $\mu mol.m^{-2}.s^{-1}$ with a 1:1 light:dark cycle mode was applied. But in comparison, for four lights including blue470, white, green540 and red590 a 100 $\mu mol.m^{-2}.s^{-1}$ intensity showed to be as efficient or even more efficient than a 500 $\mu mol.m^{-2}.s^{-1}$. Simply, generally, light at 500 $\mu mol.m^{-2}.s^{-1}$, 1:1 light:dark mode showed relatively higher efficiency at all light colors. The damaging effect of very high 1000 $\mu mol.m^{-2}.s^{-1}$ intensity is also reflected in the efficiency results (Fig. 8). When a high flux of light energy is applied, the electron flow rate exceeds the capacity range of photosynthetic cycle inside cells to quench them and therefore photoinhibition effect occurs and cells stop growing.

The results of the light efficiency (Fig. 8) along with the results for maximum growth rate and obtainable biomass concentration (Fig. 6) suggests 500 $\mu mol.m^{-2}.s^{-1}$, 1:1 light:dark mode as the suitable choice for production of *Scenedesmus quadricauda* because indeed a 24 h 500 $\mu mol.m^{-2}.s^{-1}$ in most color ranges provides a higher growth rate (Fig. 6a) but the final biomass concentration (Fig. 6b) was similar to that of 1:1 lighting mode. In addition, the efficiency (Fig. 8) of a 24 h lighting mode was much lower than a 1:1 lighting mode.

Conclusion

The results of this study clearly showed the significant effect of operational factors such as light intensity, photoperiodic cycles and wavelength on the growth of the strain Scenedesmus quadricauda.

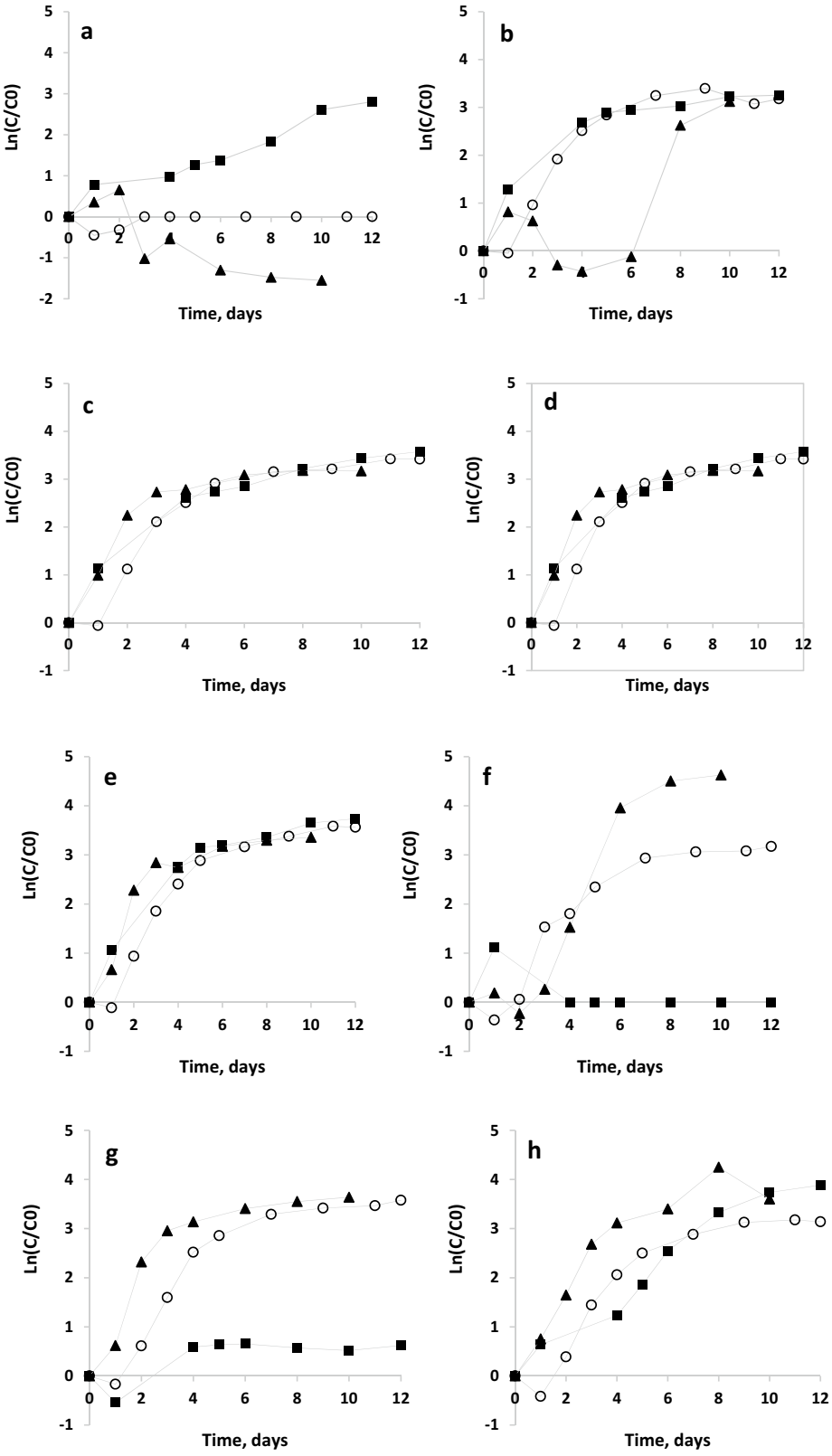

Fig.7 Microalgae growth curves under 1000 µmol.m^{-2}.s^{-1} light intensity at different photoperiods (○:1 h-1 h; ■: 12 h-12 h and ▲:24 h) and different wavelengths (**a**: 405 nm, **b**: 450 nm, **c**: 470 nm, **d**: white, **e**: 540 nm, **f**: 590 nm, **g**: 660 nm and **h**: 730 nm)

We conclude from this study that the cultivation of microalgae under light conditions controlled is a very effective method for optimizing growth or biochemical cells.

From this analysis, there is an interactive effect of intensity and wavelength on the growth of microalgae. In our case, we reveal the following:

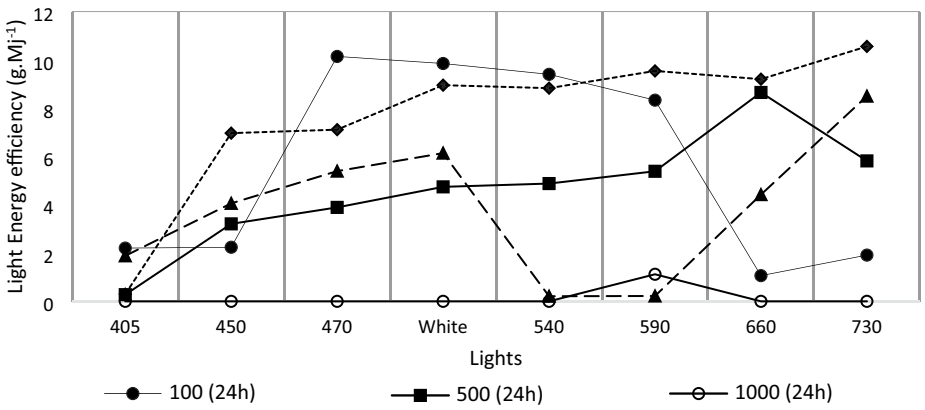

Fig. 8 Light energy efficiency at different light colors, intensities and photoperiod

I. The intensity 500 has generated the best growth with all wavelengths except with 405 nm.
II. Intensity 1000 is considered as damaging at all wavelengths except with 590 nm light.
III. The intensity 100 is below the absorption threshold (no growth) for 660 nm, 730 nm and 450 nm for the other wavelength there is a weak growth.

The exceptional cases mentioned above reveal a more complex phenomenon as regards the response of algae at different wavelengths.

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Availability of data and material Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest There are no conflicts of interests to be declared.

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