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Photoautotrophic Growth and Cell Division of *Euglena gracilis* with Mixed Red and Blue Wavelengths

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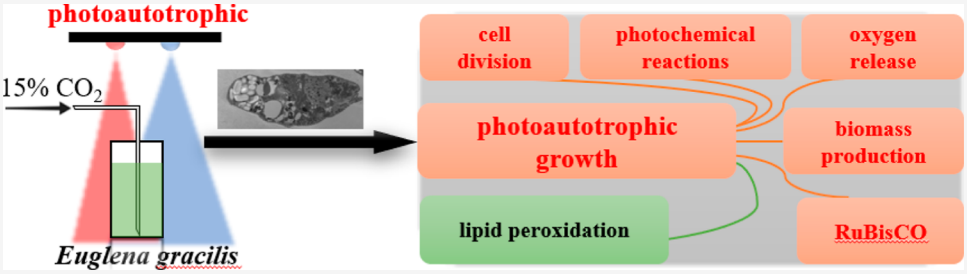

ABSTRACT: To improve flue gas CO_2 fixation of *Euglena gracilis* under a photoautotrophic environment, mixed red and blue wavelengths were used to promote photosynthetic growth and cell division with 15% CO_2. The biomass dry weight increased by 385% to 1.31 g/L with a peak ribulose-1,5-bisphosphate carboxylase/oxygenase enzyme activity and oxygen release, when the ratio of red to blue wavelength decreased to 2:3. *E. gracilis* mainly engaged in photochemical reactions with accumulated photosynthetic pigment to focus on light conversion by optimizing the carotenoid/chlorophyll ratio and chlorophyll *a*/*b* ratio. The energy capture efficiency of open photosystem II (PSII) reaction centers and quantum efficiency of charge separation in PSII reaction centers were improved to maintain active photosynthesis. Blue light facilitated transition from G1 to S phase, while red light promoted transition from S to G2 phase. The malondialdehyde content decreased to a valley of 1.36 nmol/mg protein to keep efficient photosynthetic apparatus with carbon fixation.

1. INTRODUCTION

Photosynthetic microalgae can achieve CO_2 fixation and carbon reduction by absorbing CO_2 and converting it into proteins, lipids, and pigments. Furthermore, microalgae are photosynthetic autotrophs, which are endowed with many significant advantages such as high photosynthetic efficiency,[1] short growth cycle, and strong environmental adaptability.[2] Therefore, the use of microalgae to reduce carbon emissions is a green, efficient, and recyclable carbon capture, utilization, and sequestration technology that is very promising.[3] Because they are photoautotrophs, the growth of microalgae for carbon sequestration is greatly influenced by changes in light irradiation, which is a key environmental factor for microalgae. While algal cells typically absorb all radiation in the visible spectrum, only a fraction of it can be used for photosynthesis.[4] Among the spectrum, red light and blue light usually have the greatest effect on cell photosynthesis and metabolism.[5−7] However, the absorption and utilization of light wavelengths by microalgae show remarkable species specificity.[8,9] The effects of red and blue light differ and can even be diametrically opposed for different microalgal cells. For example, the growth rate of *Spirulina platensis* increased significantly under red light, while that of *Nannochloropsis* sp. increased slightly under blue light and was the lowest under red light.[10,11] Therefore, research on the effect of different light conditions on cell growth and photosynthesis for specific algal species and light optimization is beneficial for improving the photosynthetic carbon fixation rate and biomass production of microalgae. The results provide guidance for the implementation and promotion of industrial CO_2 fixation by microalgae.

As unicellular microalgae with superior environmental adaptability, *Euglena gracilis* can survive harsh environmental conditions such as low pH, high salt, and high-energy ionizing radiation.[12] Especially at a pH of 3.0, *E. gracilis* obtains the maximum specific growth rate value and cannot be easily contaminated.[13] The ability of *E. gracilis* to grow rapidly in such acidic environments is a unique advantage over other algal species such as *Chlorella* sp., *Arthrospira platensis*, and

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Nannochloropsis oceanica, which can currently be farmed on a large scale. And, *E. gracilis* is projected to offer substantial advantages and potential for reducing high CO_2 concentrations from industrial flue gas. In addition, *E. gracilis* cells play an increasingly important role in the field of nutritional food supplementation and anti-inflammatory,[14,15] antitumor,[16] and anti-human immunodeficiency virus[17] medicines because of their rich nutrient content, high digestibility, high absorption rate without cell walls,[18] and valuable production of the carbohydrate paramylon. Improving the photosynthetic carbon fixation rate and biomass production of *E. gracilis* may soon become a new research hotspot.

Several studies have been conducted to examine the effects of different light qualities on *E. gracilis*. Bolige and Goto were the first to demonstrate phytochrome-like responses in *Euglena* and derive their action spectra. This result suggests that *E. gracilis* can sense and respond to spectral changes in their photic environment.[19] In a different study, the cis−trans isomerization of ζ-carotene was induced by blue-light irradiation, although the photoreceptor responsible for this effect was not identified.[20] In *E. gracilis*, β-carotene accumulation is promoted by irradiation with low-intensity blue or red light. However, high-intensity blue light induced the accumulation of zeaxanthin and all-*trans*-diatoxanthin.[21] These studies have mainly uncovered phenomena and potential mechanisms by which the spectral distribution affects the sensing and synthesis of phytochromes, often from an organelle perspective and at the molecular level.[22,23] However, the influence of the spectral distributions, especially mixed spectral distributions, on the growth of autotrophic *E. gracilis* for carbon fixation has not been examined to date. Further, research on the growth characteristics of red−blue mixed spectral distributions—which are considered to drive photosynthesis most effectively—on *E. gracilis* and especially on the fixation of high CO_2 concentrations is nonexistent.

In this study, artificial light-emitting diode (LED) lamp beads were employed to obtain specific different ratios of red and blue mixed light. The effect of the mixed spectral distribution on the growth characteristics of *E. gracilis* cells was examined in a high-carbon environment (15% CO_2). By comparison of the photosynthetic pigment content and fluorescence parameters of cells under these different light conditions and combined with the analysis of key enzyme activities, the potential utilization mechanism of red and blue light and self-regulation in *E. gracilis* were explored. Based on flow cytometry, cell cycle assays were used to examine the effects of red and blue mixed light changes on different stages of cell proliferation in *E. gracilis*. This work shows how photosynthetic carbon fixation and biomass production of *E. gracilis* can be improved by optimizing the incident light spectrum. This is not only a useful supplement to the understanding of the light response mechanisms of *E. gracilis* cells but also provides a new theoretical basis and facilitation method for microalgae to efficiently fix flue gas CO_2.

2. MATERIALS AND METHODS

2.1. Microalgal Strain and Cultivation. The *E. gracilis* strain used for the experiments was a CCAP 1224/5Z strain that was grown in autoclaved Cramer−Myers medium.[24] Cells were cultivated in 400 mL column photobioreactors with a 300 mL working volume. In an artificial greenhouse, cultures were maintained at a constant temperature of 24 ± 1 °C. Furthermore, 15 vol % CO_2 (85 vol % nitrogen) was supplied continuously as carbon source, with the flow rate controlled at 8 mL·min^{-1} through a flow meter (SevenStar CS-200, China).

In the present experiment, an artificial hybrid light source was manufactured from two types of LED lamp beads, namely, blue light (430−470 nm) and red light (620−680 nm). *E. gracilis* was cultivated under continuous red light or continuous mixed red and blue light (tested red and blue light intensity ratios were 4:1, 2:3, and 1:4). The experimental photosynthetic photon flux density (PPFD) was 200 μmol·m^2·s^{-1}, which was measured using a spectral color illuminometer (Hopoo, OHSP-350P, China).

2.2. Measurement Methods. *2.2.1. Cell Biomass and pH.* The optical density (OD) of the microalgal suspension was measured at 750 nm (OD_{750}) using an ultraviolet/visible spectrophotometer (Unico UV2600, USA). The biomass dry weight of the algal cells was measured using the heat drying method.[25]

The pH of the microalgal suspensions was measured daily with a pH meter (Mettler Toledo, FE20, Switzerland).

2.2.2. Pigment Content, Photosynthetic Fluorescence, and Photosynthetic Oxygenation. Pigment extraction and determination were conducted as previously reported:[26] chlorophyll *a* (Chl *a*), *b* (Chl *b*), and carotenoids' contents were measured by ethanol extraction and spectrophotometry.[27]

The photosynthetic fluorescence parameters of *E. gracilis* cells (such as Fs, Fm′, Fo, Fm, and Fv/Fm) were directly measured by a pulse modulation fluorometer (FMS-2, Hansatech, UK). Prior to measurement, the samples were kept in the dark for 15 min. The ΦPSII and nonphotochemical quenching (NPQ) were calculated according to the following formulas

$$\Phi\mathrm{PSII} = \frac{\mathrm{Fm}' - \mathrm{Fs}}{\mathrm{Fm}'} \quad (1)$$

$$\mathrm{NPQ} = \frac{\mathrm{Fm} - \mathrm{Fm}'}{\mathrm{Fm}'} \quad (2)$$

The oxygen release concentration of *E. gracilis* cells was measured using an oxygen electrode (Chlorolab 2+, Hansatech, UK) with a liquid-phase oxygen electrode system.

2.2.3. Key Enzyme Expressions. Preparation of enzyme extracts was conducted as previously reported:[26] Crude enzyme extracts were obtained using an ultrasonic cell disruptor (UH-S2, AUTO Science, China) for enzyme assays.[28]

Total protein concentrations were tested using the total protein assay kit (bicinchoninic acid method). The ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) was determined with the RuBisCO assay kit. Malondialdehyde (MDA) was measured via the thiobarbituric acid method using an MDA assay kit. Superoxide dismutase (SOD) was determined via the water-soluble tetrazolium salt (WST-1) method using the SOD assay kit.[29] All of the above-mentioned kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2.4. Cell Cycle. The cell cycle of *E. gracilis* cells was detected using a cell cycle detection kit (Nanjing, China). Cells were collected by centrifugation (2000 rpm, 10 min) and washed twice with phosphate-buffered saline (PBS). The cells were resuspended by adding 0.3 mL of PBS. Next, 1.2 mL of −20 °C anhydrous ethanol was added, and the mixture was refrigerated at −20 °C overnight. After the fixation stage, the mixture was centrifuged at 2000 rpm for 5 min. Pellets were

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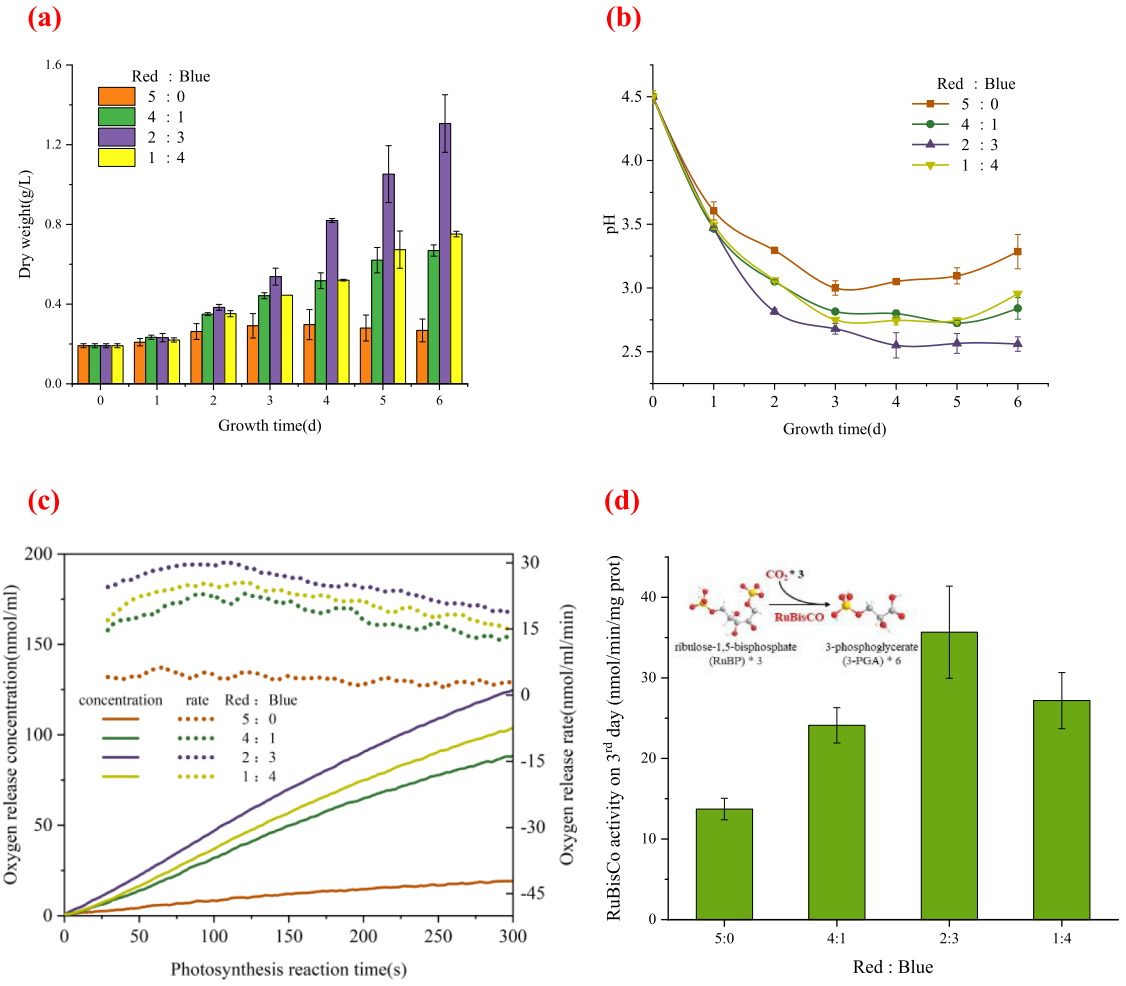

Figure 1. Effects of various ratios of mixed red and blue wavelengths on (a) dry biomass weight, (b) pH value, (c) oxygen release, and (d) RuBisCO activity (on the 3rd day) of *Euglena gracilis*.

resuspended in 100 μL of RNase A and incubated for 30 min at 37 °C. Then, 400 μL of propidium iodide was added, and the 30 min reaction was carried out at 4 °C and protected from light. The cell cycle of the sample was examined on a flow cytometer (FACSCalibur, BD, USA).

2.2.5. Paramylon Content. Paramylon extraction of *E. gracilis* cells was conducted as previously reported.[26] The paramylon content was tested using glucose as standard.[30]

2.2.6. Statistical Analyses. All experiments were independently repeated three times. The data represent the mean value and standard error. Statistical analysis was performed using Student's *t*-test in Microsoft Office Excel Software (version 2016).

3. RESULTS AND DISCUSSION

3.1. Effects of Various Ratios of Mixed Red and Blue Wavelengths on the Photosynthetic Growth of *E. gracilis*. Different ratios of mixed red and blue light affect the photosynthetic growth characteristics of *E. gracilis* directly. As shown in Figure 1, the dry weight of *E. gracilis* biomass increased and then decreased with an increasing proportion of blue light; the pH value first decreased and then increased. When the PPFD was 200 μmol·m^{2}·s^{-1}, the red and blue mixed light of 2:3 ratio achieved the best promotion effect on biomass accumulation. The dry weight of *E. gracilis* biomass reached 1.31 g/L at a red/blue light ratio of 2:3, which was 385, 96, and 75% higher than that at red/blue light ratios of 5:0 (0.27 g/L), 4:1 (0.67 g/L), and 1:4 (0.75 g/L), respectively. It is noteworthy that when the red/blue light ratio was 5:0, i.e., when *E. gracilis* received only high-intensity red light during incubation, the biomass accumulated only slightly in the first 3 days and then gradually decreased; the pH also started to increase after the third day. In addition to daily monitoring data showing impaired photosynthetic growth of *E. gracilis* under irradiation with pure red light, a gradual whitening and precipitation of cells were also observed under this light exposure at a later culture stage. These results indicate that irradiation with pure red light could not sufficiently drive complete photosynthesis. The cells were continuously depleted of biomass in short supply, and red light causes cell damage,[5] which was accelerated by the high PPFD. In addition, while blue light is irreplaceable in initiating a certain linear link of photosynthesis in *E. gracilis*, an excessive proportion of blue light will lead to a mismatch in the efficiency of the reaction link, resulting in a declined growth rate.

Figure 1c shows the oxygen release from *E. gracilis* cells on the third day of incubation under all four tested light conditions. The concentration of oxygen and the oxygen release rate were lower in *E. gracilis* cells that were irradiated with pure red light, while the photosynthetic oxygen release activity of cells maintained a high level at a red−blue light ratio

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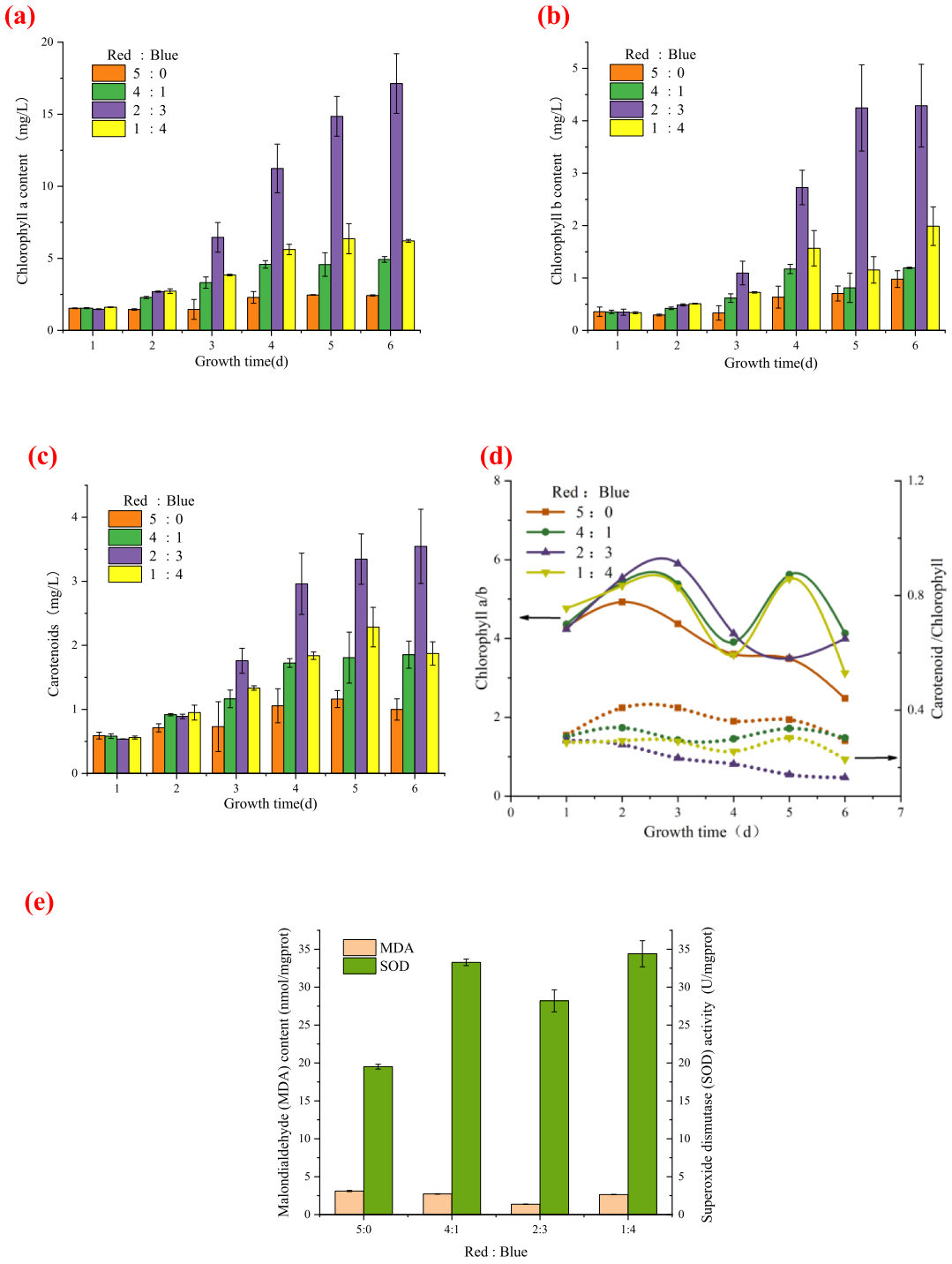

Figure 2. Photosynthetic pigment content (a–d), damage extent, and antioxidant capacity (e) of *E. gracilis* with various ratios of mixed red and blue wavelengths.

of 2:3. In oxygenic photosynthesis, the formation of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) and adenosine 5′-triphosphate (ATP) through noncyclic electron transfer and photophosphorylation is accomplished by the mutual coordination of photosystem (PS) I and PS II in series. Oxygen is produced in PS II, while the formation of NADPH depends on PS I. The PS II reaction center is excited by photons to complete the charge separation reaction, while the mobile plastocyanin transfers electrons to the PS I reaction center, which is also in the excited state. The PS II reaction center then captures electrons at the oxygen-evolving complex to release oxygen from water molecules to start the next round of the charge separation reaction. In this process, red light preferentially excites PS II, while blue light preferentially excites PS I.[31] Therefore, under irradiation with pure red light, PS II is excited by photons, while the PS I reaction center is less active. Thus, the downstream electron transfer demand is significantly reduced, leading to an overall decline of efficiency of the two photoreactions in series. Furthermore, when the proportion of blue light increases, the charge separation of the PS II reaction center is excited continuously, the PS II/PS I ratio gradually increases, the

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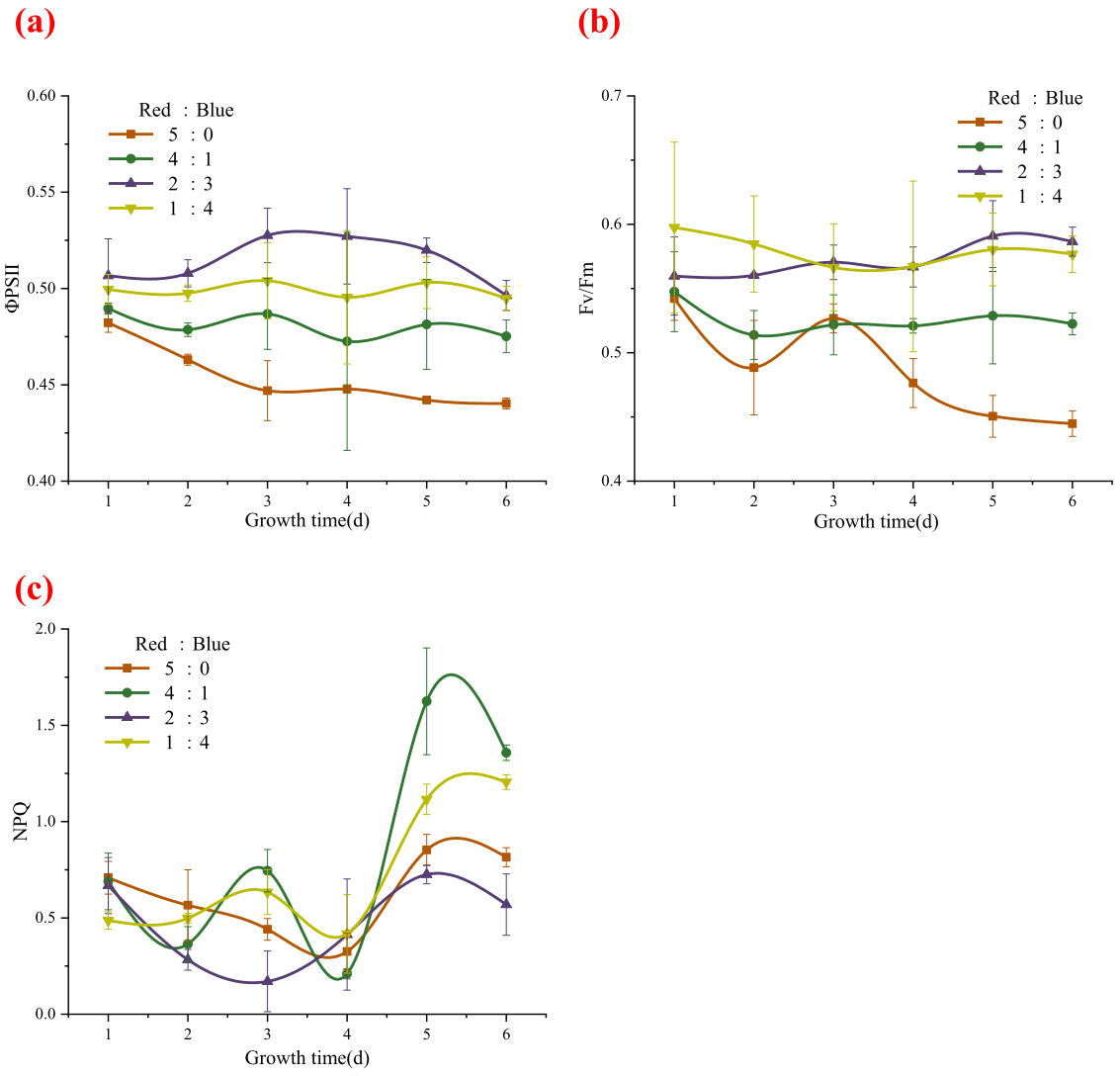

Figure 3. Photochemical parameters of (a) ΦPSII, (b) Fv/Fm, and (c) NPQ of *E. gracilis* with various ratios of mixed red and blue wavelengths.

noncyclic electron transfer process is improved, and the photosynthetic oxygen release efficiency increases. However, if the proportion of blue light is increased blindly, leading to an imbalance with excessively high PS II/PS I ratio, oxygenic photosynthesis and downstream carbon assimilation would also be affected. The Calvin cycle uses NADPH and ATP produced by light reactions for CO_2 fixation. This process involves 13 different enzymatic reactions, with RuBisCO playing a crucial role as the key enzyme for carbon fixation. As shown in Figure 1d, on the third day, the RuBisCO activity of *E. gracilis* cells first increased and then decreased with an increasing blue light proportion. Irradiation with blue light relieved the photoreactive excitation pressure caused by irradiation with high-intensity red light, significantly promoted RuBisCO activity, and thus improved the CO_2 fixation efficiency. The reason may be a specific regulation of photosynthesis-related enzymes by blue light.[32] Appropriate irradiation containing blue light (e.g., red and blue light ratios of 4:1 and 2:3) can improve the interaction between the light and dark reactions. Such a light ratio can also improve the orderly coordination of photosystems and enzyme systems, which promotes cell growth and the synthesis of organic matter. In contrast, an excessively high ratio of blue light may affect the PS II/PS I balance in addition to the stability of photosynthesis-related mRNA synthesis.[33] Consequently, this leads to a decrease in the activity of key carbon fixation enzymes and the rate of biomass production.

3.2. Effects of Various Ratios of Mixed Red and Blue Wavelengths on Pigment Synthesis and Antioxidant Capacity of *E. gracilis*. Photosynthetic pigments provide the basic guarantee for light-energy absorption and photochemical reactions in photoautotrophic *E. gracilis*. Chl *a* is the core pigment for oxygenic photosynthesis, and Chl *b* broadens the range of light absorption.[31,34] Carotenoids, as accessory pigments, not only absorb light that is not absorbed by chlorophyll and transfer it to chlorophyll but also protect the photosynthetic apparatus.[35,36] As shown in Figure 2a–c, the contents of Chl *a*, Chl *b*, and carotenoids were notably higher under mixed red and blue light than under pure red light. The maximum concentrations of Chl *a*, Chl *b*, and carotenoids were 17.1 4.3, and 3.5 mg/L, respectively, which were obtained under light conditions with a 2:3 ratio of red and blue light. These results indicate that the introduction of blue light to the spectrum can noticeably increase the contents of photosynthetic pigments, especially Chl *a*. This increase can be attributed to the beneficial regulation by blue light on

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chloroplast differentiation and photosynthetic enzyme activity during photosynthesis.[32] In addition, the increasing Chl *b* content led to a shift of the maximum light absorption peak of chlorophyll to shorter wavelengths, thus allowing the cells to better adapt to the increasing blue light.[32] Red light exposure can regulate the synthesis of photopigment protein complexes at the transcriptional level.[37] Therefore, when the ratio of red light is too low (e.g., a 1:4 red/blue light ratio), the synthesis of photosynthetic pigments may be affected first, followed by the photosynthetic carbon sequestration of cells. As shown in Figure 2d, the Chl *a*/*b* ratio under irradiation with blue light was higher than that under irradiation with red light, which corresponds to the ability of blue light to effectively increase the Chl *a* content (as mentioned above). As the most important pigment in photosynthesis, Chl absorbs and converts light and directly participates in photosynthesis. At the early culture stage, cells irradiated with blue light had a higher Chl *a*/*b* ratio, indicating that these cells invested more in light conversion. This is because Chl *b* is involved only in light absorption by the antenna and not in the light conversion at the reaction center. Notably, the Chl *a*/*b* ratio of cells under optimal light conditions (red and blue light ratios of 2:3) fluctuated less after the fourth day in late culture. This suggests that the cells were able to adapt to current light exposure and reach a relatively stable balance of light energy absorption and conversion. This was different from the cases of 1:4 and 4:1 red-to-blue light ratios.

Carotenoids are photosynthetic accessory pigments and play an important role in defending the photosynthetic apparatus from photo-oxidative damage. Carotenoids can not only quench the three-wire excited chlorophyll but also directly play the role of an antioxidant to quench reactive oxygen species. Consequently, they prevent lipid peroxidation in the membrane system and stabilize and protect the photosynthetic membrane system such as thylakoid membrane.[38] As shown in Figure 2c, the carotenoid content of *E. gracilis* cells increased significantly under irradiation with blue light. At red and blue light ratios of 1:4, 2:3, and 4:1, the carotenoid contents of cells were 1.85, 3.5, and 1.87 mg/L, respectively, which were 85, 250, and 87% higher than contents under red light conditions (1 mg/L). Correspondingly, the MDA content of *E. gracilis* cells under blue light irradiation was also reduced significantly, while the SOD activities were all higher than those under pure red light. At a ratio of red and blue light of 2:3, the MDA content of cells reached the minimum value of 1.36 nmol/mg prot, which was only 43.9% of that under red light (3.1 nmol/mg prot). The SOD activity of cells reached 28.19 U/mg of prot, which was 44.5% higher than that under red light (19.51 U/mg of prot). These results indicate that *E. gracilis* cells that were irradiated with blue light had higher levels of antioxidant capacity, lower levels of lipid peroxidation and photooxidative damage, and more stable and efficient photosynthetic membrane systems. All of these changes enabled faster growth of photosynthetic carbon fixation. Moreover, it is worth noting that despite the high carotenoid content of *E. gracilis* cells irradiated by blue light, their carotenoid/chlorophyll ratio was not higher than that of cells exposed to pure red light. The carotenoid/chlorophyll ratio is related to the light utilization efficiency of cells.[39] A lower carotenoid/chlorophyll ratio indicates that under blue light irradiation, *E. gracilis* cells were still predominantly engaged in photochemical reactions. This growth trend (i.e., focusing more on photosynthesis rather than photoprotection) was more conducive to CO_2 fixation and biomass accumulation in microalgal cells.

3.3. Effects of Various Ratios of Mixed Red and Blue Wavelengths on Photosynthetic Fluorescence Parameters of *E. gracilis*. Fluorescence analysis of *E. gracilis* cells was performed under four light conditions, including photochemical efficiency parameters ΦPSII and Fv/Fm and the fluorescence quenching parameter NPQ. The photochemical efficiency parameter curves (Figure 3a,b) show that both the effective quantum yield and maximum quantum yield of photochemical energy conversion of PS II increased under blue light irradiation. This result is basically consistent with the photosynthetic oxygen release rate and the biomass accumulation of cells. At a ratio of red to blue light of 2:3, the maximum values of ΦPSII and Fv/Fm of *E. gracilis* cells were 0.53 and 0.59, respectively, which were 17.8 and 34.1% higher than those at a ratio of red to blue light of 5:0 with the same period. While the ΦPSII and Fv/Fm of cells that had been exposed to lower or higher ratios of blue light decreased, they maintained higher levels compared to those of red light exposure. These parameters suggest that irradiation with blue light could effectively promote the quantum efficiency of charge separation in PSII reaction centers and, therefore, increase the effective photosynthetic electron transport rate. The energy capture efficiency of the open PSII reaction center can be improved, which means that photosynthesis can be maintained at a relatively active level for an extended time.

As a reliable NPQ parameter, NPQ represents the decrease of fluorescence yield caused by the thermal dissipation process, and its variation reflects the change of thermal dissipation. As shown in Figure 3c, at a ratio of red to blue of 2:3, NPQ was at the lowest level, indicating that the total light energy absorbed by cells and photochemical reactions maintained a high level. NPQ is an important way to regulate photosynthetic quantum efficiency in plants, and the downregulation of NPQ is generally considered to be an effective strategy for improving the light use efficiency of microalgae biomass production.[40] NPQ is not a fixed parameter, and from the fourth day, NPQ began to be upregulated under all light conditions, which was speculated to be related to the attenuation of light intensity in microalgal suspensions caused by the increase of cell density in the late culture period. However, the growth rate and cell density of *E. gracilis* under the red−blue ratio of 2:3 increased the most, which may lead to the increase of its NPQ from the third day, and it was slightly higher than that of the other three groups that began to decline at the fourth day. At ratios of red to blue light of 4:1 and 1:4, the NPQ process became active and fluctuated sharply at the end of the culture. This sharp fluctuation may also be related to the mismatch between the absorbed light and the light utilized by the photosynthetic reaction center caused by the imbalance imposed by the ratio of red to blue light. The photochemical reaction, thermal dissipation, and fluorescence emission, which compete with each other, and the fluctuation of the photochemical reaction also directly affect the change in thermal dissipation. It is worth noting that under the pure red light conditions, NPQ was not at the highest level in the thermal dissipation curves. This may be due to the low growth activity of *E. gracilis* cells under this specific light condition and the overall poor light energy absorption and utilization capacity caused by the oxidative damage of photosynthetic apparatus. Therefore, the energy consumed by the NPQ was low.

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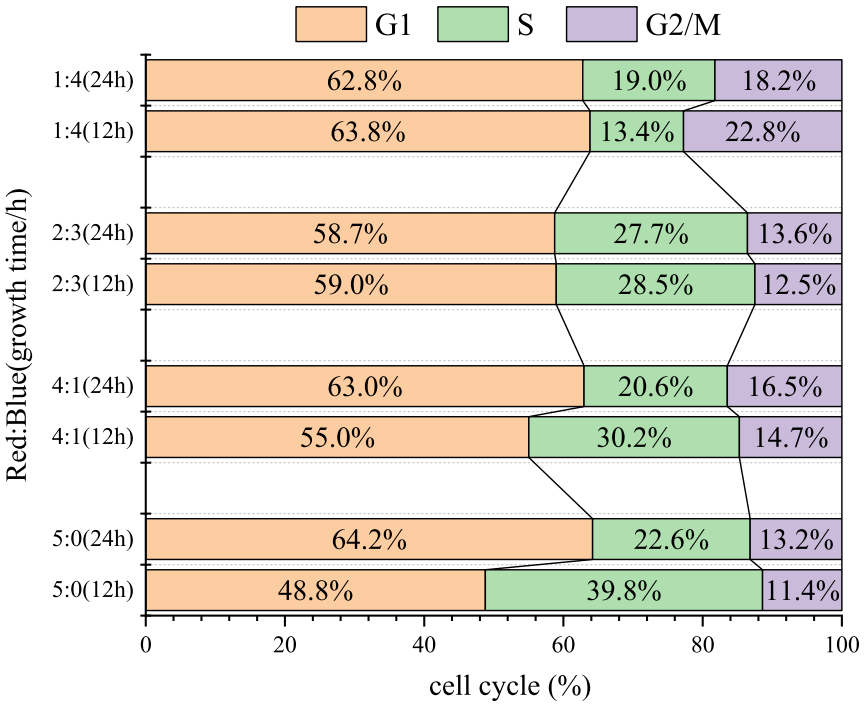

Figure 4. Cell cycle of *E. gracilis* with various ratios of mixed red and blue wavelengths.

3.4. Effects of Various Ratios of Mixed Red and Blue Wavelengths on Cell Division in *E. gracilis*. The cell cycle includes the G1 phase, S phase, G2 phase, and M phase.[41] Light quality interacts with cell division, often at the beginning of the culture. After 12 and 24 h of culture, the cell cycle of *E. gracilis* cells under four light conditions was assessed, and the results are shown in Figure 4. When the incubation time was prolonged from 12 to 24 h, the percentage of the G1 phase increased by 15.4% under pure red light and by 8% under a red/blue light ratio of 1:4. The percentage of the G1 phase decreased by 0.3 and 1% under red/blue light ratios of 2:3 and 4:1, respectively. Changes in these parameters indicated that the proportion of cells blocked in the G1 phase decreased with an increasing proportion of blue light. It can be inferred that irradiation with blue light facilitates the transition of *E. gracilis* cells from the G1 phase to S phase and exerted a promoting effect on cell proliferation. It is possible that blue light had a beneficial effect on the expression of key cell cycle proteins such as Cln3p or cyclin E.[42,43] These proteins directly act on G1-S,[44] the core regulatory point of the cell cycle, causing cells to move out of the division-stalled G1 phase early and enter the S phase, initiating division. In addition, as shown in Figure 4, when the incubation duration was prolonged from 12 to 24 h, the percentage of the G2 phase increased by 1.8% under both red/blue light ratios of 5:0 and 1:4 and only increased by 1.1% under a red/blue light ratio of 2:3; however, it decreased by 4.6% under a red/blue light ratio of 4:1. The changes of G2 phase parameters showed that the proportion of cells that could enter the G2 phase decreased with decreasing red light proportion. This means that the transition from the S to G2 phase was hindered, indicating that the red light irradiation was able to promote the transition from the S to G2 phase of *E. gracilis* cells, which may be related to the promotion of DNA synthesis.[45] Finally, the total ratio of the S phase and G2 phase of cells under the four tested light conditions was compared after 24 h of incubation. The value reached a maximum of 41.3% at a ratio of red and blue light of 2:3, indicating that the red and blue mixed light at this ratio had the best promoting effect on cell proliferation.

3.5. Effects of Various Ratios of Mixed Red and Blue Wavelengths on the Paramylon Content of *E. gracilis*. Paramylon is a linear β-1,3-glucan that is synthesized in large quantities, mainly during the exponential period, and accumulates intracellularly in the form of granules.[17] In past studies, although attention was directed to the effect of light on paramylon accumulation, most studies focused on light intensity,[18] while the effect of light quality on paramylon content has not been examined. Therefore, in addition to studying the effects of mixed red and blue light on photosynthetic oxygen release and growth carbon sequestration in *E. gracilis*, this paper also focused especially on the changes in paramylon content, which is a special high value-added component of *E. gracilis*. In this study, the trend of paramylon accumulation under the four tested light conditions was generally consistent with the photosynthetic growth of the cells. After 6 days of culture, the paramylon content of cells was as high as 0.33 g/L at a ratio of red to blue light of 2:3, while the paramylon contents were only 0.05, 0.21, and 0.20 g/L at ratios of red to blue light of 5:0, 4:1, and 1:4, respectively. Unlike paramylon accumulation, the change in the ratio of intracellular paramylon to dry biomass weight differed from the trend of cellular biomass accumulation. As shown in Figure 5, among the four tested light conditions, the highest paramylon ratio was 4:1 for red and blue light, reaching 30.7%, followed by 2:3 and 1:4 for red and blue light, reaching 25.4 and 26.1%, respectively. When the red and blue light was 2:3, the accumulation of paramylon was the highest, which benefited from the domestication process for the promotion of the growth rate of the linear growth phase. For photoautotrophic cultures of *E. gracilis*, the accumulation of paramylon, although influenced by the temperature and light intensity, depends mainly on the growth phase. Paramylon was synthesized as a

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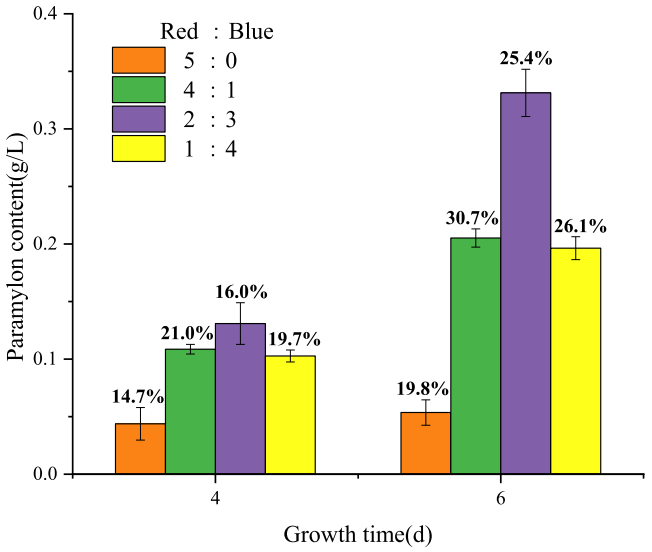

Figure 5. Paramylon content of *E. gracilis* with various ratios of mixed red and blue wavelengths.

storage polysaccharide that corresponds only to excess energy. When the ratio of red to blue is 2:3, cells grow rapidly, and a lot of energy is used for plastid formation, cell division, photosynthetic carbon sequestration, and biomass accumulation, which should be an important reason for the low proportion of paramylon. The lowest paramylon ratio was found under the pure red light condition, which was probably due to slow cellular growth and lack of excess energy for paramylon synthesis.[46] The paramylon content of *E. gracilis* produced under photoautotrophic conditions is still low. Because *E. gracilis* is continuously developed and cultivated as biological carbon fixation media with high potential, the intrinsic mechanism of how the light quality influences paramylon accumulation under the photoautotrophic mode and the method of paramylon augmentation may become a valuable direction for future exploration.

4. CONCLUSIONS

The biomass dry weight increased by 385% to 1.31 g/L with a peak RuBisCO enzyme activity and oxygen release, when the ratio of red to blue wavelength decreased to 2:3. *E. gracilis* mainly engaged in photochemical reactions with the accumulated photosynthetic pigment to focus on light conversion. The energy capture efficiency of open PSII reaction centers and the quantum efficiency of charge separation in PSII reaction centers were improved. Blue light facilitated transition from the G1 to S phase, while red light promoted transition from the S to G2 phase. The MDA content decreased to a valley of 1.36 nmol/mg-protein to keep an efficient photosynthetic apparatus. Although an artificial LED light does not have the zero carbon footprint of natural sunlight, it guides the rational design and optimization of light sources in industry, such as the use of rated filters, which can simulate the spectral ratio explored by LED light sources in the laboratory, thereby optimizing the natural light spectrum, improving the yield of microalgae biomass, reducing carbon emissions, and providing a more feasible solution for the engineering application of microalgae biomass energy.

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Notes

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