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Improvement in lipid production and biodiesel quality of *Pavlova* sp. by monochromatic illumination*

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Abstract *Pavlova* sp. is not only one of the most popular microalgae in aquaculture, but also a source of high-quality biodiesel feedstock. With white light as a control (W), *Pavlova* sp. was cultured in this study under varying light quality, including monochromatic red light (R), blue light (B), and combinations thereof with different proportions (illuminators of *m*R*n*B, comprised of *m* red light units and *n* blue ones, *m*+*n*=7), to examine the effects of illuminating light quality on biocomponent production and biodiesel quality. The results show that combined monochromatic light, especially 2R5B, 3R4B, 4R3B, and 5R2B, could improve the growth of *Pavlova* sp. The dry weight of harvested algae powder in the 5R2B group reached 418.03 mg/L, and was 22.65% higher than that in the control group (W). Lipid production under combined monochromatic light of 4R3B reached 107.86 mg/L, and was 25.61% higher than in the control (W). In addition, illumination using 4R3B increased the proportion of C16∶0 (palmitic acid) and C16∶1 (palmitoleic acid) fatty acids in *Pavlova* sp. by 15.55% and 21.94%, respectively, which translates into improved biodiesel quality. All cetane numbers (CN) for 4R3B–6R1B were over 51, while iodine values (IV) and degrees of unsaturation (DU) were reduced, leading to more stable biodiesel suitable for long-term storage. In addition, protein production under 6R1B was as high as 31.56 mg/L, 1.8 times greater than under W. Light quality is proposed as an effective tool to regulate biocomponent production by microalgae.

Keyword: *Pavlova* sp.; light quality; growth; lipid; biodiesel

1 INTRODUCTION

Due to increasing combustion of fossil fuels, the atmospheric concentration of CO_2 has been increasing for decades. It has led to global climate change and further threatens ecological stability, food security, and social welfare (Christenson and Sims, 2011). As a result, sustainable and renewable energy sources have been developed as alternatives, such as first-, second-, third-, and fourth-generation biofuels. Single cell oil produced by microalgae is considered one of the most effective raw materials for third-generation biodiesel production; the advantages of microalgal biodiesel production include fast growth, high oil productivity, and low arable land demand (Chisti, 2007; Lowrey et al., 2015). Compared to traditional oilseed crops, their simple unicellular structure allows for potentially high oil productivity. Microalgae cultivated using brine or saltwater may obtain nutrients from wastewater and waste CO_2 streams, and do not compete for resources with traditional agriculture (Chisti, 2007; Mata et al., 2010).

Triacylglycerides (TAGs) serve as energy storage in microalgae which can be easily converted into biodiesel through transesterification reactions (Fukuda et al., 2001). TAGs share a common structure, usually in which three long-chain fatty acids (FAs) are coupled to a glycerol molecule to form a triple ester. When glycerol is displaced with three small alcohol (e.g., methanol) molecules by transesterification reactions, mono-alkyl esters of

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linear-chain fatty acids will be produced as biodiesel (Meher et al., 2006). Biodiesel offers comparable performance to conventional petroleum diesel, but is less harmful in terms of toxic emissions, less volatile, and safer in terms of transportation and storage (flash point>150 °C) (Nascimento et al., 2013). Consequently, biodiesel is non-toxic, biodegradable, and an excellent alternative to petroleum diesel.

As a result of their balanced cellular protein and lipid composition, microalgae have been widely used as aquatic feed (Sørensen et al., 2016). Microalgal culture is a necessary step in seed production for bivalve hatcheries in particular. In order to ensure a better balance of essential nutrients for the seed, microalgae are commonly used in plurispecific diets consisting of one diatom species and one or more dinoflagellate species (Robert and Gérard, 1999). A large number of experimental results have proved that *Pavlova* sp. has a positive effect on the cultivation of shellfish seed when used together with one or more diatom species in a plurispecific diet. Accordingly, *Pavlova* sp. is one of the most commonly used species in bivalve shellfish hatcheries (Ponis et al., 2006).

Pavlova sp. is a biflagellated, brown/golden single-celled microalga with no cell wall and with enriched TAGs. During recent years, *Pavlova* sp. has received increasing attention in the field of aquaculture as one of the most popular microalgae providing nutrients for fish, mollusk, and shrimp farming (Thiyagarajan et al., 2020). Since *Pavlova* sp. has no cell wall, it can even be commercialized as a natural food using intact cells, without the need for extraction of intracellular materials. In other words, *Pavlova* sp. is considered a good producer of high-value products (Kanamoto et al., 2021). Therefore, given its high content of TAGs, there is potential for coproduction of valuable products using *Pavlova* sp. such as polyunsaturated fatty acids (PUFAs), pigments, antioxidants, pharmaceuticals, and biomass for animal feed, fertilizer, or energy, in addition to biodiesel production (Mata et al., 2010). However, current research on *Pavlova* sp. is mainly focused on aquaculture. There are limited quantitative reports on biodiesel and biocomponent production by *Pavlova* sp., especially with regard to optimization of illumination conditions.

In this study, *Pavlova* sp. was cultured under monochromatic red light, blue light, and combinations thereof at different proportions, with white light as a control. The effects of various light quality on growth, as well as pigment, protein, polysaccharide, and lipid production, fatty acid composition, and biodiesel quality of *Pavlova* sp. were analyzed. The results of this study can assist in the optimization of illumination conditions for *Pavlova* sp. to improve the productivity and quality of biodiesel and, simultaneously, to obtain a variety of valuable coproducts.

2 MATERIAL AND METHOD

2.1 Algal strain and culture conditions

The *Pavlova* sp. was obtained from the Key Laboratory of Marine Aquaculture, Ministry of Education, Ocean University of China.

Seven units (1 Watt each) emitting white light (W, continuous spectrum), monochromatic red light (R, peak wavelength 660 nm), or monochromatic blue light (B, peak wavelength 455 nm) were assembled into an illuminator. The control comprised seven white-light emitting units (W). Illuminators in the treatments were *m*R*n*B (*m*=0, 1, 2, 3, 4, 5, 6, or 7, *m*+*n*=7), i.e., *m* red-light emitting units (R) combined with *n* blue-light emitting units (B). In this system, an illuminator of 0R7B emits only monochromatic blue light, while 7R0B emits only monochromatic red light (Jin et al., 2021). The spectral quality in terms of relative photon flux density was examined using a plant lighting analyzer (PLA-30, EVERFINE Corporation, China) for each illuminator and is presented in Fig.1.

Pavlova sp. in the logarithmic growth phase was inoculated into 150 mL of silicon-free seawater f/2 medium in a 250-mL Erlenmeyer flask with the initial optical density ($OD_{690\ nm}$) of 0.03. The algae were cultivated under the above illuminators with a standardized light intensity of 65±2 μmol/(m^2·s) by adjusting the distance of the illuminator from the culture flask at temperature of 22±2 °C, with light-dark cycle 16 h:8 h and without aeration. The culture period was 14 d, shaking once each morning and evening (Jin et al., 2021; Yu et al., 2022). All the treatments were performed with at least 3 replicates.

2.2 Determination of algal growth and dry weight of biomass

A volume of 200 μL of algal culture was transferred to a 96-well plate every two days. The $OD_{690\ nm}$ was measured with a microplate reader to obtain a growth curve. Cell density was calculated according to the standard curve for $OD_{690\ nm}$ vs. cell density of *Pavlova* sp. The specific growth rate μ (/d), the doubling rate K (/d), and the generation time T (d) were calculated (Kong et al., 2010; Ben

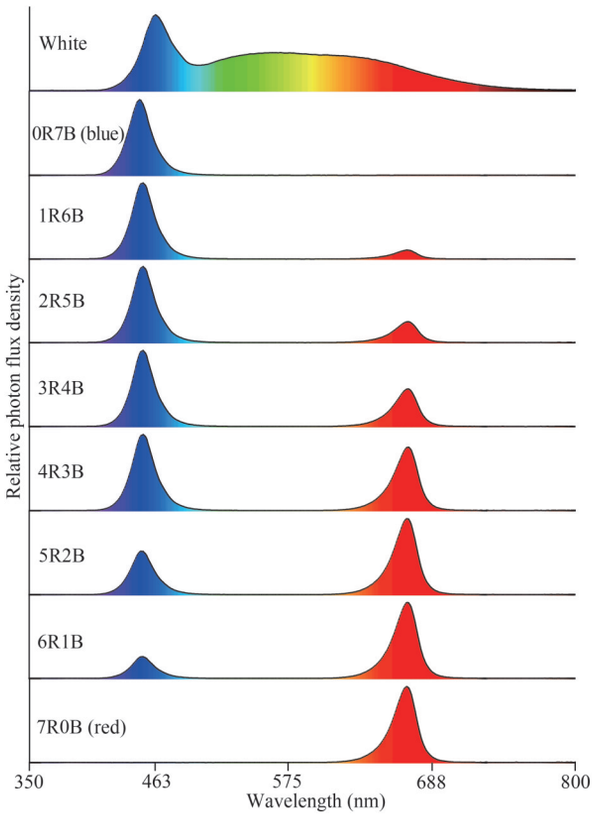

Fig.1 Spectral quality of the illuminators used in this study

Garali et al., 2016).

After cultivation, 100 mL of algal culture was centrifuged (3 700 r/min, 10 min). The pellet was resuspended and washed using 10 mL of distilled H_2O before being centrifuged again. The precipitate was transferred to a clean glass bottle and lyophilized in a freeze dryer for the biomass dry weight measurement (Yu et al., 2022).

2.3 Biocomponent content measurement

Pigment content was determined by the absorbance method (Sulochana and Arumugam, 2016). The algal culture (5 mL) was centrifuged (4 000 r/min, 10 min, 4 °C). The pellets were suspended with 5 mL of 90% acetone and left standing overnight at 4 ℃ in the dark. Pigments were extracted by ultrasonication (700 W, 10 min, and ice bath) and centrifugation (4 000 r/min, 10 min, 4 °C). The optical density of the supernatant was measured at 470-(A_{470}), 644-(A_{664}), and 661-nm (A_{661}) wavelengths. The pigment concentrations were calculated as chlorophyll *a* (mg/L)=11.24×A_{661}–2.404×A_{644} and carotenoids (mg/L)=[1000×A_{470}–1.9×chlorophyll *a*–63.14×(20.13×A_{644}–4.19×A_{661})]/214 (Sulochana and Arumugam, 2016).

Total protein and carbohydrate content were determined by the Kaumas blue method with bovine serum albumin as the standard and the phenol-sulfuric acid method with D-glucose as the standard (Jin et al., 2021). The algal culture (30 mL) was centrifuged (4 000 r/min, 10 min, 4 °C). The pellets were suspended with 30 mL of distilled water. Cell contents were lysed on ice using an ultrasonic cell disruptor (700 W, 10 min, and ice bath). Cell debris was removed by centrifugation (4 000 r/min, 10 min, 4 °C) and the supernatant was collected for protein or carbohydrate content measurement.

The lipid content and lipid production were measured following our previous method (Yu et al., 2022). About 20 mg of lyophilized algal powder was suspended with 3.8 mL of a mixed solution (water∶chloroform∶methanol=4∶5∶10, v/v/v), and was extracted by ultrasonication (200 W, 8 min, and ice bath). After addition of 1 mL of water and 1 mL of chloroform, the sample was vibrated and centrifuged. The chloroform phase was washed with distilled water, centrifuged for stratification, and transferred for total lipid weight measurement after solvent evaporation. The lipid content was calculated using the obtained lipid weight divided by the measured weight of the algal powder, while lipid production was calculated using the lipid content multiplied by the dry weight of biomass harvested (Yu et al., 2022).

2.4 Fatty acid composition analysis

Fatty acid methyl esterification was performed using the KOH-methanol method (Cha et al., 2011). Fatty acid composition was analyzed using the GC-MS method described in our previous study (Yu et al., 2022). A Thermo Scientific ITQ900 GC-MS with a TR-5MS (30 m×0.25 mm×0.25 μm) was employed with a constant flow of 0.8-mL/min helium (99.999%) as the carrier gas. The sample (1 μL) was injected in splitless mode (230 °C). The temperature program for GC was as follows: 120 °C for 1 min, increased to 240 °C at 3 °C/min, held for 10 min. The temperature of the EI ion source of MS was 220 °C. Solvent delay was 4 min, and mass spectra were acquired in full-scan mode with a mass range from 50 to 800 amu. Peaks with percentages of peak areas >1% were recorded and subjected to peak identification. Based on the standards and NIST Mass Spectral Library (2008), each fatty acid methyl ester (FAME) was identified. The proportion of each fatty acid was calculated by the area normalization method (Qari and Oves, 2020; Yu et al., 2022).

2.5 Biodiesel quality analysis

Biodiesel properties such as cetane number (CN), iodine value (IV, in g I_2/100 g), degree of unsaturation (DU, in %), and cold filter plug point (CFPP, in °C) were determined by empirical equations according to the molecular weight of the fatty acid, the number of double bonds, and the proportion of each fatty acid component (Francisco et al., 2010; Wu and Miao, 2014).

IV and CN were calculated by Eqs.1 & 2:

$$\mathrm{IV}=\sum\frac{254DN}{M}, \quad (1)$$

$$\mathrm{CN}=46.3+\frac{5\,458}{\sum\frac{560N}{M}}-(0.255\times\mathrm{IV}), \quad (2)$$

where M is the molecular weight of the fatty acid, D is the number of double bonds, and N is the proportion of each fatty acid component.

DU was calculated by Eq.3:

$$\mathrm{DU}(\%)=\mathrm{MUFA}+(2\times\mathrm{PUFA}), \quad (3)$$

where MUFA (monounsaturated fatty acid) and PUFA are the weight proportion (wt%) of the monounsaturated and polyunsaturated fatty acids, respectively.

CFPP was calculated by Eq.4:

$$\mathrm{CFPP}=3.417\times[(0.1\times C16)+(0.5\times C18)]-16.477, \quad (4)$$

where *C16* and *C18* are the weight proportion (wt%) of the saturated fatty acids of the respective chain lengths.

2.6 Data analysis

IBM SPSS22 software was used for one-way analysis of variance (one-way ANOVA). Multiple comparisons were conducted by the least significant difference (LSD) or Dunnett's T3 test. Uppercase letters represent extremely significant differences ($P\leq0.01$), while lowercase letters represent significant differences ($P\leq0.05$) in all figures (Jin et al., 2021).

3 RESULT

3.1 Effects of light quality on the growth of *Pavlova* sp.

The effects of different light quality on the growth of *Pavlova* sp. are shown in Fig.2a. *Pavlova* sp. grew under all tested light quality. The growth of *Pavlova* sp. under the white-light control (W) was better than under monochromatic red light (7R0B) or blue light (0R7B), but lower than most of those under combined monochromatic light (except 1R6B). Accordingly, the cell density under W was 40.38×10^6 cells/mL, while cell densities under monochromatic blue light (0R7B), red light (7R0B), and 1R6B treatment were only 38.59, 38.65, and 38.65×10^6 cells/mL, respectively (Fig.2b). The cell density of *Pavlova* sp. under monochromatic light combinations 2R5B to 6R1B was significantly higher than under W ($P<0.01$). The highest cell density under 5R2B, was 44.84×10^6 cells/mL, which was 11.05% higher than under the control (W). The specific growth rates under monochromatic light combinations of 2R5B, 3R4B, 4R3B, 5R2B, and 6R1B were all higher than 0.170/d, and were also significantly higher than those of W, 0R7B, 7R0B, and 1R6B. The generation times of *Pavlova* sp. under W and 0R7B were 4.15 d and 4.23 d, respectively. The shortest generation time was observed under the monochromatic light combination 5R2B, was only 3.97 d (Fig.2c). The biomass dry weight under W was 340.83 mg/L (Fig.2d). Those under the monochromatic light combinations of 2R5B, 3R4B, 4R3B, and 5R2B were all greater than 390 mg/L, and were significantly higher than those of W, 0R7B, 7R0B, and 1R6B. The highest biomass dry weight was under 5R2B, which was 418.03 mg/L and 22.65% higher than under W. The biomass dry weights under 0R7B, 7R0B, and 1R6B were 325.58, 334.43, and 372.20 mg/L, respectively, all lower than under W. Therefore, for biomass production, the monochromatic light combinations of 2R5B, 3R4B, 4R3B, and 5R2B are superior to W, while W is superior to the two monochromatic light treatments of 0R7B and 7R0B.

3.2 Effects of light quality on pigment, protein, carbohydrate, and lipid accumulation of *Pavlova* sp.

Pigment production of *Pavlova* sp. under different light quality is shown in Fig.3a–b. Chlorophyll *a* and carotenoid production under control W was 0.337 and 0.407 mg/L, respectively. Both chlorophyll *a* and carotenoid production under W, 0R7B, and 7R0B was generally higher than that under combined monochromatic light, but the difference was not significant.

Protein production in *Pavlova* sp. was significantly influenced by light quality (Fig.3c; $P<0.05$). The lowest protein production, 18.00 mg/L, was observed under W. The protein content under 1R6B, 6R1B, and 7R0B was higher than 30 mg/L. The highest protein content was 31.60 mg/L under 6R1B, 1.75 times that under W.

Carbohydrate production in *Pavlova* sp. was also

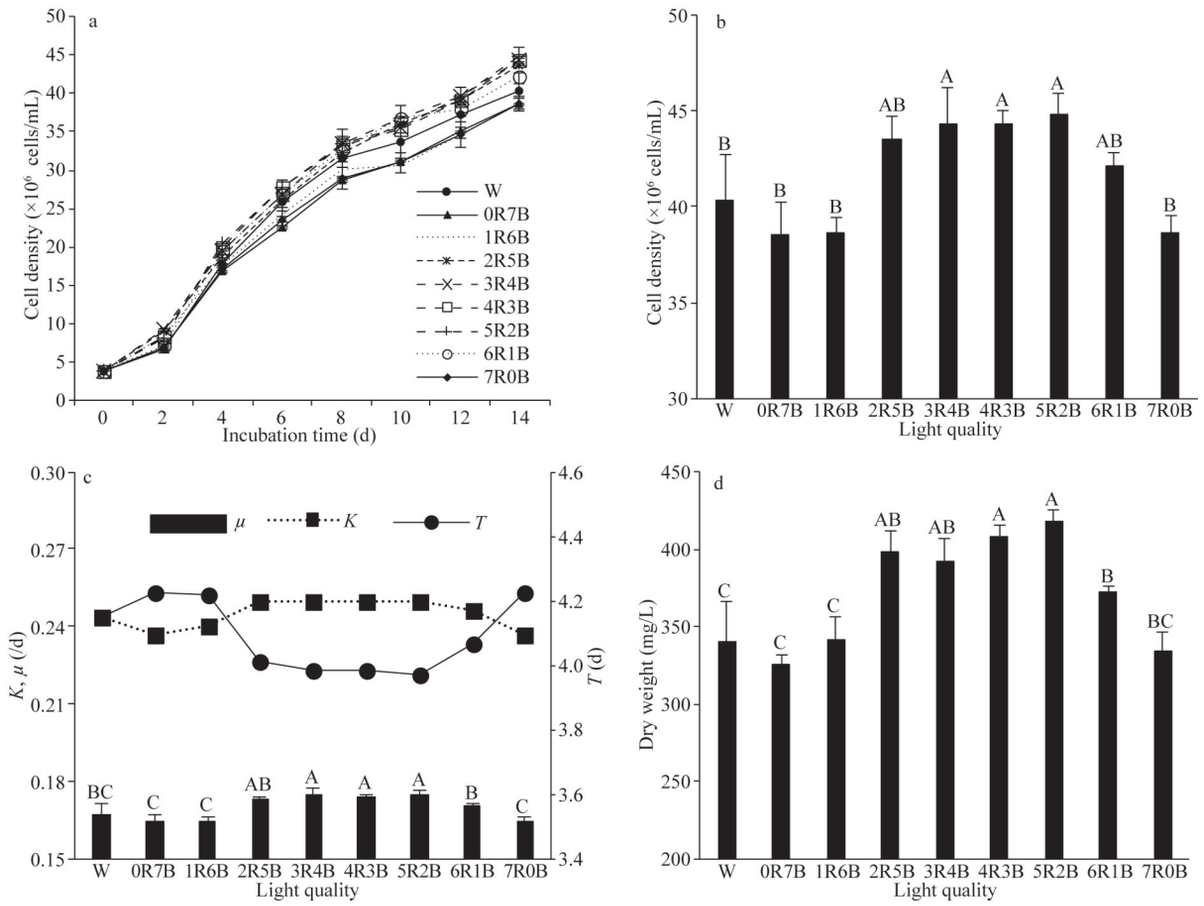

Fig.2 Effects of light quality on growth of *Pavlova* sp. (means±SD, *n*=3)

a. growth curve; b. cell density at harvest; c. growth rate (μ), doubling rate (K), and generation time (T); d. dry weight at harvest. Different letters indicate a statistical difference as determined by an LSD test or Dunnett's T3 test, uppercase letters indicate $P<0.01$.

significantly affected by light quality (Fig.3d); it was 53.33 mg/L under W, while under 7R0B it was significantly higher, 71.19 mg/L. Both 0R7B and combined monochromatic light were not conducive to the accumulation of carbohydrates, especially from 3R4B to 5R2B, as their production was much lower than W. The lowest value was only 30.12 mg/L in 4R3B, which was 56.5% of that under W and 42.31% of that under 7R0B.

Differing light quality had little effect on the lipid content (DW, in%) of *Pavlova* sp., which was 21.28%–26.42% without significant difference among the nine treatments (Fig.3e); however, lipid production depends on both the dry weight and lipid content of algal cells. Combined monochromatic light promoted growth of *Pavlova* sp., thus obtaining a higher dry weight of algal powder. Lipid production was only 85.87 mg/L under W (Fig.3f). The monochromatic light combinations 2R5B, 3R4B, 4R3B, and 5R2B significantly increased lipid production, and the maximum value observed was 107.86 mg/L under 4R3B, which was 25.61% higher than under W. Lipid production under 7R0B and 0R7B was lower than under W, only 71.06 and 81.32 mg/L, respectively.

The variation in biocomponent production of *Pavlova* sp. resulting from monochromatic light combinations versus the control W is shown in Fig.4. In general, combined monochromatic light promotes dry weight, protein, and lipid production in *Pavlova* sp., for which the amplitude of variation was all positive (Fig.4). By contrast, the amplitude of variation in carotenoid, chlorophyll *a*, and carbohydrate production for the majority of the combined monochromatic light treatments was negative. The monochromatic red and blue light treatments also led to positive variation for protein production, similar to that under combined monochromatic light. However, the same trends were not observed for other biocomponent production under monochromatic red and blue light treatments, leading to negative variation in lipid and dry weight production, positive variation in carotenoid production,

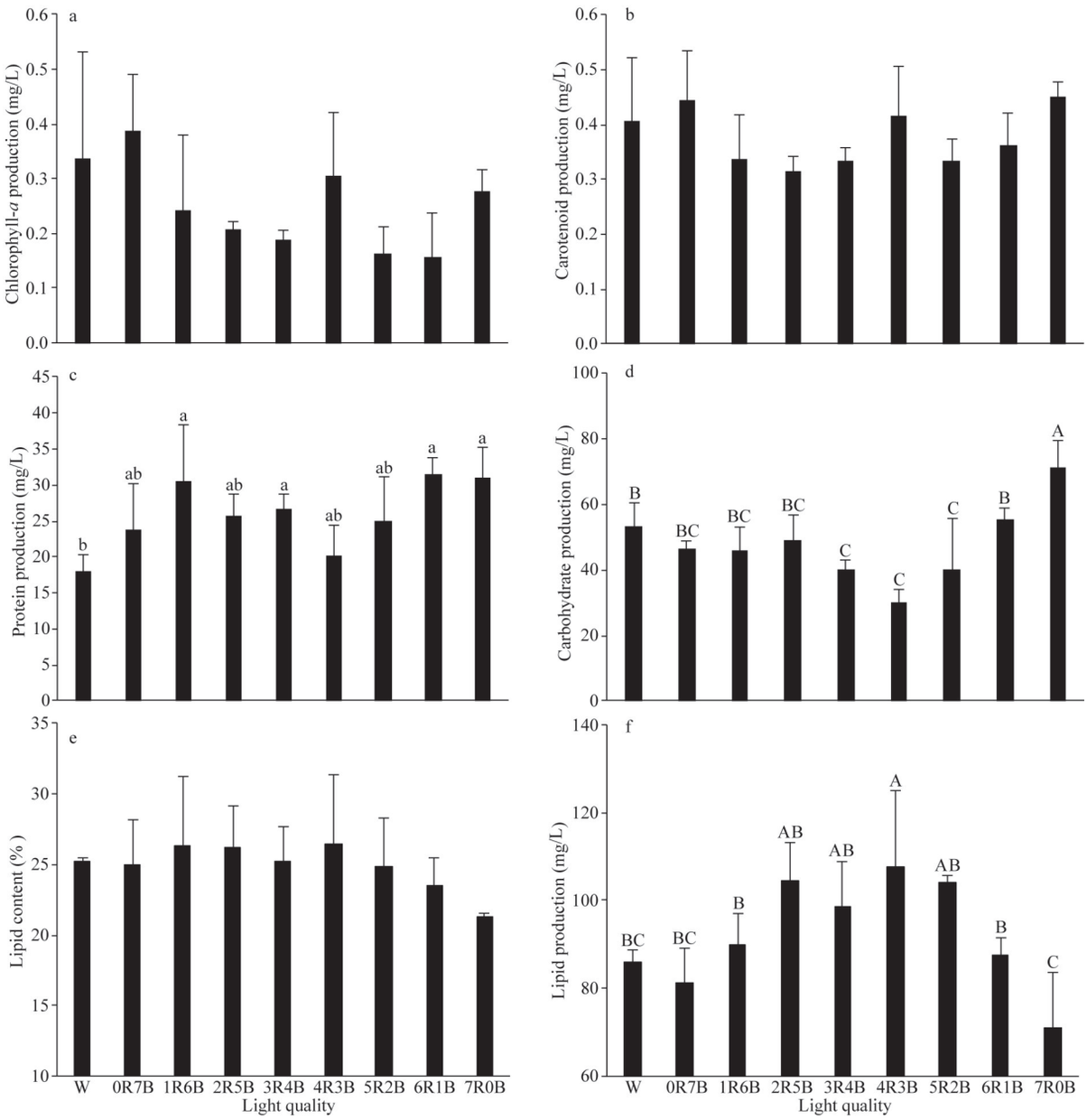

Fig.3 Effects of light quality on pigment, protein, carbohydrate, and lipid production of *Pavlova* sp. (means±SD, *n*=3)

a. chlorophyll-*a* production; b. carotenoid production; c. protein production; d. carbohydrate production; e. lipid content; f. lipid production. Different letters indicate statistical difference as determined by an LSD test or Dunnett's T3 test; uppercase letters indicate $P<0.01$ and lowercase letters indicate $P<0.05$.

and positive/negative variation in chlorophyll *a* and carbohydrate production.

3.3 Effects of light quality on composition of fatty acids in *Pavlova* sp.

The fatty acid components of *Pavlova* sp. are dominated by the following six (Table 1): C14∶0 (myristic acid), C16∶0 (palmitic acid), C16∶1 (n-7) (palmitoleic acid), C18∶0 (stearic acid), C18∶1 (n-9) (oleic acid), and C20∶5 (n-3) (eicosapentaenoic acid, EPA).

The two dominant fatty acids in *Pavlova* sp. are C16∶0 and C16∶1, which together account for nearly 70% of the total. The next most significant fatty acid was C20∶5 (EPA), accounting for 10% to 20%. Saturated fatty acid (SFA) content accounted for about 40% of all fatty acids, including C14∶0, C16∶0, and C18∶0, of which C16∶0 accounted for the

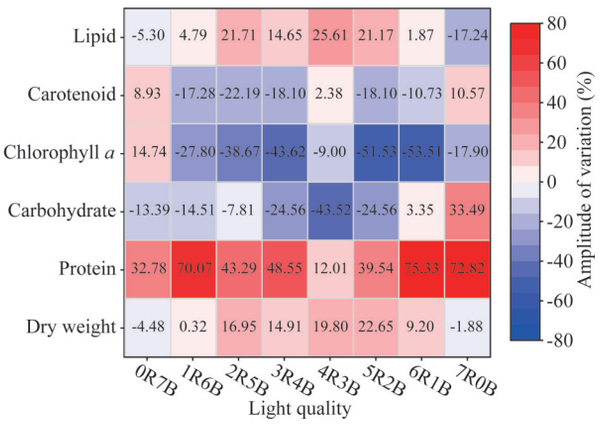

Fig.4 Variation amplitude (%) of the biocomponent production by *Pavlova* sp. under different monochromatic light combinations compared to that under control white light

The variation (%) in production of each biocomponent was the difference between the production in treatment and control divided by production in control.

majority. The combined monochromatic light significantly increased the proportion of C16:0, especially 4R3B and 5R2B, which increased C16:0 by 15.55% and 13.69% compared with W, respectively (Fig.5). Both monochromatic light and combined monochromatic light were not conducive to the accumulation of the other two saturated fatty acids, C14:0 and C18:0, which show negative variation in Fig.5. The accumulation of C14:0 and C18:0 under 7R0B decreased by 23.05% and 28.08%, respectively. Contrastingly, combined monochromatic light led to slight increases in C16:0 (Fig.5). Therefore, the content of SFA under combined monochromatic light 2R5B to 6R1B was higher than W, and extremely significantly higher than 7R0B, 0R7B, and 1R6B (Table 1). MUFA content accounted for more than 40% of all fatty acids, including C16:1 and C18:1, of which C16:1 accounted for the majority, as shown in Table 1. All monochromatic light and combined monochromatic light significantly increased the proportion of C16:1, which experienced an increase of over 20% under 1R6B, 4R3B, 6R1B, and 7R0B (Fig.5). However, the proportion of C18:1 either increased or decreased depending on the light combination, with the largest decrease under 4R3B (28.96% decrease), and the largest increase under 7R0B (36.22% increase). As the result, the MUFA content under monochromatic light and combined monochromatic light was significantly higher than under W, with the content under 7R0B the highest (Table 1). The main PUFA present was C20:5. The content of C20:5 under W was significantly higher than under any other light quality, accounting for 16.78% of fatty acids. No monochromatic light or combined monochromatic light treatment promoted its accumulation; for example, 6R1B reduced its content by 55.18%, while the content of C20:5 under 0R7B decreased by 2.98% (Fig.5). In overall terms of fatty acid composition, combined monochromatic light showed a significant effect of increasing C16:0 and C16:1, which would have a remarkable impact on the quality of biodiesel.

3.4 Effects of light quality on the biodiesel quality of *Pavlova* sp.

Figure 6 shows the quality indexes of biodiesel derived from the lipids of *Pavlova* sp. under different

Table 1 Fatty acid composition of *Pavlova* sp. under differing light quality

Light	FAME (%)								
	C14:0	C16:0	C16:1 (n-7)	C18:0	C18:1 (n-9)	C20:5 (n-3)	SFA	MUFA	PUFA
W	1.93±0.08^{A}	35.25±1.39^{AB}	30.09±1.88^{C}	5.87±1.26	10.09±4.24	16.78±1.46^{A}	43.05±1.69^{BC}	40.18±3.10^{C}	16.78±1.46^{A}
0R7B	1.89±0.09^{AB}	33.94±0.37^{B}	35.15±0.99^{AB}	4.69±0.40	8.05±2.07	16.28±0.95^{AB}	40.52±0.74^{C}	43.20±1.10^{BC}	16.28±0.95^{AB}
1R6B	1.90±0.06^{A}	34.96±0.76^{B}	36.31±1.45^{AB}	4.37±0.39	8.56±1.61	13.91±0.41^{B}	41.23±0.47^{C}	44.86±0.22^{B}	13.91±0.41^{B}
2R5B	1.73±0.27^{AB}	37.61±0.42^{AB}	35.21±2.44^{AB}	5.33±1.01	10.56±3.50	9.56±1.94^{C}	44.66±0.54^{A}	45.78±1.41^{B}	9.56±1.94^{C}
3R4B	1.72±0.15^{AB}	38.00±1.38^{AB}	33.62±2.86^{B}	5.49±1.91	9.68±0.73	11.49±1.74^{BC}	45.20±2.69^{A}	43.31±3.01^{BC}	11.49±1.74^{BC}
4R3B	1.76±0.06^{AB}	40.73±0.99^{A}	36.69±0.84^{A}	4.63±0.16	7.16±1.54	9.02±1.64^{C}	47.12±1.16^{A}	43.86±1.12^{B}	9.02±1.64^{C}
5R2B	1.69±0.02^{A}	40.08±0.98^{A}	34.64±1.00^{AB}	4.48±0.57	10.95±3.76	8.18±2.62^{C}	46.24±1.49^{A}	45.58±3.58^{B}	8.18±2.62^{C}
6R1B	1.63±0.16^{AB}	38.68±0.91^{AB}	36.21±1.80^{AB}	4.31±0.87	11.66±1.97	7.52±0.40^{D}	43.86±2.26^{B}	47.05±1.87^{AB}	7.52±0.40^{D}
7R0B	1.48±0.03^{B}	34.02±0.02^{B}	36.19±1.41^{AB}	4.22±0.75	13.74±2.48	10.35±1.90^{C}	39.73±0.74^{C}	49.93±1.22^{A}	10.35±1.90^{C}

The fatty acid methyl esters (FAME) of C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (n-7) (palmitoleic acid), C18:0 (stearic acid), C18:1 (n-9) (oleic acid), C20:5 (n-3) (eicosapentaenoic acid), SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), and PUFA (polyunsaturated fatty acid) of *Pavlova* sp. are shown as means±SD (n=3). Different letters indicate statistical difference as determined by an LSD test or Dunnett's T3 test; uppercase letters indicate P<0.01.

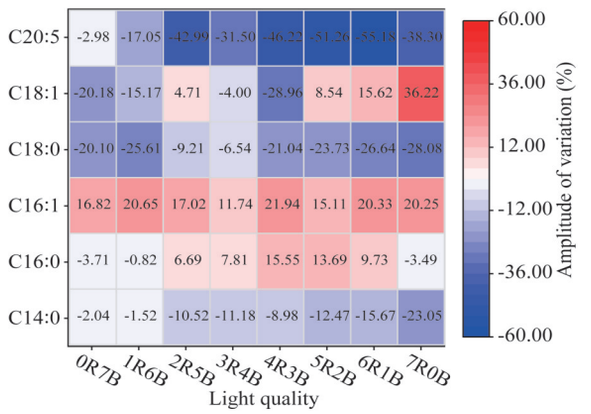

Fig.5 Variation amplitude (%) for FAMEs of *Pavlova* sp. under different monochromatic light compared to control white light

The identified fatty acids include C14:0 (myristic acid), C16:0 (palmitic acid), C16 : 1 (n-7) (palmitoleic acid), C18 : 0 (stearic acid), C18:1 (n-9) (oleic acid), and C20:5 (n-3) (eicosapentaenoic acid). The variation (%) in content of each fatty acid content was calculated as the variation between the treatment and control divided by the control.

light quality. The CN values for biodiesel of *Pavlova* sp. are significantly higher in the combined monochromatic light and 7R0B treatments (P<0.01; Fig.6a). The lowest CN value observed was 43.8 under 0R7B, followed by 44.2 under W and 45.8 under 1R6B. There was no significant difference among these three lowest CN values. CN values for 7R0B and combined monochromatic light (except 1R6B) were significantly higher than for W. The CN values for 4R3B, 5R2B, and 6R1B were even greater than 51. The IV was 109.7 g I_2/100 g under W, which was close to the value under 0R7B (Fig.6b). IVs under 7R0B and combined monochromatic light (except 1R6B) were significantly lower than under W, ranging from 78 to 92 g I_2/100 g (P<0.01). The DU value under W was 73.7%, which was close to those values under 0R7B, 1R6B, and 7R0B (Fig.6c). DU values under 4R3B, 5R2B, and 6R1B were significantly lower than under W (P<0.01), at only 61.9%, 61.9%, and 62.9%, respectively (Fig.6c). The CFPP of biodiesel of *Pavlova* sp. under all light quality was between 2.3 °C and 5.8 °C, and its fluidity was good at low temperatures (Fig.6d). The CFPP values for W, monochromatic light, and combined monochromatic light treatments were not significantly different.

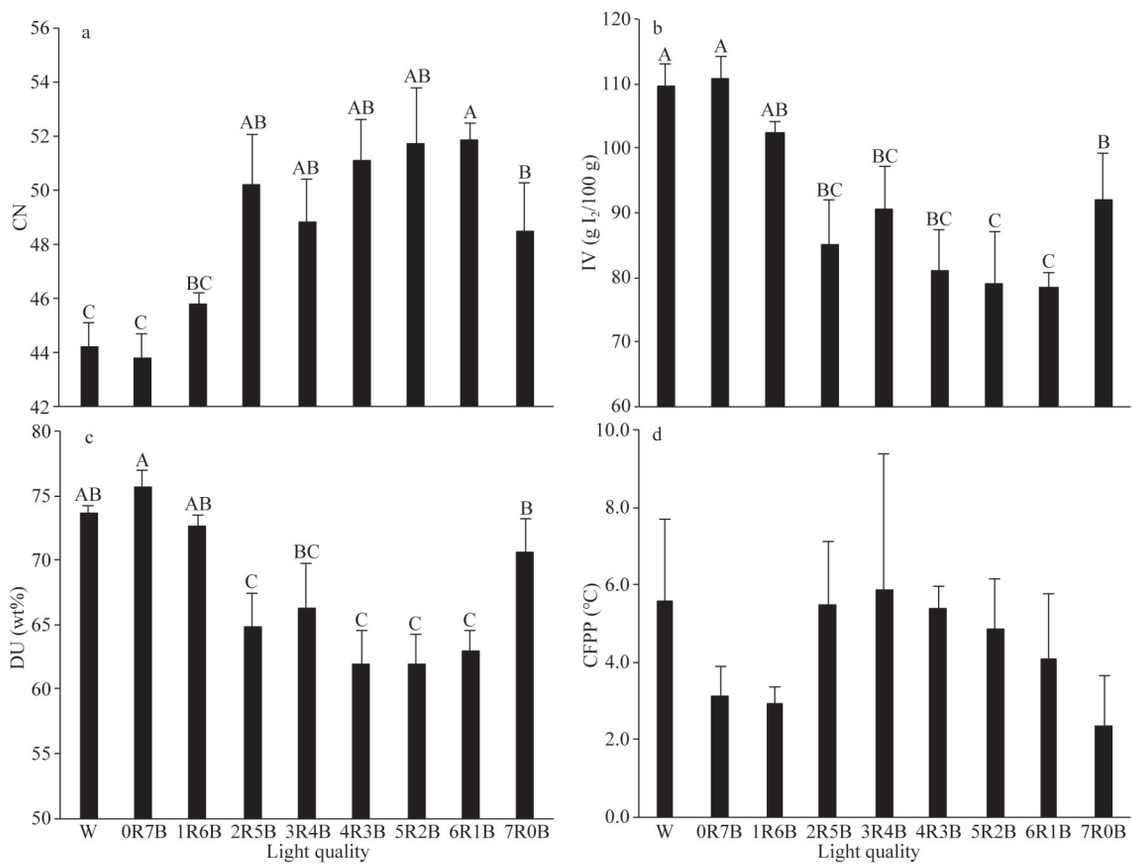

Fig.6 Biodiesel quality of *Pavlova* sp. under differing monochromatic light (means±SD, n=3)

a. CN; b. IV; c. DU; d. CFPP. Different letters indicate statistical difference as determined by an LSD test or Dunnett's T3 test; uppercase letters indicate P<0.01.

4 DISCUSSION

In a microalgal cultivation system, the appropriate employment of illumination conditions is a key consideration, since light is critical for algal growth, photosynthesis, and intracellular metabolism (Li et al., 2020; Sharma et al., 2020). In this study, *Pavlova* sp. grew faster and a higher dry weight of algal powder was harvested under combined monochromatic light versus white light (W). In terms of growth of *Pavlova* sp., combined monochromatic light was the best light source, followed by white light, while the effect of the two monochromatic lights alone performed the poorest. This is consistent with previous results for *Dunaliella salina* and *Nannochloris oculata*, which showed that these two green microalgae grew better under combined monochromatic light, while the growth effect was poorest under monochromatic light, especially red light (Jin et al., 2021; Yu et al., 2022). In this experiment, growth of *Pavlova* sp. under blue light and red light was inferior to that under white light, and there was little difference between blue light and red light treatments. In nature, red light is thought to be rarely experienced by microalgae since blue-green light predominates in the water column while red light is strongly attenuated by surface water. Therefore, blue light sensors are likely of great importance for algal light perception (Jungandreas et al., 2014). Red light has actually been shown to be detrimental for microalgae; such results are available for *D. salina* (Jin et al., 2021). A similar result for the diatom *Phaeodactylum tricornutum* was reported, in which the growth rate under white light was higher than under red light and blue light, and photosynthetic efficiency was lowest under red light (Costa et al., 2013). However, another study suggested that growth rate, cell dry weight, and chlorophyll *a* content of *P. tricornutum* was not significantly different between red and blue light (Jungandreas et al., 2014). The green algae *N. oculata*, *D. salina*, and *Scenedesmus* sp. were reported to show higher growth under combined monochromatic light but poor growth under red light in comparison with white light (Kim et al., 2013; Jin et al., 2021; Yu et al., 2022). This species-specific preference for light quality may reflect differences in the ability of these photoautotrophs to adapt and self-adjust their pigment composition when subjected to different light quality (Cerff and Posten, 2012; Kim et al., 2013; Li et al., 2019). In this study, no significant difference was observed in pigment production under white versus combined monochromatic light, which indicates that *Pavlova* sp. does not display remarkable ability to adjust its pigment composition. As in Chrysophyta, the growth performance of *Pavlova* sp. under monochromatic light was inferior to that under white light in the present study, but there was little difference between blue light and red light. It is suggested that the efficiency of *Pavlova* sp. in utilizing monochromatic light might be different from that of green algae or diatoms. Numerous prior studies have also shown that various algae are sensitive to the spectral quality of illumination light (Vadiveloo et al., 2015; Altunoz et al., 2017; Li et al., 2019). By using a graded series of red-blue light combinations under the same illumination intensity, the present study supports the claim that an appropriate species-specific illumination spectrum is very important for microalgae cultivation, particularly in a photobioreactor (Altunoz et al., 2017; Li et al., 2020).

In this study, combined monochromatic light benefited not only growth and biomass accumulation, but also the accumulation of lipids. Lipid content under combined monochromatic light was slightly higher than that of white light; correspondingly, lipid production under 2R5B to 5R2B with an increased dry weight was also the highest, significantly higher than under white light, especially under 4R3B, which was 25.6% higher than under white light. Conversely, the lipid content and dry weight of algal powder were low under monochromatic blue light and red light, with lipid production also low. Lipid production of *Pavlova* sp. under red light was only 71.06 mg/L, which was the lowest among all light treatments. The results show that monochromatic light, especially red light, is not conducive to lipid production in *Pavlova* sp. One report showed that *Chlorella* sp. achieved higher carbohydrate accumulation under red light, which was also observed in this study. Carbon allocation between carbohydrates and lipids inclined toward carbohydrates under red light, which may have resulted in the low lipid production observed under that treatment (Li et al., 2019). This is different from the results obtained with the alga *P. tricornutum*, in which the lipid content after being cultured under red light for 10 days increased by 27.8% compared with white light (Sharma et al., 2020). For microalgae, light quality does not only have a direct effect on chloroplast migration, zygote germination, and the light acclimation reactions of photosynthesis,

but also influences the metabolic network (Li et al., 2019). The changes within *P. tricornutum* under differing light quality with respect to cell growth, photosynthetic efficiency, and macromolecular and metabolic profiles by shifting the illuminating light from red to blue and vice versa have been investigated. The results showed that light quality changes led to two functional responses, including the regulation of activities of enzymes for nitrogen and carbon assimilation and gene transcription related to carbon redistribution (Jungandreas et al., 2014). In the present study, varying light quality also caused changes in carbon allocation by *Pavlova* sp. Combined monochromatic light increased not only lipid production, but also protein production, which was accompanied by a decrease in carbohydrate production.

The total quantity and profiles of fatty acids of *Pavlova* sp. in this study were similar to those reported by Ponis et al. (2006). The major fatty acids found were the saturated C16∶0, the monounsaturated C16∶1, and the n-3 polyunsaturated C20∶5 (EPA). These three fatty acids accounted for more than 80% of the total fatty acids. Some differences among cultures under varying light quality were detected in terms of proportions of fatty acids. Combined monochromatic light significantly increased the content of SFA and MUFA, a result also observed in *Chlorella* sp. and *N. oculata* under monochromatic light (Li et al., 2019; Yu et al., 2022). All monochromatic light and combined monochromatic light significantly increased the proportion of C16∶1, and the increase was greater than for C16∶0 (Fig.5). MUFA content of monochromatic and combined monochromatic light was significantly higher than that of white light, but the content of both C14∶0 and C20∶5 (EPA) under white light was significantly higher than under other light quality.

Biodiesel properties such as cetane number (CN), kinematic viscosity described by CFPP, oxidative stability evaluated by IV and DU, etc., depend upon the fatty acid profile in raw materials of microalgae and are taken into account by international standards and specifications (Ramos et al., 2009). Light quality regulates algal carbon distribution and the composition of fatty acids and therefore has the potential to improve the quality of biodiesel (Li et al., 2019). Fatty acids from microalgae can include PUFAs and FAMEs with four or more double bonds. The high content of esters from SFAs leads to an increase in the CN, and a sufficient amount of esters from MUFAs significantly improves flow properties of biodiesel at low temperatures. Thus, microalgae with a high content of SFAs and MUFAs are most promising for the production of biodiesel fuel (Gao et al., 2017). This study revealed that the content of SFA and MUFA in *Pavlova* sp. was 80%–90% under various light treatments, making it a high-quality raw material for biodiesel production. CN value is a principal indicator of biodiesel quality related to ignition delay time and combustion quality, and is contributed to by longer carbon chains and high saturation of fatty acids (Ramos et al., 2009). A higher CN value for biodiesel means better ignition and engine performance; the reference standard criteria for biodiesel application by European Standard EN 14214 and ASTM International Standard D6751 were CN>51 and CN>47, respectively (Gharajeh et al., 2020). The C16∶0 (palmitic acid) and C16∶1 (n-7) (palmitoleic acid) content of *Pavlova* sp. under combined monochromatic light was significantly higher than under white light and monochromatic light, which has obvious advantages in improving the quality of biodiesel from *Pavlova* sp. Combined monochromatic light, especially from 4R5B to 6R1B, produced lipids with a CN value of more than 51, which meets the biodiesel standard, allowing cells produced under this light quality to be used as direct biodiesel feedstock (Gharajeh et al., 2020). DU and IV, which indicate the oxidative stability of biodiesel, are both measured based on proportions of monounsaturated and polyunsaturated fatty acids (Francisco et al., 2010; Wu and Miao, 2014). The unsaturation of fatty acids affects the feasibility of long-term storage of a given biodiesel. Polyunsaturated fatty acids are more easily oxidized than monounsaturated fatty acids and tend to produce biodiesel with less oxidation stability (Ramos et al., 2009; Wu and Miao, 2014). The proportion of C16∶1 (n-7) (palmitoleic acid) under combined monochromatic light was increased, resulting in low DU and IV, high oxidation stability, and better long-term storability. *Pavlova* sp. cultivated under combined monochromatic light is rich in SFA and MUFA, potentially producing biodiesel with higher CN and higher oxidation stability.

5 CONCLUSION

Combined monochromatic light outperforms white light and monochromatic light for cell growth and biomass accumulation of *Pavlova* sp. Combined monochromatic light, especially 6R1B, is the best illuminating spectrum for producing protein. Monochrome red light is best for producing

pigments, especially carotenoids.

Combined monochromatic light is superior to white light and monochromatic light in lipid accumulation by *Pavlova* sp. Among the combinations, 4R3B is best for lipid production.

All combined monochromatic light except 1R6B enhances C16:0 (palmitic acid) accumulation in *Pavlova* sp., while monochromatic light and all combined monochromatic light promote accumulation of C16:1.

Combined monochromatic light, especially 4R3B, 5R2B, and 6R1B, helps to further improve the biodiesel quality of *Pavlova* sp., with higher CN values (>51) and lower DU and IV values.

6 DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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