RESEARCH PAPER

A comprehensive study on the effect of light quality imparted by light-emitting diodes (LEDs) on the physiological and biochemical properties of the microalgal consortia of *Chlorella variabilis* and *Scenedesmus obliquus* cultivated in dairy wastewater

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Abstract

The effect of light wavelengths on the physiological, biochemical and lutein content of the microalgal consortia Chlorella variabilis and Scenedesmus obliquus was evaluated using different light sources. Among different light treatments, cool-white fluorescent light produced the highest biomass of 673 mg L^{-1} with a specific growth rate of 0.75 day^{-1} followed by blue (500 mg L^{-1}; 0.73 day^{-1}). The chlorophyll content was enhanced under blue light (10.7 mg L^{-1}) followed by cool fluorescent light (9.3 mg L^{-1}), whereas the lutein productivity was enhanced under cool fluorescent light (7.22 mg g^{-1}). Protein content of the microalgal consortia was enhanced under all light treatments with the highest protein accumulation under cool-white fluorescent light (~56% of dry mass) closely followed by amber light (52% of dry mass), whereas the carbohydrate content was higher under amber light (~35% of dry mass). The results revealed that the consortia could grow well on diluted dairy wastewater thereby reducing the cost of algal production when compared with the use of inorganic media and a two-phase culture process utilizing cool fluorescent and amber light could be employed for maximizing algal biomass and nutrient composition with enhanced lutein production. The study also emphasizes on the economic efficiency of LED lights in terms of biomass produced based on the modest electricity consumed and the importance of using amber light for cultivating microalgae for its nutrient content which has seldom been studied.

Keywords Biomass productivity · Economic efficiency · Lutein · Amber · Nutrient content

Introduction

Marginal biomass productivity is one of the fundamental constraints that prevent algal technology from reaching appreciable commercialization. Various environmental factors are known to influence microalgal growth and light is considered as one of the primary factors [1]. Sunlight is the most cost-effective light source and is mainly used for outdoor microalgal production (raceway ponds); however, electrical lighting is gaining importance owing to the fact that it can provide better control of the lighting intensity and wavelengths for photobioreactors [2]. Electrical lighting for

microalgal cultivation is generally derived from three major light sources: high-intensity discharge lamps, fluorescent lamps and light-emitting diodes (LEDs). Among the sources listed, fluorescent lamps are commonly used by researchers for microalgal production as they cover a wide range of wavelengths. In recent years, LEDs have been gaining popularity because of their compactness, longer lifetime, high electrical conversion efficiency and low heat emission [2, 3].

Microalgae, similar to plants, capture light energy (light-harvesting antennas) and produce electrons in the reaction center of the photosystems. For efficient photosynthesis, preserving an excitation balance between the two photosystems (PSI and PSII) is of prime importance. To serve the purpose, microalgae possess specific light-harvesting antennas for expanding the available wavelength of light. Certain groups of algae contain accessory pigments which help in efficiently harvesting light for photosynthesis [4]. Green algae, in particular, possess a chlorophyll–protein complex which

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is comprised of chlorophyll a and b and carotenoids for carrying out the photosynthesis [5]. Light quality and intensity highly influence the photosynthetic process in microalgae and also induce modifications in their biochemical composition [6]. Light quality, in particular, has a strong influence on the growth rate in both microalgae and macroalgae [7]. Besides this, light quality is also known to induce a significantly high amount of mature cells in the culture [8]. Red and blue wavelengths in particular are known to induce high photosynthetic machinery due to the high absorbances of photosystems I and II for these wavelengths. Previous studies have also reported that blue/red light highly influences the algal growth rate and pigment synthesis [9, 10]. Apart from photosynthesis, blue light also regulates enzyme activation and gene transcription and the cells damaged by exposing the algae to red light could be repaired by exposing them to blue light. Therefore, for achieving maximum growth and biomass productivity, microalgae require light conditions that are ideal and that fall within a narrow band of spectrum [11].

Zhong et al. [12] reported that the microalgae *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Scenedesmus quadricauda* and *Scenedesmus obliquus*, grown under blue light, have the potential to be used in the production of nutritional supplements for human consumption as well as a biofuel feedstock, due to increased biomass productivity and lipid composition. The authors from the same study concluded that blue light is the best wavelength for enhancing algal growth rates followed by red and white lights. Another study put forward by Teoa et al. [13] on the algal species *Nannochloropsis* and *Tetraselmis*, under the mixture of red and blue lights with a 24 h photoperiod, revealed that blue wavelength had a positive effect on the biomass productivity. Therefore, adequate selection of a light wavelength can enhance the growth and biomass productivity of microalgal species, but this differs not only from species to species but also from one strain to another. This response of microalgae to light quality can be attributed to the variation in light-harvesting pigments and membranes [4, 6, 14].

However, the light wavelengths ranging between 500 and 600 nm have rarely been studied because of their low electrical conversion and radiation efficiencies [2]. The knowledge of how microalgae respond to light quality is still a question that needs to be addressed. Combination of different LEDs with varying wavelengths could promote growth rate and biomass productivity by enhancing the process of photosynthesis, but the practice requires further studying [9, 10]. In addition, the impact of light properties on different strains of algae has not been investigated extensively by researchers [6]. To address these gaps, the current study aims to provide a clear insight on how different wavelengths of light from monochromatic LEDs (470, 595, and 655 nm) including cool fluorescent, impact the growth rate, biomass productivity, chlorophyll/lutein contents and biochemical composition for the microalgal consortia

composed of *Chlorella variabilis* and *Scenedesmus obliquus* when cultivated on synthetic dairy wastewater, while keeping the light intensity and temperature at 40 ± 3 µmol m^{-2} s^{-1} and 24 ± 2 °C, respectively, under a 24 h photoperiod agitated at 90 rpm on an orbital shaker. The major reason for choosing the algal consortium for this study was to prevent culture crashes that may occur when using monocultures. In addition, algal consortium is considered to be stable and produce higher biomass with stronger resistance to contaminants such as pathogens and grazers [15]. The above strains were chosen as there has been no study reported till date employing this microalgal consortium consisting of the strains *Chlorella variabilis* and *Scenedesmus obliquus* for enhancing biomass productivity, biochemical composition and lutein content using varying light treatments. Furthermore, the study on the effect of amber light and amber + blue light (mixture of amber + blue was used to improve the electrical and radiation efficiency) on the biomass growth, chlorophyll/lutein contents, biomass productivity and biochemical composition of this microalgal consortia will open a new avenue for researchers to focus on. The study also reveals the effect of light quality on lutein content which is considered to be a high value-added product.

Materials and methods

Microalgal strains and culture conditions

Axenic algal strains of *Chlorella variabilis* and *Scenedesmus obliquus* were obtained from the Culture Collection of Algae and Protozoa. The stock cultures were maintained in 125 mL Erlenmeyer flasks containing culture medium at 24 ± 2 °C under a 24 h photoperiod. There are several reports on the effect of 24 h photoperiod on the growth and biomass productivity of the algal species. Specifically, a study on *Botryococcus braunii* KMITL 2 showed that a photoperiod of 24 h enhanced the biomass concentration to almost four times when compared to the 12:12 photoperiod. Therefore, longer duration of light increased the specific growth rate and biomass concentration of the algal species and a linear trend was observed with increasing photoperiods [16]. This experiment was accordingly carried out under a 24 h photoperiod with continuous illumination. Lower irradiance of 40 ± 3 µmol m^{-2} s^{-1} was used to reduce the economic costs incurred while using continuous illumination. Overhead lighting for the flasks was provided using cool-white fluorescent light (4200 K, F72T8CW, Osram Sylvania, MA, US) at a photosynthetic photon flux density (PPFD) of 40 ± 3 µmol m^{-2} s^{-1}. The flasks were agitated on an orbital shaker at 90 rpm. Sub-culturing was done regularly after every 10 days to maintain live algal culture. MN8 medium was used for sub-culturing of *Chlorella variabilis*, while Blue Green medium (BG11) was used for sub-culturing

of *Scenedesmus obliquus*. The compositions of the media were obtained from Cheng et al. [17] and Rippka et al. [18]. The glassware for culturing the algal strains was sterilized at 105 °C for 15 min in a hot air oven. Similarly, the media for sub-culturing the algal strains was autoclaved at 121 °C for 30 min prior to inoculation.

Cultivation of the microalgal consortia with diluted dairy wastewater under different light wavelengths

The microalgal consortia (20 mL) were inoculated into a 500 mL Erlenmeyer flask containing 180 mL culture medium (diluted dairy wastewater). The dilution of the dairy wastewater was carried out due to high turbidity and determined based on the results obtained from a feasibility study that was carried out using undiluted, 25% and 10% wastewater (results not reported here). The simulated dairy wastewater was prepared in the laboratory using 2 g L^{-1} dried milk powder, 2.8 g L^{-1} NH_4Cl, 0.1 g L^{-1} $MgSO_4.7H_2O$, 0.076 g L^{-1} $CaCl_2.H_2O$, 2 g L^{-1} KH_2PO_4 and 4 g L^{-1} $NaHCO_3$ [19]. Sodium bicarbonate was the carbon source for the algal consortia cultivated on simulated dairy wastewater. The LED tubes were installed above the orbital shaker (~0.5 m on a wooden board) and the temperature was maintained at 24 ± 2 °C. The flasks were then exposed to different light wavelengths treatments: blue (450 nm, VanqLED, Shenzhen, Guangdong, China), amber (595 nm, VanqLED), amber + blue (ratio 1:1), red (650 nm, VanqLED), and cool-white fluorescent light (Sylvania Cool White, USA). The spectra of the electrical lighting systems used in this study are presented in Fig. 1 where wavelength (nm) is plotted against relative spectral photosynthetic photon flux (µmol m^{-2} s^{-1}). The flasks were illuminated at 40 ± 3 µmol m^{-2} s^{-1} for 16 days with a 24 h photoperiod on an orbital shaker agitated at 90 rpm. The photosynthetic photon flux density (PPFD) and spectrum of the lighting systems were measured using a spectroradiometer (PS-300, Apogee, Logan, UT). The temperature was continuously monitored using a temperature sensor (Raytek Minitemp, MT6).

Estimation of growth rate and biomass productivity

The growth rate of the microalgal consortia was measured in terms of optical density, chlorophyll content and dry mass. Optical density was measured using a UV spectrophotometer (Ultrospec 2100 pro) at 680 nm [20]. The dry mass was determined by filtering 5 mL of the culture through a Whatmann filter paper followed by drying in an oven at 80 °C for 24 h and cooling in a dessicator until a constant weight was achieved [21]. Chlorophyll content was measured using a modified procedure put forward by Parsons and Strickland [22]. In brief, 5 mL of the algal suspension was taken and centrifuged at 3500 rpm for 10 min. The supernatant was discarded and the pellet was resuspended in 96% methanol followed by vortexing for 2 min. The tubes were then kept on a hot plate for 30 min at 60 °C. The resulting mixture was centrifuged again at 3500 rpm for 10 min and the supernatant was collected separately. The steps were repeated until the chlorophyll was completely extracted. The absorbance of the supernatant
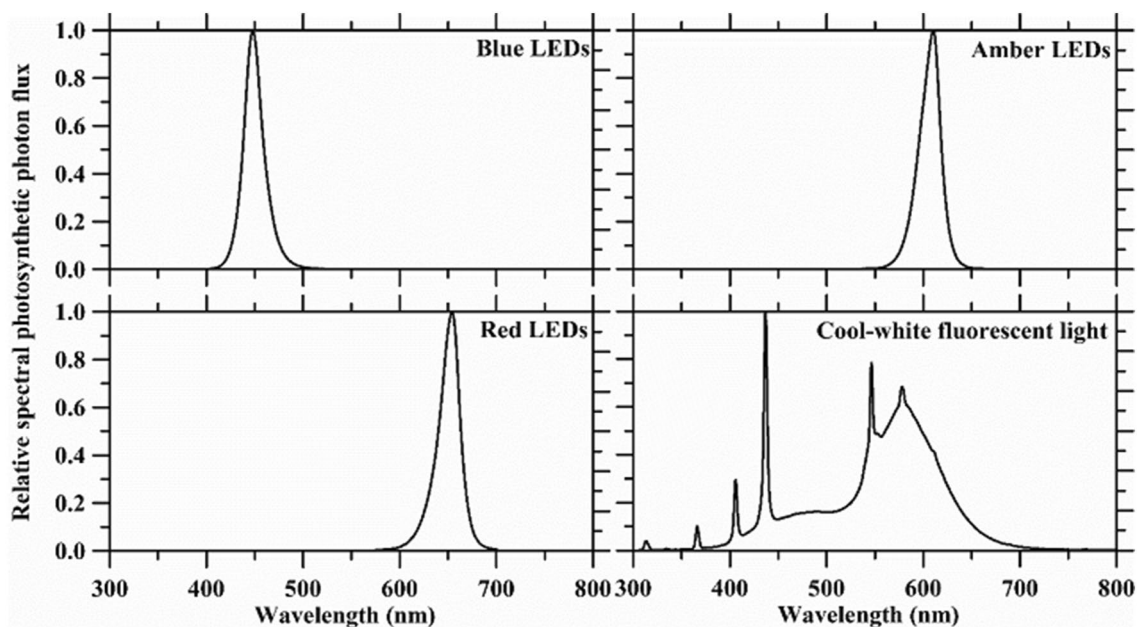

Fig. 1 Spectral compositions of the lighting systems used in this study

was read at 650 nm and 665 nm and the chlorophyll present was calculated using modified Eq. (1) [23]. The chlorophyll extraction was carried out at 60 ℃ with respect to a study that suggested that the highest extraction yield of chlorophyll from *Nannochloropsis gaditana* and *Dunaliella salina* was obtained at 60 ℃ [24]. Similarly, another study by Sartory and Grobbelaar [25] found that the chlorophyll extraction from *Scenedesmus quadricauda*, *Selenastrum capricornutum* and *Microcystis aeruginosa* was maximized by boiling the algae in 95% ethanol or methanol at 100 ℃ for 5 min [25, 26]. In this study, no chlorophyll degradation for the microalgal mixture was observed at temperature of 60 ℃. The variation in pH was monitored regularly using a Fischer Scientific pH meter (Accumet Basic, AB15). The analyses were carried out every alternate day for 2 weeks, except for dry mass which was determined at the end of the 16-day experiment.

Chlorophyll content (CC) was determined using the following Eq. (1) [23]:

$$CC = 22.12 \times A650 + 2.71 \times A665, \tag{1}$$

where A represents absorbance.

Specific growth rate (μ) was determined using the following Eq. (2):

$$\mu = \frac{\ln\left(\frac{X_2}{X1}\right)}{(t_2 - t_1)}, \tag{2}$$

where X_2 represents the dry mass at time t_2 and X_1 represents the dry mass at time t_1 [11].

Doubling time (D) was determined using the following Eq. (3):

$$D = \frac{0.693}{\mu}, \tag{3}$$

where μ represents specific growth rate [27].

Biochemical analysis

After 16 days, the algal samples were centrifuged at 5000 rpm for 20 min to assess the biomass yield. This was followed by freeze drying of the biomass at − 55 °C for 2 days which was then utilized for carbohydrate, protein and lipid analyses using modified methods described by Waghmare et al. [28], Slocombe et al. [29] and Folch et al. [30].

In brief, for the carbohydrate analysis, 5 mg of the freeze dried biomass was taken and hydrolyzed with 5 mL of 2.5 N hydrochloric acid at 100 °C for 3 h. After cooling, sodium carbonate was added to the solution until effervescence ceased and made up to 100 mL with distilled water and centrifuged at 4000 rpm for 10 min. To 1 mL of the above solution, 4 mL of anthrone-sulphuric acid reagent was added.

The solutions were heated at 100 °C in a hot water bath for 10 min. The heated solutions were then cooled to room temperature and subjected to spectrophotometric analysis at 630 nm. The observations were noted and the unknown concentration of carbohydrate present was calculated directly from the standard curve [28].

For protein analysis, about 5 mg of freeze dried algal biomass was taken and 0.2 mL of 24% (w/v) TCA (tri-chloroacetic acid) was added. The samples were then heated at 95 °C for 15 min followed by cooling to room temperature. This was followed by addition of 0.6 mL of water and the mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and the pellet was resuspended in 0.5 mL of Lowry reagent D. The pellet was then heated to 55 °C for 60 min and centrifuged again at 10,000 rpm at room temperature for 10 min. The pellet was then discarded and the supernatant was retained for further analysis using Lowry assay. In brief, 0.05 mL of sample was taken and 0.95 mL of Lowry reagent D was added and incubated for 10 min at room temperature. A 0.1 mL of Folin's reagent was added and vortexed immediately and the absorbance of the sample was read at 600 nm after 30 min. The unknown concentration of protein was obtained directly from the standard curve [29].

For lipid analysis, 10 mg of freeze dried algae was taken and suspended in 5 mL of chloroform and methanol (2:1). The mixture was incubated at room temperature after closing the opening with aluminum foil for 24 h. The mixture was then filtered using a Whatmann filter paper. The filtrate was collected in a 50 mL pre-weighed beaker which was then kept on a hot plate at 50 °C. The chloroform–methanol mixture was evaporated to dryness leaving a residue at the bottom of the beaker. The total lipid was calculated by subtracting the weight of the beaker with lipid from the weight of the empty beaker [30].

Lutein extraction and quantification

Lutein was extracted from the algal consortia by adopting a method described by Inbaraj et al. [31]. In short, 10 mg of the alga was treated with a 3 mL mixture of hexane–ethanol–acetone–toluene (10:6:7:7, v/v) in a 10 mL volumetric flask and shaken for 1 h. To the contents, 2 mL of 40% methanolic potassium hydroxide was added for saponification at 25 °C in the dark for 16 h. After saponification, 3 mL of hexane was added for partitioning, shaken for 1 min and 10% sodium sulfate solution was added and diluted to volume. The mixture was allowed to stand until two phases separated clearly. The upper layer containing lutein was collected. The extracts of upper layer was evaporated to dryness, re-dissolved in 1 mL methanol–methylene chloride (50:50, v/v), and filtered through a 0.2 μm membrane filter for HPLC analysis.

HPLC analysis and identification of peak

The separation, identification, and quantification of lutein were made by high-performance liquid chromatography analysis. The Agilent HPLC system consists of a quaternary pump with a degasser, variable wavelength detector equipped with Agilent chromatography computer software. For separation of lutein, a Discovery C18 column (250 mm × 4.6 mm I.D., 5 µm) was used. The mobile phase (A) consisted of methanol–acetonitrile–water (84:14:2, v/v/v) and the mobile phase (B) consisted of methylene chloride (100%), with a flow rate set as 0.6 mL min^{-1}. A gradient solvent system with 100% A and 0% B in the beginning, decreased to 95% A and 5% B in 8 min, 75% A and 25% B in 25 min, 100% A and 0% B in 25 min was utilized for extracting lutein. Extract injection volume was 20 µL and the response of the peak was detected at 450 nm. The identification of lutein was made by comparing the retention time of the standard with that of the sample. The lutein quantity for each sample was calculated using the following equation [32]:

$$\text{Lutein quantity (mg/g)} = \frac{\text{Lutein concentration} \left(\frac{\text{mg}}{\text{L}}\right) \times \text{Volume of solvent} (L)}{\text{Dry weight (g)}}. \tag{4}$$

Preparation of standard curve for lutein

Commercial lutein obtained from Sigma-Aldrich was used as the analytical standard. Concentrations of lutein varying from 0.5 to 7.5 µg mL^{-1} were injected and the standard curve was prepared by plotting concentration against area. The regression analysis was done using Microsoft Excel and a correlation coefficient (R^2) of 0.99 was obtained.

Statistical analysis

All the experiments were carried out in triplicates to ensure accuracy and consistency. The values obtained were expressed as means of triplicate values ± standard deviation. The optical density, chlorophyll content and dry mass were analyzed statistically using SAS MANOVA and the biochemical characteristics was analyzed using ANOVA followed by Tukey's test. MANOVA was mainly used to reduce the type I error which may occur while performing ANOVA repetitively. Besides this, MANOVA accounts for both the main effect and the interaction effects as specified in Table 1,

Table 1 MANOVA for overall treatment effect (light quality)

MANOVA test criteria and F approximations for the hypothesis of no overall light effect
H = Type III SSCP matrix for light
E = Error SSCP matrix

Parameter	Statistic	Value	F value	Num DF	Den DF	Pr > F
Growth (optical density)	Pillai's trace	3.53	5.59	32	24	<0.0001
Chlorophyll content	Pillai's trace	3.82	15.51	32	24	<0.0001
Biomass productivity (dry mass)	Pillai's trace	3.48	5.06	32	24	<0.0001

Table 2 MANOVA for days effect

MANOVA test criteria and F approximations for the hypothesis of no days effect
H = Type III SSCP matrix for days
E = Error SSCP matrix

Parameter	Statistic	Value	F value	Num DF	Den DF	Pr > F
Growth (optical density)	Pillai's trace	0.99	575.04	7	4	<0.0001
Chlorophyll content	Pillai's trace	0.99	1595.88	7	4	<0.0001
Biomass productivity (dry mass)	Pillai's trace	0.99	1083.59	7	4	<0.0001

Table 3 MANOVA for interaction effect (days*light quality)

MANOVA test criteria and F approximations for the hypothesis of no days*light effect
H = Type III SSCP matrix for days*light
E = Error SSCP matrix

Parameter	Statistic	Value	F value	Num DF	Den DF	Pr > F
Growth (optical density)	Pillai's trace	3.48	6.68	28	28	<0.0001
Chlorophyll content	Pillai's trace	3.14	3.63	28	28	0.0005
Biomass productivity (dry mass)	Pillai's trace	3.40	5.62	28	28	<0.0001

2 and 3, respectively, where light treatments and days are the main effects and days*light treatments is the interaction term [33]. The statistical analyses revealed that there was a significant effect of light treatments on the physiological and biochemical properties of the microalgal consortia with respect to days of growth at a significance level of $p < 0.05$.

Results

Effect of LED lights on the growth and biomass productivity of the microalgal consortia

The microalgal consortia was cultured on diluted dairy wastewater and exposed to varying wavelengths of light for a period of 16 days. The culture growth of the microalgal consortia differed significantly and was dependent on the wavelength of light applied. Continuous illumination was provided to stimulate the growth of the microalgal consortia. The dry mass of the microalgal consortia under cool-white fluorescent light and LED light treatments is presented in Fig. 2. A linear correlation was observed between optical density and dry mass for different light treatments and is presented in the form of equations below.

$$\text{For cool fluorescent}: y = 390.81x - 3.1825 \left(R^2 = 0.96\right), \tag{5}$$

$$\text{For blue}: y = 332.79x - 8.2146 \left(R^2 = 0.96\right), \tag{6}$$

$$\text{For red}: y = 352.07x - 46.295 \left(R^2 = 0.96\right), \tag{7}$$

$$\text{For amber + blue}: y = 428.4x - 0.8102 \left(R^2 = 0.95\right), \tag{8}$$

$$\text{For amber}: y = 174.37x + 19.829 \left(R^2 = 0.93\right). \tag{9}$$

The wavelengths of light had a crucial effect on the microalgal biomass yield (dry biomass obtained after 16 days of inoculation) and productivity (dry biomass obtained per day with respect to the day zero). Among the wavelengths of light used for this study, a maximum biomass yield of 673 mg L^{-1} was observed under cool-white fluorescent light followed by blue, amber + blue, red and amber light with 500 mg L^{-1}, 380 mg L^{-1}, 300 mg L^{-1}, 280 mg L^{-1} and a biomass productivity of 0.042 g L^{-1} day^{-1}, 0.031 g L^{-1} day^{-1}, 0.024 g L^{-1} day^{-1}, 0.019 g L^{-1} day^{-1}, 0.018 g L^{-1} day^{-1}, respectively. This suggests that cool-white fluorescent light was the best light source for maximal growth and biomass productivity of this microalgal consortia followed by blue, amber + blue, amber and red. The results for statistical analyses revealed that optical density, chlorophyll content and dry mass were significantly different with respect to the light treatments (cool fluorescent, blue, red, amber + blue and amber) which is considered to be the main effect at $p < 0.05$ and can be seen in Table 1. Similarly, the optical density, chlorophyll content and dry mass were different with respect to days (main effect) as well, at $p < 0.05$, and the same is presented in Table 2. The interaction term days*light was also significant at $p < 0.05$ as shown in Table 3 and this reveals that the growth rate and dry mass were affected by both the main effects (light treatments and days). In addition, it can also be seen that all the dependent variables (optical density, chlorophyll content and dry mass) contributed to the significant overall effect which can be seen in Tables 1, 2 and 3, respectively [33].

The specific growth rate under different light treatments is presented in Fig. 3. The specific growth rate of the microalgal consortia under cool-white fluorescent and blue light in the growth phase (up to 4 days) was higher ($\mu = 0.75$ and 0.73 day^{-1}) than that of amber, amber + blue and red
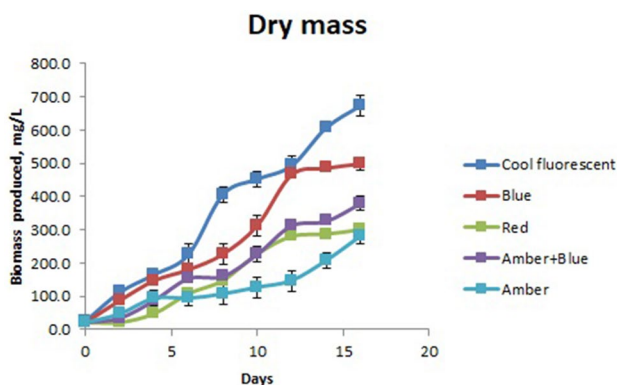

Fig. 2 Dry mass for microalgal consortia under cool fluorescent blue, red, amber + blue, and amber lights. Data are expressed as mean ± SD, $n = 3$
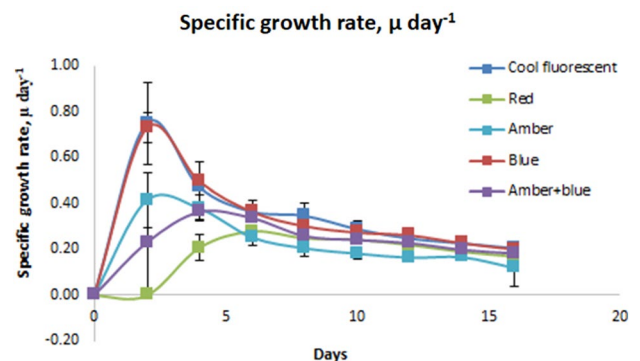

Fig. 3 Specific growth rate of the microalgal consortia under cool fluorescent blue, red, amber + blue, and amber lights. Data are expressed as mean ± SD, $n = 3$

$(0.41 \ \text{day}^{-1}, 0.37 \ \text{day}^{-1} \text{ and } 0.21 \ \text{day}^{-1})$. The results also revealed that the biomass doubling time was highly influenced by the wavelength of light under study. The biomass doubling time of the microalgal consortia was observed to be 22.2 h under cool fluorescent light followed by blue (22.8 h), amber (40.6 h), amber + blue (45.0 h) and red (79.2 h).

Chlorophyll content of the microalgal consortia with respect to light treatments

An increase in chlorophyll content was observed under blue LED (10.7 mg L^{-1}), followed by cool-white fluorescent (9.3 mg L^{-1}), amber (4.6 mg L^{-1}), red (4.5 mg L^{-1}) and amber + blue (4.3 mg L^{-1}). The results thus revealed that the microalgal consortia behaved differently under different wavelengths of light as presented in Fig. 4 and the statistical analyses for chlorophyll content seems to be significant at $p < 0.05$ (data presented in Tables 1, 2 and 3).

Effect of light wavelength on the nutritional composition of the microalgal consortia

The effect of varying wavelengths of light on the composition of the microalgal biomass is presented in Fig. 5. Cool-white fluorescent light had the best nutritional composition in term of proteins followed by amber, blue, red and amber + blue lights. To the contrary, carbohydrate composition was higher under amber light, whereas the lipid composition was higher under red light. The results also revealed that the protein composition of the microalgal consortia was higher under all wavelengths of light when compared to the carbohydrate and lipid composition thereby indicating the potential use of the microalgal biomass as a protein source for animal feed and/or as an ingredient for the production of value-added products.
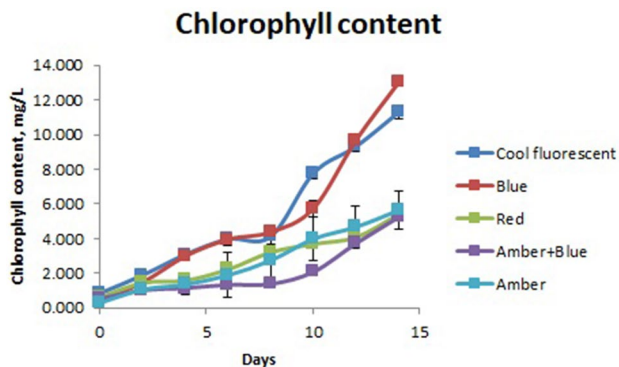
Fig. 5 Nutritional composition of the microalgal consortia under cool fluorescent, blue, red, amber + blue and amber lights. Data are expressed as mean ± SD, $n = 3$

Economic efficiency of energy consumption related to biomass production

The economic efficiencies of different light sources were evaluated based on the cost of energy consumption and the amount of biomass produced. The equation to assess the economic efficiency of energy used to biomass produced (E_c) is as follows:

$$E_c = \frac{DMn - Dm0}{k \times T \times P}, \tag{10}$$

where DMn and DMo are the dry mass at day n and at day zero, k is the cost per unit of electricity consumed (\$/W), T is the time (days) and P is the power supplied by the light source based on the information provided by the supplier (W) [34]. The results revealed that blue light had the highest energy to biomass efficiencies (12.5 g L^{-1})/(\$) as compared to other light treatments and the efficiencies are presented in Fig. 6. The amber light had the lowest energy to biomass efficiency (2.26 g L^{-1})/(\$) and this can be attributed to the lower electrical conversion efficiency of amber light.

Fig. 4 Chlorophyll content of microalgal consortia under cool fluorescent blue, red, amber + blue, and amber lights. Data are expressed as mean ± SD, $n = 3$
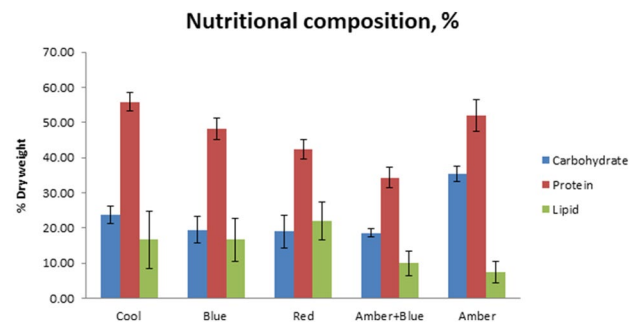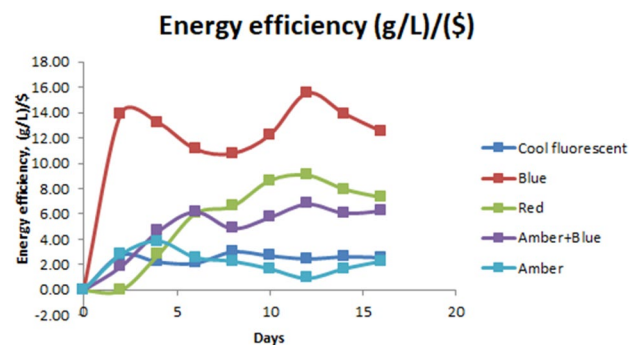
Fig. 6 Economic efficiencies for various light sources such as cool fluorescent, blue, red, amber + blue and amber

Effect of light quality on lutein concentration

The effect of light quality on lutein concentration was significant and the results revealed that cool fluorescent light had the highest lutein content of (7.22 mg g^{-1}) followed by amber + blue (6.50 mg g^{-1}), amber (5.96 mg g^{-1}), blue (1.82 mg g^{-1}) and red (1.68 mg g^{-1}). The lutein content reported in this study is the highest lutein concentration reported for any microalgal consortia grown under an autotrophic mode of cultivation till date. For optimum separation of a compound, the k value should be between 1 and 10 and in case of a complex mixture it should be between 2 and 10. Since this study has only one compound eluting at a retention time of 9.6 as shown in Fig. 7, the k value of the lutein peak obtained was calculated to be approximately 1 indicating that optimum separation of the compound was achieved and the strength of the solvent used for preparing mobile phase was acceptable [35]. Figure 7 represents a chromatogram of crude lutein obtained by extraction from the algal biomass grown under cool fluorescent light after saponification, plotted as retention time against absorbance with the quantity of lutein calculated using Eq. (4).

Discussion

Different wavelengths of light highly influence the synthesis of chlorophyll and pigments in algae [10]. In addition, light wavelengths along with light intensity and photoperiod are considered to be the major driving factors in enhancing the growth and biomass productivity of algal species [11, 36]. Emission of energy by a particular light source is dependent on its wavelength. In short, energy is inversely proportional to wavelength [37, 38]. Therefore, shorter wavelengths of light possess higher energy when compared to light of longer wavelengths under the same PAR values. This implies that blue light, with its shorter wavelength, has more energy and photosynthetic efficiency

and could enhance biomass production when compared to other light wavelengths within the spectrum [39].

However, in this study, a higher growth rate and biomass productivity were observed with cool-white fluorescent light followed by blue, amber + blue, amber and red at 40 μmol m^{-2} s^{-1}. This implies that the microalgal consortia utilized cool-white fluorescent light better than any other wavelength treatments and this can be explained by the fact that cool-white fluorescent light had a balanced mix of blue and red light as shown in Fig. 1. According to Foyer [37] and Kommareddy and Anderson [38], the mechanism for efficient transfer of energy for photosynthesis occurs when the molecule excited is closer to the first singlet stage. This implies that the actual photon energy required for efficient photosynthetic reaction is provided by light wavelengths occurring between 600 and 700 nm without any heat loss. Similarly, light wavelengths between 400 and 500 nm could also be effectively utilized for photosynthesis if other pigments like β-carotene, lutein, violaxanthin or neoxanthin are present. Since this study employs green algae, the most important pigments present include chlorophyll and lutein. This explains the reason for cool fluorescent light to outperform other wavelengths of light as chlorophyll absorbs energy in 600–700 nm wavelength range and lutein absorbs energy in the 400–500 nm range thereby increasing the overall photosynthetic rate, as there are more light-harvesting antennas to collect light. Though there are several reports of the impact of light wavelengths on the productivity and growth rate of *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Scenedesmus quadricauda, Nannochloropsis Phaeodactylum tricornutum*, *Isochrysis galbana*, *Nannochloropsis salina* and *Nannochloropsis oceanica*, no study using the consortium of algal strains *Chlorella variabilis* and *Scenedesmus obliquus* has been reported to date [3, 9, 12]. The results obtained in this study therefore indicate that the microalgal consortia grown under cool-white fluorescent light can generate more biomass when compared to other wavelengths of light.

Fig. 7 Chromatogram of the crude lutein obtained by extraction after saponification
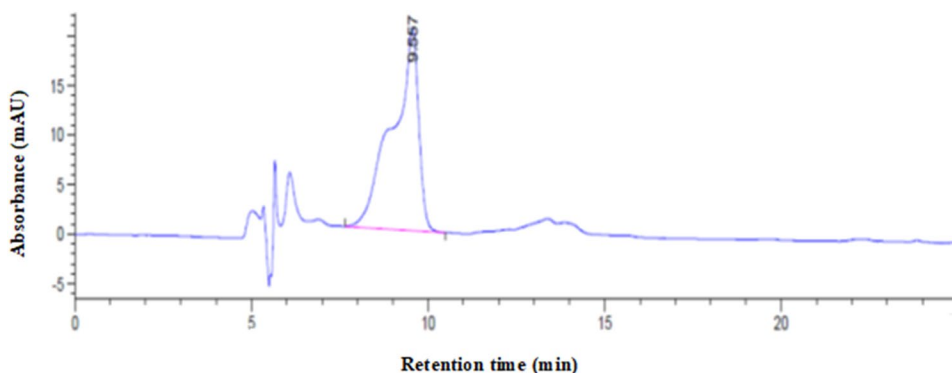
The chlorophyll content, however, was higher under blue light when compared to other wavelengths of light tested. This could be attributed to the increase in the number/size of light-harvesting units under light-limiting conditions when the consortium was exposed to blue light. The higher chlorophyll content obtained under blue light could also be associated with a mechanism known as chromatic adaptation which helps the algal species to maximize their photosynthetic ability by utilizing the available light efficiently under light-limiting conditions [40]. The outcome obtained from the study was in accordance with the results put forward by Mercado et al. [41], wherein blue light exhibited higher chlorophyll content when compared to white light in four out of the five strains of algae with no significant effects on the growth rates. In addition, the absorption bands of chlorophyll were significantly present in the blue wavelength of light favoring photosynthesis [34].

Numerous studies have reported that the response of algae to varying light wavelengths is species dependent or more specifically strain dependent due to the variation in their light-harvesting mechanisms/membranes. As a result, manipulating light wavelengths can alter the biochemical composition of algae [4]. Based on the results obtained here, it can be seen that the protein content of the algal consortia was higher under all wavelengths of light with white light exhibiting the highest concentration. This increase in protein content could be attributed to the enhancement of the structural protein of PS-II (has not been demonstrated conclusively) and the amount of nitrogen present in the wastewater. Availability of nitrogen, in excess, results in higher protein content and vice versa [42, 43]. The lipid content was enhanced under red light and this could be explained by the increase in enzyme activity of carbonic anhydrase, Rubisco and respiratory enzymes. The accumulation of triglycerides is highly dependent on the activity of enzymes, i.e., the higher the enzyme activity, the higher the amount of triglycerides produced and vice versa. The carbohydrate content was enhanced under the amber wavelength of light and this could signify a decrease in enzyme activity as the concentration and activity of enzymes is directly proportional to the rate of breakdown of carbohydrates. In this study, the microalgal consortia grown under all the other light wavelengths (red, amber + blue, blue and cool fluorescent) except amber had a reduction in carbohydrate content owing to the increase in enzyme activity (more catabolism of carbohydrates) [4, 11].

Taking into consideration the amount of biomass produced per unit of electricity consumed, it can be clearly seen that blue light had a biomass production (500 mg L^{-1}) with higher economic efficiency of 12.5 (g L^{-1}) \$$^{-1}$ when compared to the other light treatments after 16 days of inoculation. Though cool fluorescent light had the highest biomass productivity, it was found to have the least economic efficiency owing to the needed increased electricity consumption to produce the required intensity of light [34].

Some microalgae have been known in recent years for producing interesting quantities of lutein. For instance, the optimal conditions for producing lutein from *Scenedesmus almeriensis* have been studied and were considered to produce much higher lutein content (~ 5 mg g^{-1}) than the traditional source from marigold flowers (~ 1 mg g^{-1}), thereby making microalgae a potential source for the extraction of lutein [44]. Similarly, another strain of microalga *Chlorella vulgaris* produced approximately 0.7 mg g^{-1} of lutein [32]. In the current study, it was revealed that the microalgal consortia of *Chlorella variabilis* and *Scenedesmus obliquus* had a maximal lutein productivity of 7.22 mg g^{-1} as compared to around 5 mg g^{-1} for *Scenedesmus almeriensis* and 0.7 mg g^{-1} for *Chlorella variabilis,* thereby making it a promising consortium for biotechnological purposes. Finally, based on our results, tailoring light quality can significantly alter the biomass productivity and biochemical composition including lutein content.

Conclusion

Light wavelengths have a profound impact on the growth, biomass productivity, chlorophyll/lutein and nutrient contents of the microalgal consortia composed of *Chlorella variabilis* and *Scenedesmus obliquus* and the data obtained from this study support this claim. This work clearly states that the microalgal consortia could grow well on diluted (10%) dairy effluent and the most effective light for enhancing the growth and biomass productivity is white light (380–700 nm). However, blue light had the highest energy to biomass conversion (highest economic efficiency) as compared to white light which could mean that blue light can also serve as a light source for enhancing growth rate and biomass productivity. On the other hand, the most effective light for enhancing chlorophyll content is blue light (470 nm), while for optimized nutrient content, amber light (595 nm) is the best. Thus, a two-phase culture process of cultivating the microalgal consortia under cool-white fluorescent light, followed by exposure to amber light could maximize the benefits in terms of enhancing biomass productivity and nutrient content with higher lutein productivity within 16 days of growth. The biomass produced could be either used as animal feed or as a feedstock for bio-ethanol production due to its high protein and carbohydrate content. Furthermore, this study also opens up a new avenue of research for the targeted cultivation of algae for nutraceutical production (lutein) under amber light wavelength (500–630 nm) which has long been neglected by researchers.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Palacios YM, Vonshak A, Beardall J (2018) Photosynthetic and growth responses of *Nannochloropsis oculata* (Eustigmatophyceae) during batch cultures in relation to light intensity. Phycologia 57:492–502
2. Schulze PSC, Barreira LA, Pereira HGC, Perales JA, Varela JCS (2014) Light emitting diodes (LEDs) applied to microalgal production. Trends Biotechnol 32:422–430
3. Ma R, Thomas-Hall SR, Chua ET, Netzel ME, Netzel G, Lu Y, Schenk PM (2018) LED power efficiency of biomass, fatty acid, and carotenoid production in *Nannochloropsis* microalgae. Biores Technol 252:118–126
4. Vadiveloo A, Moheimani NR, Cosgrove JJ, Bahri PA, Parlevliet D (2015) Effect of different light spectra on the growth and productivity of acclimated *Nannochloropsis* sp. (Eustigmatophyceae). Algal Res 8:121–127
5. Ueno Y, Aikawa S, Kondo A, Akimoto S (2019) Adaptation of light-harvesting functions of unicellular green algae to different light qualities. Photosynth Res 139:145–154
6. Krzemińska I, Pawlik-Skowrońska B, Trzcińska M, Tys J (2014) Influence of photoperiods on the growth rate and biomass productivity of green microalgae. Bioprocess Biosyst Eng 37:735–741
7. Figueroa FL, Aguilera J, Niell FX (1995) Red and blue light regulation of growth and photosynthetic metabolism in *Porphyra umbilicalis* (Bangiales, Rhodophyta). Eur J Phycol 30:11–18
8. Schulze PS, Pereira HG, Santos TF, Schueler L, Guerra R, Barreira LA, Perales JA, Varela JC (2016) Effect of light quality supplied by light emitting diodes (LEDs) on growth and biochemical profiles of *Nannochloropsis oculata* and *Tetraselmis chuii*. Algal Res 16:387–398
9. Ra CH, Sirisuk P, Jung JH, Jeong GT, Kim SK (2018) Effects of light-emitting diode (LED) with a mixture of wavelengths on the growth and lipid content of microalgae. Bioprocess Biosyst Eng 41:457–465
10. Wu H (2016) Effect of different light qualities on growth, pigment content, chlorophyll fluorescence, and antioxidant enzyme activity in the red alga *Pyropia haitanensis* (Bangiales, Rhodophyta). BioMed Res Int 20:1–8
11. Atta M, Idris A, Bukhari A, Wahidin S (2013) Intensity of blue LED light: a potential stimulus for biomass and lipid content in fresh water microalgae *Chlorella vulgaris*. Biores Technol 148:373–378
12. Zhong Y, Jin P, Cheng JJ (2018) A comprehensive comparable study of the physiological properties of four microalgal species under different light wavelength conditions. Planta 248:489–498
13. Teoa CL, Idrisa A, Wahidinb S, Laic LW (2014) Effect of different light wavelength on the growth of marine microalgae. J Technol 67:97–100
14. Gatamaneni BL, Orsat V, Lefsrud M (2018) Factors affecting growth of various microalgal species. Environ Eng Sci 35:1037–1048
15. Newby DT, Mathews TJ, Pate RC, Huesemann MH, Lane TW, Wahlen BD, Mandal S, Engler RK, Feris KP, Shurin JB (2016) Assessing the potential of polyculture to accelerate algal biofuel production. Algal Res 19:264–277
16. Ruangsomboon S (2012) Effect of light, nutrient, cultivation time and salinity on lipid production of newly isolated strain of the green microalga, *Botryococcus braunii* KMITL 2. Biores Technol 109:261–265
17. Cheng YS, Labavitch J, VanderGheynst JS (2015) Organic and inorganic nitrogen impact *Chlorella variabilis* productivity and host quality for viral production and cell lysis. Appl Biochem Biotechnol 176:467–479
18. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier R (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology 111:1–61
19. Vidal G, Carvalho A, Mendez R, Lema JM (2000) Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters. Biores Technol 74:231–239
20. Koreivienė J, Valčiukas R, Karosienė J, Baltrėnas P (2014) Testing of *Chlorella/Scenedesmus* microalgae consortia for remediation of wastewater, CO_2 mitigation and algae biomass feasibility for lipid production. J Environ Eng Landsc Manag 22:105–114
21. Qin L, Sun Y, Shu Q, Feng P, Zhu L, Xu J, Yuan Z (2016) Microalgae consortia cultivation in dairy wastewater to improve the potential of nutrient removal and biodiesel feedstock production. Environ Sci Pollut Res 23:8379–8387
22. Parsons TR, Strickland JDH (1963) Discussion of spectrophotometric determination of marine plant pigments, with revised equation for ascertaining chlorophylls and carotenoids. J Mar Res 21:155–163
23. Porra R, Thompson W, Kriedemann P (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta Bioenerget 975:384–394
24. Macias-Sanchez MD, Mantell Serrano C, Rodriguez Rodriguez M, Martinez de la Ossa E, Lubian LM, Montero O (2008) Extraction of carotenoids and chlorophyll from microalgae with supercritical carbon dioxide and ethanol as cosolvent. J Sep Sci 31:1352–1362
25. Sartory DP, Grobbelaar JU (1984) Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia 114:177–187
26. Hosikian A, Lim S, Halim R, Danquah MK (2010) Chlorophyll extraction from microalgae: a review on the process engineering aspects. Int J Chem Eng 20:1–11
27. Difusa A, Talukdar J, Kalita MC, Mohanty K, Goud VV (2015) Effect of light intensity and pH condition on the growth, biomass and lipid content of microalgae *Scenedesmus* species. Biofuels 6:37–44
28. Waghmare AG, Salve MK, LeBlanc JG, Arya SS (2016) Concentration and characterization of microalgae proteins from *Chlorella pyrenoidosa*. Bioresour Bioprocess 3:16
29. Slocombe SP, Ross M, Thomas N, McNeill S, Stanley MS (2013) A rapid and general method for measurement of protein in micro-algal biomass. Biores Technol 129:51–57
30. Folch J, Lees M, Sloane Stanley G (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509
31. Inbaraj BS, Chien JT, Chen BH (2006) Improved high performance liquid chromatographic method for determination of carotenoids in the microalga *Chlorella pyrenoidosa*. J Chromatogr A 1102:193–199

32. D'Este M, De Francisci D, Angelidaki I (2017) Novel protocol for lutein extraction from microalga *Chlorella vulgaris*. Biochem Eng J 127:175–179

33. French A, Macedo M, Poulsen J, Waterson T, Yu A (2008) Multivariate analysis of variance (MANOVA):1–8

34. Wang CY, Fu CC, Liu YC (2007) Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. Biochem Eng J 37:21–25

35. Gupta P, Sreelakshmi Y, Sharma R (2015) A rapid and sensitive method for determination of carotenoids in plant tissues by high performance liquid chromatography. Plant Methods 1:5

36. Amini Khoeyi Z, Seyfabadi J, Ramezanpour Z (2012) Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae *Chlorella vulgaris*. Aquacult Int 20:41–49

37. Foyer (1984) Little light shed on photosynthesis. Photosynthesis 4:90135–90137

38. Kommareddy A, Anderson G (2003) Study of light as a parameter in the growth of algae in a photobioreactor (PBR). ASAE meeting Paper No. 034057: 1-23

39. Das P, Lei W, Aziz SS, Obbard JP (2011) Enhanced algae growth in both phototrophic and mixotrophic culture under blue light. Biores Technol 102:3883–3887

40. Ahluwalia A, Rai R, Kumar H (1980) Chromatic adaptation and photoreversal in blue-green alga *Calothrix clavata* West. J Biosci 2:63–68

41. Mercado JM, del Pilar S-S, Correa-Reyes G, Lubián L, Montero O, Figueroa FL (2004) Blue light effect on growth, light absorption characteristics and photosynthesis of five benthic diatom strains. Aquat Bot 78:265–277

42. Safafar H, Uldall Nørregaard P, Ljubic A, Møller P, Løvstad Holdt S, Jacobsen C (2016) Enhancement of protein and pigment content in two *Chlorella* species cultivated on industrial process water. J Mar Sci Eng 4:84

43. Marchetti J, Bougaran G, Jauffrais T, Lefebvre S, Rouxel C, Saint-Jean B, Lukomska E, Robert R, Cadoret JP (2013) Effects of blue light on the biochemical composition and photosynthetic activity of *Isochrysis* sp. (T-iso). J Appl Phycol 25:109–119

44. Sánchez JF, Fernández JM, Acién FG, Rueda A, Pérez-Parra J, Molina E (2008) Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis*. Process Biochem 43:398–405