Influence of light quality on *Chlorella* growth, photosynthetic pigments and high-valued products accumulation in coastal saline-alkali leachate

Xiao-ya Liu, Yu Hong and Wen-ping Gu

ABSTRACT

Using saline-alkali leachate to cultivate microalgae is an effective way to realize the utilization of wastewater and alleviate the shortage of water resources. Light source is usually used as an optimized parameter to further improve the cultivation efficiency of microalgae. In this work, the influence of light qualities on the growth and high-valued substances accumulation of *Chlorella* sp. HQ in coastal saline-alkali leachate were investigated. The specific growth rate of *Chlorella* in coastal saline-alkali leachate was 0.27–0.60 d^{-1}. At the end of cultivation, the algal density under blue light reached $8.71 \pm 0.15 \times 10^7$ cells·mL^{-1}, which was significantly higher than the other light groups. The lipid content in the biomass was 29.31–62.95%, and the highest lipid content and TAGs content were obtained under red light and blue-white mixed light, respectively. Percentages of total chlorophylls (0.81–1.70%) and carotenoids (0.08–0.25%) were obtained in the final biomass of the coastal saline-alkali leachate. In addition, the contents of photosynthetic pigments and three high-valued products under mixed light were higher than those of monochromatic light, and the protein, total sugar and starch content under blue-red mixed light was 1.52–3.76 times, 1.54–3.68 times and 1.06–3.35 times of monochromatic blue light and red light, respectively.

Key words | *Chlorella*, high-valued products, light quality, pigments, saline-alkali leachate

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HIGHLIGHTS

- *Chlorella* in coastal saline-alkali leachate exhibited better growth curve under blue light.
- The highest total lipid content per unit biomass of *Chlorella* was obtained under red light.
- Mixed LEDs light can improve the photosynthetic ability of *Chlorella* in coastal saline-alkali leachate.
- High-valued substances content of *Chlorella* under mixed LEDs light was higher than that of monochromatic light.

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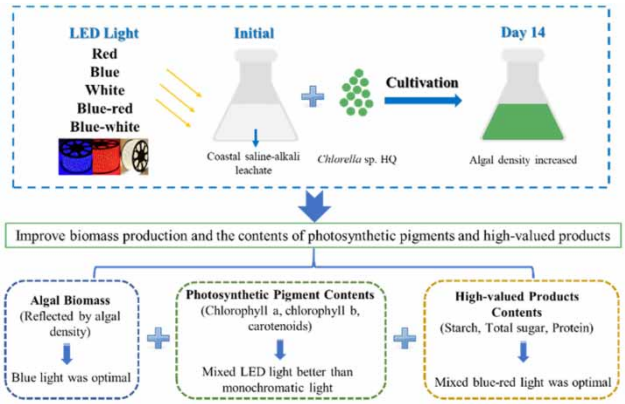
GRAPHICAL ABSTRACT



INTRODUCTION

Soil salinization is a widespread environmental problem worldwide, and there are approximately 954 million hectares of saline-alkali land in the world (Yue *et al.* 2020). Coastal saline-alkali soils, have a high content of exchangeable sodium, which is easy to cause soil consolidation, and the high salinity in the soil prevents plant roots from absorbing water and obtaining nutrients (Tang *et al.* 2020). In addition, as saline-alkali land is an important reserve land resource for food production, the restoration of saline-alkali land is essential to global food demand. Nowadays, the improvement of saline-alkali land often adopts various methods such as physical restoration, chemical modification and bioremediation, and the common practiced method in the world is the combination of irrigation and drainage, which removes the salt in the soil through leaching (Wang *et al.* 2018; Meena *et al.* 2019). Although this method can improve the soil quality, it also produces a large amount of wastewater with high salinity. The wastewater contains inorganic salts, which is an abundant source of energy to be recovered and reused, but if it is directly discharged, it may cause groundwater pollution and deterioration of water quality (Fang *et al.* 2019). At present, the global water consumption is gradually increasing, and a large part of the population still lives in water-scarce areas (Zhu & Dou 2018; Archer *et al.* 2019). Given the above, the resource utilization of saline-alkali leachate is essential to alleviate the shortage of water resources and maintain the sustainable development of the social economy. Our previous studies have shown that the coastal saline-alkali leachate can be used to cultivate three oleaginous microalgae at low cost to realize the conversion of wastewater resources (Liu *et al.* 2020).

As a global emerging industry, microalgae cultivation has been extensively researched and marketed in recent years due to its enormous economic and social potential (Mata *et al.* 2010). Microalgae are highly efficient photosynthetic cell factories, which contain high amount of high-valued substances, such as proteins, pigments, carbohydrates and lipids. Among the possible applications of the microalgae, they can be used in the food, feed, life sciences and renewable fuels, for example carbohydrates and fatty acids can be converted into alcohol and biodiesel (Chew *et al.* 2017; Chandra *et al.* 2019). However, the cost of large-scale production of microalgae and related biological products is still high, which has brought challenges to the development of microalgae biotechnology. Current researches are aimed at increasing the biomass of microalgae, improving the contents of lipid and high-valued substances, or reducing the cost of microalgae cultivation (Chen *et al.* 2009). How to improve the cultivation efficiency of microalgae in saline-alkali leachate and the content of high-valued substances is of great significance for

reducing the production cost of microalgae-based biological products.

It is known that environmental factors such as light, nutrients, temperature and CO_2 concentration can significantly affect the algal growth and lipid accumulation including content and composition (Ananthi *et al.* 2021). Light, as the energy source of microalgae photosynthesis, is one of the important factors affecting the growth of algae and the accumulation of active substances. In indoor microalgae cultivation, artificial light sources such as fluorescent light are commonly used as energy sources, which undoubtedly increase the power consumption and may exacerbate the greenhouse phenomenon (Sun *et al.* 2017). Light emitting diode (LED) has been widely used in microalgae cultivation due to its advantages of narrowing specific wavelengths with low power consumption, small chip size, and long duration (Kim *et al.* 2014). It has been reported that the microalgal photosystem II and photosystem I can be enhanced or induced by red and blue light wavelengths, respectively (Ravelonandro *et al.* 2008). In addition, since pigments such as chlorophyll a and chlorophyll b are sensitive to light of different wavelengths, it would be pertinent to select LEDs to regulate the distribution of various products in microalgae (Schulze *et al.* 2014). Shu *et al.* (2012) found that light quality has a significant effect on algae cell growth and product formation, and this effect is dependent on the algae species. Abiusi *et al.* (2014) investigated the influence of light quality on algae cell size, growth, productivity, photosynthetic efficiency of *Tetraselmis suecica* F&M-M33, and found that red light can be profitably used for the production of this marine microalga for aquaculture.

Therefore, in order to further improve the cultivation efficiency of *Chlorella* in the coastal saline-alkali leachate and the content of high-valued substances, the effects of five different light sources (including monochromatic blue, red, white LED light and blue-red mixed light, blue-white mixed light) on the growth of *Chlorella* and the accumulation of lipid, pigments, protein, total sugar and starch were explored. The findings can provide a theoretical guidance on how to accurately use light quality to make *Chlorella* in coastal saline-alkali leachate obtain higher content of high-valued substances. This will not only provide a resource utilization method for the coastal saline-alkali leachate, but also reduce the production cost of microalgae-based biological products.

MATERIALS AND METHODS

Microorganism

The microalga *Chlorella* sp. HQ (No. GCMCC7601) was used in the experiment, which was isolated in our previous study and has been kept in China General Microbiological Culture Collection Center. The strain was routinely cultivated in an axenic Selenite Enrichment (SE) medium, which contained 250 mg·L^{-1} of $NaNO_3$, 75 mg·L^{-1} of $K_2HPO_4{\cdot}3H_2O$, 75 mg·L^{-1} of $MgSO_4{\cdot}7H_2O$, 25 mg·L^{-1} of $CaCl_2{\cdot}2H_2O$, 175 mg·L^{-1} of KH_2PO_4, 25 mg·L^{-1} of NaCl, 5 mg·L^{-1} of $FeCl_3{\cdot}6H_2O$, 0.81 mg·L^{-1} of $FeCl_3$, 10 mg·L^{-1} of Na_2EDTA, 2.86 mg·L^{-1} of H_3BO_3, 1.81 mg·L^{-1} of $MnCl_2{\cdot}4H_2O$, 0.22 mg·L^{-1} of $ZnSO_4{\cdot}H_2O$, 0.079 mg·L^{-1} of $CuSO_4{\cdot}5H_2O$, and 0.039 mg·L^{-1} of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$. All chemicals were of analytical grade. Prior to the experiments, cultures were maintained in Erlenmeyer flask at 25 ± 1 °C, 4800 lux incident light intensity in a 14:10 h light/dark cycle and the flasks were shaken for three times a day.

Cultivation setup

The coastal saline-alkali soil samples used in this study were taken from Dongying, Shandong Province, China. The detailed preparation method of the coastal saline-alkali leachate was described by Liu *et al.* (2020). The obtained leachate was filtered through 0.45-μm filtration membrane and sterilized at 121 °C for 30 min, and was stored after cooling to room temperature for later use.

The experiments were performed in a climate chamber equipped with LEDs as the light source. *Chlorella* sp. HQ was cultivated in 250 mL Erlenmeyer flasks with 180 mL sterile coastal saline-alkali leachate and initial cell density of 2×10^5 cells·mL^{-1}, and the temperature was maintained at 25 ± 1 °C. The illumination was supplied at an average light intensity of 4500 lux and a photoperiod of 14:10 h light/dark. Based on light intensity, two mixed LED light treatments (red:blue = 1:2; blue:white = 1:2) and three monochrome LED light treatments (monochrome red; blue; and white

LED) were set up in this study. In order to avoid the influence of the light source on the other experimental groups, each treatment group was separated by aluminum foil.

In the experiment, the algal density was measured every other day, and the biomass, lipids, TAGs, total sugar, starch, protein, and photosynthetic pigment content of the microalgae were measured at the end of cultivation. The Erlenmeyer flask was manually shaken three times a day to ensure that the algae cells and the culture medium were thoroughly mixed, and to reduce experimental errors.

Analytical methods

Microalgal growth and mathematic model analysis

Algal density was determined using a Neubauer hemocytometer. To determine the yield of biomass, a 20 mL algae solution sample was filtered through a pre-weighed 0.45-μm membrane. The membrane was washed twice with distilled water, dried at 110 °C for 24 h until completely dehydrated and weighed again, then the biomass yield was calculated.

A generalized Logistic model was fitted to the measured data (cell density) with Origin software.

$$N_t = \frac{K}{1 + e^{a-rt}} \tag{1}$$

$$\frac{dN}{dt} = rN\left(\frac{K-N}{K}\right) \tag{2}$$

$$R_{max} = \frac{rK}{4} \tag{3}$$

In the above formula, N_t (cells·mL^{-1}) is cell density at cultivation time t (d). K, a and r represent the maximum cell density during the whole cultivation period, the relative position from the origin and algae cell intrinsic growth rate, respectively. We determined the parameters (K, a, r) of each light treatment group through non-linear regression, and R_{max} (maximum population growth rate, in which case N equals to half of K) were calculated (Equation (3)).

Lipid and triacylglycerols determination

The lipid content was determined based on the method of modified chloroform-methanol extraction (Bligh & Dyer 1959). Using a high-speed refrigerated centrifuge (CR22G, HITACHI, Japan), 40 mL of algae liquid was centrifuged at 12,000 rpm, 4 °C for 10 min, and concentrated to 0.8 mL. For extraction, 2 mL of chloroform, 2 mL of methanol, and 1 mL of distilled water were added to the above concentrated algae solution in this order, and mixed well. After extraction, centrifuged again for 10 min (4,000 rpm, 4 °C), then transferred the bottom chloroform layer to the pre-weighed dry glass tubes, blown it to a constant weight with a nitrogen blower. Finally, the microalgal lipid was calculated by gravimetric method. In addition, after the measurement of lipid was completed, 0.4 mL of isopropyl alcohol was added to the glass tube, and the triacylglycerols (TAGs) was determined by the enzyme colorimetric kit method (A110-1 GPO-PAP M Enzyme Method Single Reagent Type) (Li et al. 2010).

Photosynthetic pigments determination

The photosynthetic pigments analysis followed the modified procedure as used from Kirk & Allen (1965). A 5 mL sample was centrifuged at 8,000 rpm for 5 min. The pellets were re-suspended in 5 mL of 80% acetone solution at 4 °C for 24 h in darkness. After centrifugation at 4 °C, 4,000 rpm for 15 min, the obtained supernatant was measured for absorbance at 480 nm, 645 nm, and 663 nm, respectively. The 80% acetone solution was used as the blank. The photosynthetic pigments content was calculated as shown below:

$$\text{Chlorophyll a (mg·L}^{-1}) = 12.7 \times OD_{663} - 2.69 \times OD_{645} \tag{4}$$

$$\text{Chlorophyll b (mg·L}^{-1}) = 22.9 \times OD_{645} - 2.69 \times OD_{663} \tag{5}$$

$$\text{Carotenoids (mg·L}^{-1}) = OD_{480} + (0.114 \times OD_{663}) - (0.638 \times OD_{645}) \tag{6}$$

where OD_λ is optical density at wavelength λ (nm).

Protein, total sugar and starch determination

Total sugar, starch and protein were determined using modified standard methods previously described by Liu et al. (2019).

Statistics

All experiments were carried out in triplicates, and the data were the mean values ± standard deviation from three independent experiments. All statistical analyses were conducted using SPSS software. One-way ANOVA analysis and Duncan's multiple range test were performed to assess the significant differences among the light source treatments ($p < 0.05$).

RESULTS AND DISCUSSION

Algal growth performance in coastal saline-alkali leachate under different light sources

In order to improve the growth efficiency of *Chlorella* in coastal saline-alkali leachate, LED lights with different light qualities were used as the light source for the growth of *Chlorella*. The growth curves of *Chlorella* sp. HQ in the coastal saline-alkali leachate under different light sources are shown in Figure 1. The five treatments exposed to blue, red, white, blue-red and blue-white mixed light, *Chlorella* sp. HQ maintained high speed growth, and the cell density exceeded 1.00×10^7 cells·mL^{-1} after 5 days of cultivation. After the end of cultivation, it was found that the algal density under blue light was the highest, reaching $8.71 \pm 0.15 \times 10^7$ cells·mL^{-1}, which was about 2–3 times that of the other treatment groups. In addition, *Chlorella* reached the log phase earlier under blue light than the other treatment groups, which indicated that the growth of *Chlorella* was significantly affected by light quality. Meanwhile, the biomass yield of *Chlorella* in the coastal saline-alkali leachate was determined at the end of cultivation under five different light sources (Figure 2). The results showed that biomass yield of *Chlorella* grown under monochromatic blue and white light were significantly higher than that of other groups ($p < 0.05$). And, the algal biomass obtained by *Chlorella* using white light in coastal saline-alkali leachate was 1.76 times higher than that using red light, which indicated that choosing a light source with a specific light quality can significantly increase the biomass of microalgae. At present, there are currently studies on increasing the biomass of microalgae by adjusting other parameters. Gao *et al.* (2019) achieved the purpose of increasing the biomass of *Chlorella* sp. G-9 by adjusting the initial organic carbon-nitrogen ratio of simulated wastewater, and the results showed the algal biomass productivities obtained in wastewater with TOC/TN ratio of 1 and 3 were 1.2 and 1.5 times higher than the value in photoautotrophic condition (TOC/TN = 0).

Based on the the experimental data of microalgae cultivation exhibited in Figure 1, a Logistic model was used to describe the light sources effects on the growth of *Chlorella*

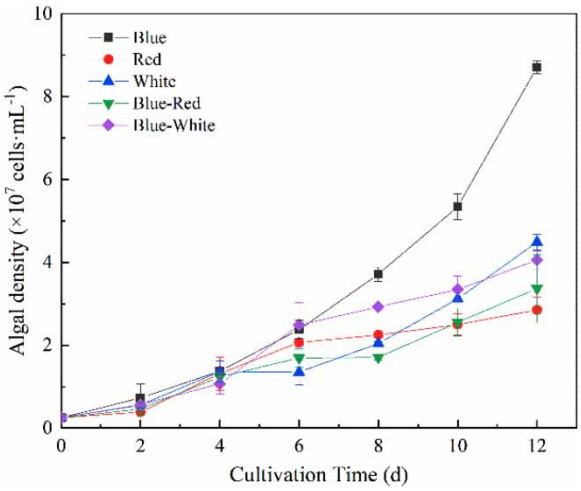


Figure 1 | Growth curves of *Chlorella* sp. HQ in coastal saline-alkali leachate under different light sources.

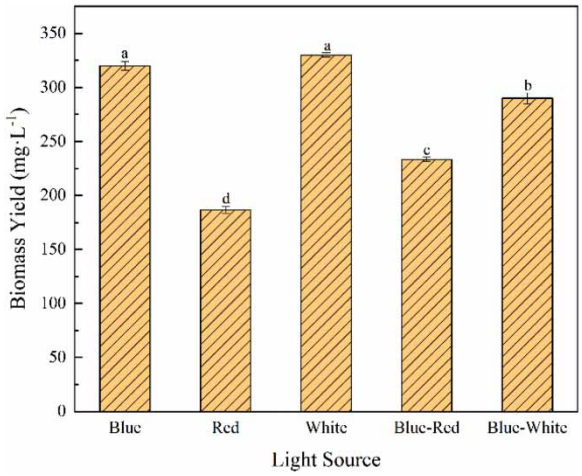


Figure 2 | The biomass yield of *Chlorella* in coastal saline-alkali leachate under different light sources. Different letters above each column indicate significant differences between values ($p < 0.05$).

sp. HQ. Through the model fitting, the fitting parameters of K, r and R_{max} are listed in Table 1. The results showed that using red light as the light source, the specific growth rate of *Chlorella* sp. HQ in coastal saline-alkali leachate was the highest, reaching 0.60 d^{-1}. Maaitah *et al.* (2020) used olive oil washing wastewater to provide a low-cost substrate for algal biomass production and found that the maximum specific growth rate of *C. pyrenoidosa* was 0.4872 d^{-1}, which was lower than the maximum specific growth rate obtained in this experiment. Compared with r and K, R_{max} is more suitable for predicting the growth potential of microalgae (Campos *et al.* 2014). In this study, the R_{max} values of *Chlorella* in the monochromatic blue, white light, and blue-white mixed light groups were higher, which was consistent with the law of algal density at the end of cultivation. In contrast, the blue-red mixed light group gave the lowest growth performances in view of the R_{max}. This showed that *Chlorella* sp. HQ was more suitable to use blue light as a light source to obtain higher algal biomass in the coastal saline-alkali leachate.

Several studies have shown that the growth ability of microalgae under monochromatic blue or red light is similar or enhanced compared to white light (Mouget *et al.* 2004; Abiusi *et al.* 2014). Herein, monochromatic blue light was the optimal light source for *Chlorella* sp. HQ growth because it achieved the highest cell density and the highest value of R_{max}. Similarly, Atta *et al.* (2013) found that *Chlorella vulgaris* grew faster under blue light compared to white light with the light intensity of 200 μmol photons $m^{-2}{\cdot}s^{-1}$. In another study, *Nannochloropsis* sp. and *Tetraselmis* sp. were cultured for 14 d under blue, red, red-blue LED light and white fluorescent light, based on the results of optical density measurements, it was found that microalgae exhibited better growth curve under blue light (Teo *et al.* 2014). Researchers have reported that blue light not only participates in photosynthesis and energy activation, but also helps regulate gene transcription and enzyme activation (Ruyters 1984).

However, many studies have obtained different results, that is, red light is more suitable for the growth of microalgae. This may be due to the different responses of different species to light, which is reflected in product formation and cell growth of microalgae (Shu *et al.* 2012). Wang *et al.* (2007) found that the biomass yield and specific growth rate of *Spirulina platensis* under red light were higher than those of blue light. Xu *et al.* (2013) found that under different C/N ratios, the amount of algal dry weight exposed by the red, white, and yellow light groups was similar, much higher than that of the purple and blue light groups.

Photosynthetic pigment contents of *Chlorella* in coastal saline-alkali leachate under different light sources

The generation of photosynthetic pigments is related to the growth of microalgae. Generally, the percentage of pigments in the algal biomass that is finally harvested from the culture medium is constant (Maaitah *et al.* 2020). In this study, the effect of five different light sources on pigment contents of *Chlorella* sp. HQ in the coastal saline-alkali leachate are presented in Figure 3. The total chlorophylls content and total carotenoids content of *Chlorella* sp. HQ in the coastal saline-alkali leachate were 0.81–1.70% and 0.08–0.25%, respectively. It has been reported that the relative proportion of photosynthetic pigments changes with the spectral quality of light (Rochet *et al.* 1986). The photosynthetic pigments content of *Chlorella* in the coastal

Table 1 | The fitting parameters of the logistic model for *Chlorella* in coastal saline-alkali leachate under different light sources

Light sources	Maximum population density K (10^7 cells·mL^{-1})	Intrinsic growth rates r (d^{-1})	Maximum population growth rates R_{max} (10^7 cells·mL^{-1}·d^{-1})	R^2
Blue light	8.86 ± 0.97	0.36 ± 0.02	0.80 ± 0.10	0.9984
Red light	2.69 ± 0.14	0.60 ± 0.12	0.41 ± 0.08	0.9716
White light	11.54 ± 2.30	0.27 ± 0.02	0.78 ± 0.16	0.9993
Blue-Red light	3.90 ± 0.46	0.32 ± 0.02	0.31 ± 0.04	0.9403
Blue-White light	4.04 ± 0.33	0.48 ± 0.09	0.49 ± 0.09	0.9720

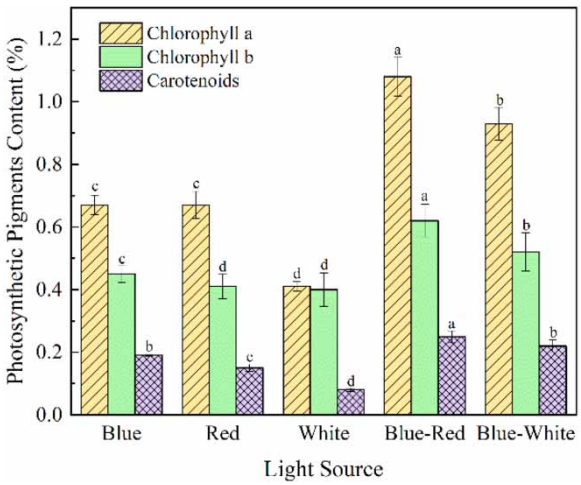


Figure 3 | The photosynthetic pigments content of *Chlorella* in coastal saline-alkali leachate under different light sources at the end of cultivation. Different letters above each column indicate significant differences among different light sources in pigments content ($p < 0.05$).

saline-alkali leachate under the blue-red mixed light was significantly higher than that of monochromatic blue and red light ($p < 0.05$). The chlorophyll a content of *Chlorella* in the blue-white mixed light group was 1.38 times and 2.27 times that of the monochromatic blue and white light groups, respectively. Similarly, the content of chlorophyll b and carotenoids in the blue-white mixed light group was also higher than that in the monochromatic blue and white light groups. The above results showed that compared with the monochromatic light group, the mixed light group was more conducive to the accumulation of pigments of *Chlorella* sp. HQ in the coastal saline-alkali leachate. Pereiraa & Oterob (2019) reported that blue light can increase the total carotenoid content in *Dunaliella salina*, and when it was mixed with red light, due to the synergistic effect, the carotenoid content was significantly higher than the other two monochromatic light experimental groups. The explanation on the higher production of chlorophyll and carotenoid under mixed LED light treatments (blue-red and blue-white mixed light) can be related to complementary chromatic adaptation. According to Emerson and Brody's theory, complementary chromatic adaption is the process of adjusting relative content of major light harvesting pigments which have similar absorption spectra patterns to that of the incident light. And complementary chromatics adaptation can improve the algae cells photosynthetic ability (Rochet *et al.* 1986; Mouget *et al.* 2004).

In addition, the chlorophyll b and carotenoids content in the blue light group were higher than those in the red light group, while the content of chlorophyll a was not significantly different ($p < 0.05$). The reason may be that chlorophyll a in microalgae can absorb blue and red light, while other auxiliary pigments (such as carotenoids) absorb blue light (Kandilian *et al.* 2013). Similarly, Vadiveloo *et al.* (2015) found that the content of chlorophyll a in *Nannochloropsis* sp. exposed the blue light was higher than that of red light. Tamburic *et al.* (2014) found that the microalgae had a higher photosynthetic rate when exposed to monochromatic blue light than to monochromatic red light, which due to that blue light was more easily absorbed by microalgae than red light and was more efficiently transmitted to photosystem II under light-saturated illumination. Therefore, the photosynthetic efficiency of *Chlorella* under blue light was higher than red light, and higher biomass productivity can be obtained. In this work, we obtained similar results, that is, the biomass yield of *Chlorella* in the coastal saline-alkali leachate under blue light was higher than that of red light (Figure 2).

Comparison of high-valued products accumulation of *Chlorella* in coastal saline-alkali leachate under different light sources

Figure 4 demonstrates the total lipid per algal biomass and TAGs content per total lipids yield of *Chlorella* sp. HQ in

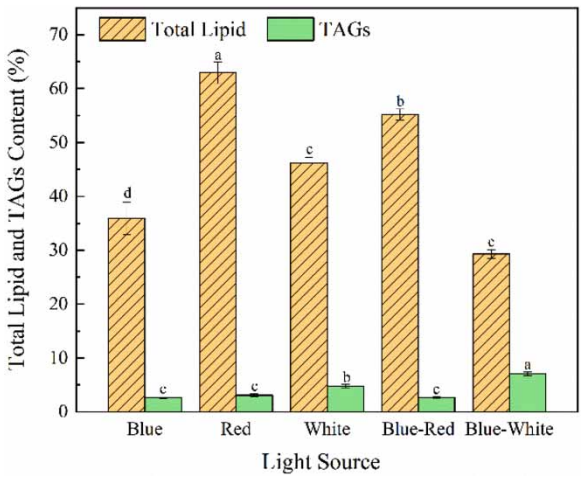


Figure 4 | The total lipids content per algal biomass and TAGs content per total lipids yield of *Chlorella* in coastal saline-alkali leachate under different light sources at the end of cultivation. Different letters above each column indicate significant differences among different light sources in total lipid content and TAGs content ($p < 0.05$).

the coastal saline-alkali leachate with different light sources at the end of cultivation. The results showed that the lipid content of *Chlorella* in the coastal saline-alkali leachate was 29.31–62.95%, and the value under red light was the highest, which was significantly higher than the blue light group and the blue-red mixed light group ($p < 0.05$). Maaitah *et al.* (2020) found that using different dilutions of olive oil washing wastewater as the culture medium of *Chlorella pyrenoidosa*, the lipid content of *Chlorella* under undiluted conditions was the highest, reaching 51.5%, which was lower than the highest lipid content of *Chlorella* sp. HQ in the coastal saline-alkali leachate.

The TAGs content per lipid yield of *Chlorella* was the highest under blue-white mixed light, reaching 7.10 ± 0.35%, which were 2.73 and 1.48 times that of monochromatic blue and white light, respectively. The accumulation of lipids in algal biomass is often associated with a decrease in biomass productivity (Duarte *et al.* 2019). In this experiment, the total lipid content per unit biomass under red light was significantly higher than the blue light and blue-red mixed light, while the biomass under red light was the lowest (Figures 2 and 4). This indicated that red light was conductive to lipid accumulation but was not good to the growth of biomass of *Chlorella* in coastal saline-alkali leachate.

In addition, in the previous study, the fluorescent lamp was used as light source, the protein, total sugar and starch content of *Chlorella* sp. HQ in coastal saline-alkali leachate were higher than those of SE medium, indicating that the accumulation of high-valued substances can be improved under salinity stress (Liu *et al.* 2020). In this study, under blue light conditions where *Chlorella* sp. HQ can obtain higher biomass in saline-alkali leachate, the protein and total sugar content of *Chlorella* was 50.09% and 24.73%, respectively. Rashid *et al.* (2019) found that *Ettlia* sp. and *Chlorella* sp. HS-2 was co-cultured in BG11, and its protein and carbohydrate content were 44 and 33%, respectively. This indicated that the coastal saline-alkali leachate can obtain a higher content of high-value substances under the adjustment of suitable light quality. As shown in Figures 5 and 6, the accumulation characteristics of high-value substances of *Chlorella* under different light qualities have been further explored. The results showed that at the end of experiment, the protein, total sugar and starch contents in blue-red mixed light cultures were higher than those in monochromatic red and blue light, which were

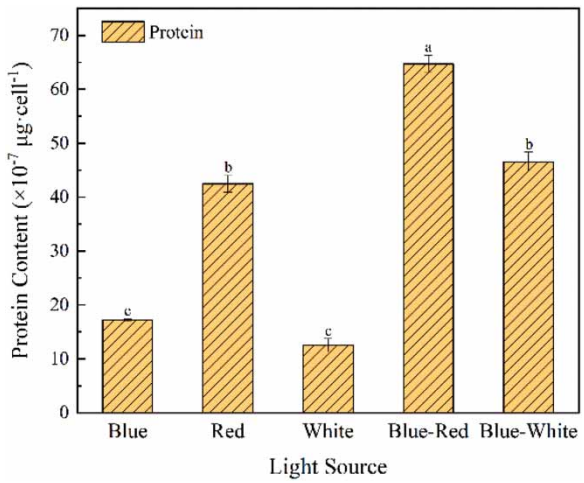


Figure 5 | The protein content of *Chlorella* in coastal saline-alkali leachate under different light sources at the end of cultivation. Different letters above each column indicate significant differences between values ($p < 0.05$).

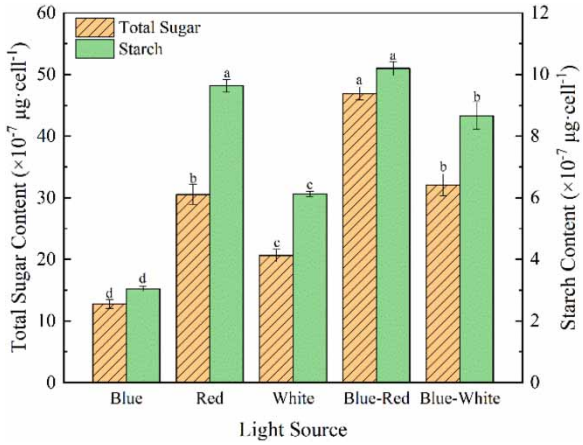


Figure 6 | The total sugar and starch content of *Chlorella* in coastal saline-alkali leachate under different light sources at the end of cultivation. Different letters above each column indicate significant differences in total sugar content and starch content among different light sources ($p < 0.05$).

$64.71 \pm 1.63 \times 10^{-7}$ μg·cell^{-1}, $46.92 \pm 1.05 \times 10^{-7}$ μg·cell^{-1} and $10.20 \pm 0.23 \times 10^{-7}$ μg·cell^{-1}, respectively. In addition, statistically significant differences were observed among blue-white mixed light, monochromatic blue, and white light groups, the protein, total sugar and starch content in blue-white mixed light cultures remaining higher than the monochromatic blue and white light groups ($p < 0.05$). This indicated that mixed light treatments (blue-red and blue-white) were more appropriate for microalgal protein, total sugar and starch accumulation than monochromatic LED light groups (monochromatic red, blue, and white) in the coastal saline-alkali leachate.

The total sugar and starch content of *Chlorella* in the coastal saline-alkali leachate under blue light lower than those of other light groups, which may be due to the fact that blue light can promote the production and activity of respiratory enzymes in microalgae. The increase in the concentration and activity of respiratory enzymes accelerates the rate of decomposition of carbohydrates, resulting in a reduction in carbohydrate content (Kowallik 1982; Ruyters 1984). Similarly, Vadiveloo *et al.* (2015) found that the carbohydrate content of *Nannochloropsis* sp. under blue light was lower than that of other light treatments. However, several studies have shown that under different light qualities, the light-harvesting pigments and membranes of microalgae are changed due to various types of algae, resulting in different aspects responses of microalgae to light spectra (Saavedra & Voltolina 1994).

Besides, the effect of light quality on the microalgae growth is a complex process, which is not only related to photosynthesis pathways, but also stimulates photoreceptors, affects microalgae cell division, enzyme activation, gene transcription, and so on (Oldenhof *et al.* 2006; Abiusi *et al.* 2014). For example, in this paper, the total lipid content of *Chlorella* sp. HQ in the coastal saline-alkali leachate under red light was significantly higher than that of monochromatic blue and white light, while another study found that *Nannochloropsis* sp. had significantly higher lipid content under blue light than that of red light and white light (Vadiveloo *et al.* 2015). The reason may be that the activities of carbonic anhydrase and ribulose biphosphate carboxylase/oxygenase which play a vital role in the regulation of the microalgal carbon cycle are enhanced under blue light (Eskins *et al.* 1991).

In general, by optimizing the light quality of the light source required for culturing *Chlorella* in saline-alkali leachate, the light conditions can be controlled more accurately, and the high yield of algal biomass and the maximum utilization of coastal saline-alkali leachate resources can be achieved.

CONCLUSION

Based on the experimental results obtained in this study, optimizing light quality can harvest higher algal biomass from the coastal saline-alkali leachate. Compared with monochromatic lights, blue-red mixed light and blue-white mixed light were more conducive to the accumulation of photosynthetic pigment of *Chlorella*, and complementary chromatics adaptation can improve the algae cells photosynthetic ability. While the highest total lipid content per unit biomass was achieved under monochromatic red light. In addition, mixed LED light (blue-red light and blue-white light) can be used to improve the production of high-valued products. Therefore, if obtaining more biomass and high-valued substances is the desired goal, then a two-stage control of light quality can be considered, such as using blue light first and then using blue-red mixed light as the light source, so as to maximize the benefits of saline-alkali leachate resources utilization.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Abiusi, F., Sampietro, G., Marturano, G., Biondi, N., Rodolfi, L., D'Ottavio, M. & Tredici, M. R. 2014 Growth, photosynthetic efficiency, and biochemical composition of *Tetraselmis suecica* F&M-M33 grown with LEDs of different colors. *Biotechnology and Bioengineering* **111** (5), 956–964. https://doi.org/10.1002/bit.25014.

Ananthi, V., Brindhadevi, K., Pugazhendhi, A. & Arun, A. 2021 Impact of abiotic factors on biodiesel production by microalgae. *Fuel* **284**. https://doi.org/10.1016/j.fuel.2020.118962.

Archer, J., Luffman, I., Joyner, T. & Nandi, A. 2019 Identifying untapped potential: a geospatial analysis of Florida and California's 2009 recycled water production. *Journal of*

Water Reuse and Desalination **9** (2), 173–192. https://doi.org/10.2166/wrd.2018.012.

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*. *Bioresource Technology* **148**, 373–378. https://doi.org/10.1016/j.biortech.2013.08.162.

Bligh, E. G. & Dyer, W. J. 1959 A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**, 911–917. https://doi.org/10.1139/o59-099.

Campos, H., Boeing, W. J., Dungan, B. N. & Schaub, T. 2014 Cultivating the marine microalga *Nannochloropsis salina* under various nitrogen sources: effect on biovolume yields, lipid content and composition, and invasive organisms. *Biomass and Bioenergy* **66**, 301–307. https://doi.org/10.1016/j.biombioe.2014.04.005.

Chandra, R., Iqbal, H. M. N., Vishal, G., Lee, H. S. & Nagra, S. 2019 Algal biorefinery: a sustainable approach to valorize algal-based biomass towards multiple product recovery. *Bioresource Technology* **278**, 346–359. https://doi.org/10.1016/j.biortech.2019.01.104.

Chen, P., Min, M., Chen, Y., Liang, W. & Ruan, R. 2009 Review of biological and engineering aspects of algae to fuels approach. *International Journal of Agricultural & Biological Engineering* **2** (4), 1–30. https://doi.org/10.3965/j.issn.1934-6344.2009.04.001-030.

Chew, K. W., Yap, J. Y., Show, P. L., Suan, N. H., Juan, J. C., Ling, T. C., Lee, D. & Chang, J. 2017 Microalgae biorefinery: high value products perspectives. *Bioresource Technology* **229**, 53–62. https://doi.org/10.1016/j.biortech.2017.01.006.

Duarte, J. H., de Souza, C. O., Druzian, J. I. & Costa, J. A. V. 2019 Light emitting diodes applied in *Synechococcus nidulans* cultures: effect on growth, pigments production and lipid profiles. *Bioresource Technology* **280**, 511–514. https://doi.org/10.1016/j.biortech.2019.02.009.

Eskins, K., Jiang, C. Z. & Shibles, R. 1991 Light-quality and irradiance effects on pigments, light-harvesting proteins and Rubisco activity in a chlorophyll- and light-harvesting-deficient soybean mutant. *Physiologia Plantarum* **83** (1), 47–53. https://doi.org/10.1111/j.1399-3054.1991.tb01280.x.

Fang, S. B., Tu, W. R., Mu, L., Sun, Z. L., Hu, Q. Y. & Yang, Y. 2019 Saline alkali water desalination project in Southern Xinjiang of China: a review of desalination planning, desalination schemes and economic analysis. *Renewable and Sustainable Energy Reviews* **113**. https://doi.org/10.1016/j.rser.2019.109268.

Gao, F., Yang, H. L., Li, C., Peng, Y. Y., Lu, M. M., Jin, W. H., Bao, J. J. & Guo, Y. M. 2019 Effect of organic carbon to nitrogen ratio in wastewater on growth, nutrient uptake and lipid accumulation of a mixotrophic microalgae *Chlorella* sp. *Bioresource Technology* **282**, 118–124. https://doi.org/10.1016/j.biortech.2019.03.011.

Kandilian, R., Lee, E. & Pilon, L. 2013 Radiation and optical properties of *Nannochloropsis oculata* grown under different irradiances and spectra. *Bioresource Technology* **137**, 63–73. https://doi.org/10.1016/j.biortech.2013.03.058.

Kim, D. G., Lee, C., Park, S. & Choi, Y. 2014 Manipulation of light wavelength at appropriate growth stage to enhance biomass productivity and fatty acid methyl ester yield using *Chlorella vulgaris*. *Bioresource Technology* **159**, 240–248. https://doi.org/10.1016/j.biortech.2014.02.078.

Kirk, J. T. O. & Allen, R. L. 1965 Dependence of chloroplast pigment synthesis on protein synthesis: effect of actidione. *Biochemical & Biophysical Research Communications* **21** (6), 523–530. https://doi.org/10.1016/0006-291X(65)90516-4.

Kowallik, W. 1982 Blue light effects on respiration. *Annual Review of Plant Physiology* **33**, 51–72. https://doi.org/10.1146/annurev.pp.33.060182.000411.

Li, X., Hu, H. Y., Yang, J. & Wu, Y. H. 2010 Enhancement effect of ethyl-2-methyl acetoacetate on triacylglycerols production by a freshwater microalga, *Scenedesmus* sp. LX1. *Bioresource Technology* **101** (24), 9819–9821. https://doi.org/10.1016/j.biortech.2010.07.103.

Liu, X., Hong, Y., He, Y. & Liu, Y. 2019 Growth and high-valued products accumulation characteristics of microalgae in saline-alkali leachate from Inner Mongolia. *Environmental Science and Pollution Research* **26**, 36985–36992. https://doi.org/10.1007/s11356-019-06842-z.

Liu, X., Hong, Y., He, Y. & Gu, W. 2020 Comparison of oleaginous microalgal growth and lipid accumulation in saline-alkali leachate: a case from Shandong Province. *Desalination and Water Treatment* **187**, 390–398. https://doi.org/10.5004/dwt.2020.25477.

Maaitah, M., Hodaifa, G., Malvis, A. & Sánchez, S. 2020 Kinetic growth and biochemical composition variability of *Chlorella pyrenoidosa* in olive oil washing wastewater cultures enriched with urban wastewater. *Journal of Water Process Engineering* **35**. https://doi.org/10.1016/j.jwpe.2020.101197.

Mata, T. M., Martins, A. A. & Caetano, N. S. 2010 Microalgae for biodiesel production and other applications: a review. *Renewable and Sustainable Energy Reviews* **14** (1), 217–232. https://doi.org/10.1016/j.rser.2009.07.020.

Meena, M. D., Yadav, R. K., Narjary, B., Yadav, G., Jat, H. S., Sheoran, P., Meena, M. K., Antil, R. S., Meena, B. L., Singh, H. V., Meena, V. S., Rai, P. K., Ghosh, A. & Moharana, P. C. 2019 Municipal solid waste (MSW): strategies to improve salt affected soil sustainability: a review. *Waste Management* **84**, 38–53. https://doi.org/10.1016/j.wasman.2018.11.020.

Mouget, J., Rosa, P. & Tremblin, G. 2004 Acclimation of *Haslea ostrearia* to light of different spectral qualities–confirmation of `chromatic adaptation' in diatoms. *Journal of Photochemistry and Photobiology B: Biology* **75**, 1–11. https://doi.org/10.1016/j.jphotobiol.2004.04.002.

Oldenhof, H., Zachleder, V. & Van Den Ende, H. 2006 Blue- and red-light regulation of the cell cycle in *Chlamydomonas reinhardtii* (Chlorophyta). *European Journal of Phycology* **41** (3), 313–320. https://doi.org/10.1080/09670260600699920.

Pereiraa, S. & Oterob, A. 2019 Effect of light quality on carotenogenic and non-carotenogenic species of the genus

Dunaliella under nitrogen deficiency. *Algal Research* **44**. https://doi.org/10.1016/j.algal.2019.101725.

Rashid, N., Ryu, A. J., Jeong, K. G., Lee, B. & Chang, Y. K. 2019 Co-cultivation of two freshwater microalgae species to improve biomass productivity and biodiesel production. *Energy Conversion and Management* **196**, 640–648. https://doi.org/10.1016/j.enconman.2019.05.106.

Ravelonandro, P. H., Ratianarivo, D. H., Joannis Cassan, C., Isambert, A. & Raherimandimby, M. 2008 Influence of light quality and intensity in the cultivation of *Spirulina platensis* from Toliara (Madagascar) in a closed system. *Journal of Chemical Technology & Biotechnology* **83** (6), 842–848. https://doi.org/10.1002/jctb.1878.

Rochet, M., Legendre, L. & Demers, S. 1986 Photosynthetic and pigment responses of sea-ice microalgae to changes in light intensity and quality. *Journal of Experimental Marine Biology & Ecology* **101** (3), 211–226. https://doi.org/10.1016/0022-0981(86)90264-9.

Ruyters, G. 1984 Effects of blue light on enzymes. Blue light effects in biological system. *Proceedings in Life Sciences* 283–301. https://doi.org/10.1007/978-3-642-69767-8_32.

Saavedra, M. D. P. S. & Voltolina, D. 1994 The chemical composition of *Chaetoceros* sp. (Bacillariophyceae) under different light conditions. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **107** (1), 39–44. https://doi.org/10.1016/0305-0491(94)90222-4.

Schulze, P. S. C., Barreira, L. A., Pereira, H. G. C., Perales, J. A. & Varela, J. C. S. 2014 Light emitting diodes (LEDs) applied to microalgal production. *Trends in Biotechnology* **32** (8), 422–430. https://doi.org/10.1016/j.tibtech.2014.06.001.

Shu, C., Tsai, C., Liao, W., Chen, K. & Huang, H. 2012 Effects of light quality on the accumulation of oil in a mixed culture of *Chlorella* sp. and *Saccharomyces cerevisiae*. *Journal of Chemical Technology & Biotechnology* **87** (5), 601–607. https://doi.org/10.1002/jctb.2750.

Sun, B., Fan, X., Ye, H., Fan, J., Qian, C., van Driel, W. & Zhang, G. 2017 A novel lifetime prediction for integrated LED lamps by electronic-thermal simulation. *Reliability Engineering & System Safety* **163**, 14–21. https://doi.org/10.1016/j.ress.2017.01.017.

Tamburic, B., Szabó, M., Tran, N. T., Larkum, A. W. D., Suggett, D. J. & Ralph, P. J. 2014 Action spectra of oxygen production and chlorophyll a fluorescence in the green microalga *Nannochloropsis oculata*. *Bioresource Technology* **169**, 320–327. https://doi.org/10.1016/j.biortech.2014.07.008.

Tang, J. W., Zhang, S. D., Zhang, X. T., Chen, J. H., He, X. Y. & Zhang, Q. Z. 2020 Effects of pyrolysis temperature on soil-plant-microbe responses to *Solidago canadensis* L.-derived biochar in coastal saline-alkali soil. *Science of the Total Environment* **731**. https://doi.org/10.1016/j.scitotenv.2020.138938.

Teo, C. L., Atta, M., Bukhari, A., Taisir, M., Yusuf, A. M. & Idris, A. 2014 Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. *Bioresource Technology* **162**, 38–44. https://doi.org/10.1016/j.biortech.2014.03.113.

Vadiveloo, A., Moheimani, N. R., Cosgrove, J. J., Bahri, P. A. & Parlevliet, D. 2015 Effect of different light spectra on the growth and productivity of acclimated *Nannochloropsis* sp. (Eustigmatophyceae). *Algal Research* **8**, 121–127. https://doi.org/10.1016/j.algal.2015.02.001.

Wang, C., Fu, C. & Liu, Y. 2007 Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. *Biochemical Engineering Journal* **37** (1), 21–25. https://doi.org/10.1016/j.bej.2007.03.004.

Wang, R., Wan, S., Sun, J. & Xiao, H. 2018 Soil salinity, sodicity and cotton yield parameters under different drip irrigation regimes during saline wasteland reclamation. *Agricultural Water Management* **209**, 20–31. https://doi.org/10.1016/j.agwat.2018.07.004.

Xu, B., Cheng, P., Yan, C., Pei, H. & Hu, W. 2013 The effect of varying LED light sources and influent carbon/nitrogen ratios on treatment of synthetic sanitary sewage using *Chlorella vulgaris*. *World Journal of Microbiology & Biotechnology* **29** (7), 1289–1300. https://doi.org/10.1007/s11274-013-1292-6.

Yue, Y., Shao, T., Long, X., He, T., Gao, X., Zhou, Z., Liu, Z. & Rengel, Z. 2020 Microbiome structure and function in rhizosphere of Jerusalem artichoke grown in saline land. *Science of the Total Environment* **724**. https://doi.org/10.1016/j.scitotenv.2020.138259.

Zhu, Z. & Dou, J. 2018 Current status of reclaimed water in China: an overview. *Journal of Water Reuse and Desalination* **8** (3), 293–307. https://doi.org/10.2166/wrd.2018.070.

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