Original Research Article

EFFECTS OF LIGHT ON THE GROWTH AND *β-carotene* ACCUMULATION IN THE GREEN ALGAE *Dunaliella salina*

ABSTRACT

The marine microalgae *Dunaliella salina* holds significant economic value due to its rich content of *β-carotene*, a natural pigment with high antioxidant capacity, immune-stimulating properties, and a crucial role in antioxidant reactions with vitamins C and E. This study evaluated the effects of light factors on the growth and biosynthesis of *β-carotene* in the *Dunaliella salina* strain isolated from coastal waters in the central region of Vietnam. The results indicated that after six days of static culture in f/2 medium at a room temperature of 25°C, using red LED light (650 nm) at a light intensity of 40.5 µmol photon/m2/s combined with a light/dark cycle of 24/0, the highest yield of *β-carotene* was achieved at 1.65 ± 0.07 mg/L/day. These findings provide important scientific data for further research to identify the optimal conditions for increasing biomass and stimulating the accumulation of valuable secondary compounds of the microalgae *Dunaliella salina* in Vietnam.

Keywords: Dunaliella salina; β-carotene; Light; Growth; Accumulation

1. INTRODUCTION

β-carotene is a yellow-orange pigment synthesized by various plants and microalgae in nature. This pigment plays a significant role in photosynthesis and offers cell protection against photooxidation [1]. Moreover, *β-carotene* is a vital nutrient as it can be converted into vitamin A. It also acts as a peroxyl radical scavenger and an immune response booster [2]. In the market, *β-carotene* has been extensively applicated as a coloring agent, food additive, antioxidant, and a preventive agent for cancer and heart disease, as well as eye and skin health promotion [3].

Despite most commercial *β-carotene* being chemically synthesized, the trend to use natural sources of this compound is growing due to rising health concerns. Studies have shown that the marine green microalgae *Dunaliella salina* contains high levels of *β-carotene* (up to 10% of the dry weight [4]). This is an abundant and safe natural source of *β-carotene* that can be exploited to develop into health supplements for humans and seafood species [5]. *Dunaliella salina* belongs to the Chlorophyceae family of unicellular green algae and can accumulate carotenoids under various adverse conditions, such as high salinity, high light intensity, or low growth temperature [6]. Therefore, currently, several studies have been carried out to screen and select algae strains, and optimize the culture environment and culture process to enhance the commercial production of natural *β-carotene* from this species [7].

Among the environmental factors affecting *β-carotene* biosynthesis, light is considered to be the most significant [8]. Light is the primary energy source for phototrophic algae, and the growth and metabolism of algae, particularly *D. salina*, are influenced by light conditions (i.e., light source type, light intensity, light spectrum, lighting mode, etc.) [6]. Therefore, selecting suitable light conditions for algae culturing is very important for the production of biomass and compounds from microalgae. This study was conducted to investigate the impact of light intensity and spectrum on the growth and accumulation of *β-carotene* in the microalgae *D. salina*. The findings from this study will serve as the scientific basis for the development of a *D. salina* culture process for the production of natural *β-carotene* compounds.

2. MATERIAL AND METHODS

2.1. Microalgae strain

Dunaliella salina was isolated from coastal waters in the central region of Vietnam by the authors and kept in the Algae Technology laboratory belonging to the Faculty of Biology and Environmental Science, The University of Science and Education, Danang University. The microalgae strain was cultured in f/2 medium at 25°C and illuminated with white LED light with an intensity of 30 µmol photon/m2/s with a light/dark cycle of 16/8.

2.2. Experimental layout

To investigate the effect of light spectrum on the biological characteristics of *D. salina*, three light spectrum treatments, including blue, red, and white, were arranged with a constant intensity of 30 µmol photon/m2/s. Meanwhile, for the experiments to evaluate the effect of light intensity, three intensity levels of 13.5, 27.0, and 40.5 µmol photon/m2/s were examined. Except for the investigated factors, the remaining experimental conditions were maintained in the same way as the rearing conditions. The experiments used Light Emitting Diode (LED) as the light source, which is commonly used in microalgae culture due to its ability to provide a specific narrow wavelength range and low power consumption compared to fluorescent lamps. Each treatment was repeated three times, and the input microalgae density of each flask was 100 × 103 cells/mL.

The experiments lasted for six days. Algae densities were examined every two days and *β-carotene* levels were identified at the end of the experiments (day 6).

2.3. Determination of density and growth rate

The algae cells were observed under a 4X objective microscope, photographed on a Neubauer counting chamber, and counted using ImageJ software. The growth rate (d-1) was calculated using the formula:

$$\mu = \frac{ln(N_2) - ln(N_1)}{t_2 - t_1}$$

where N_1 and N_2 (cells/mL) are the cell density of microalgae at the beginning (t_1) and the end (t_2) time of the culture, respectively.

2.4. Determination of *β-carotene* content

The content of *β-carotene* was determined according to Shaish's method [9]. Specifically, 1 mL of algae solution was taken and centrifuged at 5000 rpm for 5 minutes. The supernatant was removed, and the residue was extracted with a 3 mL mixture of ethanol and hexane with a volume ratio of 2:1. Then, 2 mL of H2O and 4 mL of n-hexane were added and the mixture was centrifuged at 5000 rpm for 5 min. After that, the extract in the n-hexane phase was taken and measured the optical density (OD) at a wavelength of 450 nm using a Jasco V750.

The content of *β-carotene* of the extract was calculated using the formula:

[*β-carotene*] (µg/ml) = A450 × 25,2

where A450 is the value measured at wavelength 450.

The content of *β-carotene* in each microalgal cell was determined by the formula:

[*β-carotene*tb] (pg/cell) = [*β-carotene*] / Algae density

2.5. Data analysis

Descriptive statistics and data processing were performed using R software. One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to test the significant difference between the mean values of different treatments.

3. RESULTS

3.1. Effect of light spectrum on growth and *β-carotene* accumulation in *Dunaliella salina*

The microalgae *D. salina* grown under different light spectrum conditions exhibited varying growth patterns (Figure 1). Under red light, the microalgae cell density increased sharply on day 2 and continued to rise until the end of the experiment (day 6), reaching 278 ± 7.04 × 103 cells/mL. In contrast, under blue light and white light, the algae exhibited slow growth until day 4, and then entered the equilibrium phase of the growth curve, with densities of approximately 180 × 103 cells/mL on day 6 for both spectra.

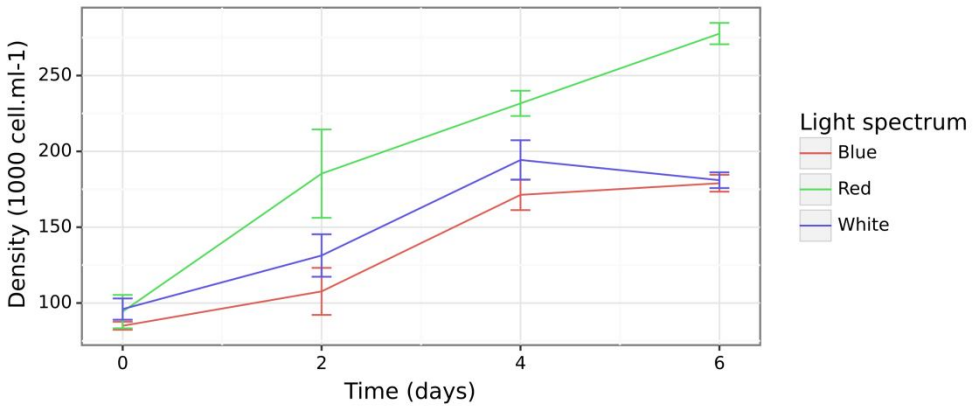

Fig. 1. The cell density of *D. salina* under different light spectra over time

The average growth rate of the microalgae *D. salina* during the 6-day culture period was highest in the red light spectrum treatment with a value of 0.18 ± 0.019 d-1, significantly higher than the other two treatments (*p-values* < 0.05) (Figure 2). The average growth rates under blue and white light were 0.125 ± 0.007 d-1 and 0.106 ± 0.01 d-1, respectively. However, this difference was not statistically significant (*p-values*> 0.05).

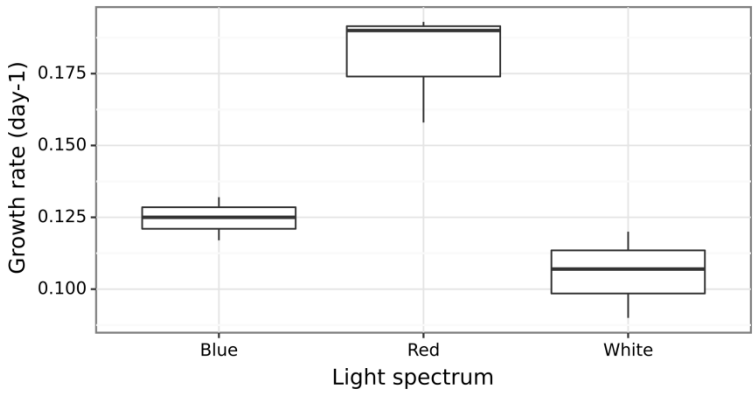

Figure 2. The average growth rate of *D. salina* under different light spectra

Regarding *β-carotene* accumulation, blue light had the most positive effect on *D. salina* (Figure 3). The average *β-carotene* content per algal cell under the blue light was 35.89 ± 4.15 pg/cell, higher than that under red light (30.74 ± 2.68 pg/cell) and white light (29.14 ± 2.58 pg/cell). However, the differences were not statistically significant (*p-values* > 0.05).

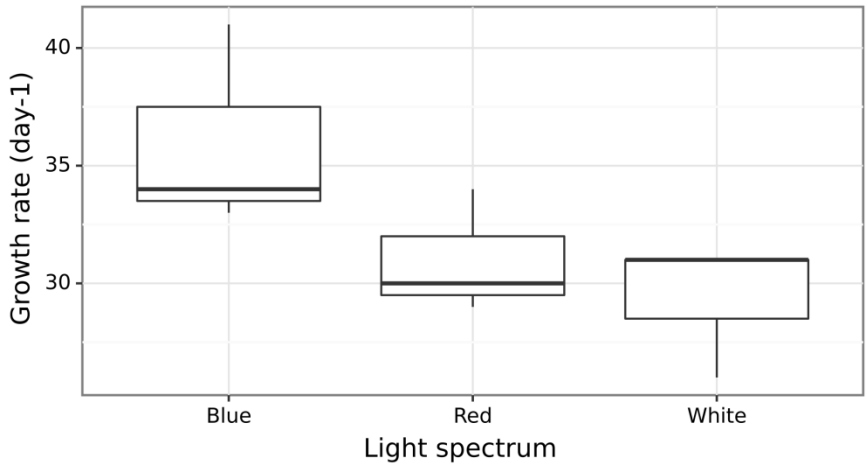

Figure 3. *β-carotene* content in *D. salina* cells under different light spectra

3.2. Effect of light intensity on growth and *β-carotene* accumulation in *Dunaliella salina*

Light intensity had a significant impact on the growth of microalgae *D. salina*, as shown in Figure 4. The density of microalgae increased continuously throughout the experiment under the treatment with an intensity of 40.5 µmol photon/m2/s, whereas in the treatment with 13.5 µmol photon/m2/s, the density increased sharply from the 2nd day onwards. All treatments did not show signs of reaching the equilibrium phase at the end of the experiment. The cell densities on day 6 were 435 ± 11.9 × 103 cells/mL, 304 ± 20.2 × 103 cells/mL, and 242 ± 38.8 × 103 cells/mL for respectively light intensities of 40.5, 27.0, and 13.5 µmol photon/m2/s.

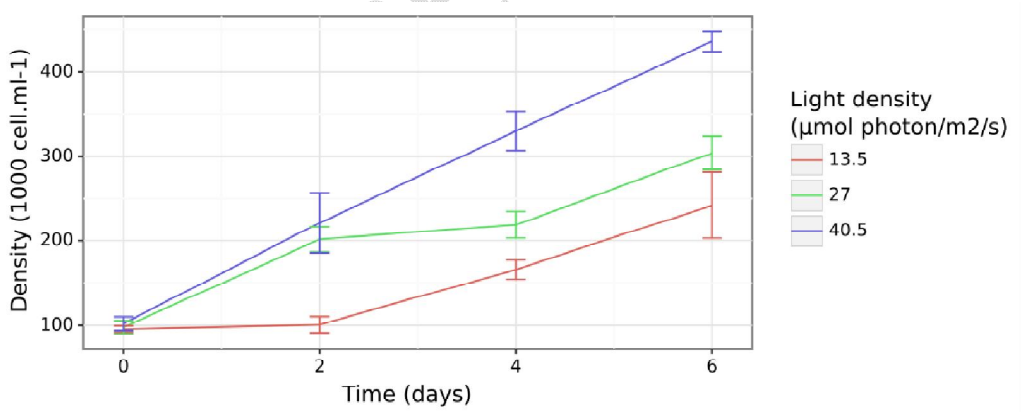

Figure 4. The cell density of *D. salina* at different light intensities over time

The average growth rate of *D. salina* was proportional to the light intensity in the surveyed range (Figure 5). At the lowest experimental light intensity (13.5 µmol photon/m2/s), the algae grew at 0.15 ± 0.03 d-1. Meanwhile, the average growth rate was obtained highest at 0.24 ± 0.01 d-1 in the treatment with 40.5 µmol photon/m2/s, significantly higher than that in the other two treatments (*p-values* < 0.05).

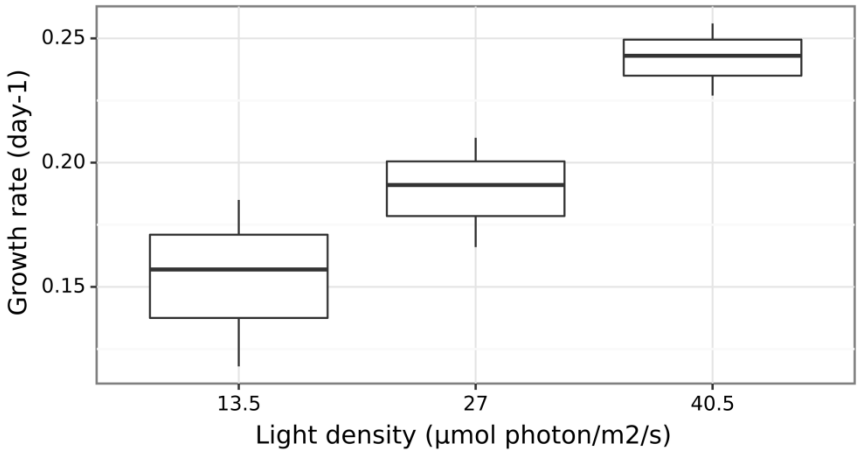

Figure 5. The average growth rate of *D. salina* at different light intensities

Contrary to the growth rate, *β-carotene* accumulation displayed a decreasing trend with increasing light intensity. Specifically, the average *β-carotene* content per microalgal cell was highest in the treatment with 13.5 µmol photon/m2/s (33.4 ± 4.41 pg/cell), followed by 27 µmol photon/m2/s (29.19 ± 4.41 pg/cell), and lowest at 40.5 µmol photon/m2/s (26.34 ± 0.76 pg/cell). However, the difference between treatments was not statistically significant (*p-values* > 0.05).

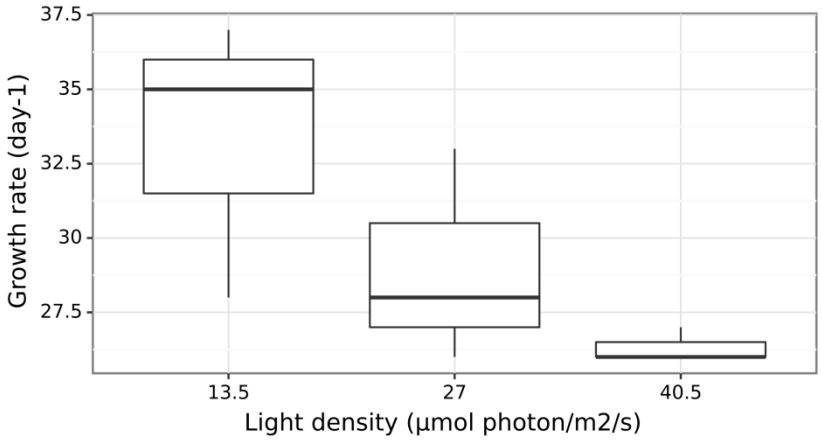

Figure 6. *β-carotene* content in *D. salina* cells at different light intensities

4. DISCUSSION

Carotenoids are a group of pigments synthesized in photosynthetic organisms that are characterized by orange, yellow, or red colors. Their main functions are to capture light, protect cells against light-induced damage, and stabilize pigment-protein complexes and photosynthetic reaction centers on the thylakoid membrane [1]. Therefore, under different lighting conditions, the response of microalgae is not expected to be the same. In this study, that response was evaluated by measuring their growth rate parameters and *β-carotene* content.

Our results showed that red light had the best growth-stimulating effect, while blue light promoted *β-carotene* accumulation in *D. salina*. This is in agreement with the results of Han et al. (2019) who reported that *D. salina* grows best in red light and less well in white and blue light [10]. The emission spectrum of the red LED (625–680 nm) coincides with the

photosynthetic photon absorption capacity of chl-a and chl-b pigments [11]. In *D. salina*, photosynthesis was shown to be maximal in the red absorption band of the chlorophyll pigments [8]. Therefore, the wavelength range of the red LED gives the highest growth rate in this algae. Meanwhile, blue light, which is absorbed by the group of carotenoid pigments, is not suitable for photosynthesis to generate biomass (growth) [12]. However, blue light has a short wavelength and high photon energy, thus it is easy to damage *D. salina* cells [10]. Under such adverse conditions, the microalgae tends to accumulate more *β-carotene* to protect and maintain cell life [4]. The transcriptome analysis conducted by Li et al. (2020) also showed that red light is most suitable for algal growth, with stronger expression of growth-control genes compared to blue light. In contrast, cellular carotenoid content as well as the expression of reactive oxygen species (ROS) synthase genes were enhanced under blue-spectrum illumination [13].

High light intensity often stresses microalgae cells, inhibits photosynthesis, and directs the flow of carbon and energy to the synthesis of storage compounds rather than to protein synthesis [14]. However, the threshold for light stress depends on the species, strain and cell composition characteristics. For example, the strain *D. salina* CCAP 19/30 was observed to decrease the photosynthesis rate when grown under illumination conditions of 200-500 µmol photon/m2/s due to photoinhibition, but at a higher intensity (1500 µmol photon/m2/s), when algae have synthesized intracellular glycerol to stabilize and protect the photosynthetic apparatus, the rate of photosynthesis increased to the maximum [14]. In our study, the growth rate of *D. salina* increased proportionally with light intensity. This may be because our survey range is quite low (13.5 - 40.5 µmol photon/m2/s), has not reached the light saturation point for *D. salina*. Therefore, when the amount of energy provided to the algae increases, the algae can perform photosynthesis better without photoinhibition.

Most algae have light saturation points in the range of 26 - 400 µmol photon/m2/s, although some strains of *D. salina* are able to adapt to extremely high light due to their good defense mechanism, for instance, some strains of *D. salina* showed the optimal growth at light intensities of more than 1000 µmol photon/m2/s [15]. The accumulation of carotenoids in Dunaliella generally increases with increasing light intensity. Wu et al. (2020) reported that increasing the light intensity from 100 to 200 µmol photons/m2/s increased the accumulation of *β-carotene* in the *D. salina* Y6 strain by 31.5% [16]. In another study, Dunaliella strains (DF15, DF17, DF40, and UTEX 253) showed *β-carotene* accumulation under light intensity conditions of more than 200 µmol photon/m2/s with a maximum yield of 3.5 mg/L/day recorded at an intensity of 1500 µmol photon/m2/s [15].

In this study, when the light intensity increased from 13.5 to 40.5 µmol photon/m2/s, the *β-carotene* content tended to decrease, although not significantly. This may be because the primary function of carotenoids pigments is to aid in light capture, carry out photosynthesis and protect cells from damage caused by harsh light conditions. As such, under such a favorable intensity range, algae still prioritize using energy to grow populations over maintaining cell survival.

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