The Effect of Different Light Spectra on Beta-Carotene Production by *Dunaliella salina*

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ABSTRACT: *This study focused on the effect of different light intensities and spectra on the beta-carotene production within Dunaliella salina cells (green eukaryote microalgae) which are purified from Urmia Lake in northwest Iran. For this purpose, four LED light spectra (white light: 360–760 nm, red light: 620–645 nm, yellow light: 587–595 nm, and blue light: 460–475 nm) were used in this experimental research. The light intensity of 200 µmol/(m^2 s) was considered for each LED light spectra. The highest beta-carotene content extracted under a sequential combination of colored light (white, blue, red, and yellow respectively) was 16.93 µg of beta-carotene per mg of cell dry weight and the highest accumulated beta-carotene within the cells among single-colored light was 15.16 µg /mg when the cells were cultivated under yellow light.*

KEYWORDS*: Microalgae, Dunaliella salina, Beta-carotene, Colored lights, Salinity.*

INTRODUCTION

Microalgae are located at the bottom of the food chain in aquatic ecosystems. They take up CO_2 and H_2O (with the aid of sunlight) and convert them into complex organic compounds such as proteins, carbohydrates, lipids, etc. [1-4]. They supply a large portion of the world's oxygen demand [5]. Although they have a lower photosynthetic efficiency than higher plants, they have better growth rates and oil-producing capabilities compared to others [6].

Dunaliella salina is a unicellular photosynthetic green microalga with two equal flagella. The cell's forms are ovoid, spherical, and ellipsoid. The shape of the cells changes in different environmental conditions. The cell size varies from 5 to 25 µm in length and from 3 to 13 µm in width [7]. These halo-tolerant eukaryote cells have no cell wall and can withstand a wide range of salinities (0.5-5 M NaCl) [8-9]. These cells accumulate large amounts of beta-carotene under stressed growth conditions, such as high light intensity, high salinity, nutrient deprivation, etc. [10-13].

Light is the most important factor in microalgae growth because it is the main energy source for photosynthetic activities. The light intensity and the light wavelength

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constitute the key parameters. It should be noted that very high light intensities could lead to a photoinhibition phenomenon and, consequently, cause a growth decrease [14, 15].

Beta-carotene is a lipophilic hydrocarbon ($C_{40}H_{56}$) with an unsaturated chain that gives it a yellow-orange color. This molecule has an important role in the human diet because of its characteristic of acting as pro-vitamin A and a radical quencher. More than 90% of commercial beta-carotene is obtained by chemical methods [16]. *Dunaliella salina* cells have a beta-carotene content of 0.5-1% of their dry weight under regular environmental conditions but it could go up to 12% of their dry weight under stress conditions and could be accumulated within oil globes in the cup-shaped chloroplast of the cells [17]. Beta-carotene has a major protective role against excessive irradiance by suppressing the formation of reactive oxygen species and quenching singlet radical oxygen and performing as a light filter [18]. Hence, it could be predicted that large amounts of beta-carotene would accumulate within the cells by increasing light irradiation to the cells and changing cell conditions to stress situations.

In the present study, the impact of several spectra of light and the combination of different light packages (white, yellow, red, and blue) on biomass production and beta-carotene content in *Dunaliella salina* cells were investigated.

EXPERIMENTAL SECTION

Organisms and culture medium

Dunaliella salina (Urmia Lake species) was provided by the Iranian Biological Resource Center (IBRC) and cultivated in modified Johnson's culture medium. This medium contained 1M salt concentration with the following ingredients:

$MgCl_2{\cdot}6H_2O$ 1.5 g, $MgSO_4{\cdot}7H_2O$, 0.5 g, KCl 0.2 g, $CaCl_2{\cdot}2H_2O$ 0.2 g, KNO3 1 g, $NaHCO_3$ 0.043 g, KH_2PO_4 0.035 g, Fe-solution 10 mL, Trace-element solution 10 mL, Fe solution (for 1 L), Na_2EDTA 189 mg, $FeCl_3{\cdot}6H_2O$ 244 mg, Trace-element solution (for 1 L) H_3BO_3 61 mg, $(NH4)_6Mo_7O_{24}{\cdot}4H_2O$ 38 mg, $CuSO_4{\cdot}5H_2O$ 6 mg, $CoCl_2{\cdot}6H_2O$ 5.1 mg, $ZnCl_2$ 4.1 mg, $MnCl_2{\cdot}4H_2O$ 4.1 mg [19].

Culture conditions

For the initial step of favorable microalgal growth, it was necessary to find the optimum salinity for the best growth procedure. Hence, five samples of *Dunaliella salin*a with five different salinities from 0.5 to 2.5 M NaCl concentration were considered. Each sample was under 135 µmol/(m^2 s) intensity of white light with continuous aeration for 24 days. As indicated in the next part, the optimum salinity of 1 M was reported for the best *Dunaliella salina* growth pattern. So, this salinity was chosen as the basic salinity for all the samples that were cultivated in these experiments. For the assessment of light effects on microalgae growth patterns and beta-carotene production, 11 *Dunaliella salina* samples (each sample volume: 1 L) were cultivated in 1 M salt concentration, with continuous aeration, at an average temperature of 25±2 ºC, and a culture period of 24 days. Four samples were under the illumination of fixed colored lights that included white light (360–760 *nm*, with the intensity of 200 µmol/(m^2. s)), red light (620–645 *nm*, 200 µmol/(m^2. s)), yellow light (587–595 *nm*, 200 µmol/(m^2. s)), and blue light (460–475 *nm*, 200 µmol/(m^2. s)) during the entire culture period (24 days). Furthermore, seven samples of microalgae culture medium were located under various spectra with different light intensities during the cultivation time. The first sample (W6–Y18) was put under white light for 6 days and then shifted to a yellow light for 18 days. The second sample (W12–Y12) was fixed for 12 days under white light and then was transferred to yellow light for 12 days. The third sample (W18–Y6) was under white light for 18 days and then moved to a yellow light for 6 days. Sample 4 (W6–B6–Y12) was under white light for 6 days and was then put under blue light for 6 days, and then located under yellow light for 12 days. The positioning and shifting procedure of samples 5, 6, and 7 were as follows: Sample 5 (W6–R6–Y12), first six days under white light, then six days under red light, and, finally, 12 days under yellow light. Sample 6 (W6–B6–R6–Y6), first six days in white, then six days in blue, then six days in red, and, for the last step, six days under yellow light. Sample 7 (W6–R6–B6–Y6), six days in white light, then six days in red light, followed by six days in blue light, and, finally, six days under yellow light. Continuous aeration was considered for each culture sample. For microalgae growth investigation, the Optical Density (OD) was recorded for each sample at 560 *nm* by a spectrophotometer every two days. Table 1. gives the duration of each sample, which remained under specific light spectrums. It should be stated that all the cultures are replicated at least two times per treatment.

Table 1: Timetable of different samples under various light spectrums.

t/day / Sample and Color Symbol	1-6 day	6-12 day	12-18 day	18-24 day	24-30 day
White (W)	W W6-Y18 W12-Y12 W18-W6 W6-B6-Y12 W6-R6-Y12 W6-B6-R6-Y6 W6-R6-B6-Y6	W W12-Y12 W18-W6	W W18-W6	W	W
Yellow (Y)	Y	Y W6-Y18	Y W6-Y18 W12-Y12 W6-B6-Y12 W6-R6-Y12	Y W6-Y18 W12-Y12 W18-W6 W6-B6-Y12 W6-R6-Y12 W6-B6-R6-Y6 W6-R6-B6-Y6	Y W6-Y18 W12-Y12 W18-W6 W6-B6-Y12 W6-R6-Y12 W6-B6-R6-Y6 W6-R6-B6-Y12
Red (R)	R	R W6-R6-Y12 W6-R6-B6-Y6	R W6-B6-R6-Y6	R	R
Blue (B)	B	B W6-B6-Y12 W6-B6-R6-Y6	B W6-R6-B6-Y6	B	B

Cell dry weight measurement

The cell's dry weight values were obtained with the method of *Delavari Ameri et al*. [20].

Beta-carotene extraction

Beta-carotene extraction was accomplished by using the solvent method (hexane, ethanol): 1 mL of *Dunaliella salina* culture centrifuged at 3000 rpm for 10 minutes. Then, the supernatant was removed and 2 mL ethanol, 1 mL hexane, and 2 mL water were added to the sediment. After vigorous shaking, the solution was placed in a centrifuge device at 3000 rpm for 10 minutes again. After centrifugation, the supernatant phase was divided into two distinct phases. At the end of the process, the upper phase (hexane and beta-carotene compound) separated, and beta-carotene determination was done by spectrophotometer at 450 *nm*. By Eq. (1), the amount of beta-carotene was calculated in µg/mL [21].

$$\text{Beta-carotene content (µg/mL)}: 25.2 \times A_{450} \quad (1)$$

RESULTS AND DISCUSSION

Dunaliella salina growth assessment

Based on the aforesaid information, five different salinity samples were cultivated to evaluate the optimum growth rate of the *Dunaliella salina* culture. Fig. 1 shows the cell dry weight versus the time for the different salinities (o.5–2.5 M). The result that showed the optimum salinity for D. salina growth was 1 M NaCl. (Fig. 1).

These results had desirable conformity with the salinity that was reported by Hashemi et al. [19]. As shown in Fig. 2 during the growth process, *D. salina* was exposed to a single-colored spectrum of white light (360–760 *nm*), red light (620–645 *nm*), yellow light (587–595 *nm*), and blue light (460–475 *nm*) during the entire culture period (24 days) and the highest produced biomass was in the red, white, blue and yellow light spectra, respectively. (Fig. 2)

Helena et al. [22] assessed the effect of red and blue LED light spectra on the cell density of *Dunaliella salina* and reported that the red light had a higher cell density compared to the blue light. Besides, *Li et al.* [23] studied the effect of red and blue light on the growth pattern of *Dunaliella salina* species. The results revealed that the red light could promote biomass production. *Xu* and *Harvey* [24] reported that no significant differences were observed on the growth pattern of *Dunaliella salina* under blue, red, and white light spectra. *Han et al.* [25] investigated the effect of *Dunaliella salina* growth procedure under 50 µmol/(m^2 s) red, blue, and white light. Their results obviously demonstrated that red light had the best impact on the biomass production of *Dunaliella salina*. As described in the previous part (cultivation conditions), seven particular samples were considered for cultivation

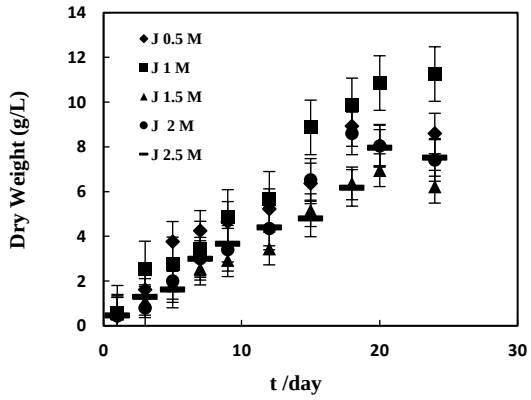

Fig. 1: Cell dry weight versus time for different salinities.

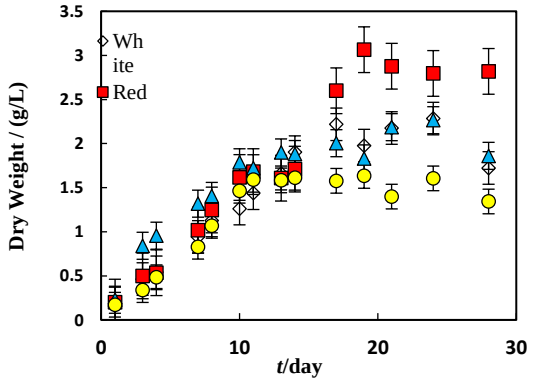

Fig. 2: Cell dry weight versus time under single colored light: red, white, blue, and yellow at 1 M NaCl.

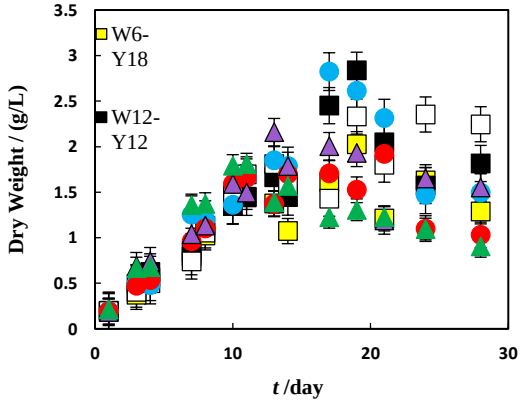

Fig. 3: Cell dry weight versus time with various wavelengths in particular periods at 1 M NaCl (W: white light, B: blue light, R: red light, Y: yellow light, each number after abbreviations indicated several days that each sample remained under a particular light spectrum).

under different spectra of colored lights on various consecutive days. According to Fig. 3 the best result for biomass production belonged to Sample 7 (W6–R6–B6–Y6), which was exposed for four-time limits of six days under white, red, blue, and yellow light spectra, respectively. Because of the extensive wavelength of white light, it was vitally recommended that the cells be exposed to white light on the first days of cultivation for a better growth process (Fig. 3).

Evaluation of extracted beta-carotene

The beta-carotene concentration production is shown in Fig. 4 In experimental research, the amount of accumulated beta-carotene, obtained as a result of single-colored light irradiation, was measured. The highest beta-carotene content was recorded in the yellow light spectrum with 15.16 µg of beta-carotene per mg of the dry weight of microalgae. According to the previous section, the lowest biomass production was recorded for yellow light (Fig. 2) but, as seen in Fig. 4 the highest amount of beta-carotene was achieved in the yellow light spectrum, and this result was in accordance with the literature. Under high light irradiation, *D. salina* cells can't use adequate light energy. This additional amount of light energy can increase the formation of ROS (reactive oxygen species) molecules. These molecules can cause peroxidation reaction, DNA mutation, chlorophyll bleaching, etc. within microalgae cells [26-28]. Beta-carotene and other carotenoids protect the cells against reactive oxygen molecules. So under excessive light irradiation and some other environmental stresses, beta-carotene accumulation within the cells increases significantly. According to the results, the lowest biomass production and the highest extracted beta-carotene content were determined for the yellow light. This happened due to a stressed condition, in which the growth rate dropped and the production of secondary metabolites, such as beta-carotene, increased. Beta-carotene accumulation in *Dunaliella sp.* is primarily triggered when the cell division rate diminishes due to the impact of stressful factors [29-31]. (Fig. 4)

According to the data recorded for single-colored light, the highest beta-carotene content was obtained under yellow light irradiance. So, it was obvious that all the effort was focused on using this spectrum at the end of the logarithmic phase of growth. In the preliminary days of cultivation, it was necessary to use white light for optimal

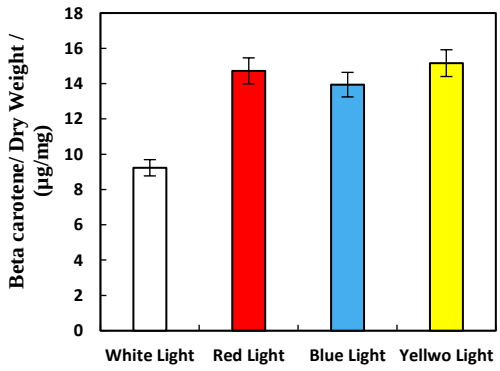

Fig. 4: Beta-carotene per dry weight of cells (µg/mg) for single-colored white, red, blue, and yellow light at 1 M NaCl.

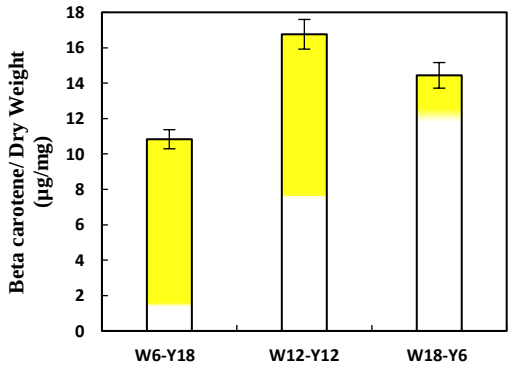

Fig. 5: Beta-carotene per dry weight of cells (µg/mg) for consecutive combination of colored light, white and yellow spectrums at 1 M NaCl.(W: white light, Y: yellow light, each number after abbreviations indicated the number of days that each sample remained under particular light spectrum).

growth due to the extensive spectra of light. Therefore, Sample (W12–Y12), which was under white light for 12 days and for 12 days under yellow light irradiation had the highest beta-carotene content in comparison to the Samples 1 (W6–Y18) and 3 (W18–Y6) (Fig. 5). *Hejazi* and *Wijffels* [32] studied three different light intensities: 1.5×10^{-8} (low), 2.7×10^{-8} (intermediate), and 4.5×10^{-8} (high) µmol/s per cell in *D. salina* species. The results showed that with increasing light intensities, the beta-carotene, which was accumulated in the cells, increased. Also, the beta-carotene extraction rate was enhanced by increasing the light exposure. *Li et al.* [23] reported that the red light could improve accumulated carotenoid content within *D. salina* cells compared to the blue and white light spectra. *Xu* and *Harvey* [24] stated that *D. salina* cells under red light had the maximum carotenoid content compared with the cells that were under white and blue (lowest content) light spectra. It should be noteworthy to mention this point that in all cases, more than 80 percent of the total carotenoid content was beta-carotene. *Han et al.* [25] assessed beta-carotene synthesis in *D. salina* cells under 50 µmol/(m^2 s) red, blue, and white light and revealed that the highest beta-carotene accumulation happened under red light spectra. *Gordillo et al.* [33] used various light intensities ranging from darkness to 1500 µmol/(m^2.s) to investigate the changes in pigment composition in *Dunaliella viridis.* A rise in irradiance from 700 to 1500 µmol/(m^2.s) did not cause further considerable changes in pigment composition. Moreover, *Orset* and *Young* [34] studied the culture characteristics of *D. salina* grown under light irradiation of 50 to 1,250 µmol/(m^2. s) after eight days of treatment. The highest carotenoid content was at 1250 µmol/(m^2. s) with the amount of 10.35 ± 0.89 mg/L. Fu *et al.* [35] used a combination of red *LED* (75%) with blue *LED* (25%) and found that this mixture allowed growth at a *higher* total photon flux in *D. salina* species, and additional blue light, instead of red light, could cause an increase in beta-carotene synthesis within the cells. *Hashemi et al.* [19] investigated the beta-carotene production by *D. salina* within the hybrid tubular photobioreactor under white light spectra and various salt concentrations. They stated that the highest beta-carotene production was 4.85 µg /mg under white light spectra and 2.5 M salt concentration. (Fig. 5)

From a beta-carotene production point of view, it was shown that the best single-colored light for beta-carotene synthesizing within the cells was yellow. It declared that the use of white light was necessary for a better microalgae growth process in the early days of cultivation. As shown in Fig. 6 the highest beta-carotene content (16.93 µg of beta-carotene per mg of cell dry weight) was recorded in a combination of four colored lights: white, blue, red, and yellow respectively, each in equal periods of six consecutive days. It could be concluded that for the best beta-carotene synthesis, the cells should be exposed to white and blue light in the early days of cultivation for a favorable growth pattern (lag phase and early stages of logarithmic phase), and in the latter stages of the log phase to the stationary phase, red light and then yellow light

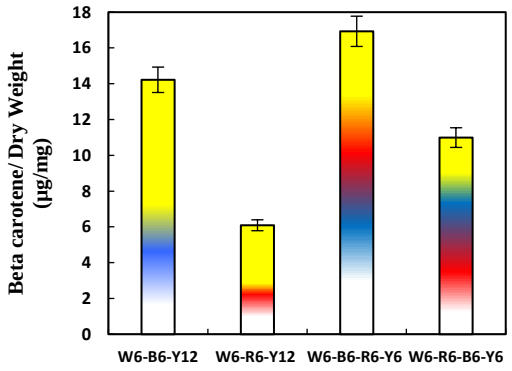

Fig. 6: Beta-carotene per dry weight of cells (µg/mg) for a consecutive combination of colored light, white, red, blue, and yellow spectrums at 1 M NaCl (W: white light, B: blue light, R: red light, Y: yellow light, each number after abbreviations indicated the number of days that each sample remained under a particular light spectrum).

could create the highest beta-carotene content within the chloroplast of the cells as they did under a single-colored light. (Fig. 6)

CONCLUSIONS

The investigation of beta-carotene production within *D. salina* cells showed that the single-colored lights had a better effect on the beta-carotene synthesis of the cells in comparison with the single white light (yellow and red were better than the blue light spectra). Also, in the sequential combination of colored light, the W6-B6-R6-Y6 and W12-Y12 samples had the highest, and W6-R6-Y12 samples had the lowest beta-carotene production, respectively. It should be noted that the W6-R6-Y12 sample had the lowest accumulated beta-carotene level even in comparison with the single-colored white light. So it was found that this light spectra sequence didn't have a suitable effect on the beta-carotene production within *D. salina* cells. It could be concluded that the red light wasn't a good choice in the initial days of cultivation and logarithmic phase of growth Concerning high beta-carotene production.

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REFERENCE

[1] Tandeau de Marsac N., Houmard J., Adaptation of Cyanobacteria to Environmental Stimuli: New Steps Towards Molecular Mechanisms, *FEMS. Microbiol. Lett*., **104(1-2)**: 119-189 (1993).

[2] Hashemi A., Pajoum Shariati F., Sohani E., Azizi S., Hosseinifar S.Z., Delavari Amrei H., CO_2 Biofixation by *Synechococcus Elongatus* from the Power Plant Flue Gas under Various Light–Dark Cycles. *Clean. Technol. Envir.*, 1-9 (2020),

[3] Tavakoli O., Hajinajaf N., Fallahi A., Sarafzadeh M.H., Cultivation of Mixed Microalgae Using Municipal Wastewater: Biomass Productivity, Nutrient Removal, and Biochemical Content. *Iran. J. Biotechnol.* (2020),

[4] Azizi S., Hashemi A., Pajoum Shariati F., Bonakdarpour B., Safamirzaei M., Fouling Identification in Reciprocal Membrane Photobioreactor (RMPBR) Containing *Chlorella Vulgaris* Species: Hydraulic Resistances Assessment. *J. Chem. Technol. Biotechnol*., (2020),

[5] Norton T.A., Melkonian M., Andersen R.A., Algal Biodiversity. *Phycologia.*, **35(4)**: 308-326 (1996).

[6] Rodolfi L., Chini Zittelli G., Bassi N., Padovani G., Biondi N., Bonini G., Microalgae for Oil: Strain Selection, Induction of Lipid Synthesis and Outdoor Mass Cultivation in a Low- Cost Photobioreactor, *Biotechnol. Bioeng.*, **102(1)**: 100-112 (2009).

[7] Ben-Amotz A., Glycerol Production in the Alga *Dunaliella*. Biochemical and Photosynthetic Aspects of Energy Production, 191-208 (1980).

[8] Jahnke L. S., White A. L., Long-term Hyposaline and Hypersaline Stresses Produce Distinct Antioxidant Responses in the Marine Alga *Dunaliella Tertiolecta*, *J. Plant. Physiol.* **160(10)**: 1193 (2003).

[9] Phadwal K., Singh P., Isolation and Characterization of an Indigenous Isolate of *Dunaliella* sp. for β-carotene and Glycerol Production from a Hypersaline Lake in India, *J. Basic Microbiol*., An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms. **43(5)**: 423-429 (2003).

[10] Aasen A., Eimhjellen K., Liaaen-Jensen S., An Extreme Source of Beta-Carotene, *Acta. Chem. Scand.*, **23(7)**: 2544-2545 (1969).

[11] El-Baky H. A., El-Baz F. K., El-Baroty G. S., Production of Antioxidant by the Green Alga *Dunaliella salina*, *Int. J. Agric. Biol.* **6(1)**: 1560-8530 (2004).

[12] Hashemi A., Pajoum Shariati F., Delavari Amrei H., Heydarinasab A., Growth Pattern and β-Carotene Production of *Dunaliella salina* Cells in Different Salinities. *J. Food. Technol. Nutr,* **16(4)**: 45-50 (2019).

[13] Hashemi A., Pajoum Shariati F., Delavari Amrei H., Heydarinasab A., The Effect of Instantaneous and Slow Release Salt Stress Methods on Beta-Carotene Production within *Dunaliella Salina* Cells. *Journal of Chemistry and Chemical Engineering (IJCCE),* **40(5):** 1642-1652 (2021).

[14] Chisti Y., Biodiesel from Microalgae, *Biotechnol. adv.,* **25(3)**: 294-306 (2007).

[15] Azizi S., Bayat B., Tayebati H., Hashemi A., Pajoum Shariati F., Nitrate and Phosphate Removal from Treated Wastewater by *Chlorella Vulgaris* under Various Light Regimes within Membrane Flat Plate Photobioreactor. *Environ. Prog. Sustain. Energy.*, (2020).

[16] Leon R., Martın M., Vigara J., Vilchez C., Vega J.M., Microalgae Mediated Photoproduction of β-Carotene in Aqueous–Organic Two Phase Systems, *Biomol. Eng.*, **20(4-6)**: 177-182 (2003).

[17] Del Campo J.A., García-González M., Guerrero M.G., Outdoor Cultivation of Microalgae for Carotenoid Production: Current State and Perspectives, *Appl. Microbiol. Biotechnol.*, **74(6)**: 1163-1174 (2007).

[18] Telfer A., What is β–Carotene Doing in the Photosystem II Reaction Centre?, Philosophical Transactions of the Royal Society of London B: Biological Sciences. **357(1426)**: 1431-1440 (2002).

[19] Hashemi A., Monire M., Pajoum Shariati F., Delavari Amrei H., Beta-Carotene Production within *Dunaliella Salina* Cells under Salt Stress Condition in an indoor Hybrid Helical Tubular Photobioreactor. *Can. J. Chem. Eng.* **98(1)**: 69-74 (2020).

[20] Delavari Amrei H., Nasernejad B., Ranjbar R., Rastegar S., An Integrated Wavelength Shifting Strategy for Enhancement of Microalgal Growth Rate in PMMA-and Polycarbonate-Based Photobioreactors, *Eur. J. Phycol.*, **49**: 324-331 (2014).

[21] Morowvat M. H., Ghasemi Y., Culture Medium Optimization for Enhanced β-Carotene and Biomass Production by *Dunaliella Salina* in Mixotrophic Culture, *Biocatal. Agric. Biotechnol.*, **7**: 217-223 (2016).

[22] Helena S., Zainuri M., Suprijanto J., Microalgae Dunaliella Salina (Teodoresco, 1905) Growth Using the LED Light (Llight Limiting Dioda) and Different Media. *Aquat. Procedia*, **7**: 226-230 (2016).

[23] Li Y., Cai X., Gu W., Wang G., Transcriptome Analysis of Carotenoid Biosynthesis in *Dunaliella Salina* under Red and Blue Light. *J. Oceanol. Limnol*, **38(1)**: 177-185 (2020).

[24] Xu Y., Harvey P.J., Carotenoid Production by *Dunaliella Salina* under Red Light, *Antioxid*, **8(5)**: 123 (2019).

[25] Han S. I., Kim S., Lee C., Choi Y. E., Blue-Red LED Wavelength Shifting Strategy for Enhancing Beta-Carotene Production from Halotolerant Microalga, *Dunaliella Salina*, *J. Microbiol*, **57(2)**: 101-106 (2019).

[26] El Baz F.K., Aboul-Enein A.M., El-Baroty G.S., Youssef A Abdel-Baky H.H., Accumulation of Antioxidant Vitamins in *Dunaliella Salina*, **2**(4)**:** 220-223 (2002).

[27] Leshem Y., Seri L., Levine A., Induction of Phosphatidylinositol 3- kinase- Mediated Endocytosis by Salt Stress Leads to Intracellular Production of Reactive Oxygen Species and Salt Tolerance, *The. Plant. J.*, **51**(2)**:** 185-197 (2007).

[28] Tammam A.A., Fakhry E. M., El-Sheekh M., Effect of Salt Stress on Antioxidant System and the Metabolism of the Reactive Oxygen Species in *Dunaliella Salina* and *Dunaliella Tertiolecta*, *Afr. J. Biotechnol.*, **10(19)**: 3795-3808 (2011).

[29] Ben-Amotz A., Industrial Production of Microalgal Cell-Mass and Secondary Products-Major Industrial Species, Handbook of Microalgal Culture: *Biotechnol. Appl. Phycol.*, **273 (**2004).

[30] Hosseini Tafreshi A., Shariati M., *Dunaliella* Biotechnology: Methods and Applications, *J. Appl. Microbiol.* **107(1)**: 14-35 (2009).

[31] Lamers P. P., Janssen M., De Vos R. C., Bino R. J., Wijffels R. H., Carotenoid and Fatty Acid Metabolism in Nitrogen-Starved *Dunaliella Salina*, a Unicellular Green Microalga, *J. Biotechnol.*, **162(1)**: 21-27 (2012).

[32] Hejazi M., Wijffels R., Effect of Light Intensity on β-Carotene Production and Extraction by *Dunaliella Salina* in Two-Phase Bioreactors, *Biomol. Eng.*, **20(4-6)**: 171-175 (2003).

[33] Gordillo F. J., Jiménez C., Chavarría J., Niell F. X., Photosynthetic Acclimation to Photon Irradiance and its Relation to Chlorophyll Fluorescence and Carbon Assimilation in the Halotolerant Green Alga *Dunaliella Viridis*, *Photosynth. Res.*, **68(3)**: 225-235 (2001).

[34] Orset S. C., Young A. J., Exposure to Low Irradiances Favors the Synthesis of 9-Cis β, β-Carotene in *Dunaliella Salina* (Teod.), *Plant Physiol.*, **122(2)**: 609-618 (2000).

[35] Fu W., Guðmundsson Ó., Paglia G., Herjólfsson G., Andrésson Ó.S., Palsson B. Ø., Enhancement of Carotenoid Biosynthesis in the Green Microalga *Dunaliella Salina* with Light-Emitting Diodes and Adaptive Laboratory Evolution, *Appl. Microbiol. Biotechnol.*, **97(6)**: 2395-2403 (2013).