Physiological and Biochemical Responses of Dinoflagellate *Symbiodinium* sp. to Different Light Intensities

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Abstract

Symbiotic dinoflagellates create valuable bioactive compounds such as carotenoids and lipids, which are used in various industrial fields. However, a small amount of their biomass can be produced by using the suspension-based closed photobioreactors. The present study tried to obtain high lipid and total carotenoid contents from *Symbiodinium* sp. by creating optimal light intensity conditions using a Twin-layer photobioreactor. In this regard, growth rate, biomass, chlorophyll a concentration, and total carotenoid and lipid contents were examined at the light intensities of 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ for 16 days. Based on the results, biomass productivities ranged from 35.7 to 72.0 $g.m^{-2}$ at 50 to 250 µmol photons $m^{-2}s^{-1}$, respectively. In addition, the highest linear growth rates were 2.03, 3.27, and 5.85 $g.m^{-2}d^{-1}$ at the light intensity of 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ between 12-16 days, respectively. Further, the maximum amount of total carotenoids, chlorophyll a, and total lipid was attained 0.85 and 0.96 $g.m^{-2}$, and 27.77% at the light intensity of 250 $\mu mol.m^{-2}s^{-1}$, respectively. The results represented that the immobilization of algal cells in the photobioreactor biofilm resulted in producing high biomass, total carotenoids, and lipids.

Keywords: Biomass, Chlorophyll a, Total carotenoids, Total lipids, Twin-layer photobioreactor, Symbiotic alga.

Introduction

The light quality and quantity affect microalgae physiology significantly, providing the energy needed for photosynthesis (Etheridge and Roesler, 2005). The light leads to variations in the growth dynamics, ultrastructure, biophysics, and physiology of algae for their adaptation to change environmental conditions (Schlöter et al., 2000). Based on the results of the various studies, algal bioactive compounds such as pigments, lipids, and fatty acids react to an increase or decrease in light intensity differently (Treignier et al., 2008; Valenzuela-Espinoza et al., 2011). For example, an enhancement in light intensity can inhibit algae growth (Parkhill and Cembella, 1999) or improve the quality of bioactive com-

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pounds such as polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in dinoflagellates (Fuentes-Grünewald et al., 2009).

The biomedical, biological, and biophysical researchers identified dinoflagellates as the most promising subject due to their two valuable compounds of carotenoids and fatty acids (Benstein et al., 2014; Sugawara et al., 2009). Peridinin is considered as one of the main carotenoids in dinoflagellates, which exists only in this group. Additionally, it is a part of the membrane attached to light receptor complexes (LHCs) in PCP and AcpP complexes. Both complexes play a role as an optical shield, as well as transferring energy to chlorophyll a, which result in increasing absorption range in optical products and transferring excited energy with 95% efficiency to chlorophyll a (Pinto et al., 2000).

Microalgae have attracted much attention due to their higher lipid content compared to the higher plants (Grünewald, 2012). Under adverse and stressful conditions, microalgae enhance lipid production and alter their lipid biosynthetic pathway to lipid formation and accumulation (Hu et al., 2008; Zhang et al., 2014). The results of the previous studies indicated that 20-70% of the total biomass of microalgae can be lipid (Fuentes-Grünewald et al., 2013). Microalgae lipids have high potential for pharmaceutical, chemical, and food industries, and are considered as a suitable source for biodiesel production (Gordillo et al., 1998). The highest percentage of total fatty acid content was observed in *Symbiodinium microadriaticum* among the assessed symbiotic algae (Mansour et al., 1999). Light intensity can improve lipid metabolism and profile (Valenzuela-Espinoza et al., 2011; Yeesang and Cheirsilp, 2011). However, only a small amount of biomass is obtained from culturing the symbiotic dinoflagellates which can produce valuable bioactive compounds by using the suspension-based closed photobioreactors. The present study sought to produce a high lipid and total carotenoid content by providing optimal conditions for culturing *Symbiodinium* sp. by using a Twin-layer photobioreactor.

Material and methods

Preparation and culture conditions in a Twin-layer photobioreactor

Symbiodinium sp. (strain CCATM-210) was prepared from the culture collection of algae in the Department of Marine Biology, Tarbiat Modares University. In addition, culture medium ASP12 was used (Benstein et al., 2014), which was sterilized by autoclaving at 121 ˚C for 20 min. Further, the suspension culture was performed in a 50-ml Erlenmeyer flask with 200×10^3 cells. ml^{-1} at a light intensity of 100 $\mu mol.m^{-2}s^{-1}$ at 23 ± 1 ˚C and photo-period was adjusted to 9 h dark and 15 h light. The symbiotic algae were cultivated in a 50-ml Erlenmeyer flask until reaching the density of 8×10^6 cells. ml^{-1}. Furthermore, the algal inoculant was transferred to 2lit of the prepared medium. The biomass was centrifuged at 500rpm when culture stock in 2 liters reached 4×10^6

cells.ml^{-1} again. Finally, microalga biomass was transferred into the culture discs of a Twin-layer photobioreactor and cultivated at 23±1 ˚C at the light intensity of 50, 100, and 250 µmol.m^{-2}s^{-1} for 16 d (Benstein et al., 2014). During culturing in a Twin-layer photobioreactor, the medium was exchanged every 3 d to compensate evaporation and avoid nutrient depletion.

Determination of growth rate and cell biomass

In order to evaluate the dry weight of microalga, samples were collected every four days and dried in a freeze-drier (Model FD-5010-BT) for 2 h. The specific growth rate was calculated by using Guillard (1973) equation.

$\mu = (\ln X_t - \ln X_0)/t$, day^{-1}

where X_0 demonstrates initial cell density and X_t denotes its density after t days.

Additionally, the biomass concentration (B) of samples was obtained by using the following equation.

$B = W_t - W_{m/a}$ (g.m^{-2})

in which W_t and W_m are respectively considered as total (biomass + membrane) and membrane weight, and a illustrates inoculation area (m^2).

Determination of chlorophyll a concentration and total carotenoid content

Algal cells were extracted by pure methanol and 2% ammonium acetate solvent. To this end, 4.5 ml of pure methanol was added into extracted biomass and vortexed for 120 s. In addition, the supernatant was placed in ice bath under dark conditions for 2 h. Further, 0.5 ml of 0.5 M ammonium acetate (pH: 7.2) was poured, vortexed again, and stored at -20 ˚C overnight. Furthermore, the samples were vortexed for 10 s over three 30 min periods. In the next stage, the insoluble impurities were removed by double centrifugation at 3500 rpm at 4 ˚C for 10 min. Then, the concentration of chlorophyll a was measured by using a 5100-Vis spectrophotometer at 665 nm and calculated by using the following equation.

$A = \varepsilon\lambda.c.d$

where A represents the absorbance of chlorophyll at wavelength (λ), which can be obtained by using a UV–Vis absorbance spectrometer. Additionally, d indicates the path length of cuvette (cm), ε refers to molar extinction coefficient, and c demonstrates an accurate molar concentration (Porra et al., 1989).

The carotenoid content was determined as follows. First, 5 ml of 90% acetone solution was added to the extract and vortexed for 2 min. Additionally, the pellets were placed in an ultrasonic bath at 4 ˚C for 15 min. Further, the material was kept in an ice bath under dark conditions for 2 h. Furthermore, the samples were vortexed for 4 s over four 30-min periods and stored at 20 ˚C overnight. Finally, the acetone extract was measured using a spectrophotometer at 470 nm and obtained by some researchers (Jeffrey and Haxo, 1968; Jeffry et al., 1975; Lira et al., 2017; Prezelin and Haxo, 1976).

Measurement of total lipid content

In the study, lipid was extracted from the culture. For this purpose, 50 mg of each sample was soaked in 4 ml of distilled water

and homogenized for 1min. After pouring 10ml of methanol and 5 ml of chloroform the samples were restored for 5 min and homogenized. In addition, the mixture was rested for 15 min, 5 ml of chloroform was added, and the resultant mixture was again rested for 15 min to obtain lipid extract. Further, the sample was vigorously stirred after adding 5 ml of distilled water and transferred to a decanter container for 12 h in order to help separate two phases. Furthermore, the lower chloroform phase containing lipid was poured to the pre-weighed 50-ml Erlenmeyer flasks and the level of their solutions was equalized by adding chloroform. Then, lipid solution was dried in the boiling water by nitrogen. Finally, concentrated sediments were weighed and total lipid content was calculated (Bligh and Dyer, 1959; Nigam et al., 2011; Zhukova, 2007).

Statistical analysis

Experimental treatments were compared in three replicates, data were analyzed by SPSS and Excel software, and their homogeneity was examined through Kolmogorov-Smirnov test. Further, one-way ANOVA and Duncan statistical test were utilized to compare the differences between 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ light intensities. The significance of each parameter was reported at 95% ($p<0.05$).

Results

The effect of different light intensities on the productivity of *Symbiodinium* sp. biomass was measured at 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ on days 4, 8, 12, and 16 (Fig. 1A). Based on the results, biomass productivities increased over the time and a significant difference was observed between the obtained biomass at all three light intensities ($P< 0.05$). Additionally, the highest and lowest biomass was produced at 250 and 50 $\mu mol.m^{-2}s^{-1}$, respectively. Fig. 1B displays the linear growth rate of *Symbiodinium* sp. As demonstrated, the maximum linear

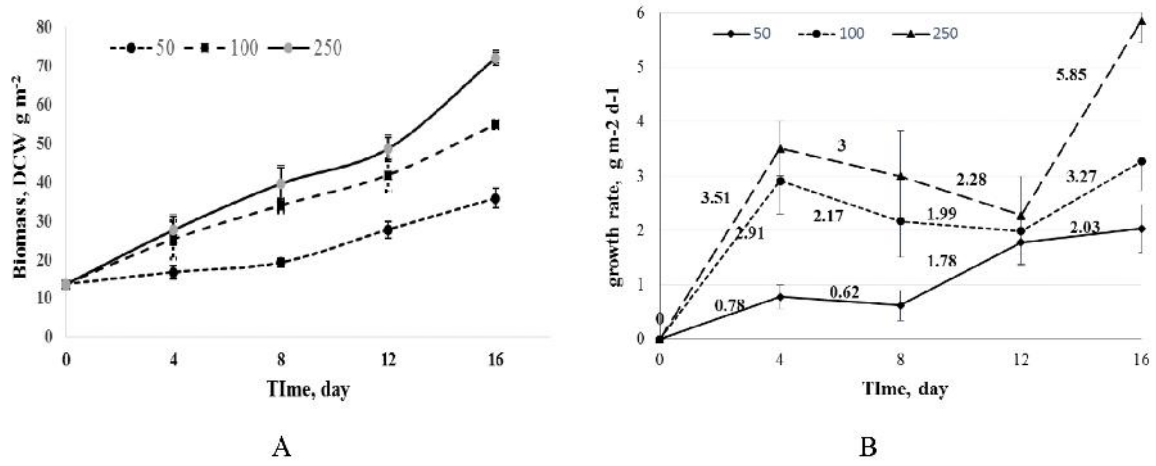

Fig. 1. The effect of different light intensities on the dry biomass of *Symbiodinium* sp. ($g.m^{-2}$) (A) and linear growth rate of *Symbiodinium* sp. ($g.m^{-2}d^{-1}$) (B) under the light intensities under study by the 16th day (mean ± SD, n = 6, six replicate filters)

growth rate was respectively determined 2.03, 3.27, and 5.85 $g.m^{-2}d^{-1}$ at the light intensity of 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ between 12-16 days. Under 50 µmol photons $m^{-2}s^{-1}$, an increase in linear growth rate from 0.78 by 2.6 times results in decreasing biomass density to 35.7 $g.m^{-2}$ within 16 days. Further, light intensity increased up to 100 µmol photons $m^{-2}s^{-1}$ in Twin-layer photobioreactor. Furthermore, *Symbiodinium* sp. represents a linear growth rate from 3.5-5.85 g dry weight $m^{-2}d^{-1}$ to 72 $g.m^{-2}$ in vertical growth surface after 16 days at high photon fluence rate (250 µmol photons $m^{-2}s^{-1}$). Based on the Guillard's equation, mean specific growth rate was obtained 0.06, 0.08, and 0.10 d^{-1} at the light intensities of 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$, respectively. In general, biomass density and growth rate increased by raising light intensity.

The evaluation of the bioactive compounds produced by *Symbiodinium* sp. indicated the effec tiveness of low and high light intensities on biomass production. In addition, the maximum and minimum concentration of chlorophyll a was 0.96 and 0.69 $g.m^{-2}$ at the light intensities of 250 and 50, respectively. Further, an increase in light intensity resulted in enhancing chlorophyll a concentration (Fig. 2A). Furthermore, the total carotenoid content was determined 0.36, 0.61, and 0.85 $g.m^{-2}$ at the light intensities of 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ by the 16th day, respectively (Fig. 2B). Thus, total carotenoid content developed by rising light intensity. The statistical results demonstrated a significant difference in the amount of total carotenoid content and chlorophyll a concentration at the intended light intensities by the 16th day ($P< 0.05$)

Finally, total lipid percentage was significantly different ($p<0.05$) at the light intensities under study by the day 16, the lowest and highest of which were 19.5 and 27.77%, respectively, at dry weight at 50 and 250 $\mu mol.m^{-2}s^{-1}$ light intensities (Fig. 3). Therefore, the amount of total lipid production improved by increasing light intensity.

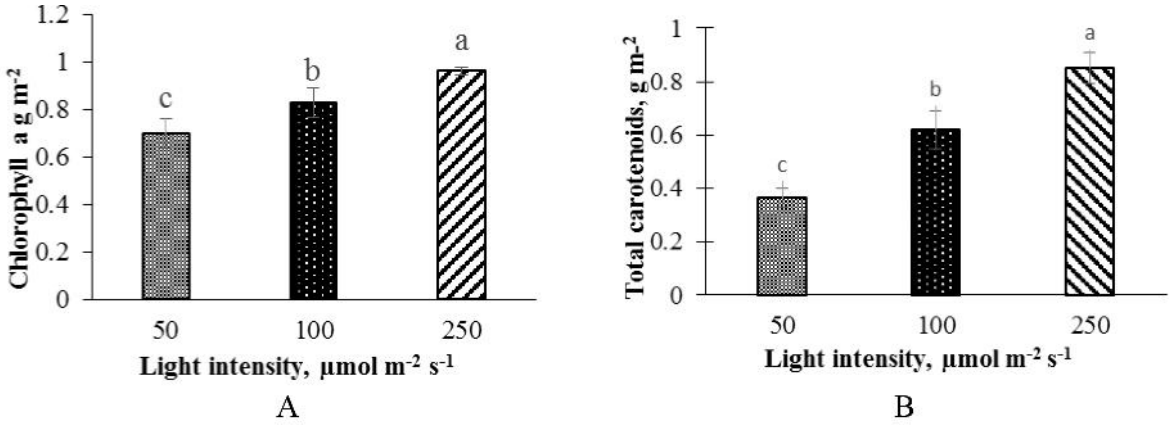

Fig. 2. (A) Chlorophyll a and (B) total carotenoid contents in *Symbiodinium* sp. at 50, 100, and 250 µmol photons $m^{-2}s^{-1}$ (a, b, c, standard deviation, n = 6, six replicate filters)

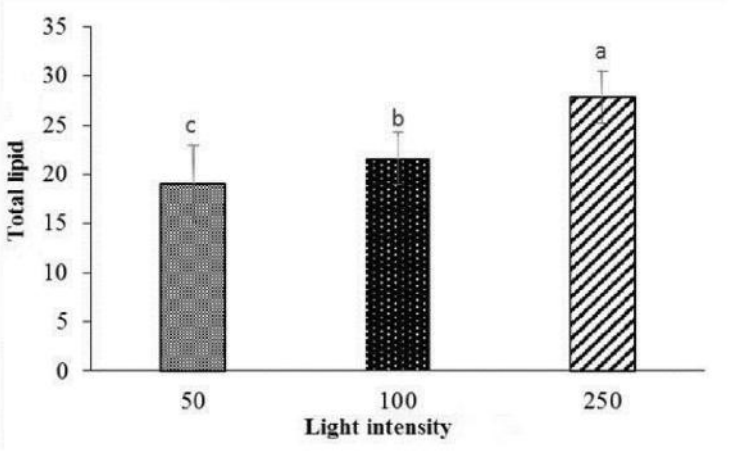

Fig. 3. Total lipid percentage in *Symbiodinium* sp. under the intended light intensities (a, b, c, standard deviation, n = 6, six replicate filters)

Discussion

The light intensity influences the growth efficiency of symbiotic dinoflagellates in vivo (Fuenes-Grünewald, 2012; Graham et al., 2010). Wilkerson et al. (1983) assessed the growth rate of *Symbiodinium microadriaticum* in different hosts and reported specific growth rates from 0.01 to 0.1 d^{-1}. Obviously, growth rate in the laboratory-controlled culture conditions can be several times higher than that in the natural environment. In the present study, the growth rate of the symbiotic microalga *Symbiodinium* sp. under study enhanced under light from 0.6 to 0.1 d^{-1}. Additionally, linear growth rate kept increasing by raising light intensity to 250 $m^{-2}s^{-1}$, which demonstrates that growth inhibition has to be occur at the light intensities above those under study. The 470 $\mu mol.m^{-2}s^{-1}$ light intensity can prevent dinoflagellates from growing (Parkhill and Cembella, 1999) although the issue was not considered in the present study. In general, the range of the biomass productivity of the *Symbiodinium* sp. under study was similar to that of other microalgae immobilized on Twin-Layers under comparable conditions (Benstein et al., 2014; Podola et al., 2017). Based on the results of the present study, biomass content enhanced linearly under all three light intensities although growth rate at the light intensity of 50 $\mu mol.m^{-2}s^{-1}$ was lower than that of two others, which may be related to the difference in biofilm division into two photosynthetic activities (Kiperstok, 2016; Li et al., 2015).

The absorption of light wavelengths by protective pigments increases under the high light intensity. When affecting microalgae such as *Symbiodinium* sp. by high light, their photosynthetic apparatus prevents damage from photosynthetic active radiation (Hoegh-Guldberg and Jones, 1999). The results of cultivating microalga under the low light illumination (50 $\mu mol.m^{-2}s^{-1}$) represented that the total carotenoid con-

tent was determined 0.36 $g.m^{-2}$, while the immobilized cells accumulated up to 0.85 $g.m^{-2}$ of total carotenoids under the higher photon fluency (250 $\mu mol.m^{-2}s^{-1}$). Further, the obtained carotenoid pattern indicated an enhancement in total carotenoid such as peridinin production because of improving light intensity. Furthermore, a relationship was observed between raising light intensity and increasing total carotenoids linearly with its protective role in maintaining photosynthetic system and biomass growth process (Khalesi and Lamers, 2010; Kiperstok, 2016; Schlöter et al., 2000).

Regarding the cultivation of most species under limited and optimal light, the concentration of chlorophyll a and photosynthetic pigments is linearly related to growth rate (Goericke and Montoya, 1998). The results of the present study reflected an improvement in growth rate and chlorophyll a by enhancing light illumination. The changes in chlorophyll a concentration are based on the variations in PSU count (Prézelin, 1976). However, the stimulating effect of light intensity on microalgae reduces the function of photosystem II, electron transfer, and chlorophyll a, and consequently decreases growth (Lesser, 1996).

Algae adapt to environmental conditions by altering lipid metabolism. In general, microalgae increase lipid content under unfavorable and stressful conditions (Gordillo et al., 1998; Nigam et al., 2011). Comparatively, the biomass and total lipid contents up to 27% related to some of the dinoflagellates cultured in a photobioreactor are quite similar to those of the green algae such as *Chlorella minutisima* and *Scendesmum obliquus*, as well as the different species of *Botryococcus*, which are often used to produce biodiesel (Fuentes-Grünewald et al., 2013; Rodolfi et al., 2009; Yeesang and Cheirsilp, 2011). The results of the present study regarding the effect of light intensity on total lipid content in *Symbiodinium* sp. demonstrated that high intensity led to greater total lipid content. In addition, the maximum and minimum total lipid yield at 250 and 50 $\mu mol.m^{-2}s^{-1}$ were 27.83 and 19.31% of dry weight, respectively. Comparing the total lipid content of *Symbiodinium* sp. and *Alexandrium catenella* (27%), *Amphidinium* sp. (18.9%), *Scrippsiella* sp. (16%), *Symbiodinium microadriaticum* (15%), *Gymnodinium* sp. (22.6%), *Gymnodinium sanguineum*, and *Fragilidium* sp. (13%) of dry weight indicated that the studied species can be considered as a prospective species with fairly high lipid content (Fuentes-Grünewald et al., 2009; Islam et al., 2013; Mansour et al., 1999).

Further, light intensity particularly affects growth and bioactive compounds. Furthermore, the content of biomass, chlorophyll a, carotenoid, and total lipid improved following an enhancement in light intensity, and *Symbiodinium* sp. is considered as light intensity-resistance. At the same time, it seems that greater lipid percentage is a protective mechanism against high light intensity. Finally, the immobilization of algal cells in the biofilm of photobioreactor resulted in producing maximum biomass and bioactive compounds through biofilm culture method

because of overcoming the hydrodynamic stress of suspension culture on microalgae cells.

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