**Quantitative Analysis on Photon Numbers Received per Cell for Triggering β-Carotene Accumulation in *Dunaliella salina***

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**ABSTRACT**

Accumulation of β-carotene in *Dunaliella salina* is highly dependent on light exposure intensity and duration, but quantitative analysis on photon numbers per cell for triggering β-carotene accumulation is not available so far. In this study, experiment results showed that significant β-carotene accumulation occurred with at least 8 hours illumination at 400 µmol photons·m-2·s-1. To quantifying the average number of photons received per cell (APRPC), correlation between light attenuation with light path, biomass concentration, and β-carotene content was built with both Lambert-Beer and Cornet models, and the latter provided a better simulation. With Cornet model, APRPC was calculated and proposed as a parameter for β-carotene accumulation. It was found that once APRPC reached 0.7 µmol photons cell-1, β-carotene accumulation was triggered, and it was saturated at 9.9 µmol photons cell-1. This study showed that APRPC can be used as an important parameter in *D. salina* cultivation process, to accurately simulate and control β-carotene production.

**Key words:** β-carotene; average irradiance; *D. salina*; photons received per cell

**INTRODUCTION**

β-carotene has wide applications in the nutraceutical, cosmetic and food industries, and its current global consumption is about 1000 tons per year (Gong & Bassi, 2016). There has been a rising demand for natural β-carotene, instead of synthetic products, due to the advantages of its mixed stereoisomers of all-*trans* and 9-*cis* β-carotene, which are more fat-soluble and less crystallizable than synthetic β-carotene (Combe et al.,2015). Microalgae can serve as promising feedstock for natural β-carotene production, among which, green alga *Dunaliella salina* has been regarded as the best candidate because of its high β-carotene content (up to 14%) (Fachet et al., 2016; Rabbani et al., 1998).

A two-stage cultivation strategy is usually applied for β-carotene accumulationin *D. salina*. In the first stage, also known as ‘green stage’, nutrient replete medium and optimal light intensity are adopted to obtain as many as green vegetative cells. Then in the second stage (also refer to as ‘red stage’ or ‘induction stage’), green cells are subjected to various stress conditions such as high irradiance, high salt and nitrate/phosphate deprivation to stimulate β-carotene accumulation (Kleinegris et al., 2011; Lamers et al., 2012; Masojidek et al., 2009). Up till now, such stress-induced *D. salina* β-carotene accumulation is mostly qualitatively investigated (Bonnefond et al., 2017; Fachet et al., 2016; Gong & Bassi, 2016; Phadwal & Singh, 2003), while the exact quantitative relationship between stress and β-carotene accumulation is so far unknown.

Among various stresses, light irradiance is considered to be the most important environmental parameter for β-carotene induction (Lamers et al., 2010). Many efforts have been made on the coordination between incident light intensity and carotenoids accumulation in *D. salina* (Fachet et al., 2016; Gomez & Gonzalez, 2005; Wu et al., 2016; Xu et al., 2016). However, since light penetration logarithmically decreases with cell growth and β-carotene accumulation, the relationship between initial light intensity at the culture medium surface and final β-carotene content in *D. salina* cells is ambiguous, thus cannot be applied as real-world β-carotene production guidance, which unfortunately takes place in various cultivation configurations and at different culturing densities. Quantifying photon numbers received per cell to initiate β-carotene accumulation, therefore, is necessary for establishing a meaningful relationship between light irradiance and *D. salina* β-carotene production.

Clarifying average light irradiance (number of photons) reaching each cell in the culture is the prerequisite to realize the above goal. According to previous studies, incident light intensity, microalgae cell density, light path, and light extinction coefficient impacted the average irradiance for microalgae (Huang et al., 2014; McHardy et al., 2018; Yun & Park, 2001), whereas the correlation between light extinction coefficient and average irradiance is complicated by pigment accumulation (especially β-carotene) during the induction stage (Bechet et al., 2013; Fernandez et al., 1997; Martinez et al., 2012). Although the influence of pigment content on light extinction coefficient is available for other microalgal strains (e.g. astaxanthinin *Haematococcus pluvialis*) (Gao et al., 2017; Martinez et al., 2012), little effort has been focused on the impact of β-carotene content on the light attenuation during β-carotene accumulation in *D. salina* suspension, which requires in-depth investigation.

The objective of this study is to quantify the effect of light availability on β-carotene accumulation. Firstly, light distribution in the *D. salina* suspension during β-carotene accumulation stage was simulated using Lambert-Beer model and Cornet model, then the complex relationship between β-carotene accumulation and light absorption coefficient and scattering coefficient was comprehensively investigated. The present study quantified “light stress” via a physical variable, namely the average number of photons received per cell (APRPC). Critical values of APRPC for high β-carotene accumulation in *D. salina* was identified. Results of this study would be of reference value on average irradiance modeling and optimization of culture condition, and could facilitate indoor and outdoor massive *D. salina* β-carotene production.

**MATERIALS AND METHODS**

**Strain and medium**

Themicroalgal *Dunaliella salina* CCAP 19/18was purchased from Culture Collection of Algae and Protozoa (Windermere, United Kingdom). The strain was previously maintained in the medium of modified Artificial Sea Water (ASW), with composition of 1.5M NaCl, 5 mM KNO3, 0.45 mM MgCl2·6H2O, 0.05 mM MgSO4·7H2O, 0.3 mM CaCl2·2H2O, 0.13 mM K2HPO4, 0.02 mM FeCl3, 0.02 mM EDTA, and 1 mL of trace elements stock containing 50 mM H3BO3, 10 mM MnCl2·4H2O, 0.8 mM ZnSO4·7H2O, 0.8 mM CuSO4·5H2O, 2 mM NaMoO4·2H2O, 1.5 mM NaVO3, and 0.2 mM CoCl2·6H2O, and the pH was adjusted to 7.5 by adding 40 mM of Tris-buffer (Doddaiah et al., 2013). Stock culture was performed in 500 mL conical flasks at 50 µmol photons·m-2·s-1 light intensity. After reaching steady state, the microalgal biomass was inoculated into a set of multi-device-equipped flat-plate photobioreactors (PBRs) named “Algal Station” (Cao et al., 2019).

**Cultivation condition**

Incident light intensities on the PBR surface were controlled by white LEDs. Light paths of the PBR were 0.025 m, 0.05 m and 0.10 m. The cultivation temperature was automatically controlled at 25±0.5℃, while pH level was maintained at 7.5±0.2 by pulsed CO2 mixed with air. Cultures in the PBR were agitated with 0.2 µm membrane filtered air at 400 mL·min-1. Incident light intensities, and transmitted light intensities were recorded online*.*

**Experimental design**

**Light transfer modeling in PBRs**

To model the light distribution inside the culture, a set of experiments were conducted at incident light intensity 400 µmol photons·m-2·s-1 with various biomass concentrations (0.10, 0.25, 0.50, 0.75, 1.0 and 1.5 g·L-1) and light paths (0.025 m, 0.05 m and 0.10 m). Light intensity inside the culture, along with biomass concentration, was measured at different light paths. This data was used to model light attenuation as a function of biomass concentration and light path, the measurements were carried out in triplicate.

**APRPC calculation**

The average number of photons received per cell was calculated, termed as APRPC, calculated by Eq. (1)

*APRPC =*  (1)

where *APRPC* is average number of photons received per cell (μmol photons·cell-1), *Iav* is the volume-averaged irradiance (μmol photons·m-2·s-1), *T* is the induction time (s), *S* is the area of illumination surface (0.051 m2), and *C* is cell number (cell·L-1)

**Short-term effect APRPC on β-carotene accumulation**

*D. salina* were cultivated for different time lengths and under different average irradiances, each treatment was repeated three times. A set of experiments were conducted at various average irradiance levels (100, 200, 400, 800, 1200 and 1600 µmol photons·m-2·s-1) and induction times (2, 4, 8, 12, 16, 24 h). In this experiment, the average irradiance inside the PBRs was kept constant through adjusting incident light intensity with increased cell concentration during the whole induction periods, which was realized by a self-controlled system named as “Algal Station” developed by Cao, et al. (2019), and the ratio of incident irradiance and biomass concentration was used to optimize APRPC in batch cultures, thus APRPC could be controlled during the β-carotene accumulation induction stage. Pigment content and biomass measurement was conducted within 24 hours of sampling. In general, when β-carotene content was 2 times higher than the initial content, it was regarded as significant β-carotene accumulation.

**Long-term effect of APRPC on β-carotene accumulation**

In order to get higher β-carotene content, long-term effect of APRPC on β-carotene accumulation in *D. salina* was investigated. A set of experiments were conducted at various average irradiance levels (50, 100, 400, 800, and 1200 µmol photons·m-2·s-1) and induction times (24, 48, 72, 96, 120, 144 h), the measurements were carried out in triplicate.

**Analytical methods**

**Dry weight determination**

Dry weight was determined using pre-weighed Whatman GF/C filters (47 mm diameter). 10 mL cultures were filtered and washed three times with 2 mL 0.5 M ammonium bicarbonate and then were dried at 60°C for over 16 h until the weight was constant, dry weight (g∙L-1) of the microalgae cells was calculated by subtracting the clear filter weight from the final weight (Chi et al., 2016).

**Pigment measurement**

For determining the amount of pigments including chlorophyll (Chla and Chlb) and carotenoids, about 10 mg of dried biomass was extracted with 1 mL 90% (v/v) acetone, vortexed for 20 s, and then centrifuged at 10,000 g for 2 min. The above pigment extraction procedure was repeated until the solution was colorless. The absorbance of Chla, Chlb and carotenoids content was measured at 665 nm, 645 nm, and 470 nm respectively, using a UV/VIS spectrophotometer (Jasco V-530, JASCO Corporation, Japan).

*CChla* (mg∙L-1) *=11.75 (A665)-2.35 (A645)*  (2)

*CChlb* (mg∙L-1) *=18.61 (A645)-3.96(A665)*  (3)

*Total carotenoids* (mg∙L-1)= *(1000A470-2.270 CChla-81.4 CChlb)/198* (4)

*C*: pigment concentration (mg∙L-1) , *Ax* is absorbance at x nm wavelength.

*Pigment content* (%) *=*  (5)

*M*: dry cell weight (mg).

The modified spectrophotometric method was used to determine β-carotene content in biomass (Zhu et al., 2018). 1 mL of cell suspension was centrifuged at 10,000 rpm for 2 min. After centrifugation, the supernatant was discarded and 3 mL dodecane was added. The sample was shaken vigorously to re-suspend the algae pellets. Then, 9 mL of methanol was added to completely break up the cells and the tube was shaken vigorously again, then centrifuged for 2 min at 10,000 rpm. The dodecane-containing lipophilic carotenoids (upper layer) were measured with a spectrophotometer (Jasco V-530, JASCO Corporation, Japan) at 453 nm and 665 nm with dodecane as reference. β-carotene concentration was calculated as Eq. (6):

*Cβ-car* (mg∙L-1) *= (A453- A665/3.91)×3.657×3×X*  (6)

where: *(A453−A665/3.91*) is the absorbance of β-carotene corrected for chlorophyll contamination, 3.657 is the calibration factor derived from HPLC analysis of β-carotene concentration ([Zhu et al., 2018](#_ENREF_33)), 3 is the amount of milliliters of dodecane added for extraction, and *X* is the dilution factor to measure absorbance on spectrophotometer.

The amount of β-carotene in the algae biomass was calculated according to Eq. (7).

*β-carotene (%) =*  (7)

*Cβ-car* is the β-carotene content (mg∙L-1)，*DW* is the cell dry weight (g·L-1).

**Light distribution model establishment**

**Light attenuation analysis**

Two models were adopted to analyze light attenuation inside the microalgae suspension, i.e., Lambert-Beer model (Eq. (8)) (Bechet et al., 2013), and Cornet model (Eqs (9-11)) (Fernandez et al., 1997).

= (8)

where *I* is the local light intensity (μmol photons·m-2·s-1), *I0* is the incident light intensity (µmol·photons·m-2·s-1), *Ka* is the extinction coefficient (m2·g-1), *X* is the microalgae concentration (g·L-1), *b* is the fitting constant (m−1) and *L* is the light path (m).

*=* (9)

(10)

(11)

where *Ea* is the mass absorption coefficient (m2·g-1), and *Es* is the mass scattering coefficient (m2·g-1), *L* is the light path (m), and *X* is the microalgae concentration (g·L-1). *α1* and *α2* represent the correlation between *Ea* and *Es*. Matlab 2014 was employed to estimate the parameters of Lambert-Beer model and Cornet model.

**Average irradiance calculation**

In the flat plate reactor, the average light intensity can be calculated as Eq. (12) (Suh & Lee, 2001).

*Iav =* (12)

where *Iav* is the volume-averaged irradiance (μmol photons·m-2·s-1), *V* is the volume of the PBR (L), and *I* is the local light intensity (μmol photons·m-2·s-1).

**RESULTS AND DISCUSSION**

**Light attenuation in *D. salina* cell suspension**

Light distribution in the *D. salina* suspension with incident light intensity of 400 µmol photons·m-2·s-1 is shown in Fig. 1. For all light paths, as the PBR was well-mixed, local light intensity attenuated exponentially with the increase of the cell concentration, and longer light paths displayed much faster light attenuation. At 0.25 g·L-1 cell concentration, the local light intensity at 0.025 m, 0.05 m, 0.10 m light paths respectively decreased by 51.8%, 68.60% and 89.2% of the incident light intensity. While at 0.5 g·L-1 cell concentration, corresponding local light intensities were respectively reduced by 65.34%, 83.36% and 96.17%. At 1.50 g·L-1 algae concentration, 22.34, 5.31 and 0.30 µmol photons·m-2·s-1 light intensity were measured at 0.025 m, 0.05 m, and 0.10 m light paths, respectively, holding only 0.08-5.6% of incident light intensity. It was found in previous study that less than 10 µmol photons·m-2·s-1 local light intensity limited microalgae growth (Naderi et al., 2015; Naderi et al., 2017), therefore, the areas inside the PBR that experience <10 µmol photons·m-2·s-1 light intensities can be considered as dark zone. Results in this study showed that there was no dark zone when cell concentration was ≤ 0.5 g·L-1, but when cell concentration increased to 0.75 g·L-1, dark zone occurred at ≥0.05 m light path. Such phenomenon showed strong influence of *D. salina* cell concentration on light attenuation, especially at longer light paths. This agreed with the results of Fachet et al. that after 3 days of cultivation under low light conditions, all supplied light was absorbed by cells (Fachet et al., 2014).

Fig.2 demonstrates the variation of transmittance (*I/I0*) with different cell concentrations at three different light paths. The results show that at low cell concentrations (˂ 0.75 g·L-1) light transmittance reduced significantly with increasing cell concentrations. When cell concentration > 0.75 g·L-1, however, *I/I0*leveled off at 0.1 for 0.025 m light path and almost zeroed at 0.05 m and 0.10 m light paths. Results of these experiments are consistent with those of Naderi et al. (Naderi et al., 2017), who developed a light distribution model for estimating local light intensity inside PBR culturing *Chlorella vulgaris* and found that, when the biomass increased from 0.09 g·L-1 to 1.34 g·L-1, light intensity attenuated rapidly by 77% over very short light paths (0.01 m).

The parameters calculated in this study were in accordance with the classical Lambert-Beer model and Cornet model on light attenuation evaluation. Light attenuation fitted well with the Lambert-Beer model (*Ka*=0.08 ± 0.006 m2·g-1, *b*=1.280 ± 0.175 m−1, *R2*=0.984), and it fitted slightly better with the Cornet model (*Ea* =0.023 ± 0.002 m2·g-1, *Es*=0.749 ± 0.051 m2·g-1, *R2*=0.993). Previous studies revealed that light attenuation in *Haematococcus pluvialis* culture was better explained using Cornet model rather than Lambert-Beer model (Garcia-Malea et al., 2006; Sheng et al., 2018),whereas light attenuation in *Phaeodactylum tricornutum* suspension at low biomass concentration preferred Lambert-Beer model (Fernandez et al., 2000). Such discrepancy could be because the size of the *D. salina* cells was much bigger (15-20 μm at the β-carotene accumulation stage) comparing with 3-8 μm of *P.* *tricornutum* cells, while around the same level with *H. pluvialis* cells, thus the light scattered by microalgal cells could not be neglected.

Clarifying light attenuation patterns with microalgal biomass concentration and light paths is of significant benefit for photobioreactor design/amplification, as well as for cultivation process optimization. To reach this goal, the first task is to determine the interaction between light distribution and biomass concentration, and the light attenuation phenomenon is thought to be due to absorption by photosynthetic and accessory pigments and by microalgal cells themselves at specific wavelengths. However, besides absorption, light scattering at selective wavelengths also take place. In addition to increased biomass concentration, β-carotene accumulation in *D. salina* can also cause light attenuation in the reactor. In order to quantify the light availability inside the PBR, it is necessary to measure the extinction coefficient at different cell β-carotene contents as well.

**Effect of β-carotene content on light attenuation**

During β-carotene accumulation stage of *D. salina*, β-carotene is the dominant pigment, and its content has an impact on light attenuation (Sheng et al., 2018). Thus， the impact of β-carotene content on the light attenuation in the *D. salina* culture was investigated based on the assumption that the size and shape of all microalgae cells were consistent. The parameters in the Lambert-Beer model and Cornet model were estimated using data at different β-carotene contents, and were displayed in Table 1. From Table 1, it was revealed that the extinction coefficient *Ka* in the Lambert-Beer model and the scattering coefficient *Es* in the Cornet model were positively related to β-carotene content, whereas the absorption coefficient *Ea* was negatively related to β-carotene content. *Ka*, *Es* and *Ea* as a function of β-carotene content are respectively displayed in Eqs.(13)-(15).

*Ka* = 0.0466+0.0029×Xβ-carotene content (R2 = 0.98, r = 0.99) (13)

*Es* = 0.70+0.06×Xβ-carotene content (R2 = 0.95, r = 0.98) (14)

*Ea* = 0.02+0.05×EXP(-2.62×Xβ-carotene content)(R2 = 0.90, r = -0.82) (15)

Where Xβ-carotene content is the dry weight content of β-carotene, while r is the Spearman's correlation coefficient, generally, there is a strong correlation between two events if the Spearman correlation coefficient exceeds 0.8 (Zhang et al., 2019), and R2 is the regression coefficient. This was not in accordance with previous observation that decreases in pigment concentrations led to decrease in absorption coefficient (Razmig Kandilian et al, 2014). Indeed, the absorption coefficient was also considered accounting for average amount of light absorbed by the cells. The scattering coefficient was greater than the absorption coefficient, and this difference between both coefficients could be due to the great changes in cellular composition, as well as pigment concentration of the cells (Kandilian et al., 2014). It is noteworthy that scattering coefficients are greater than the absorption coefficients owing to high pigment contents in *D. salina* cells. The result was in accordance with existing studies, indicating that high pigment content would increase light attenuation in low density microalgae suspension (Huang et al., 2014; Sheng et al., 2018).

Fig. 3 displayed the effect of β-carotene content on light attenuation under different biomass concentration, using Cornet model, with relatively low (0.5 g∙L-1) and high (1.5 g∙L-1) biomass concentrations respectively investigated. It was revealed that β-carotene content had a greater influence on light attenuation at low biomass concentration (Fig. 3a), while such influence was not as obvious at high biomass concentration (Fig. 3b). This is the first time to investigate the relationship between β-carotene content and light attenuation with different biomass concentrations in *D. salina.* From these results, the average irradiance in the culture can be accurately simulated and calculated under different β-carotene content and biomass concentrations.

**The relationship between APRPC and β-carotene accumulation**

In order to calculate APRPC during β-carotene accumulationstage, it was necessary to analyze the effect of average irradiances (Iav) and induction times on β-carotene accumulation. A contour-colour fill plot of the variation in β-carotene content with various average irradiances and induction times is displayed in Fig. 4. From the results, it can be seen that β-carotene content increased with Iav and induction time. At 2 h induction time and 100 μmol photons·m-2·s-1 Iav, low β-carotene content (0.70%) was obtained, implying that β-carotene accumulation had not started yet under this culture condition. When induction time was more than 8 h, and Iav was higher than 400 μmol photons·m-2·s-1, more than twice β-carotene content was obtained. Highest β-carotene content (2.83%) was reached at 24 h induction time and Iav of 1600 μmol photons·m-2·s-1.

APRPC varied greatly under different induction times and irradiances (see Table 2), and was a function of β-carotene content for both short-term (≤24 h) and long-term (24~144 h) cultivation, as displayed in Fig.5. Regarding both Table 2 and Fig. 4, it was found that when APRPC reached 0.7 µmol photons·cell-1, β-carotene content was doubled, comparing with original level. As displayed in Fig. 5a, within 24 h of induction, β-carotene content increased with APRPC showing positive correlations. Thus, the quantitative relationship between APRPC and *D. salina* β-carotene accumulation was built. The data displayed in Fig. 5a is also helpful for optimal short-term β-carotene induction condition (average irradiance and induction time) estimation, which can benefit efficient β-carotene production on the whole.

A long-term (24~144 h) light induction strategywas applied to get higher β-carotene content, with results displayed in Fig. 5b. The highest β-carotene content of 7.24% was observed at APRPC of 9.9 μmol photons·cell-1 at the end of cultivation, followed by 7.10% at 8.8 and 13.2 μmol photons·cell-1, and 7.0% at 11 μmol photons·cell-1, respectively. Such observation is consistent with previous findings indicating that a large number of photons were necessary for cells to accumulate large amount of β-carotene (Lamers et al., 2010), and is also consistent with the hypothesis that β-carotene synthesis pathway is activated to quench excess electrons generated in the photosynthetic electron transport chain when cells absorb large quantities of photons (Kandilian et al., 2019). Moreover, the observed inhibited β-carotene accumulation at high APRPC (>9.9 μmol photons·cell-1) could be explained by the photoinhibition and photosystem’s damage caused by high APRPC, and that β-carotene synthesis pathway was limited at longer supersaturating photons exposure, and down-regulation of electron transport through slowing of down-stream electron sinks was induced, thus non-photochemical quenching and net Photosystem II photo inactivation was also induced (Bonnanfant et al., 2019).

The above results confirm the previous statement that microalgae rely on received photons to carry out biochemical reactions (photosynthesis and inorganic carbon fixation) (R. Kandilian et al. 2014). This is the first time that expressing photon numbers received per cell is proposed in the content of β-carotene and its utility validated. Previous study calculated the carotenoid yield (mg·L-1) on absorbed light (mol-1 photons) (Fachet et al., 2016), however, β-carotene content quantified under a given light intensity in terms of APRPC was never carried out. Although Kandilian et al. (Kandilian et al., 2014; Kandilian et al., 2019) proposed a concept of “MVERA” (specific volumetric rate of energy absorption, which was a function of incident irradiance and light attenuation in the PBR depending on biomass concentration and cellular pigment content) to quantify microalgal TAG accumulation and pigment synthesis, they were conducted under nitrogen limited conditions thus MVERA was not under dynamic changes due to stagnant microalgal growth. Some other studies used nutrients sufficient medium to characterize the relationship between average irradiance inside the PBRs and pigment content in *Isochrysis galbana*, *Phaeodactylum tricornutum,* and *Haematococcus pluvialis* (Fernandez et al., 1998; Grima et al., 1994; Zhang et al., 2016). However, average volume irradiation in the PBR was difficult to keep constant because of varying biomass throughout the cultivation process. Different from these previous studies, the average irradiance inside the PBRs in the present research was kept constant by enhancing incident light intensity with increased cell concentration during the whole induction periods, and the ratio of incident irradiance and biomass concentration was used to optimize APRPC in batch cultures, thus APRPC could be controlled during the β-carotene accumulation induction stage, which was realized by a self-controlled system named as “Algal Station” (Cao et al., 2019).

Future studies should focus on developing a kinetic model that predicts β-carotene productivity during APRPC steady condition, and by optimizing the APRPC through adjusting incident irradiance and biomass concentration. Moreover, it can be validating the present observations and quantitative analysis for other microalgae species, as well as developing novel methods to optimize the instantaneous β-carotene production with respect to APRPC. The APRPC depends on biomass concentration, cell pigment content, and incident irradiance, which are dynamic and dependent upon culture conditions (e.g. medium salinity, nitrogen and carbon concentrations, pH, and temperature) and will be difficult to control in a batch cultivation system exposed to solar radiation. The methodology presented here could be extended to investigate the effects of cultivation conditions on the optimal and critical APRPC.

**CONCLUSION**

Light attenuation with biomass concentration and β-carotene content in *D. salina* was studied, and a quantitative model was proposed to reveal the effect of APRPC on β-carotene accumulation. APRPC was correlated with both biomass concentration and incident irradiance. A minimum APRPC of 0.7 µmol photons·cell-1 was necessary to trigger significant *D. salina* β-carotene accumulation. β-carotene content was saturated when APRPC reached 9.9 µmol photons cell-1. Results of this study showed significance in accurately simulating and predicting β-carotene production under different photons numbers, and will be instrumental in setting up protocols for β-carotene production in scaled-up PBRs.

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**Declaration of authors' contribution**

Yimei Xi, Zhanyou Chi designed the research. Yimei Xi, Zhanyou Chi and Jinghan Wang wrote the paper. Yimei Xi analyzed the data. Yimei Xi, Song Xue and Xupeng performed the research and provided technical support. All authors read and approved the final manuscript.

**Declaration of authors agreement to authorship and submission of the manuscript for peer review**

All authors approved the authorship and submission of the manuscript for peer review of the article.

**Conflicts of interest**

The authors declare no competing financial interest.

**Statement of informed consent, human/animal rights**

No conflicts, informed consent, human or animal rights applicable.

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TABLES

**Table 1** Variation of *Ka*, *Ea* and *Es* with different *D. salina* β-carotene contents

|  |  |  |  |
| --- | --- | --- | --- |
| β-carotene content (%) | Lambert–Beer Model | Cornet Model | |
|  | *Ka* (m2·g-1) | *Ea* (m2·g-1) | *Es* (m2·g-1) |
| 0.56 | 0.0478 ± 0.0142 | 0.0316 ± 0.0015 | 0. 718 ±0.021 |
| 0.84 | 0.0493 ± 0.0132 | 0.0242 ± 0.0010 | 0.754 ± 0.017 |
| 1.21 | 0.0504 ± 0.0175 | 0.0213 ± 0.0036 | 0.757 ± 0.014 |
| 1.93 | 0.0519 ± 0.0216 | 0.0207 ± 0.0042 | 0.846 ± 0.018 |
| 2.88 | 0.0558 ± 0.0224 | 0.0196 ± 0.0026 | 0.883 ± 0.013 |
| 4.26 | 0.0587 ± 0.0144 | 0.0167 ± 0.0032 | 0.948± 0.060 |

**Table 2** APRPC (µmol photons·cell-1) at different Iav and induction times

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Iav (μmol photons·m-2·s-1) | Induction time (h) | |  |  |  |  |
|  | 2 | 4 | 8 | 12 | 16 | 24 |
| 100 | 0.02 | 0.05 | 0.09 | 0.14 | 0.18 | 0.28 |
| 200 | 0.05 | 0.09 | 0.18 | 0.28 | 0.37 | 0.55 |
| 400 | 0.09 | 0.18 | 0.36 | 0.55 | 0.73 | 1.10 |
| 800 | 0.18 | 0.37 | 0.73 | 1.10 | 1.47 | 2.20 |
| 1200 | 0.27 | 0.55 | 1.10 | 1.65 | 2.20 | 3.30 |
| 1600 | 0.37 | 0.73 | 1.47 | 2.20 | 2.94 | 4.40 |

FIGURES

**Fig. 1**. Variation of local light intensity with microalgae concentration and light path. The measurements were carried out in triplicate. Data shown as mean ± standard deviation, n=3. Lines are simulation results and points are experimental measurements

**Fig. 2**. Experiment measured light attenuation and simulated values with a) Lambert-Beer model and b) Cornet model. In Fig.2a, *Ka* is the extinction coefficient (m2·g-1), *X* is the microalgae concentration (g·L-1), *b* is the fitting constant (m−1). In Fig.2b, *Ea* is the mass absorption coefficient (m2·g-1), and *Es* is the mass scattering coefficient (m2·g-1). Lines are simulation results and points are experimental measurements.

**Fig. 3**. Variation of local light intensity with light path at different β-carotene contents using Cornet Model (a) Microalgae concentration of 0.5 g∙L-1 (b) Microalgae concentration of 1.5 g∙L-1. Lines are simulation results and points are experimental measurements

**Fig.4**. Effect of average irradiance and induction time on β-carotene content of *D. salina*. Red color represents high β-carotene content (%), and blue for low β-carotene content (%).

**Fig.5.** Correlation between β-carotene content (%) and the APRPC in *D. salina* cell. a, the short-term (≤24 h) effect of APRPC on β-carotene content, b, the long-term (24 h≤T≤144 h) effect of APRPC on β-carotene content. Line is simulation result and points are experimental measurements.

 **Fig. 1**.



 **Fig. 2**.



 **Fig. 3**.

 **Fig. 4**.



 **Fig. 5**.