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Kinetic Modeling and Process Analysis for Photo-Production of β-Carotene in *Dunaliella salina*

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Abstract:

Dunaliella salina is a green alga with the great potential to generate natural β-carotene. However, the corresponding mathematical models to guide optimized production of β-carotene in *Dunaliella salina* is not yet available. In this study, dynamic models were proposed to simulate effects of environmental factors on cell growth and β-carotene production in *D. salina* using online monitoring system. Moreover, the identification model of the parameter variables was established, and an adaptive particle swarm optimization algorithm based on parameter sensitivity analysis was constructed to solve the premature problem of particle swarm algorithm. The proposed kinetic model is characterized by high accuracy and predictability through experimental verification, which indicates its competence for future process design, control and optimization. Based on the model established in this study, the optimal environment factors for both β-carotene production and microalgae growth were identified. The approaches created are potentially useful for microalgae cultivation and high-value compounds production.

Key words: *Dunaliella salina*, Dynamic kinetic modelling, Cultivation optimization, Environmental factors, β-carotene production

Introduction:

In the last decades, massive investments were done on microalgae industry, mainly due to their capacity to synthesize lipids for biofuel production or synthesize the carotenoid for high-value products production (Chew, 2017; Kong et al., 2018; Salome & Merchant, 2019). β-Carotene is a high-valued carotenoid pigment with wide applications in the cosmetic, pharmaceutical and food industries (Coppens et al., 2016; Gateau et al., 2017; Paillie-Jimenez et al., 2020). The supply of natural β-carotene still falls short of demand at present, since β-carotene has high antioxidant activity and significant impact on animal pigmentation (Henriquez et al., 2016). To fill this gap, green alga *Dunaliella salina* has been chosen as one of the best candidates for β-carotene production due to its high β-carotene content (up to 10%) (Benamotz et al., 1982; Xi et al., 2020a).

Microalgae require macronutrients and micronutrients for photosynthesis, which are important for growth and product accumulation. The optimal temperature and light are also critical for rapid metabolism and biomass productivity in microalgae (Alexandre Viruela, 2021; del Rio-Chanona et al., 2017). During the past years, many studies have been carried out to evaluate the optimal operating conditions for β-carotene production in microalgae. For example, high irradiance, high temperature, oxidative stress and nitrogen-deprivation have been found to significantly stimulate the accumulation of β-carotene in microalgae (Fachet et al., 2016; Wu et al., 2016;

Kim et al., 2013). Although there are extensive indoor measurements for the microalgae system (Zhu et al., 2021; Zhu et al., 2018b), much less effort has been focused on the modelling of these stages and determine the optimal operating conditions. To optimize reactor design and predict its performance, understanding of simultaneous effects of different environmental and operational variables on microalgae culture is necessary. Mathematical models can be used to study the effects of the environmental and operational variables, which are related to the output variables (e.g., biomass productivity and bio-product production), allowing the effect of changing the input variables to be studied without the need for individual experimental tests (Alexandre Viruela, 2021; del Rio-Chanona et al., 2018). In order to successfully conduct process control and make optimization, it is essential to construct a highly accurate kinetic model, which can be capable of well predicting the dynamic behavior of the underlying biosystem. Meanwhile, model-based process design is also considered to be one of the most effective tools to accomplish the transfer of bioprocess from laboratory short-term scale to industrial long-term scale (Alexandre Viruela, 2021; del Rio-Chanona et al., 2017). Despite its importance, model-based process design for β-carotene production still remains to be elucidated.

Previous modeling studies have taken on the specific challenge of modeling growth in algae systems. Although some dynamic models have been constructed to simulate this process. However, most of the environmental parameters have not been considered in biokinetics in those models, which limited their application. For example, a photobioreactor model that deals only with both light and nitrogen limitation has been

proposed through Beer-Lamberts Law and the Droop Equation, respectively (Bernard, 2009). Microalgae growth is reported to be related to light intensity and the intracellular nitrogen concentration or quota, but the effect of other relevant parameters such as temperature or inorganic carbon concentration which restrict the applicability of Microalgae have not been included. Mathematical model have been used to predict and optimize the microalgae biomass and astaxanthin production, and specific variables including light intensity, temperature, retention time and nutrients concentration have been used to monitor the process performance and construct models (Zhang et al., 2015; Zhang et al., 2016). However, due to the specificity between microalgae species and the induction stage of carotenoid, where cells stop growing and carotenoid commences to accumulate is very difficult to model.

Droop, Monod and Andrew models have been extensively applied to predict biomass growth rate under a single substrate or nutrient condition, such as phosphorus (del Rio-Chanona et al., 2017), nitrogen (del Rio-Chanona et al., 2017; Liu et al., 2018), carbon(Straka & Rittmann, 2019) and light (Holdmann et al., 2018; Zhang et al., 2015). Previous models are able to accurately estimate biomass productivity when the temperature is within a range of values enabling microalgae growth (Zhang et al., 2016). Integrated experimental-computational frameworks that have the ability to predict biomass growth and product accumulation under different growing conditions, which will help to optimize the process performance, operating conditions and scale-up of cultivation systems for commercialization and industrial applicability (Zeriouh et al., 2017).

Nevertheless, kinetic modelling of simultaneous co-limitation of growth media elements (e.g, nitrogen and carbon) and environmental factors (e.g, light and temperature) has not been reported yet. Additionally, while carotenoid accumulation has been considered to be proportional to biomass growth, the effects of abiotic stress towards enhancement of carotenoid productivity have been recently shown through a new kinetic model considering biomass growth and carotenoid accumulation as two different dynamic variables (Zhang et al., 2016). Dynamic simulation is an effective tool to determine the optimal operating conditions for both laboratory scale and industrial scale carotenoid production processes. However, the previous models are only able to accurately estimate biomass productivity when the temperature is within a range of values enabling microalgae growth (Jiang et al., 2015). Moreover, the previous models can only predict biomass and bio-compounds production when the incident light intensity is within a range of constant, but the average light intensity received by the cells in the PBRs is underestimated (Lamers et al., 2010).

To accurately simulate the dynamic process of the β-carotene induction stage, the current study aims to construct rigorous models including the effects of temperature, average light intensity, carbon and nitrogen source on algal growth and β-carotene accumulation, which to the best of our knowledge has not been reported at present. Furthermore, a sensitivity analysis was conducted to simulate various parameters on microalgae production process. The relationship between β-carotene accumulation and algal growth has also been comprehensively studied in this study, which are potentially useful for microalgae cultivation and high-value compounds production.

Material and Modeling Methodology

Microalgal strain and its preculture conditions

The microalgae *D. salina* CCAP 19/18 was purchased from Culture Collection of Algae and Protozoa (Windermere, United Kingdom). The strain was maintained in the medium of optimized Artificial Sea Water (ASW), composing of 1.5 M NaCl, 5 mM KNO_3, 0.45 mM $MgCl_2 \cdot 6H_2O$, 0.05 mM $MgSO_4 \cdot 7H_2O$, 0.3 mM $CaCl_2 \cdot 2H_2O$, 0.13 mM K_2HPO_4, 0.02 mM $FeCl_3$, 0.02 mM EDTA, 1 mL of trace elements stock per liter with 50 mM H_3BO_3, 10 mM $MnCl_2 \cdot 4H_2O$, 0.8 mM $ZnSO_4 \cdot 7H_2O$, 0.8 mM $CuSO_4 \cdot 5H_2O$, 2 mM $NaMoO_4 \cdot 2H_2O$, 1.5 mM $NaVO_3$, 0.2 mM $CoCl_2 \cdot 6H_2O$ and the pH was adjusted to 7.5 by addition of Tris-buffer (40 mM). *D. salina* was precultured in 500 mL conical flasks at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity.

Operation of photobioreactor

The microalgal cells at logarithmic phase were inoculated into a multi-device-equipped flat plate photobioreactor (also known as Algal Station), which can guarantee accurate and stable light condition control as we previously described (Cao et al., 2019). In the platform of Algal Station, the cultivation temperature was automatically controlled at 25℃, the pH was maintained at 7.5 by computer-controlled micro-addition of CO_2 in the bubbling air, and the cultures were agitated at 400 $mL \cdot min^{-1}$ with filtered air (0.2 μm porosity membrane). The culturing broth was sampled daily for analysis of dry weight and β-carotene content. The incident photon flux density and transmitted photon flux were recorded online at 20 min intervals. Each treatment was independently repeated three times.

Analytical Methods

Growth analyses

Cell density was determined spectrophotometrically using a UV/VIS spectrophotometer (Jasco V-530, Japan) at 680 nm. The microalgal dry weight (DW) was determined according to the method we previously described (Cao et al., 2019). Briefly, with pre-weighed Whatman GF/C filters, 10 mL culture broth was filtered and washed three times with 2 mL of 0.5 M ammonium bicarbonate, and then dried below 60°C for over 16 h until the weight was constant. The DW of the microalgae cells was calculated, according to the difference between final and initial filter weights and volume of the filtered sample.

The microalgal growth rate (μ_i, h^{-1}) was calculated by Equation (1)

$$\mu_i = \frac{LnDW_i - LnDW_{i-1}}{t_i - t_{i-1}} \quad (1)$$

where DW_i and DW_{i-1} ($g \cdot L^{-1}$) are the biomass concentration measured at time t_i and t_{i-1}, respectively. t_i and t_{i-1} are hour i and i-1 when the culture broth was sampled.

β-carotene content analysis

The β-carotene content was determinated by modified spectrophotometric method as we previously reported (Xi et al., 2020b; Zhu et al., 2018a). Briefly, one milliliter 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, Japan) at 453 nm and 665 nm with dodecane as reference. β-carotene concentration was calculated using Eq. (2):

$$C_{\beta\text{-car}}\ (mg{\cdot}L^{-1}) = (A_{453} - A_{665}/3.91)\times 3.657\times 3\times X \quad (2)$$

where: $(A_{453}-A_{665}/3.91)$ is the absorbance of β-carotene corrected for chlorophyll contamination, 3.657 is the calibration factor derived from HPLC analysis of β-carotene concentration, 3 is the number of milliliters of dodecane added for extraction, and X is the dilution factor to measure absorbance on spectrophotometer. The content of β-carotene in the algae biomass was calculated according to Eq. (3).

$$\beta\text{-carotene}\ (\%) = \frac{C_{\beta-car}\times 10}{DW} \quad (3)$$

where: $C_{\beta\text{-car}}$ is β-carotene concentration ($mg{\cdot}L^{-1}$), β-carotene (%) is β-carotene content, and DW is cell dry weight ($mg{\cdot}L^{-1}$).

Model construction methodology

Currently, the types of kinetic models, namely the Monod model and the Droop model（Zhang et al., 2015）, are widely used for bioprocess simulation. Due to its high accuracy and flexibility, the Monod model is selected and modified to simulate the correlation between biomass growth and consumption of nitrate and carbon.

$$\frac{dX}{dt} = \mu_0 * X - \mu_d * X^2 \quad (4)$$

$$\mu_0 = \mu_{max}\ (T, I, N, C) f(T) \left(\frac{I_{av}}{I_{av}+k_s+\frac{I_{av}^2}{k_i}}\right)\left(\frac{C}{K_C+C}\right)\left(\frac{N}{K_N+N}\right) \quad (5)$$

$$f(T) = A * e^{-\frac{E_a}{R*T}} - B * e^{-\frac{E_b}{R*T}} \quad (6)$$

Where X is biomass concentration (g· L^{-1}), μ_0 is cell specific growth rate (h^{-1}), μ_{max} is maximum cell specific growth rate (h^{-1}), I_{av} is average light intensity, μ_d is cell decay rate (h^{-1}), K_s is light saturation value produced by cell growth, K_i is photoinhibition value of cell growth, A and B are the coefficients before the index, E_a is the activation energy for cell growth, E_b is inactivation energy of cell growth, K_N is the nitrate half-velocity constant, and K_C is carbon half-velocity constant.

Equation 4 simulates the biomass growth rate. Its first term on the right-hand side represents biomass growth, and the second term represents cell respiration and decay. In terms of β-carotene production, it was reported that the uptake of culture nitrate is essential for cells to synthesize β-carotene (Lamers et al., 2012). Meanwhile, as β-carotene is a primary carotenoid, it can be consumed by cells for their growth and converted to other metabolites when necessary. Therefore, Equation 7 is constructed to simulate β-carotene production. In this equation, the first term on the right represents β-carotene synthesis rate and is originated from the Monod model, while the second term represents β-carotene consumption rate. So far there are no reports about investigating the detailed metabolic mechanisms of β-carotene consumption.

$$\frac{\mathrm{dw}}{\mathrm{dt}} = \left[b + \left(1 - \frac{K_{NW}}{N}\right)\right] * \frac{K_{NW}}{N} * \left(1 - \frac{K_{CW}}{C}\right) * \frac{K_{CW}}{C} * \left(1 - \frac{W}{W_{max}}\right) * \frac{I_{av}}{I_{av} + k_{sw} + \frac{I_{av}^2}{k_{iw}}} * \left[A_w * e^{-\frac{E_{aw}}{R*T}} - B_w * e^{-\frac{E_{bw}}{R*T}}\right] \quad (7)$$

where W is β-carotene content, b is no correlation coefficient for the growth of β-carotene production, K_{sw} is the light saturation value of β-carotene accumulation, E_{aw} is activation energy of β-carotene accumulation; w_{max} is the maximum β-carotene content, K_{NW} is nitrate half velocity constant for β-carotene synthesis, I_{av} is average

light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$), K_{iw} is β-carotene accumulation photoinhibition value, K_{CW} is carbon content half velocity constant for β-carotene synthesis, and E_{bw} is inactivation energy for β-carotene synthesis.

Parameter estimation methodology

In order to obtain the best parameters of dynamic model that can fit the experimental data, it was take the average relative error between the experimental data and the system output as the objective function, and the range of biomass and β-carotene concentration as the state constraints, and then establish a nonlinear programming problem (NLP) for the parameter variables. Considering that the particle swarm optimization (PSO) is easy to be premature, this study used an adaptive PSO based on sensitivity analysis to solve the problem. This algorithm can ensure that the required parameters can reach the approximate global optimum, and has a certain degree of robustness. The implementation in this work is programmed in the MATLAB (R2021a) optimization environment.

Results and Discussion

Effects of environmental factors on cell growth

To examine the effect of temperature, light intensity, carbon and nitrate concentration on the growth kinetics of *D. salina*, different specific sets of experiments were carried out by cultivating the cells in PBRs under different temperature, average light intensity, initial concentrations of dissolved nitrates and carbons. The experiment was setup and then monitored daily up to a total cultivation time of 96 h, when the occurrence of exponential growth was observed.

It is found that temperature remarkably influence the rate of cell growth, cell decay and bioproduct accumulation. The optimal temperature can facilitate microbial biomass growth and bioproducts synthesis (del Rio-Chanona et al., 2017; Fachet et al., 2014; Guiheneuf & Stengel, 2017). The effect of temperature on the growth rate in *D. salina* is mainly reflected in the efficiency of photosynthesis and respiration (Fachet et al., 2014). The growth rate at different temperatures was shown in Figure 1A. At the range of 10-30°C, the growth rate was positively correlated with temperature, and when the temperature rises to 30°C, the growth rate begins to decrease. In the range of 30-40°C, the growth rate has a negative correlation with temperature. The logarithmic growth phase of *D. salina* has the largest growth rate (0.164 h^{-1}) at the temperature of 28 °C. The Arrhenius equation has been widely used to describe the effects of temperature on both biomass growth (Zhang et al., 2016). The parameters of the Arrhenius model can be obtained by model fitting under different range of temperatures.

Light intensity significantly affects biomass growth rates (Bonnefond et al., 2016). In general, the effect of light intensity on cell growth can be reflected by the Aiba model (del Rio-Chanona et al., 2017). In our experiments, the Aiba model was used to replace local light intensities by an average light intensity which was calculated by Eq. 5 in a suspended reactor. The model parameters μ_{max} and K_s were fitted as shown in Figure 1B, within a certain range of light intensity, the relationship between the average light intensity and the growth rate of *D. salina* conforms to the Aiba equation. The growth rate tends to increase with the increase of light intensity stable value when the light intensity exceeds 379.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$. According to the curve, the half-saturation constant of light (K_i) is 1705 $\mu mol \cdot m^{-2} \cdot s^{-1}$. It is indicated that in most of the cases, a higher average light intensity (up to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) can result in a higher biomass growth rate, which is consistent with the observations published in previous studies (Bonnefond et al., 2016; Fachet et al., 2016).

When nitrogen concentration is the limiting factor, the relationship between the growth rate and the nitrogen concentration was shown in Figure 1C. The relationship between the nitrogen concentration and the growth rate of *D. salina* conforms to the Monod equation. The values of the model parameters μ_{max} and K_N after fitting show that within a certain range of nitrogen concentration, the growth rate μ of algal cells increased with the increase of the nitrogen concentration. The μ tends to a stable value when the nitrogen concentration exceeds 330 $mg \cdot L^{-1}$. In terms of the influence of nitrate concentration on biomass growth rate, in both sets of experiments nitrate concentration in the culture keeps increasing after the addition of dense nitrate

influent, which means that the consumption rate of nitrate due to biomass uptake was slower than its refreshment rate. By comparing biomass concentrations at the different sets of experiments, it seems that biomass growth rate was always higher in a denser nitrate concentration culture.

This tendency was also observed in the carbon experiments when the carbon content changes from 0 to 500 mM. Under the carbon limiting condition, the relationship between specific growth rate and carbon content was shown in Figure 1D. The relationship between the carbon concentration and the growth rate conformed to the Monod equation within a certain range of carbon content in *D. salina*. The growth rate (referred as μ) of the algae was improved as the increase of the carbon content when the carbon concentration was in a lower range. The μ tended to a stable value with the increase of the carbon concentration when the carbon concentration exceeds 50 mM, above which a stable of final biomass concentration was obtained, and indicated a stable biomass growth rate. Therefore, the results was suggested that the high biomass growth rate obtained with nitrate concentration 500 $mg \cdot L^{-1}$, carbon concentration 50 mM at 28℃ and the average light intensity 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Figure 1).

Effects of environmental factors on β-carotene synthesis

The β-carotene accumulation rate at different temperatures was shown in Figure 2A. We found that the β-carotene accumulation rate was negatively correlated with temperature at the range of 10-35°C, and when the temperature rises to 30°C, the β-carotene accumulation begins to increase. In the range of 30-40°C, the β-carotene

accumulation rate has a positive correlation with temperature. The *D. salina* has the largest β-carotene accumulation rate at 10 °C, which was 0.095 h^{-1}. The Arrhenius equation has been widely used to describe the effects of temperature on both bioproduct accumulation. The parameters of the Arrhenius model can be obtained by model fitting under different range of temperatures.

We found that in the different light intensity experiments, β-carotene content continuously increases with the raising average light intensity from 150 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while its maximum value falls within the range of 300 to 480 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Figure 2B). However, neither of the current observations were in agreement with the previous studies where β-carotene content was found to decrease with the increasing light intensity from 150 to 750 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fachet et al., 2014). As a result, the distinct discrepancy between current observations and previous conclusions suggests the complex metabolic mechanisms of β-carotene synthesis. It also indicated that other factors apart from light intensity can significantly affect intracellular β-carotene content. In terms of the effect of nitrate concentration on β-carotene synthesis, it was known that nitrate is essential for β-carotene synthesis, and β-carotene was a primary carotenoid which can be accumulated under nitrogen-sufficient conditions. The results showed that under the same light intensity, β-carotene content in the experiments with lower nitrate concentration was higher than that in the experiments with higher culture nitrate concentration (Figure 2B). This phenomenon was also reported in a recent study where a similar photosynthetic pigment lutein was synthesized by *Desmodesmus sp.* under a nitrogen-sufficient

condition (del Rio-Chanona et al., 2017).

Therefore, the current observation indicated that β-carotene content was higher in a lower nitrate concentration condition could also be explained as what has been demonstrated for β-carotene. Under the carbon deprivation condition, the relationship between β-carotene accumulation and carbon concentration was shown in Figure 2D, within a certain range of carbon concentration, the relationship between the carbon concentration and the specific accumulation rate conformed to the Monod equation in *D. salina*. The β-carotene accumulation rate of the algae increased with the higher concentration of the carbon. The μ tends to a stable value with the increase of the carbon concentration when the carbon concentration exceeds 200 mM.

For β-carotene production, it was found that a higher light intensity and a denser culture nitrate concentration can always lead to a higher β-carotene production as long as nitrate inhibition does not happen, as shown in Figure 2B. Such conflicting conclusion compared to that of β-carotene synthesis was reasonable, because β-carotene production is the product of both biomass concentration and β-carotene intracellular content. Although a high nitrate concentration may limit β-carotene accumulation, it can significantly facilitate green algae biomass growth. Consequently, total β-carotene production can still be increased through this condition. Nonetheless, it should be noted that low β-carotene content can remarkably elevate the bioprocess downstream separation cost, which may seriously reduce the process profitability. Hence, it was essential to guarantee an adequate β-carotene content when aiming to maximize total β-carotene production.

Results of dynamic model construction

In order to construct a highly accurate dynamic model which is capable of simulating the performance of green algal β-carotene production, and accomplish further process optimization, it is vital to understand the biochemical kinetics of the investigated system. Especially for the current process, the temperature, light intensity, carbon content and culture nitrate concentration are included in the model since previous studies have declared that they are the main factors affecting β-carotene synthesis (Bonnefond et al., 2016; Lamers et al., 2012). From the section 3.1 and 3.2, parameters in the kinetic model were calculated through single factor experiments as shown in Table 1. We found that the specific biomass decay rate not equal 0, which suggested that they have not negligible effects on the system. This can be attributed to the fact that in all the conducted experiments, biomass concentration kept steady until the end of the study, the effect of cell decay should not disguise. The parameters were used as the initial value of the parameter for further parameter identification. Considering that a single perturbation method may affect the accuracy of the results, in order to reduce the sensitivity of the parameters from the perturbation method, we randomly perturbs each parameter Q times, and draws a box plot of the objective function with respect to the perturbation percentage.

In the process of parameter simulation, the disturbances time Q = 100, disturbance range $\zeta = 5\%$, and the optimal parameters value of the system were obtained with the aid of the PSO algorithm. The optimized parameters resulted in a box plot of the objective function change under a certain perturbation percentage, as shown in the

Table 2, Figure 3 and Figure 4: E_b, A_w and b obtains the minimum value of the objective function within the perturbation range of the corresponding parameter ±5%, and E_a decreases monotonically within the perturbation range of the parameter. However, within this range, the maximum difference of the objective function value is 0.016×10^{-3}. It was suggested the system is not sensitive to changing the parameters, and the system reaches the approximate global optimal solution of the objective function at 0 perturbation of the parameters. The results show that the parameters obtained by the above algorithm can fit the experimental data well, and the parameters created in this study were an approximate global optimal solution of the nonlinear dynamic system and have certain robustness.

Validation of dynamic model predictability

To estimate the optimal operating conditions for long-term bioprocess optimization, besides accurately representing a known experiment, the model should possess great predictive capability when simulating unknown processes. For this reason, the predictive capability of the constructed model was investigated through two scenarios. In the first scenario, the model was used to predict the dynamic performance of a continuous illumination batch experiment lasting for 6 days indoors. In the second scenario, the model was applied to predict a light/dark cycle batch experiment lasting for 6 days under outdoor condition. Noticeably, due to the frequent change of light intensity, the second system becomes more complex and has a higher uncertainty compared to the first scenario. Both light intensity and initial nitrate concentration in these two experiments are different from those used for model construction. The

388 detailed operating conditions of these experiments are listed in Table 3.

389 To identify the predictability of current models for different *D. salina* strains
390 β-carotene production process, four additional experiments were carried out. The
391 experiments have the initial biomass concentration of 0.10 $g \cdot L^{-1}$, with incident light
392 intensity of 200, 600, 800 and 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and temperature of 20 and 25 °C.
393 The model was validated by Algal station system with 1.0 L PBR, steady temperature
394 and light intensity within the reactor operating conditions. Despite the added
395 variability, the model was able to accurately reproduce the process performance with
396 the same calibrated values for all the model parameters. The comparison between the
397 current model simulation results and experimental data was examined. It was shown
398 that the current models can accurately predict the dynamic performance of green algal
399 β-carotene production process with different operating conditions (Figure 5 and 6).
400 Because the current dynamic model was constructed with the aim to predict the
401 optimal operating conditions in future process design and control, it has to be
402 characterized by not only high accuracy but also good predictability. Therefore, this
403 model is used to simulate the dynamic performance of all the remaining four
404 experiments conducted in this study. We found that the current model shows great
405 predictability within a wide range of operating conditions throughout the entire
406 experimental time course. Among the all experimental data points, the majority of
407 deviation between model prediction and real experiment was far below 10%, with
408 only four exceptions shown in Figure 5A (11.4%) and Figure 6C (13.8%). Therefore,
409 it was strongly indicated the current model showed high predictability and accuracy of

the current model, as well as its great competence for further process design and optimal control.

Our model was fitted with experimental data that accounted for the most significant parameters that affect microalgae metabolism. Moreover, this model was intended to promote and assist the development of evaluation applications of microalgae at industrial level. Our model could also be used as a predictive tool to determine the combination of environmental and operational parameters that promote maximum biomass productivity in other microalgae.

Conclusions

In this study, a mathematical model was constructed to simulate the growth and β-carotene production from *D. salina*. Sensitivity analysis showed that β-carotene synthesis is more sensitive to the operating parameters of the system than cell growth. Moreover, the accuracy and predictability of kinetic model were further verified. Based on the dynamic model, optimal light intensities for cell growth and β-carotene production were proposed. The established model has high accuracy and predictive capability, which is potentially useful for further application in process control and optimization during microalgae cultivation at industrial level.

Authorship contribution statement

YX, JC and ZC designed the research. YX and FK wrote the paper. FK, YX and JZ analyzed the data. YX and TL performed the research and provided technical support. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1. Growth rate of *D. salina* under different culture conditions. (A) The relationship of temperature and cell specific growth rate (B) The relationship of light intensity and cell specific growth rate (C) The relationship of nitrogen concentration and cell specific growth rate (D) The relationship of carbon concentration and cell specific growth rate.

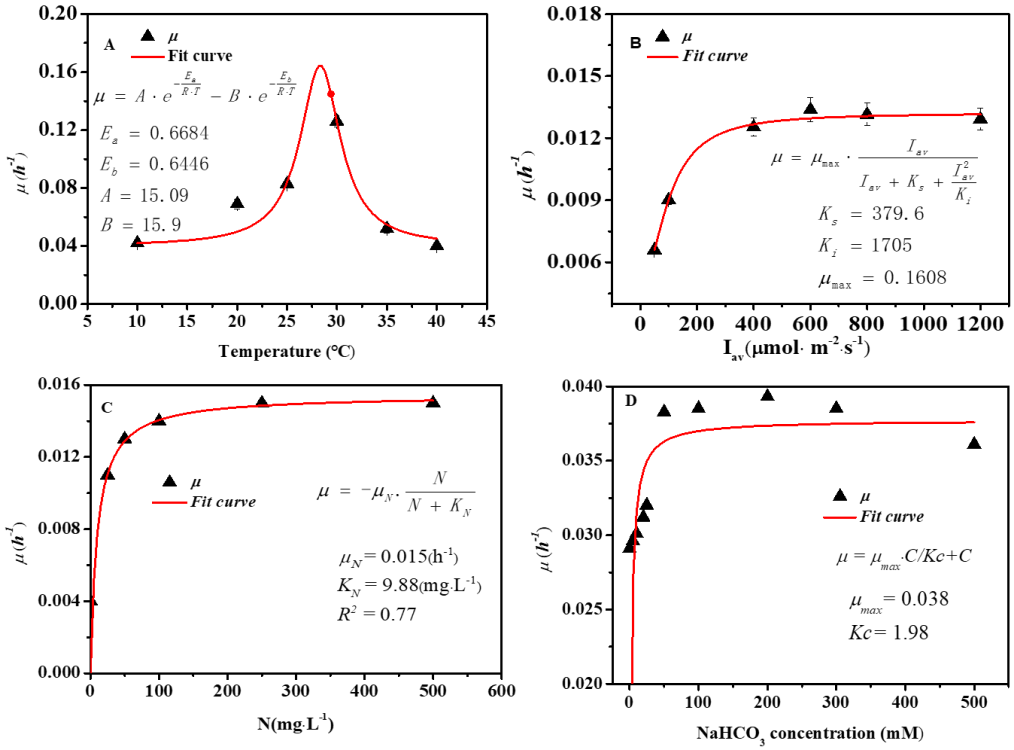


Figure 2. β -carotene accumulation rate of D. salina under different culture conditions. (A) The relationship of temperature and β-carotene accumulation rate (B) The relationship of light intensity and β-carotene accumulation rate (C) The relationship of nitrogen concentration and β-carotene accumulation rate (D) The relationship of carbon concentration and β-carotene accumulation rate.

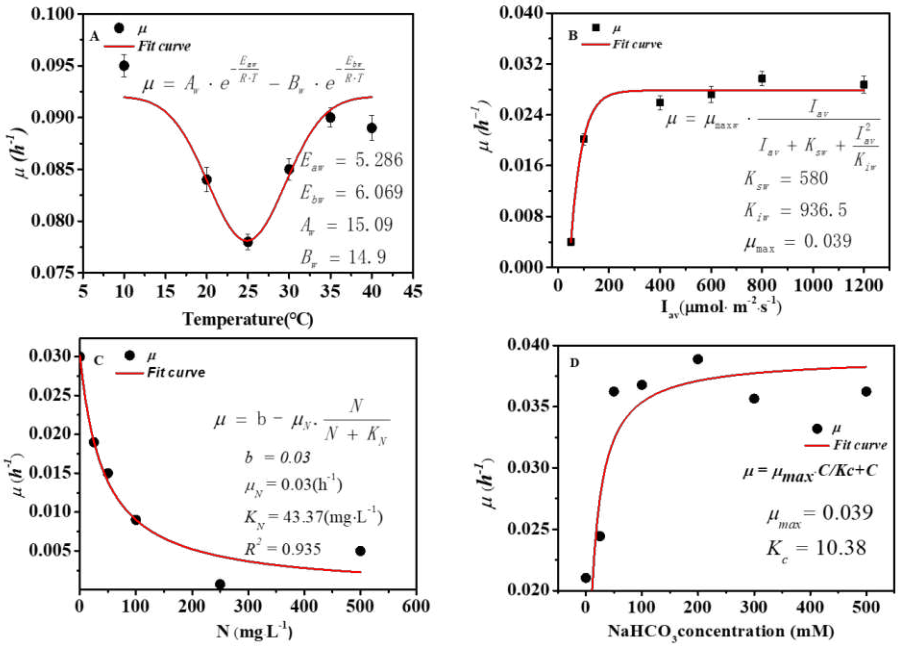


Figure 3. Optimization of model parameters for cell growth in D. salina (optimized by MATLAB). SSE: system squared error.

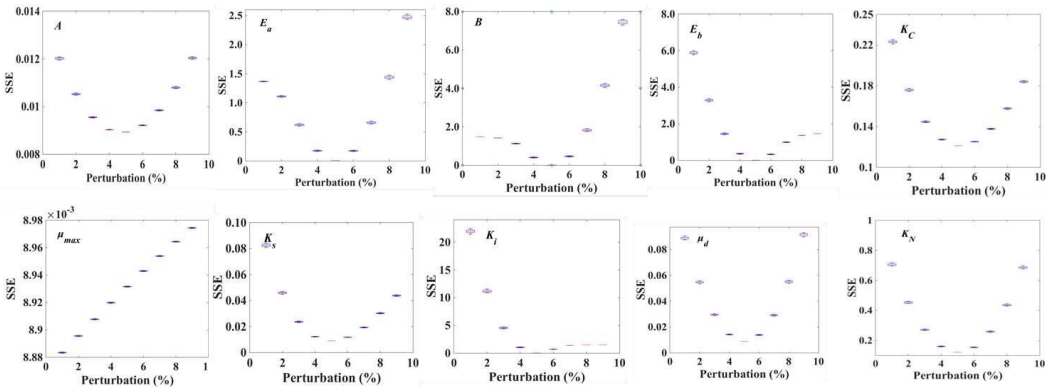


Figure 4. Optimization of model parameters for β-carotene accumulation in D. salina (optimized by MATLAB). SSE: system squared error.

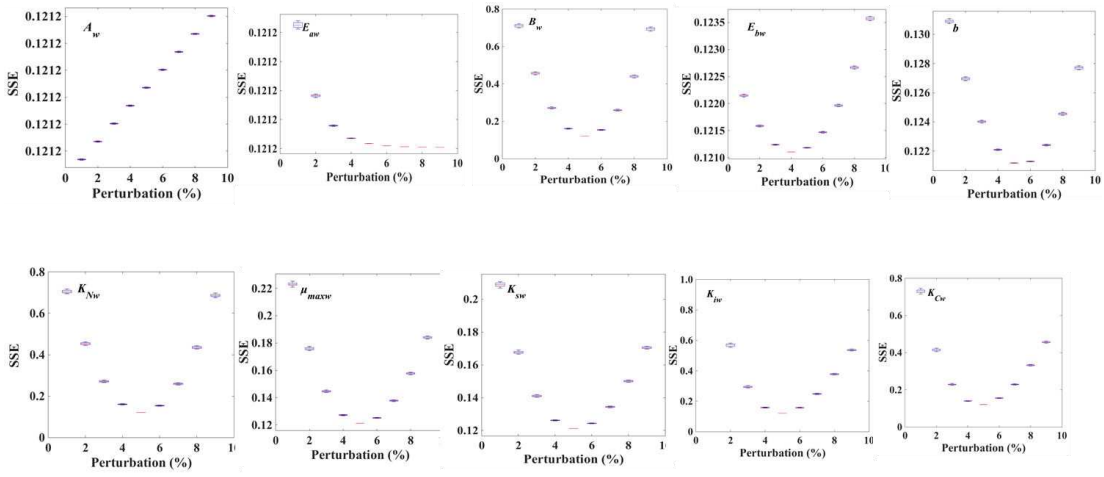


Figure 5. Comparison of model simulation results and real experimental data of biomass in *D. salina.* DW, dry weight.

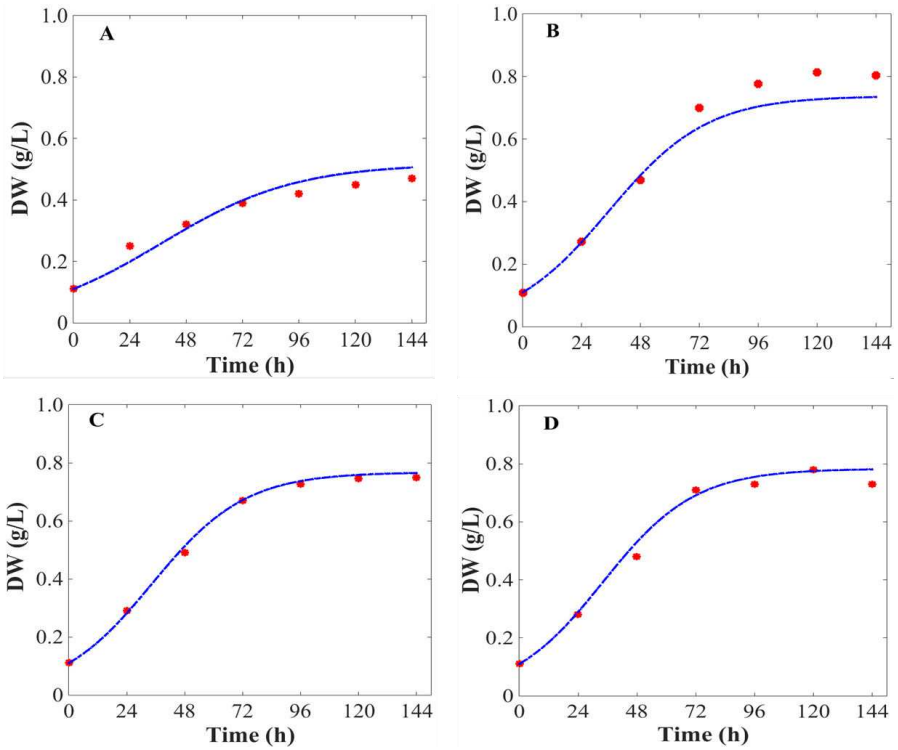
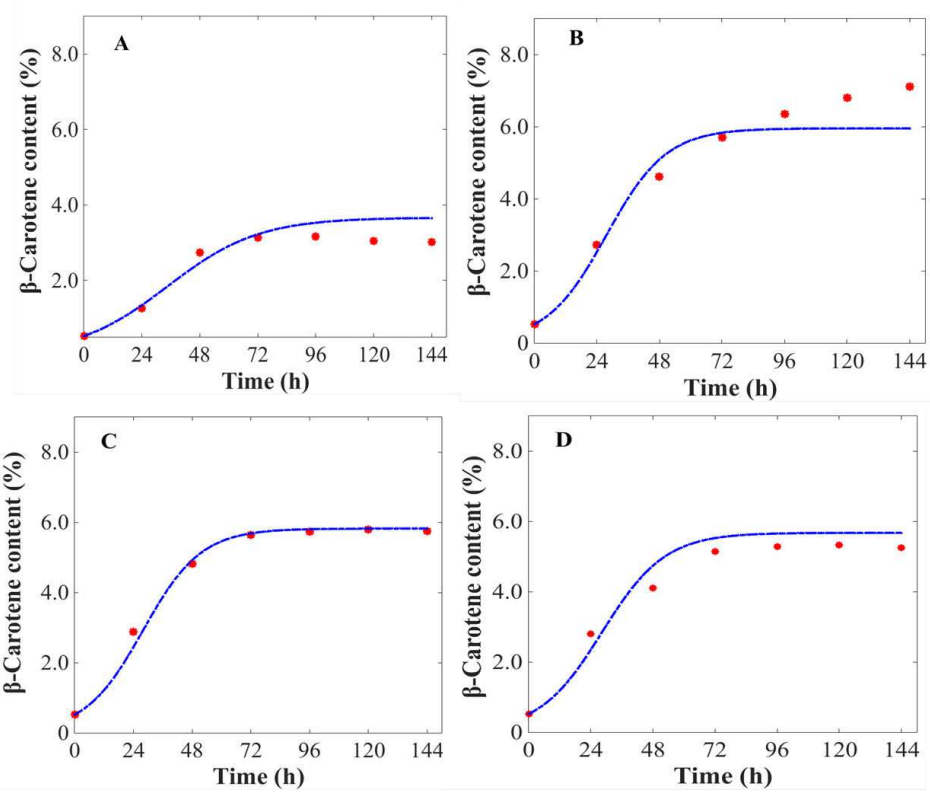


Figure 6. Comparison of model simulation results and real experimental data of β-carotene content in *D. salina.*



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665 **Tables**

666 **Table 1. Parameters based on average light intensity**

Parameter	Unit	Value
K_s	μmol m^{-2} s^{-1}	379.6
E_a	kJ mol^{-1}	0.6684
E_b	kJ mol^{-1}	0.6449
E_{aw}	kJ mol^{-1}	5.286
E_{bw}	kJ mol^{-1}	6.069
K_{sw}	μmol m^{-2} s^{-1}	580
A	h^{-1}	15.09
B	h^{-1}	14.9
A_w	h^{-1}	0.4355
B_w	h^{-1}	0.35
μ_{max}	h^{-1}	0.1608
μ_{maxw}	h^{-1}	0.039
K_i	μmol m^{-2} s^{-1R}	1705
K_{iw}	μmol m^{-2} s^{-1}	936.5
k_q		9.88
K_C		0.22
K_{CW}		10.38
k_{qw}		43.37
b		0.03
μ_d		0.3

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669 **Table 2. Optimization of model parameters (optimized by MATLAB)**

Parameter	Unit	Value (计算)	优化后
K_s	μmol m^{-2} s^{-1}	379.6	384.9
E_a	kJ mol^{-1}	0.6684	1.0026
E_b	kJ mol^{-1}	0.6449	0.5572
E_{aw}	kJ mol^{-1}	5.286	7.9290
E_{bw}	kJ mol^{-1}	6.069	3.0345
K_{sw}	μmol m^{-2} s^{-1}	580	581.6
A	h^{-1}	15.09	22.6350
B	h^{-1}	14.9	17.4630
A_w	h^{-1}	0.4355	0.6533
B_w	h^{-1}	0.35	0.1750
μ_{max}	h^{-1}	0.1608	0.20278
μ_{maxw}	h^{-1}	0.039	0.0405
K_i	μmol m^{-2} s^{-1}	1705	1707
K_{iw}	μmol m^{-2} s^{-1}	936.5	937.1
K_N		9.88	10.23
K_C		0.22	0.26
K_{CW}		10.38	10.5
K_{NW}		43.37	45.98
b		0.03	0.0150
μ_d		0.3	0.15

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672 **Table 3.** List of arbitrary cultivation condition parameter value

Photobio reactor	Initial DW ($g \cdot L^{-1}$)	Day/Night (h:h)	Temperature (℃)	Light ($\mu mol\ m^{-2}\ s^{-1}$)	Carbon (Mm)	Nitrogen ($mg \cdot L^{-1}$)
a	0.1	14:10	20	200	5	50
b	0.1	24:0	25	600	5	500
c	0.1	24:0	20	800	200	50
d	0.1	14:10	25	1000	200	500

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Supplementary Files

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