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Kinetic Modeling and Process Analysis for *Desmodesmus* sp. Lutein Photo-Production

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Lutein is a high-value bioproduct synthesized by microalga Desmodesmus sp. In the current study two aspects of this process are thoroughly investigated: identifying the complex effects of light intensity and nitrate concentration on biomass growth and lutein synthesis, and constructing an accurate kinetic model capable of simulating the entire bioprocess dynamic performance, neither of which has been previously addressed. Three original contributions are presented here. First, it is found that completely opposite to a nitrogen-limiting culture, under nitrogen-sufficient conditions a higher lutein content is caused by a higher light intensity and lower nitrate concentration. Second, contrary to lutein content, total lutein production always increases with the increasing nitrate concentration. Third, through experimental verification, the proposed kinetic model is characterized by high accuracy and predictability, indicating its competence for future process design, control, and optimization. Based on the model, optimal light intensities for lutein production and microalgae growth are identified. © 2017 American Institute of Chemical Engineers *AIChE J*, 00: 000–000, 2017
Keywords: lutein production, dynamic simulation, Desmodesmus sp. cultivation, fed-batch operation, nitrogen-sufficient culture, photobioreactor

Introduction

Lutein, a primary carotenoid synthesized by different microorganisms and plants, has recently shown great potential in the pharmaceutical and food industries due to its outstanding properties.[1–3] It has been found to effectively protect human eyes and prevent the development of cataracts,[1] and markedly delay a variety of human chronic diseases including cancer and macular degeneration.[2,3] In addition, lutein is commonly used for the pigmentation of animal tissues, and as a natural colorant for the coloration of drugs, cosmetics, and food.[4] Because of its wide application, its global market demand has been increasing significantly over the last years. For instance, in the United States its sales have been estimated to double from $150 million in 2000 to $309 million in 2018.[3,5]

However, at present the primary commercial source for lutein production is marigold, a plant which is characterized by a low lutein intracellular content of 0.03% and high process operating cost.[6] As a result, to supply the increasing lutein demand, microalgae have been considered as competent candidates to replace marigold. Microalgae such as *Chlorella zofingiensis*, *Chlorella sorokiniana*, and *Scenedesmus obliquus* have been observed to have a much higher lutein content ranging from 1.9 to 4.2 mg g^{-1}, which is about 6–15 times that of marigold.[6,7] Moreover, microalgae also have a higher growth

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Table 1. Operation Conditions of the Two Sets of Experiments

Experiment set	First (high nitrate experiment)				Second (low nitrate experiment)			
Operation conditions	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7	Exp8
Initial biomass g L^{-1}	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Initial nitrate con. mM	8.8	8.8	30	30	8.8	8.8	8.8	8.8
Inflow rate mL h^{-1}	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Influent nitrate con. M	0.5	0.5	0.5	0.5	0.1	0.1	0.1	0.1
Light intensity μmol m^{-2} s^{-1}	150	300	480	600	150	300	480	600

rate[8] and have been used in industry for the production of high-value bioproducts including astaxanthin and C-phycocyanin.[9,10]

Therefore, to further investigate the process feasibility, extensive research has been conducted within this decade. For example, different biomass cultivation modes including phototrophic, mixotrophic, and heterotrophic mode have been carried out, with the conclusion that phototrophic condition is the preferable mode for algal lutein accumulation.[6,11] Effects of culture operating conditions (e.g., light intensity and nitrate concentration) on algae biomass growth and lutein synthesis have been studied. It has been found that nitrate is essential for lutein production and a lower incident light intensity can result in a higher lutein content.[3,6,11,12] Different processes such as batch process and fed-batch process have also been designed to enhance lutein productivity and maintain cell growth.[13]

Nonetheless, there are two challenges that severely limit the industrialization of microalgal lutein production. The first challenge is the selection of desirable industrial algae species. Although several green algae species have shown high lutein productivities, it is notable that suitable lands for large scale algae biomass outdoor cultivation are mainly located in tropical and subtropical zones with a local pond water temperature easily rising up to 45°C during daytime.[14] As most of the algae species cannot survive under such a high temperature, it is vital to identify thermo-tolerant microalgae strains with high lutein productivities. A very recent study[6] has proposed *Desmodesmus* sp., a thermo-tolerant green alga with the highest reported lutein content of up to 5.0 mg g^{-1} under photoautotrophic growth conditions,[6] as a promising candidate. However, very few efforts have been focused on investigating this strain, and effects of operating conditions on its biomass growth and lutein synthesis have barely been explored.

The second challenge is to determine the optimal operating conditions and process operating mode (e.g., fed-batch process) so that lutein productivity can be maximized and process profit can be improved. However, in order to successfully conduct process control and optimization, it is essential to construct a highly accurate kinetic model capable of well predicting the dynamic behavior of the underlying biosystem.[15,16] Meanwhile, model-based process design is also considered to be the most effective tool to accomplish the transfer of bioprocess from laboratory short-term scale to industrial long-term scale.[17] Despite its importance, until now no study has focused on this aspect for lutein production and this challenge still remains.

Therefore, to resolve both of the challenges and facilitate the industrialization of microalgae based lutein production, the current study aims to thoroughly explore the effects of two key operating factors, incident light intensity and culture nitrate concentration, on both *Desmodesmus* sp. biomass growth and lutein synthesis, and to construct a kinetic model which is highly accurate and applicable in practice for future process optimization and control.

Material and Modeling Methodology

Microalgal strain and its preculture conditions

The thermo-tolerant *Desmodesmus* sp. F51 strain was donated by Professor Ching-Nen Nathan Chen of National Sun Yat-sen University, Taiwan. The medium used for the preculture of the strain is the modified Bristol's medium consisting of (g L^{-1}): $NaNO_3$, 0.75; $CaCl2{\cdot}2H_2O$, 0.025; $MgSO_4{\cdot}7H_2O$, 0.075; NaCl, 0.025; K_2HPO_4, 0.075; KH_2PO_4, 0.175; $FeCl_3{\cdot}6H_2O$, 0.005, and 1 mL of trace element solution. The trace element solution consists of (g L^{-1}): H_3BO_3, 0.061; $MnSO_4{\cdot}7H_2O$, 0.169; $ZnSO_4{\cdot}7H_2O$, 0.287; $(NH_4)_6M_{O7}O_{24}{\cdot}4H_2O$, 0.00124; $CuSO_4{\cdot}5H_2O$, 0.0025.[18] The microalgae was grown at 28°C for 5 days with continuous supply of 2.5% CO_2 at an aeration rate of 0.2 vvm and an agitation rate of 300 rpm. The microalgae culture was illuminated continuously (24 h per day) with a light intensity of 60 μmol m^{-2} s^{-1} approximately. The light intensity was measured by a Li-250 Light Meter with a Li-190SA pyranometer sensor (Li-COR Inc., Lincoln, NE).

Operation of photobioreactor

The photobioreactor (PBR) used to grow *Desmodesmus* sp. F51 is a 1 L tubular glass vessel (15.5 cm in length and 9.5 cm in inner diameter) equipped with external light sources (14 W TL5 tungsten filament lamps, Philips Co., China) fixed on both sides of the PBR. The target microalga was precultured and inoculated into the PBR with an inoculum size of 0.07 g L^{-1}. The PBR was operated at 35°C under fed-batch mode with an incident light intensity of 150–600 μmol m^{-2} s^{-1}, agitation rate of 300 rpm, and culture pH 7.5. The continuous supply of air was supplemented with 2.5% CO_2 as sole carbon source at an aeration rate of 0.2 vvm.

In the current study, eight experiments were carried out, which can be categorized into two sets based on the influent nitrate concentration. In the first set of experiments (namely high nitrate experiments, Exp 1–Exp 4), the influent nitrate concentration is 0.5 M. Initial nitrate concentration in the culture was set to be 8.8 mM when the incident light intensity is 150 and 300 μmol m^{-2} s^{-1}, and 30 mM when the light intensity is 480 and 600 μmol m^{-2} s^{-1}. In the second set of experiments (namely, low nitrate experiments, Exp 5–Exp 8), the influent nitrate concentration is 0.1 M. Initial nitrate concentration in the culture was fixed at 8.8 mM regardless of incident light intensity. The different values of initial nitrate concentration, influent nitrate concentration, and incident light intensity in these experiments were chosen from the general range of most recent microalgae studies[19–21] and assigned with the aim to explore their effects on both biomass growth and lutein synthesis. Table 1 summarizes the eight experiments implemented in the current research. All the experiments were replicated twice to guarantee the accuracy of the experimental data.

Fed-batch operation strategy

The fed-batch cultivation strategy was commenced by a batch operation with 0.07 g L^{-1} inoculum dosage and different light intensities mentioned above. When the initial nitrate in the culture was consumed, a dense nitrate solution (nitrate influent) was fed continuously by using a peristaltic pump BT100-1L (Baoding longer precision pump Co., China). In all of the eight experiments, the continuous nitrate feed was switched on from the 60th hour of the experiment. Liquid samples were collected at a fixed time interval to analyse biomass concentration, nitrate concentration and lutein production.

Analytical Procedures

Measurement of biomass concentration

The biomass concentration of microalga *Desmodesmus* sp. F51 was determined by measuring the sample optical density at a wavelength of 685 nm (denoted as OD_{685}) using a UV/Vis spectrophotometer (model UV-1780 Shimadzu, Japan) after the samples were diluted with deionized water. The OD_{685} values were converted to biomass concentration through a proper calibration equation between OD_{685} and dry cell weight.[6]

Measurement of nitrate concentration

The estimation of nitrate concentration in the medium was determined by a colorimetric method described in Ref. 22. The calibration between the absorbance and nitrogen concentration was established using sodium nitrate as standard.

Measurement of lutein content

Carotenoids were determined by modified protocols reported from Ref. 23. In general, microalgal biomass was harvested by centrifugation and its residual salts were removed by washing twice with deionized water. The biomass was dried by lyophilization. A 10 mg of lyophilized microalgae cells were disturbed by a bead-beater for 7 minutes after the addition of 1 mL 60% w/w KOH aqueous solution. After cell disruption, the mixture was kept on water bath for 40 minutes at 40°C. The carotenoids were extracted by mixing the mixture with 2 mL diethyl ether until the supernatant becomes colorless. The solvent in the combined extract was evaporated with nitrogen gas and the residue was resuspended in 3 mL acetone.

Lutein content in the extract was analyzed using a high performance liquid chromatography (Agilaten technologies, 1200 series, USA).[24] A YMC30 column operated at 4°C with 1 mL min^{-1} of mobile phase was used for all measurements. The binary mobile phase consisted of (A) 3% deionized H_2O in methanol containing 0.05 M ammonium acetate, and (B) 100% TBME (tert-butyl methyl ether). Both mobile phases contained 0.01% (w/v) butylated hydroxytoluene (BHT) and 0.05% triethylamine (TEA). The lutein content was calculated by measuring the maximal absorbance wavelength at 450 nm.

Model construction methodology

In order to construct a highly accurate dynamic model capable of simulating the performance of green algal lutein production, and to accomplish further process optimization, it is vital to understand the biochemical kinetics of the investigated system. Specific to the current process, both light intensity and culture nitrate concentration are included in the model since previous research has declared that they are the main factors affecting lutein synthesis.[19,20] Currently, two types of kinetic models, namely, the Monod model and the Droop model, are widely used for bioprocess simulation. The primary difference of these two models is that the former is predominantly applied to nutrient-limiting conditions, while the latter is mainly chosen under nutrient-sufficient conditions. Because nitrate is available and replenished in the culture throughout all of the eight experiments, and due to its high accuracy and flexibility, the Monod model is selected and modified to simulate the correlation between biomass growth and nitrate consumption.[21]

$$\frac{dc_X}{dt} = u_0 \cdot \frac{c_N}{c_N + K_N} \cdot c_X - u_d \cdot c_X \tag{1a}$$

$$\frac{dc_N}{dt} = -Y_{N/X} \cdot u_0 \cdot \frac{c_N}{c_N + K_N} \cdot c_X + F_{in} \cdot c_{N,in} \tag{1b}$$

where c_X is biomass concentration (g L^{-1}), u_0 is cell specific growth rate (h^{-1}), c_N is nitrate concentration (mg L^{-1}), K_N is nitrate half-velocity constant (mg L^{-1}), u_d is cell specific decay rate (h^{-1}), $Y_{N/X}$ is nitrate yield coefficient (mg g^{-1}), F_{in} is nitrate influent flow rate (L h^{-1}), $c_{N,in}$ is nitrate influent concentration (mg L^{-1}).

Equation 1a 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. Similarly, the first term on the right-hand side of Eq. 1b represents culture nitrate consumption due to cell growth, and the second term represents the increase of nitrate in the culture due to the addition of dense nitrate influent. Although nitrogen released by cell decay can also slightly increase the culture nitrogen concentration, this factor is not included in the current model due to its negligible amount compared to that supplied by the nitrate inflow. Because the culture volume increase caused by the addition of nitrate influent is compensated by the culture sampling once every 12 hours, the change of total culture volume is not significant in the current experiments. Thus, its effect on biomass concentration and nitrate concentration is negligible and not included in the model.

In terms of lutein production, recent research has concluded that the uptake of culture nitrate is essential for cells to synthesize lutein.[25] Meanwhile, as lutein is a primary carotenoid, it can be consumed by cells for their growth and converted to other metabolites when necessary.[25] Therefore, Eq. 1c is constructed to simulate lutein production. In this equation, the first term on the righthand side represents lutein synthesis rate and is originated from the Monod model, while the second term represents lutein consumption rate. So far there is no research investigating the detailed metabolic mechanisms of lutein consumption, hence, first-order kinetics are assumed to approximate the lutein consumption rate led by its conversion to other metabolites and degradation by cell decay.

$$\frac{dc_L}{dt} = k_0 \cdot \frac{c_N}{c_N + K_{NL}} \cdot c_X - k_d \cdot c_L \cdot c_X \tag{1c}$$

where c_L is culture lutein production (mg L^{-1}), k_0 is lutein synthesis rate constant (mg g^{-1} h^{-1}), K_{NL} is nitrate half-velocity constant for lutein synthesis (mg L^{-1}), k_d is lutein consumption rate constant (L g^{-1} h^{-1}).

Simulation of light intensity

Both cell specific growth rate (u_0) and lutein synthesis rate constant (k_0) are predominantly determined by light intensity under algal photoautotrophic growth conditions. The effect of

light intensity on biomass growth can be formulated as in Eq. 2a.[26] Similarly, its effect on lutein synthesis can be described by Eq. 2b.

$$u_0(I(z)) = u_m \cdot \frac{I(z)}{I + k_s + \frac{I^2}{k_i}} \tag{2a}$$

$$k_0(I(z)) = k_m \cdot \frac{I(z)}{I + k_{sL} + \frac{I^2}{k_{iL}}} \tag{2b}$$

where u_m is the maximum cell specific growth rate, k_m is the maximum lutein synthesis rate constant, I is light intensity, k_s and k_{sL} are light saturation terms for cell growth and lutein synthesis, respectively. k_i and k_{iL} are light inhibition terms for cell growth and lutein synthesis, respectively.

Because algae absorb photons for their growth and light is scattered by bubbles, local light intensity in the PBR keeps decreasing along the light transmission direction. As a result, local light intensities experienced by cells are much lower than the incident light intensity. To take into account light attenuation, Eq. 3, modified from the Lambert-Beer's law, is embedded into the model because of its high accuracy and convenience for parameter estimation compared to other light transmission models.[27] This equation includes the two main factors causing light attenuation in PBRs, namely, bubble scattering and cell absorption.

$$I(z) = I_0 \cdot \left(e^{-(\tau \cdot X + K_a) \cdot z} + e^{-(\tau \cdot X + K_a) \cdot (L - z)} \right) \tag{3}$$

where I_0 is incident light intensity, τ is cell absorption coefficient, K_a is bubble scattering coefficient, z is the distance from light source, and L is the width of the PBR.

However, when including this equation into the current model, the model will contain both spatial and temporal dimensions, and a partial differential equations model would be needed. To facilitate parameter estimation, process simulation and future process optimization, the 10-step Trapezoidal rule shown in Eqs. 4a and 4b is used to eliminate the spatial dimension in the model.[26] The model is then simplified into a set of ordinary differential equations. In order to further reduce the complexity of the current model, the PBR is assumed to be a column with a square cross section. This square area is assumed to be the same as that of the original circle area, which means that the width of the square is 8.40 cm. This simplification has been characterized by high accuracy and applied in recent studies.[21]

$$u_0 = \frac{u_m}{20} \cdot \sum_{n=1}^{9} \left(\frac{I_{i=0}}{I_{i=0} + k_s + \frac{I_{i=0}^2}{k_i}} + 2 \cdot \frac{I_{i=\frac{n \cdot L}{10}}}{I_{i=\frac{n \cdot L}{10}} + k_s + \frac{I_{i=\frac{n \cdot L}{10}}^2}{k_i}} + \frac{I_{i=L}}{I_{i=L} + k_s + \frac{I_{i=L}^2}{k_i}} \right) \tag{4a}$$

$$k_0 = \frac{k_m}{20} \cdot \sum_{n=1}^{9} \left(\frac{I_{i=0}}{I_{i=0} + k_{sL} + \frac{I_{i=0}^2}{k_{iL}}} + 2 \cdot \frac{I_{i=\frac{n \cdot L}{10}}}{I_{i=\frac{n \cdot L}{10}} + k_{sL} + \frac{I_{i=\frac{n \cdot L}{10}}^2}{k_{iL}}} + \frac{I_{i=L}}{I_{i=L} + k_{sL} + \frac{I_{i=L}^2}{k_{iL}}} \right) \tag{4b}$$

where I_i refers to the local light intensity at a distance of $i = \frac{n \cdot L}{10}$ from the PBR front exposure surface.

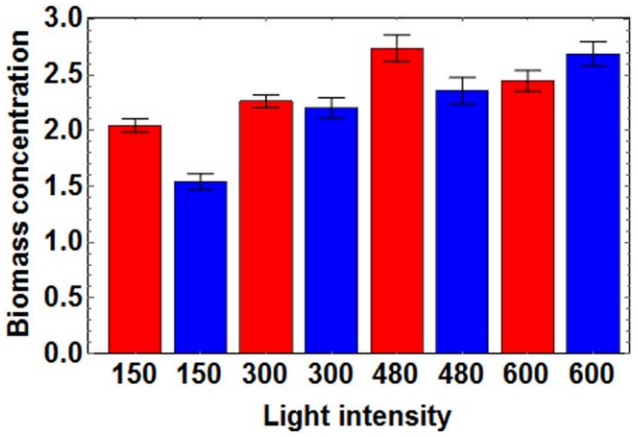

Figure 1. Final biomass concentration (g L^{-1}) in the two sets of experiments under different incident light intensity (μmol m^{-2} s^{-1}).

Red: high nitrate experiments. Blue: low nitrate experiments. [Color figure can be viewed at wileyonlinelibrary.com]

Parameter estimation methodology

Accurate estimation of parameters is crucial to guarantee an accurate and reliable model. Unreliable parameters can severely restrict the applications of models for evaluation and design purposes.[28] To this aim, a nonlinear least-squares optimization problem is formulated. The dynamic model is discretized and transformed into a nonlinear programming problem (NLP). Given the high nonlinearity and stiffness of the differential equation model, orthogonal collocation over finite elements in time is used as a discretization scheme.[29] The optimal parameters of the underlying model are determined by solving the NLP using the state-of-the-art interior point nonlinear optimization solver IPOPT.[30] The implementation in this work is programmed in the Python optimization environment Pyomo.[31]

Results and Discussion

Effects of light and nitrate concentration on cell growth

During all of the eight experiments, biomass keeps growing throughout the entire time course of operation. As initial biomass concentrations are kept the same, final biomass concentrations can be compared to analyze the effects of incident light intensity and nitrate concentration on *Desmodesmus* sp. biomass growth, which is presented in Figure 1.

From Figure 1, it can be concluded that in the low nitrate experiments (blue bars), final biomass concentration increases with the increasing incident light intensity from the experimental lower bound (150 μmol m^{-2} s^{-1}) to the upper bound (600 μmol m^{-2} s^{-1}). This tendency is also observed in the high nitrate experiments (red bars) when the incident light intensity changes from 150 to 480 μmol m^{-2} s^{-1}, above which a decrease of final biomass concentration is obtained, indicating a lower biomass growth rate. It is then concluded that in most of the cases, a higher incident light intensity (up to 600 μmol m^{-2} s^{-1}) can result in a higher biomass growth rate, which is consistent with the observations published in previous studies.[3,6,32] Furthermore, the recent research[6] has declared that photoinhibition does not appear even if the incident light intensity increases up to 750 μmol m^{-2} s^{-1} when

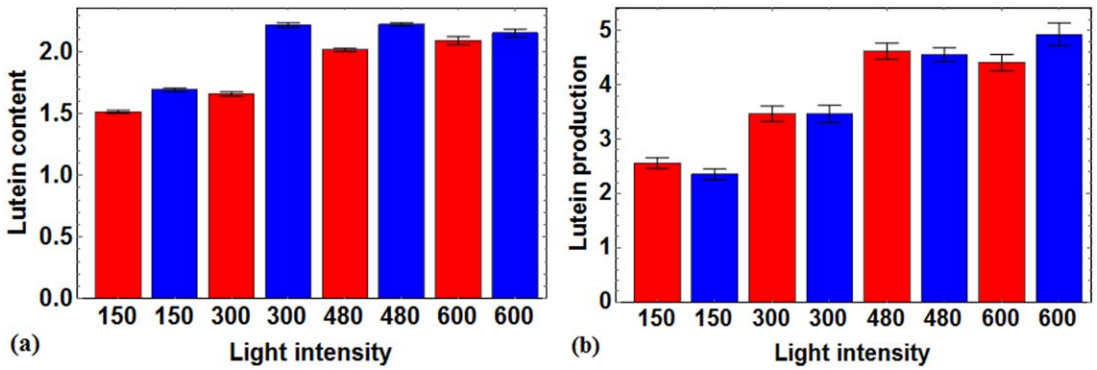

Figure 2. (a) Lutein intracellular content (mg g^{-1}) and (b) lutein total production (mg L^{-1}) in the two sets of experiments under different incident light intensity (μmol m^{-2} s^{-1}).

Red: high nitrate experiments. Blue: low nitrate experiments. [Color figure can be viewed at wileyonlinelibrary.com]

cultivating *Desmodesmus* sp., which strongly suggests the lower biomass growth rate found in the high nitrate experiments when increasing incident light intensity from 480 to 600 μmol m^{-2} s^{-1} is attributed to other factors.

In terms of the influence of nitrate concentration on biomass growth, 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 is slower than its refreshment rate. Hence, *Desmodesmus* sp. is cultivated in a nitrate-sufficient culture where the modified Monod model is valid. By comparing biomass concentrations at the same incident light intensity in the two sets of experiments, it is seen that biomass growth rate is always higher in a denser nitrate concentration culture. The only exception happens in the experiment where the culture nitrate concentration is the highest (15.8 g L^{-1}), indicating the occurrence of nitrate inhibition under such a high concentration. As a result, final biomass concentration in this experiment (high nitrate experiment, light intensity of 600 μmol m^{-2} s^{-1}) is lower than that in other experiments (low nitrate experiment with the same light intensity, and high nitrate experiment with a light intensity of 480 μmol m^{-2} s^{-1}).

Effects of light and nitrate concentration on lutein synthesis

Total lutein production and highest lutein intracellular content are presented in Figure 2 for all eight experiments. From Figure 2a, it can be seen that in the high nitrate experiments, lutein content continuously increases with the increasing incident light intensity from 150 to 600 μmol m^{-2} s^{-1}, while its maximum value in the low nitrate experiments falls within the range of 300 to 480 μmol m^{-2} s^{-1}. However, neither of the current observations is in agreement with the previous research where lutein content was found to decrease with the increasing light intensity from 150 to 750 μmol m^{-2} s^{-1}.[6] As a result, the distinct discrepancy between current observations and previous conclusions suggests the complex metabolic mechanisms of lutein synthesis. It also indicates that other factors apart from incident light intensity can significantly affect intracellular lutein content.

In terms of the effect of nitrate concentration on lutein synthesis, although it is known that nitrate is essential for lutein synthesis and lutein is a primary carotenoid which can be accumulated under nitrogen-sufficient conditions,[33] from Figure 2a it is found that under the same incident light intensity, lutein content in the low nitrate experiments (lower nitrate concentration) is higher than that in the high nitrate experiments (higher culture nitrate concentration). This phenomenon was also reported in a recent study[21] where a similar photosynthetic pigment, C-phycocyanin, is synthesized by cyanobacteria in a nitrogen-sufficient culture.

Del Rio-Chanona et al.[21] demonstrated that under nitrogen-sufficient conditions, biomass grows rapidly and thus the dense biomass concentration will result in a severe light attenuation in PBRs. Because sufficient light is one of the key factors to guarantee phycocyanin synthesis, the lack of local illumination in the culture will strongly suppress phycocyanin synthesis. Furthermore, as phycocyanin is intracellular nitrogen storage, it can be converted to other compounds for cell growth under low illumination circumstances. Therefore, opposite to that in a nitrogen-limiting culture where phycocyanin content increases with the increasing nitrate concentration (low biomass concentration due to the limited amount of available culture nitrate), in a nitrogen-sufficient culture the higher culture nitrate concentration can introduce a more severe light attenuation(biomass concentration is doubled compared to that in a nitrate-limiting culture), which will significantly lower the local light intensity that cells could experience. As a result, phycocyanin content will become much lower due to both the extremely low local light intensity which cannot effectively activate phycocyanin synthesis pathway, and its continuous conversion into other metabolites.[21] In the current study, lutein is also a photosynthetic pigment whose synthesis needs the participation of sufficient light intensity, and it serves as a primary metabolite which can be transformed to secondary metabolites for biomass growth and maintenance.[25,33] The current observation that lutein content is higher in a lower nitrate concentration under nitrate-sufficient conditions could therefore also be explained as what has been demonstrated for phycocyanin.[21]

On the contrary, for total lutein production, it is found that a higher light intensity and a denser culture nitrate concentration can always lead to a higher lutein production as long as nitrate inhibition does not happen, as shown in Figure 2b. Such conflicting conclusion compared to that of lutein synthesis is because lutein production is the product of both biomass concentration and lutein intracellular content. Although a high nitrate concentration may limit lutein accumulation, it can significantly facilitate green algae biomass growth. As a result, total lutein production can still be increased through this condition. Nonetheless, it should be noted that low lutein content can remarkably elevate the bioprocess downstream separation

Table 2. Parameters in the Current Model

Parameter	Value	Parameter	Value
u_m (h^{-1})	0.152	k_s (μmol m^{-2} s^{-1})	142.8
u_d (h^{-1})	5.95×10^{-3}	k_i (μmol m^{-2} s^{-1})	214.2
K_N (mg L^{-1})	30.0	k_{sL} (μmol m^{-2} s^{-1})	320.6
$Y_{N/X}$ (mg g^{-1})	305.0	k_{iL} (μmol m^{-2} s^{-1})	480.9
k_m (mg g^{-1} h^{-1})	0.350	τ (m^2 g^{-1})	0.120
k_d (L mg^{-1} h^{-1})	3.71×10^{-3}	K_a (m^{-1})	0.0
K_{NL} (mg L^{-1})	10.0		

cost, which may seriously reduce the process profitability. Hence, it is essential to guarantee an adequate lutein content when aiming to maximize total lutein production.

Results of dynamic model construction

Three sets of experimental data coming from the low nitrate experiments are selected to estimate the current model parameter values. These experiments were carried out under the incident light intensity of 150, 300, and 600 μmol m^{-2} s^{-1}, respectively. By transforming the parameter estimation problem into a nonlinear program (NLP), values of parameters in the current model are calculated and listed in Table 2. Figure 3 shows the model fitting result.

From Figure 3, it can be seen that the current model can well fit all sets of the experimental data, which indicates the high accuracy of the current model. From Table 2, it is found that nitrate half-velocity coefficients for both biomass growth (30.0 mg L^{-1}) and lutein synthesis (10 mg L^{-1}) are much lower than the culture nitrate concentration (higher than 300 mg L^{-1}). This suggests that biomass growth is not limited by the culture nitrate concentration and current experiments were implemented in a nitrogen-sufficient culture, which can be considered as support to the conclusion obtained in the above sections. In addition, the zero value of K_a (bubble scattering coefficient) implies that in the current PBR green algae cell absorption is the predominant factor causing light attenuation, and the effect of bubble scattering is negligible.

Validation of dynamic model predictability

Because the current dynamic model was constructed with the aim to predict the optimal operating conditions in future process design and control, it has to be characterized by not only high accuracy but also good predictability. Therefore, this model is used to simulate the dynamic performance of all the remaining five experiments conducted in the current study. Figures 4 and 5 show its prediction results compared against the real experimental data.

From the figures, it can be seen that the current model shows great predictability within a wide range of operating conditions throughout the entire experimental time course. Among the 144 experimental data points, the majority of deviation between model prediction and real experiment is far below 10%, with only four exceptions shown in Figure 4c (11.4%) and Figure 5e (13.8%). This, therefore, strongly indicates the high predictability and accuracy of the current model, as well as its great competence for further process design and optimal control.

Furthermore, based on the current model, the optimal light intensity for *Desmodesmus* sp. growth is estimated to be 175 μmol m^{-2} s^{-1}, and that for lutein production is 390 μmol m^{-2} s^{-1}. Despite the fact that these values are lower than some of the incident light intensities used in the current experiments, due to the severe light attenuation in the current PBR, average light intensities in the present experiments are much lower than the incident light intensity. Hence, photoinhibition is not observed when increasing the incident light intensity from 150 to 600 μmol m^{-2} s^{-1}.

Comparison of lutein synthesis in nitrogen-sufficient and nitrogen-limiting conditions

Finally, by comparing the results of current experiments and the previous study,[6] two conclusions can be obtained. The first one is that compared to a nitrogen-sufficient culture, under the same incident light intensity and PBR configuration,

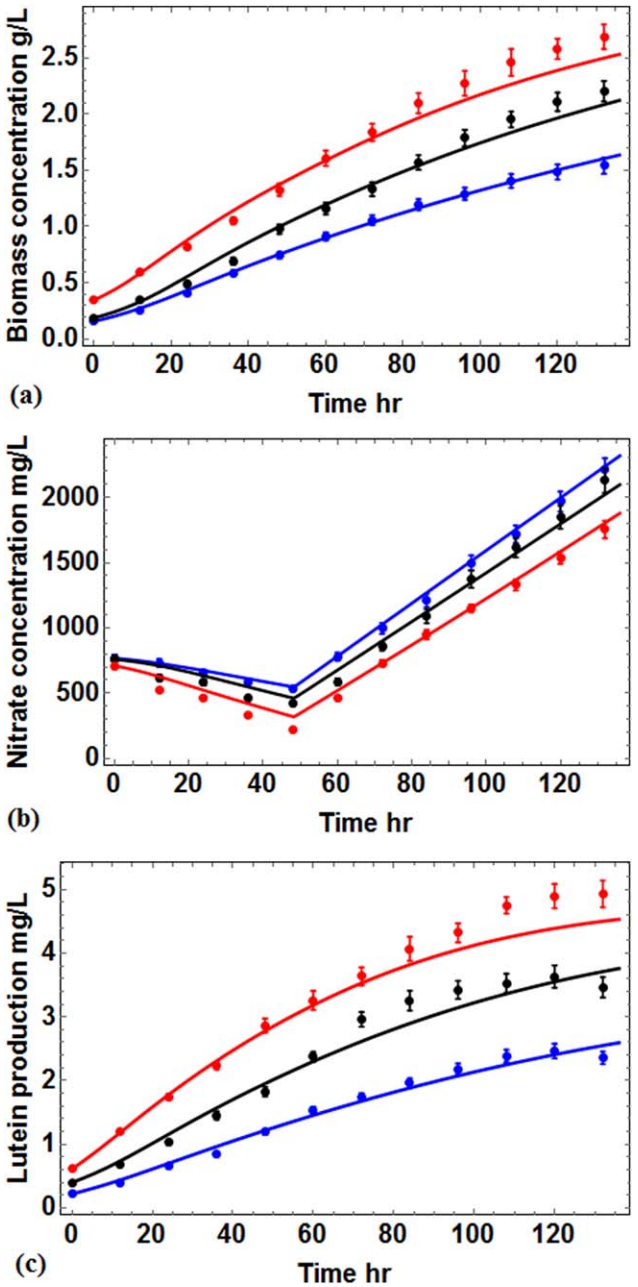

Figure 3. Model fitting results of biomass concentration (a), nitrate concentration (b), and lutein production (c).

Lines: model simulating result, points: experimental data. Blue: incident light intensity 150 μmol m^{-2} s^{-1}. Black: incident light intensity 300 μmol m^{-2} s^{-1}. Red: incident light intensity 600 μmol m^{-2} s^{-1}. [Color figure can be viewed at wileyonlinelibrary.com]

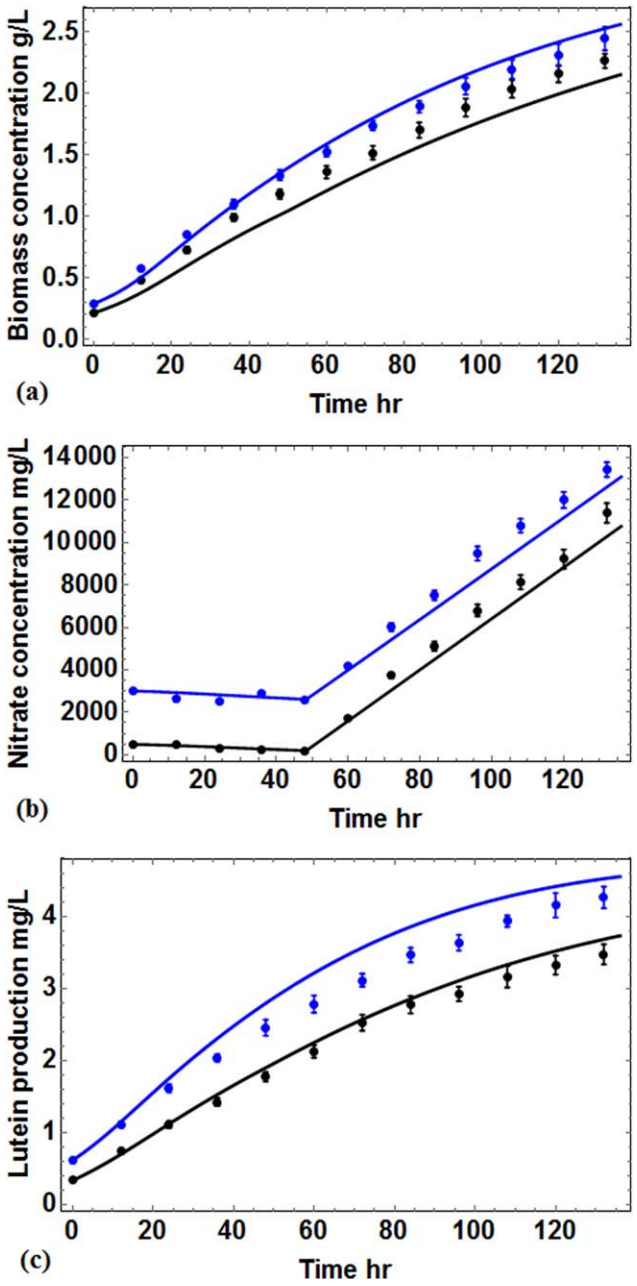

Figure 4. Model prediction results of biomass concentration (a), nitrate concentration (b), and lutein production (c) for the high nitrate experiments.

Lines: dynamic model prediction results, points: real experimental data. Black: incident light intensity 300 μmol m^{-2} s^{-1}. Blue: incident light intensity 600 μmol m^{-2} s^{-1}. [Color figure can be viewed at wileyonlinelibrary.com]

a nitrogen-limiting culture can result in a higher lutein intracellular content. The second one is that lutein content decreases more rapidly in a nitrogen-limiting culture than that in a nitrogen-sufficient culture after reaching its peak value.

For example, from the recent study[6] it is found that the highest lutein content in a nitrogen-limiting culture is 4.69 and 3.97 mg g^{-1}, corresponding to the light intensity of 150 and 600 μmol m^{-2} s^{-1}, respectively. While in a nitrogen-sufficient culture this is found to be 1.70 and 2.16 mg g^{-1}, respectively. Although the detailed metabolic mechanisms of *Desmodesmus* sp. lutein synthesis have not yet been fully identified, it has been declared that under nitrogen-limiting conditions algae prefer to convert culture nitrate into intracellular nitrogen storage for their maintenance instead of consuming it for their growth.[34–36] As a result, compared to a nitrogen-sufficient culture, cells can accumulate more nitrogen storage in a nitrogen-limiting culture. Since lutein is a nitrogen storage,[33] it is therefore possible that its content is higher in a nitrogen-limiting culture.

However, as the sufficiency of culture nitrate is essential to guarantee the rapid replenishment of intracellular nitrogen

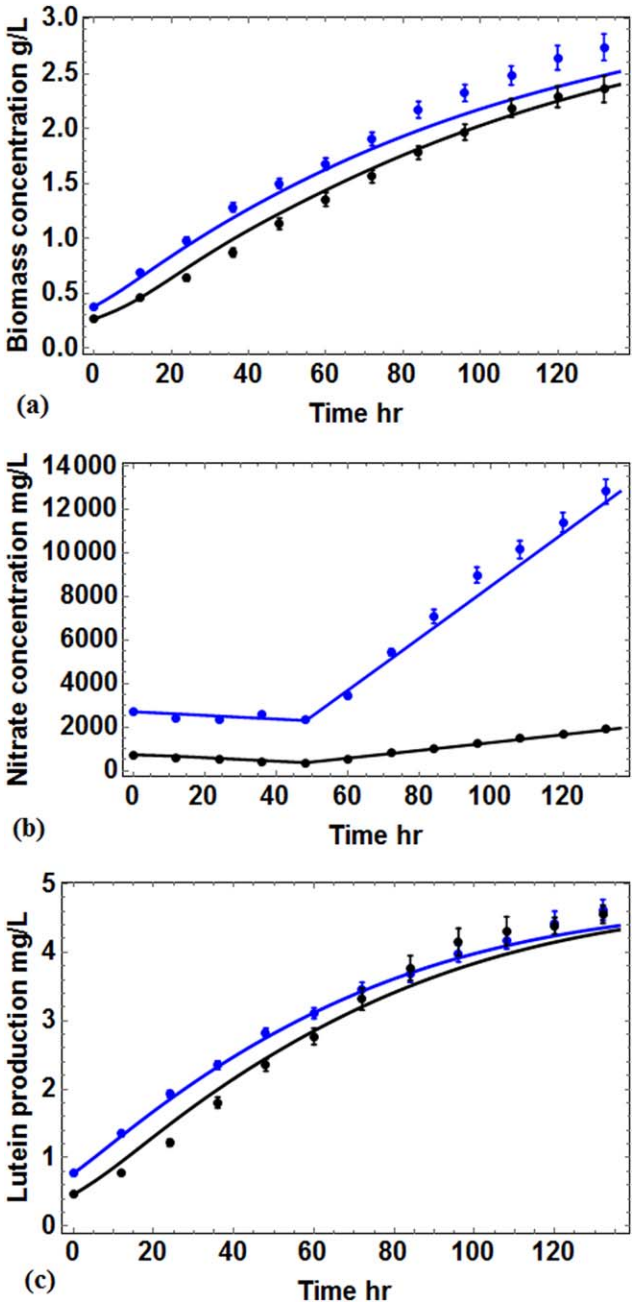

Figure 5. Model prediction results of biomass concentration (a), nitrate concentration (b), and lutein production (c) for different experiments under the incident light intensity of 480 μmol m^{-2} s^{-1}.

Lines: dynamic model prediction results, points: real experimental data. Blue: high nitrate experiment. Black: low nitrate experiment. [Color figure can be viewed at wileyonlinelibrary.com]

storage and lutein synthesis, once lutein is converted to other proteins or secondary carotenoids for cell maintenance, its content will decrease dramatically due to the limited amount of nitrate in the nitrogen-limiting culture. On the contrary, although lutein content in a nitrogen-sufficient culture is lower than that in a nitrogen-limiting culture, its decreasing rate is blunter and its value can be kept constant over a certain duration due to the excess of nitrate as observed in the current study.

Similarly, the above interpretation can explain the conflicting observations described in the above section (effects of light and nitrate concentration on lutein synthesis). In the current study, it is found that a higher lutein content is obtained under a higher incident light intensity, while in the previous research[6] a rapid drop of lutein content was found when increasing light intensity. Because a higher light intensity can facilitate biomass growth, the consumption rate of nitrogen storage into other metabolites such as proteins for cell division will be enhanced. Since nitrogen storage cannot be promptly replenished in nitrogen-limiting conditions, the content of nitrogen storage such as lutein will decrease when increasing light intensity. As a result, lutein content was found to be decreased when increasing light intensity in the previous study. In contrast, in a nitrogen-sufficient culture (e.g., the current experiments), nitrogen storage can be rapidly refilled and lutein is an accessory photosynthetic pigment, hence, its content can be kept high under high illumination conditions. Therefore, it is concluded that lutein content is determined by the complex interaction between light intensity and nitrate concentration.

Conclusion

In the current study, eight experiments were conducted to explore the effects of incident light intensity and nitrate concentration on both *Desmodesmus* sp. biomass growth and lutein production. Based on these results, the different behaviors of biomass growth and lutein synthesis under nitrogen-sufficient conditions and nitrogen-limiting cultures were thoroughly compared. It is found that microalgal lutein content under nitrogen-sufficient conditions increases with the decreasing culture nitrate concentration, opposite to that observed under nitrate-limiting conditions. However, a higher nitrate concentration can always result in a higher lutein production, due to the much-increased biomass concentration. Moreover, a kinetic model capable of simulating the dynamic performance of both cell growth and lutein production throughout the entire experimental time course of operation was proposed, and its accuracy and predictability were further verified. Based on the dynamic model, optimal light intensities for cell growth and lutein production were estimated to be 175 and 390 μmol m^{-2} s^{-1}, respectively. The high accuracy and predictive capability represented in the current proposed model therefore indicates its further application in process control and optimization.

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