Journal of Industrial Microbiology & Biotechnology (1998) 21, 283–288

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Kinetic models for phycocyanin production by high cell density mixotrophic culture of the microalga *Spirulina platensis*

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Phycocyanin production by high cell density cultivation of *Spirulina platensis* in batch and fed-batch modes in 3.7-L bioreactors with a programmed stepwise increase in light intensity program was investigated. The results showed that the cell density in fed-batch culture (10.2 g L^{-1}) was 4.29-fold that in batch culture (2.38 g L^{-1}), and the total phycocyanin production in the fed-batch culture (0.795 g L^{-1}) was 3.05-fold that in the batch culture (0.261 g L^{-1}). An unstructured kinetic model to describe the microalga culture system including cell growth, phycocyanin formation, as well as glucose consumption was proposed. The data fitted the models well ($r^2 > 0.99$). Furthermore, based on the kinetic models, the potential effects of light limitation and photoinhibition on cell growth and phycocyanin formation can be examined in depth. The models demonstrated that the optimal light intensity for mixotrophic growth of *Spirulina platensis* in batch or fed-batch cultures using a 3.7-L bioreactor was 80~160 μE m^{-2} s^{-1}, and the stepwise increase in light intensity can be replaced by a constant light intensity mode.

Keywords: *Spirulina platensis*; mixotrophic culture; light irradiance; phycocyanin production; kinetic model

Introduction

Spirulina platensis is a blue-green microalga which can produce large quantities of high value products such as phycocyanin [3]. This organism can utilize organic carbon substrates (ie, glucose, acetate, etc) for heterotrophic and mixotrophic growth [3,8]. However, heterotrophic growth is not suitable for production of phycocyanin, for the content of the alga grown heterotrophically was only 55 mg g^{-1} dry biomass [9], and heterotrophic growth of *Spirulina platensis* had an extremely long lag phase (about 200 h) and low specific growth rate of 0.0083 L h^{-1} [9]. In contrast, a lag was not observed with mixotrophic culture and there was a higher specific growth rate (0.026 L h^{-1}) as well as a greater phycocyanin content (120 mg g^{-1} dry biomass) [3].

Large-scale synthesis of phycocyanin by *Spirulina platensis* is hampered by problems associated with process design. Low productivity with typical cell densities of 0.5–1 g L^{-1} is a major obstacle to the successful commercialization of phycocyanin production [6,13]. The optimization and control of bioprocesses often requires the establishment of a mathematical model that describes the kinetics of process variables (microbial growth, substrate uptake and product formation). Despite impressive progress made recently in developing structured models for microbial growth, the unstructured models or semi-mechanistic models are still the most popular ones used in practice [25].

Fed-batch culture has been widely employed for high cell density cultivation of microorganisms [21,28]. However, little information is available concerning the kinetic models for mixotrophic microalgal culture systems involved in cell growth, glucose consumption, and product formation simultaneously, although the relationship between specific growth rate of microalgae and substrate or light intensity was sometimes involved in the investigations [4,5,10,15,17,22].

The objectives of the present study were to investigate the phycocyanin production by high cell density cultivation of *Spirulina platensis* in batch and fed-batch modes and to develop an unstructured kinetic model, which takes into account the dependence of growth and production on glucose concentration and light intensity so as to analyze the dynamics of microalgae growth and phycocyanin accumulation under mixotrophic culture conditions.

Materials and methods

Microorganism and culture medium

Spirulina platensis (UTEX 1926) was obtained from the University of Texas Culture Collection. Zarouk medium supplemented with 2 g L^{-1} glucose was used [23] and sterilized at 121°C for 15 min before use. The initial pH was adjusted to 9.5 with 1 M NaOH and 1 M HCl.

Batch culture

The experiment was carried out in a 3.7-L bioreactor (Bioengineering AG, Switzerland) containing 2.5 L of medium. The culture was agitated at 300 rpm (a mixer with flat-blade disk turbine) and sterile air was supplied to the culture at a flow rate of 100 L h^{-1}. Culture temperature was set at 30°C. The culture was illuminated at a constant light intensity of 80 μE m^{-2} s^{-1}.

Fed-batch culture

The experiment was also carried out in a 3.7-L bioreactor (Bioengineering AG, Switzerland) containing 2.5 L of medium. The culture temperature, agitation rate, and aeration rate were the same as that in the batch culture. At the

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Received 28 July 1998; accepted 8 October 1998

late exponential phase of growth (the 5th day), the sterile glucose solution (100 g L^{-1}) was fed intermittently by a peristaltic pump (2 ml min^{-1}). Light irradiance was provided by the surrounding cool white fluorescent tubes, gradually increased light intensities from 80 (the first 4 days) to 100 (days 5–6), 120 (days 7–8), 140 (days 9–10), and 160 (after day 10) μE m^{-2} s^{-1} was used.

Analytical methods

Cell dry weight concentrations, glucose concentrations, and phycocyanin content were determined according to the methods previously reported [3].

Model development

Cell growth model: The most widely used unstructured model for the specific growth rate, μ, is the Monod equation, which increases monotonically as a function of the substrate concentration C_S [11]:

$$\mu = \frac{\mu_m C_S}{K_s + C_S} \tag{1}$$

where μ_m is the maximum specific growth rate and K_S is the Monod saturation constant, a kinetic parameter which indicates how fast the maximum specific growth rate is reached. In fact, according to Eqn (1), $\mu \rightarrow \mu_m$ when $K_S \rightarrow 0$, ie the smaller K_S, the more rapidly the maximum specific growth rate is reached. Unfortunately, the Monod model often fails to account for substrate inhibition of growth at higher substrate concentrations. To overcome the drawback, another well-known model is used, the non-monotonic Haldane model as a function of the substrate concentration C_S [1]:

$$\mu = \frac{\mu_m C_S}{K_s + C_S + \frac{C_S^2}{K_i}} \tag{2}$$

where K_i is the inhibition constant. The smaller K_i, the larger the inhibition effect of the substrate.

Both the Monod model and the Haldane model only examine the effect of a single substrate and ignore the inhibition potential of other environmental factors such as light intensity, the cell itself, the product, etc.

Practically, the most important environmental factor for the algal culture is light, which primarily concerns photosynthesis. In a manner similar to that of the Tamiya model [16], a Monod-type expression $I/K_{XI} + I$ was employed to describe the effect of light. Additionally, Weiss and Ollis [20] proposed a model depending on biomass concentration only, by means of a logistic equation:

$$\mu = \mu_m \left(1 - \frac{C_X}{C_{X_m}}\right) \tag{3}$$

hence the expression $(1 - C_X/C_{X_m})$ was employed to describe the biomass inhibition. Also, the expression $(1 - C_p/C_{P_m})$ was used to describe the product inhibition, as reported in a glycerol fermentation by Zeng *et al* [24]. Thus the Haldane model may be extended as follows:

$$\mu = \mu_m \frac{C_S}{K_S + C_S + \frac{C_S^2}{K_{xi}}} \frac{I}{K_{XI} + I} \left(1 - \frac{C_X}{C_{X_m}}\right)\left(1 - \frac{C_P}{C_{P_m}}\right) \tag{4}$$

where μ is the specific growth rate (1/day), μ_m is the maximum specific growth rate (1/day), C_X, C_P, C_S are the cell concentration (g L^{-1}), the product concentration (g L^{-1}) and the glucose concentration (g L^{-1}), respectively. C_{X_m}, C_{P_m} are the maximum cell concentration (g L^{-1}) and maximum product concentration (g L^{-1}), respectively, ie they are critical concentrations for the inhibitors above which cell or product cease to grow [26]. I is light intensity (μE m^{-2} s^{-1}). K_S, K_{XI}, K_{xi} are respectively the glucose saturation constant, the light saturation constant and the glucose inhibition constant of cell growth (g L^{-1}).

Product formation model: The much-discussed kinetic model for product formation is the following equation [7]:

$$\frac{dC_P}{dt} = Y_{PX}\frac{dC_X}{dt} + \mu_{PX}C_X \tag{5}$$

The model states that the product formation rate of cells can be attributed to a growth-associated part and a non-growth-associated part. Apparently, the model does not take into account the inhibition effects of glucose, product itself and light intensity. In order to account for glucose inhibition of product formation, a Haldane-type expression $\frac{C_S}{K_{PS} + C_S + \frac{C_S^2}{K_{pi}}}$ was incorporated. In fact, Moraine and Rogovin [12] used the following equation to describe xanthan production:

$$\frac{dC_P}{dt} = \frac{\mu_{PX}C_X C_S}{K_{PS} + C_S} \tag{6}$$

Similarly, Monod-type expressions ($I/K_{PI} + I$) and the logistic expression ($C_P(1 - C_p/C_{p_m})$) were formulated accordingly to describe light influence and product inhibition, respectively. Therefore, the Luedeking-Piret equation for product formation is extended as follows:

$$\frac{dC_P}{dt} = \left(Y_{PX}\frac{dC_X}{dt} + \mu_{PX}C_X\right) \frac{C_S C_P}{K_{PS} + C_S + \frac{C_S^2}{K_{pi}}} \frac{I}{K_{PI} + I}\left(1 - \frac{C_P}{C_{P_m}}\right) \tag{7}$$

where C_X, C_P, C_{P_m}, C_S, I are the same as above. K_{PS}, K_{pi},

and K_{PI} are the glucose saturation constant, the glucose inhibition constant, and light inhibition constant of product formation (g L^{-1}), respectively. Y_{PX} is the instantaneous yield coefficient of product formation due to cell growth (g g^{-1}), which reflects the ability of product formation with the increased cell concentration. μ_{PX} is the specific formation rate of product (1/day). In fact, according to Eqn (5), $dC_P/dt \approx Y_{PX}\, dC_X/dt$ (when $C_X \rightarrow 0$), ie $Y_{PX} = dC_P/dC_X$; $\mu_{PX} = 1/C_X\, dC_P/dt$ when $dC_X/dt = 0$, ie the concentration of cells arrives at the maximum value, the cells cease to grow, so the non-growth-associated part is often considered to be related to maintenance functions of cells.

Substrate consumption model: The most widely used substrate consumption model can be expressed as:

$$-\frac{dC_S}{dt} = \frac{1}{Y_{XS}}\frac{dC_X}{dt} + \frac{1}{Y_{PS}}\frac{dC_P}{dt} + \mu_{SX}C_X \tag{8}$$

In this study, a Monod-type expression $C_S/K_{SA} + C_S$ was employed to describe the autoinhibition of substrate itself. Eqn (8) can be written as:

$$-\frac{dC_S}{dt} = \frac{C_S}{K_{SA} + C_S}\left(\frac{1}{Y_{XS}}\frac{dC_X}{dt} + \frac{1}{Y_{PS}}\frac{dC_P}{dt} + \mu_{SX}C_X\right) \tag{9}$$

which is used as glucose consumption model. C_X,C_P,C_S are the same as above. K_{SA} is the autoinhibition constant of glucose. Y_{XS} is the instantaneous yield coefficient of cells on glucose (g g^{-1}) ($Y_{XS} = -dC_X/dC_S$, when $C_X \rightarrow 0$ and $dC_P/dt = 0$). Y_{PS} is the instantaneous yield coefficient of product on glucose (g g^{-1}) ($Y_{PS} = -dC_P/dC_S$, when $C_X \rightarrow 0$ and $dC_X/dt = 0$). μ_{SX} is the specific consumption rate of glucose (1/day). In fact, $\mu_{SX} = -1/C_X\, dC_S/dt$, when $dC_X/dt = dC_P/dt = 0$, ie the concentrations of cell and product arrive at the maximum values, the cell and product cease to accumulate, so the parameter is also considered to be related to the maintenance functions of cells.

Consequently, an unstructured kinetic model (Eqns (4),(7),(9)) for phycocyanin production by mixotrophic cultivation of the microalga *Spirulina platensis* is proposed in this work. This model contains 15 parameters, which have important physiological meanings. Each one exhibits a process-state during cultivation of the microalga.

Parameter estimations were performed using a Simplex Search Method; the principal idea of this method is to search the best value of the objective function in a space of points P_i which represent the feasible solutions. The first point P_1 is arbitarily selected as the starting point (base point). The second point P_2 is chosen and compared with P_1. If P_2 is found to be a better solution than P_1, then P_2 is selected as the new base point; if not, P_1 stays as the base point. This process is continued until the best operating point is found. The details were described previously [27]. The starting values of model parameters were determined by physiologically meaningful ranges.

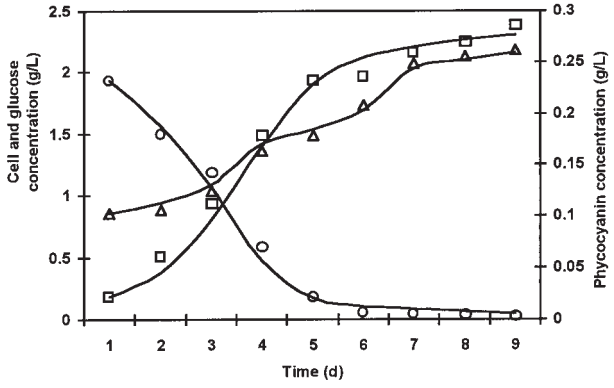

Figure 1 Time courses of cell growth, glucose consumption and phycocyanin formation for *Spirulina platensis* in batch mixotrophic culture. (□, ○, Δ) The experimental data for cell growth, glucose consumption and phycocyanin production, respectively; (—) the calculated values.

Results and discussion

The changes in cell concentration and phycocyanin production with cultivation time in batch and fed-batch cultures are plotted in Figures 1 and 2. By fitting the experimental data, the following kinetic models proposed in this work were given:

For batch culture (without light influence term, for light intensity is constant ($I = 80$)):

$$\frac{dC_X}{dt} = \frac{1.4653C_SC_X}{0.3138 + C_S + \frac{C_S^2}{96.8231}}\left(1 - \frac{C_X}{2.6278}\right)\left(1 - \frac{C_P}{0.3569}\right) \tag{10}$$

$$\frac{dC_P}{dt} = \left(0.3949\frac{dC_X}{dt} + 0.1108C_X\right)\frac{C_SC_P}{0.0136 + C_S + \frac{C_S^2}{61.8611}}\left(1 - \frac{C_P}{0.3569}\right) \tag{11}$$

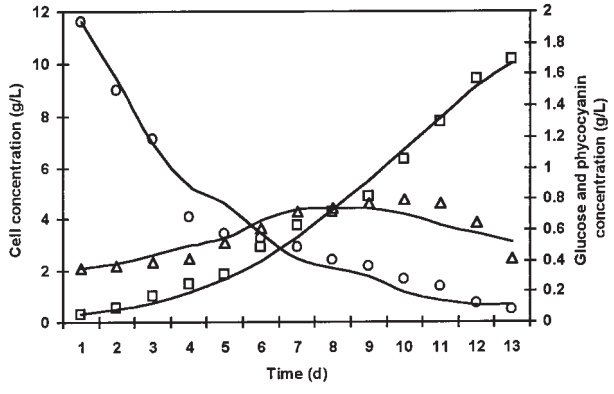

Figure 2 Time courses of cell growth, glucose consumption and phycocyanin formation for *Spirulina platensis* in fed-batch mixotrophic culture. (□, ○, Δ) The experimental data for cell growth, glucose consumption and phycocyanin production, respectively; (—) the calculated values.

$$-\frac{dC_S}{dt} = \frac{C_S}{0.9060 + C_S}\left(\frac{1}{10.2063}\frac{dC_X}{dt} + \frac{1}{0.3782}\frac{dC_P}{dt} + 0.1418C_X\right) \quad (12)$$

for fed-batch culture:

$$\frac{dC_X}{dt} = \frac{0.8831C_SC_X}{0.0972 + C_S + \frac{C_S^2}{64.3891}}\frac{I}{45.2466 + I}\left(1 - \frac{C_X}{13.7520}\right)\left(1 - \frac{C_P}{0.7386}\right) \quad (13)$$

$$\frac{dC_P}{dt} = \left(1.8533\frac{dC_X}{dt} + 0.0062C_X\right)\frac{I}{129.8042 + I}\frac{C_SC_P}{0.0484 + C_S + \frac{C_S^2}{40.3680}}\left(1 - \frac{C_P}{0.7386}\right) \quad (14)$$

$$-\frac{dC_S}{dt} = \frac{C_S}{0.2982 + C_S}\left(\frac{1}{4.0210}\frac{dC_X}{dt} + \frac{1}{0.7692}\frac{dC_P}{dt} + 0.0014C_X\right) \quad (15)$$

The experimental data fitted the models quite well (for batch culture $r^2 = 0.9940$, for fed-batch $r^2 = 0.9928$) (Figures 1 and 2). Eqns (10) and (13) demonstrated that the maximum specific growth rate was reached more slowly for batch culture than fed-batch culture, because the velocity indication parameter in batch culture ($K_S = 0.3138$ g L^{-1}) was remarkably larger than that in fed-batch culture ($K_S = 0.0972$ g L^{-1}). From Eqns (11) and (14) the instantaneous yield coefficient of product formation due to cell growth ($Y_{PX} = 1.8533$ g g^{-1}) in fed-batch culture was larger than that in batch culture ($Y_{PX} = 0.3949$ g g^{-1}); this means that the ability of phycocyanin formation with the increased cell concentration in fed-batch culture was greater than that in batch culture.

Figures 3 and 4 show the simulated results of effects of light intensity on cell growth and phycocyanin formation in fed-batch culture using the developed model.

According to Figures 1–4, it follows that the maximum cell concentration in the fed-batch culture with the variant light intensity mode (10.2 g L^{-1}) was 4.3-fold that in the batch culture with the constant light intensity (2.38 g L^{-1}), and it was much higher than that reported so far in the literature (1 g L^{-1} in outdoor culture [19]; 6.3 g L^{-1} in tubular photobioreactors [18]; 5.4 g L^{-1} by addition of protein hydrolysate [14] and 2.1 g L^{-1} in mixotrophic culture [19]). The phycocyanin concentration in the fed-batch culture increased constantly as the growth proceeded to a maximum value of approximately 0.795 g L^{-1} after approximately 10 days of cultivation and then fell away sharply, this might be due to nitrogen starvation as phycocyanin could be used as a nitrogen storage compound by *S. platensis* [2]. The resulting phycocyanin production in the fed-batch culture with the variant light intensity mode (0.795 g L^{-1}) was about 3-fold that in the batch culture with the constant light intensity mode (0.261 g L^{-1}). The difference in cell and product overall yields is due to the combined contributions of culture modes and light intensity. When light intensity is fixed ($I = 80$), the maximum cell concentration in the fed-batch culture (8.8683 g L^{-1}) was 3.73-fold that in the batch culture (2.38 g L^{-1}), and the maximum phycocyanin concentration in the fed-batch (0.6828 g L^{-1}) was 2.62-fold that in the batch culture (0.261 g L^{-1}). When the culture mode is fixed (fed-batch culture), the maximum cell concentration in the culture for

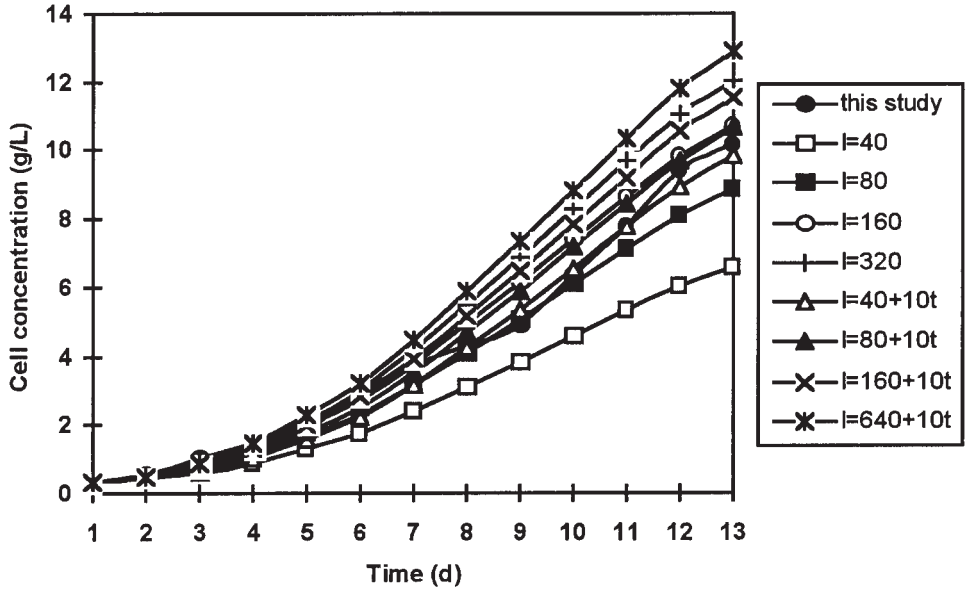

Figure 3 Simulation curves from the presented model in this paper for the effect of a different light intensity mode on cell growth in fed-batch mixotrophic culture.

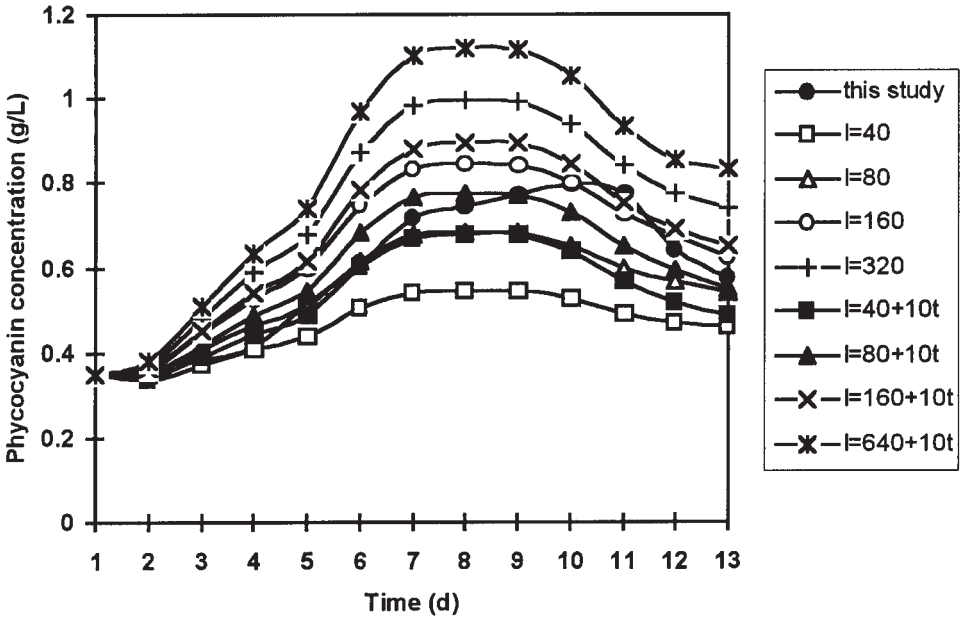

Figure 4 Simulation curves from the presented model in this paper for the effect of a different light intensity mode on phycocyanin formation in fed-batch mixotrophic culture.

$I = 40$ (6.2871 g L^{-1}) was 1.93-fold that in the culture for $I = 320$ (12.1488 g L^{-1}), and the maximum phyocyanin concentration in the culture for $I = 40$ (0.5421 g L^{-1}) was 1.84-fold that in the culture for $I = 320$ (0.9969 g L^{-1}). This showed that both culture mode (batch *vs* fed-batch) and light intensity play a role in affecting the mentioned overall yields, and the effect of culture mode is greater than that of light intensity.

Figures 3 and 4 demonstrate that the maximum cell concentration in this study was higher than that in the constant light intensity mode $I = 40$ and $I = 80$, approached the level at $I = 180$ or the variant light control mode $I = 40 + 10$ t and $80 + 10$ t; and lower than the other modes. The maximum phycocyanin concentration in this study was higher than that in the constant light intensity $I = 40$, $I = 80$, and $I = 40 + 10$ t, approached the level at $I = 160$ or the variant light control mode $I = 160 + 10$ t, and lower than the other modes. These indicated that a stepwise increase in light intensity can be replaced by a constant light intensity mode; lower light intensity (<80 μE m^{-2} s^{-1}) produces the effect of light limitation.

On the other hand, from Figures 3 and 4 it can also be seen that for the constant light intensity mode, when light intensity increases from $I = 40$ μE m^{-2} s^{-1} to $I = 160$ μE m^{-2} s^{-1}, cell concentration increased by 63%, and the phycocyanin concentration increased by 6.7%; while with the further increase of light intensity, up to $I = 320$ μE m^{-2} s^{-1}, the cell concentration only increased by 12% and the phycocyanin concentration only increased by 1.8%. For the variant light intensity mode, when light intensity changed from $I = 40 + 10$ t (μE m^{-2} s^{-1}, t = 1,2,…,13) to $I = 160 + 10$ t (μE m^{-2} s^{-1}, t = 1,2,…,13), cell concentration increased by 17.2%, and the phycocyanin concentration increased by 32.1%; while with the further increasing of light intensity, up to $I = 640 + 10$ t (μE m^{-2} s^{-1}, t = 1,2,…,13), the cell concentration only increased by 11.9% and the phycocyanin concentration only increased by 24.9%. These suggested that higher light intensities (>160 μE m^{-2} s^{-1}) result in photoinhibition.

Thus the optimal light intensity for mixotrophic growth of *S. platensis* in fed-batch culture using a 3.7-L bioreactor was 80~160 μE m^{-2} s^{-1}, and the stepwise increased light intensity mode could be replaced by a constant light intensity mode.

Acknowledgements

This research was supported by the Research Grant Council of Hong Kong and the University of Hong Kong Committee on Research and Conference Grants.

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