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Investigation on models for light distribution of *Haematococcus pluvialis* during astaxanthin accumulation stage with an application case

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ABSTRACT

Light is an important stress factor for astaxanthin accumulation in *H. pluvialis*. Hence, the light distribution in the *H. pluvialis* suspension was investigated with biomass concentration from 0.218 g/L to 2.5 g/L during the astaxanthin accumulation stage. Lambert-Beer model and Cornet model were employed to predict the light attenuation. The result showed that Cornet model could slightly better describe and predict the light distribution in *H. pluvialis* suspension. Besides, E_a (absorption coefficient) and E_s (scattering coefficient) were determined as $0.0126 \pm 0.0036\ m^2/g$ and $0.223 \pm 0.023\ m^2/g$, respectively. E_s increased with respect to astaxanthin content, whereas E_a declined with the increase of astaxanthin content. The astaxanthin content was the dominant factor at a relatively low mass concentration of *H. pluvialis* suspension. Furthermore, experiments were carried out in a 3 L-bubble-column PBR (photobioreactor) and a 12 L-bubble-column PBR. Higher volume-averaged light intensity and lower shear stress in 3 L PBR led to 66.67% higher astaxanthin content and 34.48% higher mass concentration than those of 12 L PBR during the outdoor experiment, respectively.

1. Introduction

Astaxanthin is famous for possessing superior antioxidant activity which is 38-fold higher than that of β-carotene and 500-fold higher than vitamin E [1,2]. It has important applications in the cosmetics, food, nutraceutical and feed industries [3]. Generally, astaxanthin can be derived from natural resources like microalgae, yeast and crustacean byproducts, or chemically synthesized; however, the synthesized astaxanthin is different from the natural astaxanthin in structural isomerism and bioactivity. Synthetic astaxanthin contains a mixture of three stereoisomers associated with two chiral centers, which is (3R, 3′R), (3R, 3′S) (*meso*), and (3S, 3′S), in approximately 1:2:1 proportions. The natural astaxanthin is mainly in the form of (3S, 3′S), which exhibited higher bioactivity as compared to the synthesized astaxanthin [4–6].

The freshwater unicellular alga *Haematococcus pluvialis* has been recognized as the best sources of natural astaxanthin due to its potential to accumulate astaxanthin as high as 4% of dry cell weight [2,7]. A two-stage cultivation strategy is often applied to mass culture and astaxanthin accumulation of *H. pluvialis*. In the green stage, stirred tanks or bubble columns are employed to obtain green vegetative cells under optimal light intensity and nutrient-replete media. Then in the red stage, green cells were stimulated to accumulate astaxanthin in PBRs under stress conditions like high irradiance, high salt and nitrate or phosphate deprivation, which is called induction [8–10].

In the red stage, irradiance is one of the most important parameters to induce astaxanthin biosynthesis of *H. pluvialis* [11,12]. Light penetrating in the *H. pluvialis* depends on the density of *H. pluvialis* suspension and astaxanthin content [13]. Unfortunately, light intensity decreases with the accumulation of astaxanthin, which limits final astaxanthin content of *H. pluvialis*. Therefore, it is a prerequisite to investigate the light distribution under different density of *H. pluvialis* suspension and astaxanthin content in *H. pluvialis* to improve astaxanthin content [14]. In addition, PBRs are key devices that affecting the final astaxanthin content in *H. pluvialis*. The performance of a PBR is usually determined by light characteristics and mixing characteristics. According to a previous study, the light transfer model can be employed to evaluate the light characteristic of a PBR [15]. To investigate the

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mixing characteristics in PBRs, CFD (computational fluid dynamics), as an effective and powerful tool, is used due to the advantage of saving operational cost and time [16–18]. However, little study on the application of light transfer model in *H. pluvialis* suspension during astaxanthin accumulation stage on the light characteristic of PBRs was reported.

In recent years, a few studies on light transfer models of *H. pluvialis* have been reported. Gao et al. [19] investigated the light attenuation of *H. pluvialis* in the green stage. Zhang et al. [20] investigated the light attenuation effect of the *H. pluvialis* and combine the light attenuation to propose a dynamic model of astaxanthin production. However, much less effort has been focused on the light distribution of *H. pluvialis*, the effect of astaxanthin content on the absorption coefficient and the scattering coefficient during the astaxanthin accumulation stage.

In the present study, the light distribution in the *H. pluvialis* suspension during the astaxanthin accumulation stage was investigated. Two light attenuation models (Lambert-Beer model and Cornet model) were employed to simulate the light distribution in the *H. pluvialis* suspension. The effect of astaxanthin content on the absorption coefficient and scattering coefficient was also investigated. Light attenuation model was employed to analyze the light characteristic of two bubble-column PBRs.

2. Materials and methods

2.1. Organisms and seed culture

H. pluvialis NIES-144 was obtained from the National Institute for Environmental Studies (NIES, Tsukuba, Japan). The strain was maintained at 4 °C on an agar slant of modified NIES-C medium. The modified NIES-C medium consisted of 15.0 mg $Ca(NO_3)_2{\cdot}4H_2O$, 10.0 mg KNO_3, 5.0 mg β-Na_2glycerophosphate, 4.0 mg $MgSO_4{\cdot}7H_2O$, 0.01 μg Vitamin B_{12}, 0.01 μg Biotin, 1.0 μg Thiamine-HCl, 0.3 mL PIV metals, 50.0 mg Trisaminomethane and 99.7 mL distilled water. The PIV metals consisted of 19.6 mg $FeCl_3$, 3.6 mg $MnCl_2{\cdot}4H_2O$, 2.2 mg $ZnSO_4{\cdot}7H_2O$, 0.4 mg $CoCl_2{\cdot}6H_2O$, 0.25 mg $Na_2MoO_4{\cdot}2H_2O$, 100 mg $Na_2EDTA{\cdot}2H_2O$ and 100 mL distilled water. The medium was adjusted to pH 7.5 and autoclaved at 121 °C for 15 min. The seed cells were cultured in 500 mL flasks with the above medium under continuous illumination of 25 μmol/(m^2·s) at 25 °C and 150 r/min for about 8 days and the biomass concentration can reach 0.6 g/L.

Then an 8-day old seed culture (500 mL) was inoculated into 5 L fermenter with 3 L modified NIES-C medium containing 150 mM sodium acetate for heterotrophic growth. The pH was controlled between 7.5 and 8 by pH-stat method using a concentrated medium, which was from the NIES-C culture medium by concentrating 50 multiples. The culture temperature was controlled at 25 °C. The aeration rate and agitation speeds were kept at 0.4 vvm and 40 r/min, respectively. In the exponential growth stage (heterotrophic growth for about 16 days, biomass concentration of about 8 g/L), the heterotrophic broth with high cell density was harvested and diluted in NIES-N medium for induction. The NIES-N medium was prepared by excluding N source form modified NIES-C medium, by subsisting 0.13 mg $CaCl_2{\cdot}2H_2O$ and 0.07 mg KCl for $Ca(NO_3)_2$ and KNO_3, respectively [21].

2.2. Measurement of light attenuation profile

The schematic diagram of measurement of light attenuation in microalgae suspension was shown in Fig. 1.

The outer wall and the flat bottom of the glass tube were all covered with opaque film to prevent the leakage of light from the side and the bottom. The light source should stick to the top of the glass tube to prevent the leakage of light. The incident light intensity through the flat bottom of the glass tube was measured as the initial light intensity when there was no algal broth. Due to the flat bottom, light penetrating through the bottom was still parallel light. Then algal broth was added

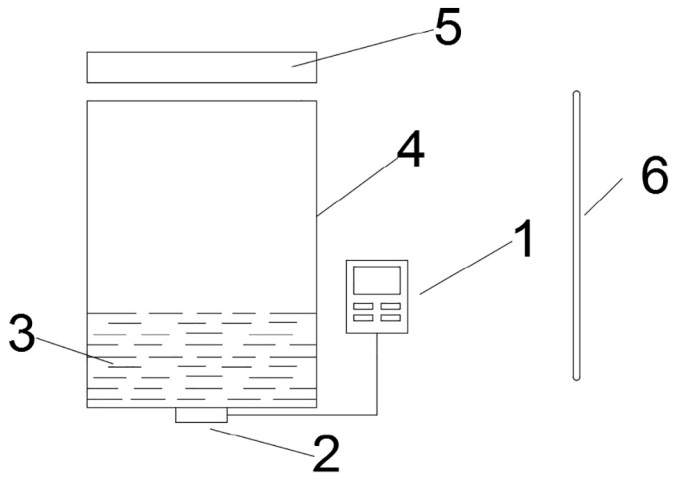

Fig. 1. The schematic diagram of experiment apparatus for measuring the light attenuation in the algal broth.

to different height. Additionally, the algal broth was stirred with the glass rod to avoid the settlement of the cells before the measurement. Illuminometer (TES-1399R, Shanghai Zhicheng Electronics Co., Ltd) was used to measure the light intensity with different light path at the flat bottom of tube. For each condition, light intensity was measured three times and the measured values were means of three replicates with standard deviation.

2.3. Analytic method

The astaxanthin content was analyzed by using high performance liquid chromatography according to the method described previously [22].

For the mass concentration measurement, a 10-mL aliquot of culture was filtered through preweighted Whatman GF/C filter paper. The filter paper was dried overnight in an oven at 80 °C until a constant weight was reached. The difference between the final weight and the weight before filtration was the mass concentration of the sample [23].

The astaxanthin content and the mass concentration were measured three times.

2.4. The light attenuation model and volume-averaged light intensity

2.4.1. The light attenuation coefficient

In general, A(X) was employed to evaluate the extent of the light attenuation and A(X) can be calculated as Eq. (1) [24].

$$A(\mathrm{X}) = \frac{\ln(I_0/I)}{L} \tag{1}$$

where A(X) is the coefficient of the light attenuation (m^{-1}), I_0 is the incident light intensity (μmol/(m^2·s)), I is the local light intensity (μmol/(m^2·s)), L is the light path (m).

2.4.2. The Lambert-Beer model and the Cornet model

Lambert-Beer model is most commonly used to simulate the light attenuation inside the microalgae suspension and it can be expressed as Eq. (2)

$$I_0 = Ie^{(\mathrm{K}_a\mathrm{X}+b)\mathrm{L}} \tag{2}$$

where K_a is the extinction coefficient (m^2/g), X is the microalgae concentration (g/m^3), b is the fitting constant (m^{-1}).

Cornet model has two assumptions: (1) throughout the whole