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Optimizing gas transfer to improve growth rate of *Haematococcus pluvialis* in a raceway pond with chute and oscillating baffles

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HIGHLIGHTS

- *H. pluvialis* flow field was optimized by up–down chute baffle and oscillating baffle.
- Mixing time decreased by 33% when the baffles were used.
- Mass transfer coefficient increased by 1.3 times when the baffles were used.
- Optimized flow field increased biomass yield by 18%.

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ABSTRACT

Up–down chute and oscillating (UCO) baffles were used to generate vortex and oscillating flow field to improve growth rate of *Haematococcus pluvialis* in a raceway pond. Effects of gas flow rate, solution velocity, and solution depth on solution mass transfer coefficient and mixing time were evaluated using online pH and dissolved oxygen probes. Mass transfer coefficient increased by 1.3 times and mixing time decreased by 33% when UCO baffles were used in the *H. pluvialis* solution, resulting in an 18% increase in biomass yield with 2% CO_2. The *H. pluvialis* biomass yield further increased to 1.5 g/L, and astaxanthin composition accumulated to 29.7 mg/L under relatively higher light intensity and salinity.

1. Introduction

Microalgae have attracted considerable research attention in CO_2 emission reduction and new energy development (Kumar et al., 2011). However, microalgal biomass production is only economically feasible when considering microalgae not only a source of microalgae energy, but foremost the extracted value-added biochemicals and liquid fuels (Šoštarič et al., 2012). An astaxanthin found in *Haematococcus pluvialis* (*H. pluvialis*), is a high-value pigment widely used in various applications, such as aquaculture and in the nutraceutical, pharmaceutical, and cosmetic industries, owing to its high anti-oxidative activity (Guerin et al., 2003). More companies can be attracted to the microalgal industry when astaxanthin yield is increased and cost of *H. pluvialis* culture is decreased. *H. pluvialis* can accumulate up to 5–6% (w/w) of astaxanthin on dry weight. The other part of *H. pluvialis* can be used as raw material to develop new energy. A new kind of biomass-based polygeneration system for the production of microalgae-based fuel can be developed for sustainable use.

Numerous studies have been conducted to improve astaxanthin by using a closed reactor (Yoo et al., 2012). To conserve energy in the production of astaxanthin by the green alga *H. pluvialis*, Katsuda et al. (2006) utilized intermittent flashing light from blue light-emitting diodes and investigated the effects of incident light intensity (2–12 μmol m^{-2} s^{-1}), duty cycle (17–67%), and frequency (25–200 Hz) of flashing on cell growth and astaxanthin production. *H. pluvialis* has been cultivated in an airlift and a bubble column photobioreactor (Ranjbar et al., 2008); the cell growth and astaxanthin production were compared to clarify the effects of liquid circulation. Results showed that the cell numbers reached 7×10^6 cells ml^{-1} after 300 h of cultivation. A thin-film photobioreactor was developed by Yoo et al. (2013), in which dry cell weight and astaxanthin reached to 4.64 g/L and 218.16 mg/L, respectively, under outdoor condition in a 15 L photo-bioreactor. Flat panel airlift photobioreactors (FP-APBR) was used by Poonkum et al. (2015); the best outdoor performance was obtained when the FP-APBR was covered with one layer of shading net, where 20.11 g/m^3 (4.47% by weight) of astaxanthin was produced.

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An economical review of *H. pluvialis* culture in FP-APBRs (Issarapayup et al., 2011) showed that better ability to resist pollution and higher microalgae biomass concentration can be reached when a closed reactor is used; however, the cost is much higher than that using a raceway pond. Li et al. (2011) found that the cost of building raceways was only about one fifteenth of the cost of photobioreactors with the same culture-carrying capacity. In addition, the maintenance and consumable cost of raceway ponds was only about 5% of that of photobioreactors. The economic assessment results were very encouraging because the estimated cost of cultivating *H. pluvialis* might be lower than chemically synthesizing astaxanthin.

Raceway reactors are commonly used because of their simplicity and scalability (Harun et al., 2010). Zhang et al. (2009) developed a two-stage growth one-step process for cultivation of *H. pluvialis* using a self-designed system that mimics an open pond in the natural environment. The characteristics of this process are green vegetative cell growth, cysts transformation, and pigment accumulation, which proceed spontaneously and successively in one open photobioreactor. Based on the laboratory work, six batch cultures of *H. pluvialis* WZ were conducted successfully to produce astaxanthin in two 100 m^2 open raceway pond by the two-stage growth one-step process. The astaxanthin content ranged from 1.61 g to 2.48 g per 100 g dry wt. Flashing light has attracted increasing interest as a potential alternative light source to increase the efficiency of photosynthesis and the productivity of algae-based products. Astaxanthin yield per photon improved by at least 60% when flashing light with an average intensity of 65.6 μE/m/s is employed (Kim et al., 2006). Baffle can be used to enhance the flashing light effect by producing vortex flow field in the raceway pond (Cheng et al., 2015b), as sunlight is the only free light source in the microalgae production. Aeration gas was broken into smaller bubbles with enhanced local solution velocity, and thus bubble generation time decreased by 27% and bubble residence time increased by 27% (Cheng et al., 2016b). However, mass transfer coefficient and mixing time were not measured with different solution depths when the up–down chute baffles were used. The main objective of boosting gas transfers between bubbles and medium is to maximize the efficiency of CO_2 transfer, i.e. minimizing CO_2 losses for economical and environmental purposes.

Industrial production of astaxanthin from *H. pluvialis* is generally based on a two-stage strategy. In the first stage, the amount of two different green cells of the flagellate (motile type) and immature resting cells (non-motile type) increase via cell multiplication (vegetative growth) under low-stress conditions. In the second stage, the two different green cells are exposed to high stress, thus inhibiting the cell proliferation (Hong et al., 2015). In general, natural solar radiation is used under outdoor conditions to save lighting costs. With raceway pond reactors, different light intensities can be applied by adjusting the depths of the culture solution. Deeper solution is needed to provide a suitable growth environment for *H. pluvialis* in the first stage to obtain higher microalgae concentration. Astaxanthin accumulation can be induced in *H. pluvialis* during transformation to the aplanospore stage as a response to various stress-inducing conditions, such as nitrogen limitation, excess acetate addition, strong light intensity, salt stress, phosphate deficiency, and addition of specific cell division inhibitors (Kang et al., 2007). Thus, in the second stage, a shallow depths is needed to produce astaxanthin from *H. pluvialis*. In a raceway pond reactor, no additional equipment is necessary in adjusting the depths of the culture solution to meet the astaxanthin production process. Thus, raceway pond reactors are very promising for high-value chemicals such as astaxanthin.

In the present study, up–down chute baffle and oscillating (UCO) baffles that generate vortex and oscillating flow field were developed to strengthen the mixing efficiency of *H. pluvialis* solution in a raceway pond. Mass transfer coefficient and mixing time were measured with different gas flow rates, as well as different velocities and depths of *H. pluvialis* solution. Results showed that the UCO baffles could improve mass transfer coefficient and decrease the mixing time, as well as microalgal growth rate, in the raceway pond.

2. Materials and methods

2.1. Measurement of mass transfer coefficient and mixing time

The experimental raceway pond was 35 cm deep, 110 cm long, and 35 cm wide. The raceway pond was divided into four flow channels with clapboards along the raceway length, and each channel was 8 cm wide. A paddlewheel was used to mix the culture solution. Each sparger was made from a rubber hose (20 cm length and 10 mm diameter) with pores placed ~2 mm apart. A schematic of one up–down chute baffles (2, 3) and one oscillating baffle (4) was shown in Fig. 1a. The chute baffles 2 and 3 were both 75 mm wide and positioned 10 and 70 mm, respectively, above the raceway pond bottom. The oscillating baffles were 320 mm long and 75 mm wide. Four triangular prism were uniformly fixed on each oscillating baffle. The cross-section of triangular prism baffle was an equilateral triangle with side length of 50 mm. One up–down chute baffle and one oscillating baffle were abbreviated as UCO baffle. Except one flow channel for paddlewheel rotation, three UCO baffles were added into the other three flow channels during the experiment (Fig. 1b). Mass transfer coefficient and mixing time were measured with different gas flow rates (0.01, 0.02, 0.03, 0.04, 0.05 vvm), as well as different velocities (10, 15, 20, 25, 30 cm/s) and depths (16, 20, 24, 28 cm) of *H. pluvialis* solution. Number of UCO baffles, gas flow rates, velocities and depths of control study were set as 0, 0.03 vvm, 20 cm/s and 20 cm depth, respectively.

The overall volumetric mass transfer coefficient k_La_L was measured and calculated as described by Mendoza et al. (2013b). *H. pluvialis* solution was used and continuously aerated with air and N_2 alternately. The rates of air and N_2 aeration were controlled and measured using a mass flow meter (SevenstarCS200, China). No air was aerated into the raceway pond when the oxygen transfer rates from atmosphere to culture were measured. The dissolved oxygen concentration was recorded as C_1 and C_2 every 1 s, respectively, in the presence and absence of the aeration gas from the gas aerator. First, the mass transfer coefficient with aeration gas was calculated as $k_{L1}a_{L1} = -1/t \cdot \ln((C^* - C_{L1})/(C^* - C_0))$, where C^* was the saturation concentration of dissolved oxygen. $C_1 = C_0$ at $t = 0$. Second, the mass transfer coefficient without aeration gas from the gas aerator was calculated as $k_{L2}a_{L2} = -1/t \cdot \ln((C^* - C_{L2})/(C^* - C_0))$, where C^* was the saturation concentration of dissolved oxygen. $C_2 = C_0$ at $t = 0$. Third, the mass transfer coefficient without the effect of atmosphere was calculated as $k_La_L = k_{L1}a_{L1} - k_{L2}a_{L2}$. All the mass transfer coefficients in this manuscript were the mass transfer coefficients without the effects of atmosphere if there was no special explanation. To simplify measurement, a concentration range of dissolved oxygen from 4 mg/L to 6 mg/L was used during calculation. Mixing time and the horizontal average solution circulation velocity (solution velocity) were defined and calculated as described by Mendoza et al. (2013a). *H. pluvialis* solution was used. During the test, the pH of the water was lowered to 6.7 ± 0.1 by adding hydrochloric acid (35% w/v). An alkalinity tracer (0.75 ml of 12 mol/L sodium hydroxide solution per 10 L culture solution) was added. The response to this pulse was measured by pH probes (InPro3253i/SG/120 mettler Toledo) at two positions in the raceway reactor (Fig. 1b). The solution velocity was calculated as $V_L = L_c/T_c$, where L_c is the length of the circula-