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Economical review of *Haematococcus pluvialis* culture in flat-panel airlift photobioreactors

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ABSTRACT

The cultivation of *Haematococcus pluvialis* NIES-144 in a flat panel airlift photobioreactor (FP-ALPBR) was examined based on its economical performance. Several cost-cutting options were proposed with the objective of maximising the profit. The use of natural lighting was inevitable to avoid the high electricity cost and replacing artificial lighting with sunlight was found to decrease the total production cost by as much as 307 US$ per 0.5 kg dry cell in the 50 L FP-ALPBR. Nevertheless, the lack of control of diurnal light intensity resulted in a drop in the growth performance with cell density decreasing from 387×10^4 to 140×10^4 cell mL^{-1}, and specific growth rate from 0.63 to 0.53 day^{-1}. Reactor size appeared to be significant for the profitability of the system, and enlarging the FP-ALPBR from 17 to 200 L required significantly lower total costs of production per year (121 US$ per 0.5 kg dry cell for 200 L culture when compared to 197 US$ per 0.5 kg dry cell for the 17 L system). Unfortunately this had to be compensated by a drop in the growth performance with cell density decreasing from 290×10^4 to 147×10^4 cell mL^{-1} and specific growth rate from 0.49 to 0.47 day^{-1}. Finally the reuse of spent medium with proper replenishment of nutrients (nitrate, phosphate, chromium, selenium and copper) caused an unexpected 30% drop in the growth rate and did not seem to provide an attractive response as the total cost per 0.5 kg dry cell was only saved by 8 US$ a year.

1. Introduction

Haematococcus pluvialis is well known as the richest natural source of astaxanthin (1.5–3% by weight) which holds superior antioxidant activity than other carotenoids (Lorenz and Cysewski, 2000). Recent studies demonstrated that airlift photobioreactors (ALPBR) were suitable for the cultivation of such alga (Ranjbar et al., 2008; Garcia-Malea et al., 2006; Kaewpintong et al., 2006; Suh et al., 2006; Vega-Estrada et al., 2005; Lee et al., 2005; Vunjak-Novakovic et al., 2005; Harker et al., 1996). Among these, the largest reported ALPBR of 55 L proposed by Garcia-Malea et al. (2006) was successfully operated with a maximum cell density of 450×10^4 cell mL^{-1} and a specific growth rate of 0.3 day^{-1}. The highest growth was reported in a 1 L reactor with a maximum cell density of 700×10^4 cell mL^{-1} but with a relatively slow specific growth rate (0.2 day^{-1}) (Ranjbar et al., 2008). Issarapayup et al. (2009) proposed a feasible alternative design of flat panel airlift photobioreactor (FP-ALPBR) where the scale up could be achieved simply by increasing the length of the reactor. It was proven that such reactor (17 L FP-ALPBR) could provide the cultivation with a specific growth rate of as high as 0.63 day^{-1} which was greater than the cultivation in a 17 L cylindrical ALPBRs with a specific growth rate of 0.32 day^{-1}. However, the system still suffered from the high energy consumption especially for the illumination devices, and from the high cost of nutrients, which rendered the system not economically attractive.

This work therefore intended to examine the economics of the cultivation process of *H. pluvialis* and to propose alternatives such as the reuse of the medium and the use of sunlight that helped reduce the production costs and improve the economics of such systems.

2. Materials and methods

2.1. Algal strain and inoculum culture preparation

H. pluvialis (NIES-144) was obtained from the Microbial Culture Collection, National Institute for Environmental Studies, Japan. Initial inoculum of *H. pluvialis* was cultivated in the sterilised F1 medium (Fabregas et al., 1998, 2000) which consists of (mg L^{-1}): 9.87 $CaCl_2{\cdot}2H_2O$, 410 KNO_3, 30 Na_2HPO_4, 2.22 $C_6H_5FeO_7{\cdot}5H_2O$, 16.41 $MgSO_4{\cdot}7H_2O$, 0.008 $CuSO_4{\cdot}5H_2O$, 0.08 $Na_2MoO_4{\cdot}2H_2O$,

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Table 1
Geometric details of flat panel airlift photobioreactors (FP-ALPBRs).

	Unit	Symbol	17 L FP-ALPBR	50 L FP-ALPBR	90 L FP-ALPBR	200 L FP-ALPBR
Total volume	L		19	60	95	210
Working volume	L		17	50	90	200
Column height	cm	H_R	50	50	50	50
Column length	cm	L_r	20	50	100	200
Column width	cm	W_R	20	20	20	20
Draft tube height	cm	H_r	30	30	30	30
Bottom clearance height	cm	H_B	10	10	10	10

0.66 MoO_3, 0.05 Cr_2O_3, 0.036 SeO_2, 0.0078 $CoCl_2{\cdot}6H_2O$, 6 $NH_4Fe(C_6H_5O_7)$, and ($\mu g\,L^{-1}$) 12 vitamin B_{12}. This culture was initially carried out in 250 mL Erlenmeyer flasks then up-scaled to 5 L and 10 L autoclavable glass bottles before transferring to the photobioreactor. Only the flagellated green cells from exponential growth phase were used as inoculums for all experiments.

2.2. Bioreactor operation

The column and draft tube were made of clear acrylic plastic with the thickness of 5 and 2 mm, respectively. Table 1 shows the geometric details of all reactors and a schematic diagram of this FP-ALPBR is displayed in Fig. 1. If not stated otherwise, the cultivation process was conducted using the following operating conditions: the ratio between the downcomer and riser cross section area (A_d/A_r) = 0.4, superficial velocity (U_{sg}) = 0.4 cm s^{-1} (Issarapayup et al., 2009). The liquid culture in the FP-ALPBR was agitated by supplying air bubbles through the porous gas sparger at the bottom of the riser section. Air from an air compressor was sterilised with a 0.22 μm Gelman filter, metered through a flowmeter. The CO_2 enriched air was mixed with air, before entering into the system. The concentration of CO_2 was 0.004 vvm, the level not found to affect the pH significantly as CO_2 was continuously taken up by the algae during the course of cultivation. The temperature of the culture was maintained at $25 \pm 5\,^{\circ}C$ in the water-evaporating cooling chamber. The FP-ALPBR was subject to sunlight that penetrated through the translucent roof of the water-evaporative chamber during the whole course of cultivation. All growth experiments were carried out in duplicate where each batch was spent roughly 15 days through the cultivation period.

Two to four vertical fluorescent lamps were allocated on both sides of the indoor airlift system. The distance of each lamp from outer surface of the column was varied depending on the light intensity required. A digital LX-5 Lux meter (model LX-50 Digicon) was employed to measure the light intensity inside the empty column and average values were reported. In the outdoor case, the system was exposed to sunlight that passed through the translucent roof and wall, and was digitally monitored by the lux meter where the intensity was collected using the data logger (model Vernier LabQuest).

2.3. Analyses

Experiments were carried out to determine the effect of design/operating parameters, i.e. the scale up of reactor (17, 50, 90 and 200 L FP-ALPBR), light supplementary (sunlight and fluorescence lamps) and re-used medium. In batch operations, the culture was grown in the bioreactor until the early stationary phase was reached.

2.3.1. Logistic law as a model to describe cell growth

In this work, the logistic function (Bellgardt, 1991) was employed to represent the growth kinetics of *H. pluvialis*. The algal cell density (X) was measured daily by microscope with an improved Neubauer hemacytometer (BOECO Germany). The growth equation in the batch mode cultivation, and the corresponding productivity (g dry cell day^{-1}) can then be calculated from:

$$\frac{dX}{dt} = kX\left(1 - \frac{X}{X_{\max}}\right) \tag{1}$$

when X = initial cell density, X_0, at $t = 0$

$$X = \frac{X_0 \exp(kt)}{1 - ((X_0(1 - \exp(kt)))/X_{\max})} \tag{2}$$

where X_0 and $X_{\max}$ (cell mL^{-1}) are initial cell density and maximum cell density at time t (day), and k (day^{-1}) is the Logistic law coefficient (or equivalent to specific growth rate). Note that the initial cell density was kept constant for all experimental runs at 1×10^5 cell mL^{-1}.

The harvest of cell was performed when cell density reached its maximum and the harvesting volume (I in Fig. 3) was calculated such that the remaining culture when mixed with the replenishing nutrient would give the initial cell concentration of 1×10^5 cell mL^{-1}. With this operation, the productivity (J in Fig. 3) could be calculated from

$$J = \frac{G \times I \times 1000}{H} \tag{3}$$

where J is productivity (g dry cell day^{-1}); G is dry weight (g L^{-1}); I is harvesting volume (m^3); H is harvesting period (day).

2.3.2. Determination of nitrate, phosphate and trace elements

Nitrate concentration (mg-NO_3/L) was analysed by the UV screening method. The sample was filtered though GF/C filters and measured by UV–visible spectrophotometer at 220 and 275 nm (AOAC, 1980). Nitrate is calculated from:

$$\text{Nitrate}\ \ (\text{mg-NO}_3/\text{L}) = \frac{(Abs_{220\,\text{nm}} - Abs_{275\,\text{nm}}) \times A}{B} \tag{4}$$

where A is concentration of nitrate in the standard curve (mg-N/L), and B is the absorbance of the standard curve (220–275 nm).

The analysis for phosphate concentration (mg-PO_4/L) follows the method proposed by Strickland and Parsons (1972). In this method, samples are filtered through GF/C filters and measured by UV–visible spectrophotometer at 885 nm.

The quantity of trace elements in the medium was measured using ICP atomic emission spectrometry (Perkin Elmer Model PLASMA-1000).

3. Results and discussion

3.1. Scale-up of FP-ALPBRs for the cultivation of H. pluvialis

Fig. 2 shows that the 17 L FP-ALPBR provided the best performance for the cultivation of such alga in terms of cell density (290×10^4 cell mL^{-1}) and specific growth rate (0.49 ± 0.03 day^{-1}). The other three FP-ALPBRs exhibited similar growth performance with the 50 L FP-ALPBR providing a slightly better growth at the maximum cell density of 140×10^4 cell mL^{-1} and the growth rate of 0.53 ± 0.05 day^{-1}, followed by the 200 L FP-ALPBR (147×10^4 cell mL^{-1} and 0.47 ± 0.04 day^{-1}) and 90 L FP-ALPBR