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# Enhanced productivity of *Chaetoceros calcitrans* in airlift photobioreactors

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### Abstract

The various modes of cultivation of *Chaetoceros calcitrans* in airlift photobioreactors (ALPBRs) were examined. The batch system illustrated that the airlift configuration was superior to the bubble column as the airlift supported the circulation of the cell within the system, leading to a better light utilization. The cultivations in both semi-continuous and continuous systems resulted in a high cell productivity, although the steady state cell concentrations in both systems were lower than that obtained from the batch system. The behavior of the large-scale airlift system was not significantly different from the conventional bubble column where the diatom could only be produced at low cell density. Despite this, among all of the systems investigated in this work, the large-scale system gave the highest productivity. The main limiting factor for the large-scale airlift culture was the availability of light. Based on economical analysis, the continuous cultivation in the 2.8 L ALPBR with a medium feed rate of 3 mL min<sup>-1</sup> was most attractive where the operation cost could be maintained at a minimum of approx.  $7.95 \times 10^{-4}$  THB L<sup>-1</sup> h<sup>-1</sup>. However, this continuous small-scale system still suffered from relatively low cell productivity (8.10 × 10<sup>4</sup> cells s<sup>-1</sup>).

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Keywords: Airlift photobioreactor; Chaetoceros calcitrans; Semi-continuous system; Continuous system; Large-scale system

#### 1. Introduction

The diatom *Chaetoceros calcitrans* has been widely used as a feed in marine hatcheries, especially in feeding shrimp larvae. The diatom is about 4–10  $\mu$ m in diameter with the box like shape and posses two long pairs of setae. The diatom is an important source of natural polyunsaturated fatty acids (PUFAs) necessary for the growth and immune function of the larvae (Belay, 1997; Borowitzka, 1999; Chiou et al., 2001). Conventionally, the cultivation of *C. calcitrans* started with the controlled cultivation in a small-scale 1 L glass bottle where a high cell density culture of approx. 2 × 10<sup>6</sup> cells mL<sup>-1</sup> could be obtained. The culture was scaled up as a low density culture at a concentration of about  $2 \times 10^5$  cells mL<sup>-1</sup> in larger tanks or opened ponds with a size of 2-10 m<sup>3</sup> until it was ready to use as feed for marine hatcheries. However, this system experienced difficulties due primarily to contamination as its low specific growth rate might provide enough time for the growth of the unneeded species which may eventually inhibit the growth of the diatom and cause harmful effects to marine culture. Although very few investigations focused on the development of photobioreactors for C. calcitrans, several types of closed photobioreactors have so far been introduced for the cultivation of microalgae such as tubular photobioreactor (del Campo et al., 2001; Grima et al., 1996; Lee and Low, 1991; Richmond et al., 1993; Tredici and Zittelli, 1998), vertical alveolar panel (Tredici et al., 1991) and flat plate bioreactor (Göksan et al., 2001; Richmond and Cheng-Wu, 2001; Richmond and Zou, 1999). Recently, we proposed the use of airlift photobioreactor (ALPBR) as an alternative cultivation system

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for *C. calcitrans* where a high maximum cell concentration of  $8.88 \times 10^6$  cells mL<sup>-1</sup> could be achieved from the batch cultivation (maximum specific growth rate =  $7.41 \times 10^{-2}$ h<sup>-1</sup> at  $u_{sg} = 3$  cm s<sup>-1</sup>) (Krichnavaruk et al., 2005). Similar airlift system was also proposed for the high-density cultivation of *Haematococcus pluvialis* where a high specific growth rate of  $0.31 d^{-1}$  was observed (Kaewpintong et al., 2007). Nevertheless, all of these work only focused on batch cultivation which is often encountered drawbacks regarding the production scheduling, reactor maintenance, etc. The aim of this work was therefore to investigate the effect of modes of operation for the cultivation of *C. calcitrans* in various types of ALPBR, i.e. batch, semi-continuous, continuous and the large-scale cultivations.

# 2. Methods

## 2.1. Preparation of culturing strain

The inoculum was prepared aseptically. Firstly, the diatom was screened as a single cell and inoculated in the 3 mL test tubes in the standard F/2 medium (Guillard, 1975) for one week. The composition of the medium was (mg L<sup>-1</sup>) 168.3 NaNO<sub>3</sub>, 12 Na<sub>2</sub>HPO<sub>4</sub> · H<sub>2</sub>O, 5.8 FeCl<sub>3</sub> · 6H<sub>2</sub>O, 20 Na<sub>2</sub>EDTA · 2H<sub>2</sub>O, 66 Na<sub>2</sub>SiO<sub>3</sub> · 9H<sub>2</sub>O, 1.96 CuSO<sub>4</sub> · 5H<sub>2</sub>O, 4.40 ZnSO<sub>4</sub> · 7H<sub>2</sub>O, 1.26 Na<sub>2</sub>MoO<sub>4</sub> · 2H<sub>2</sub>O, 36 MnCl<sub>2</sub> · 4H<sub>2</sub>O, 2.0 CoCl<sub>2</sub> · 6H<sub>2</sub>O,

0.4 vitamin  $B_1$ , and  $(\mu g L^{-1})$  2 vitamin  $B_{12}$ , 100 biotin. Next, the culture was scaled up from 250 to 500 mL flasks where dense cell culture was obtained and used as an inoculum in the airlift photobioreactor (ALPBR).

#### 2.2. Cultivation in ALPBRs

ALPBRs employed in this investigation were of concentric type (Fig. 1a) with dimensions as shown in Table 1. The column was made of clear acrylic plastic to allow effective light penetration and a clear visual observation. The cylindrical draft tube was located concentrically to the outer column with 5 cm space provided at the bottom for liquid circulation. Compressed air was supplied through a porous sparger located centrally at the base of the column and gas flow rate was measured using a calibrated rotameter. Note that the gas flow rate was converted to superficial gas velocity ( $u_{sg}$ ) based on the area of riser. Fluorescent light bulbs were supplied on both sides of the column as a light source for photosynthesis.

### 2.3. Determination of cell concentration

The cell concentration was determined by using a normal blood cell counting slide, Haemacytometer. The depth of the counting grid and the area were 0.1 mm and 25 mm<sup>2</sup>, respectively. The cells were counted in five large squares on



Fig. 1. Experimental setup for the cultivation of *C. calcitrans* (a) 17 L bubble column (BC-1) and 17 L concentric ALPBR (ALPBR-1 and ALPBR-2), (b) 170 L bubble column (BC-2), 170 L concentric ALPBR (ALPBR-3) and 170 ALPBR with multiple draft tube (ALPBR-4).

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