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Optimal growth conditions and the cultivation of *Chaetoceros calcitrans* in airlift photobioreactor

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Abstract

The optimal conditions for the growth of a diatom *Chaetoceros calcitrans* were investigated in a 2.5 L glass bubble column. The light intensity for the highest growth rate was shown to be at around 400 μmol photons $m^{-2}\,s^{-1}$. A modified standard F/2 medium with a two-fold of silica and phosphorus concentrations was illustrated to result in a better growth of this diatom. Vitamin B_{12} in the range from 1 to 3 μg L^{-1} did not significantly affect the growth. A maximum cell density obtained from this small glass bubble column was approximately 5.8×10^6 cells mL^{-1} with a maximum specific growth rate of $3.80 \times 10^{-2}\,h^{-1}$. The cultivation of *C.calcitrans* in the 17 L airlift photobioreactor (ALPBR) was illustrated to be superior to that in the bubble column with the same size. The operation with superficial gas velocity of 3 cm s^{-1} was found to give a maximum specific growth rate of $7.41 \times 10^{-2}\,h^{-1}$ with a maximum cell concentration of 8.88×10^6 cells mL^{-1} in a batch culture. A semi-continuous culture could be achieved where the harvest was performed at every 12 h. In this case, the maximum specific growth rate (μ) achievable was $9.65 \times 10^{-2}\,h^{-1}$ and the cell concentration during the harvest period was 4.08×10^6 cells mL^{-1}.

Keywords: Single cell algae; Light intensity; F/2 medium; Nutrient; Flow pattern; Growth rate; Semi-continuous culture; Bubble column; Airlift bioreactor

1. Introduction

Diatom is a basic component of marine hatchery operations because it serves as one alternative natural resource for poly unsaturated fatty acids [1]. The diatom *C.calcitrans* is considered one of the most popular strains used as a feed for shrimp larvae. *C.calcitrans* is a diatom with chlorophyll content and it is usually cultivated in a similar fashion to single cell algae where, apart from other common nutrients, light plays a significant role in controlling its growth rate. Very few investigations on the optimal condition for the growth of *C.calcitrans* are available. The only report indicated that the highest yield for this diatom was obtained at an initial silica concentration of 400 μg L^{-1} (as sodium metasilicate) [2]. However, this operation was subject to a cool environment and the optimal growth was significantly different from that in warmer locations like tropical countries such as Thailand where there is a high demand of *C.calcitrans* for marine hatcheries, particularly shrimp larvae. The development of bioreactor for mass cultivation of single cell algae or diatom always enables the adjustment of the light intensity, which leads to a successful production of high cell density culture. Examples of the novel designed photobioreactors include a tubular reactor [3,4] and the flat plate bioreactor [5]. However, these existing closed systems suffer serious drawbacks from poor mixing and gas–liquid mass transfer.

Airlift bioreactors (ALBR) have recently become an attractive alternative for cell cultivation [6]. This might be due to several main advantages such as good mixing, well-defined fluid flow pattern, relatively high gas–liquid mass transfer rate, and low capitals and operating costs. The mixing in the ALBR could be obtained without causing too much shear force in the liquid phase, which could inhibit the growth

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of the algae. In addition, it was mentioned that the well-defined circulation pattern resulted in a better light utilization particularly for the system with high density of cells [7].

The aim of this work was firstly to determine optimal conditions for the growth of *C.calcitrans* in tropical areas where the average temperature is approximately 30 °C. The standard F/2 medium [8] was employed as a standard culture medium where the amounts of silica, nitrogen, phosphorus, and Vitamin B_{12} in this medium were examined for their effects on the diatom growth. Light intensity was also manipulated to investigate its influence on the cultivation of the diatom. In addition, the semi-continuous operation of airlift photobioreactor (ALPBR) as a production system for high cell density of *C.calcitrans* was examined.

2. Materials and methods

2.1. Determination of optimal growth conditions

The investigation on the optimal conditions for the growth of *C.calcitrans* was carried out in a 2.5 L clear glass column with a diameter of 12 cm. Compressed air was provided at the bottom of the glass column at a flowrate of 3.8 L min^{-1}. The lighting was supplied through the 250 W lamps where the light intensity could be controlled from 40 to 600 μmol photons m^{-2} s^{-1} by adjusting the distance between the light source and the column. Temperature was controlled at approximately 30 °C (±2 °C). The composition of the F/2 medium was modified to examine the effect of silica, phosphorus, nitrogen, and Vitamin B_{12} on cell growth. Table 1 summarizes the variation in the controlled parameters including the range of concentrations of the selected components in the F/2 medium for this experiment. The initial cell concentration for this experiment was controlled at 5×10^5 cells mL^{-1}.

Table 1
Variation of growth factors in the determination of optimal condition for *Cheatoceros calcitrans*

Growth factor	Range
Light intensity (μmol photons m^{-2} s^{-1})	40–600
Silica concentration (mg Na_2SiO_3 L^{-1})	0–4.8
Phosphorus concentration (mg Na_2HPO_4 L^{-1})	0–3.6
Nitrogen concentration (mg $NaNO_3$ L^{-1})	0–42
Vitamin B_{12} concentration (μg L^{-1})	0–3

2.2. Production of high density culture

The experimental setup for a larger scale system is depicted in Fig. 1. The culture was grown in the 17 L airlift photobioreactor (ALPBR) along with the bubble column (BC) of the same size. Both bioreactors were made of clear acrylic plastic with a diameter of 15 cm (3 mm wall thickness). The ALPBR was equipped with a draft tube installed centrally in the column. The ratio between the cross sectional areas of downcomer and riser (A_d:A_r) was 2.63. Compressed air was provided at the bottom of the draft tube and there was a space of 5 cm between the bottom of the draft tube and the column to allow liquid circulation. The aeration rate was controlled by the calibrated rotameter where the superficial gas velocity in the riser was controlled in a range of 2–5 cm s^{-1}. Light was supplied through 12 fluorescent lamps (36 W each) at the side along the length of the columns, which yielded approximately 200 μmol photons m^{-2} s^{-1} of light intensity. The temperature was controlled at around 30 °C (±2 °C). Two modes of operations were examined here, i.e., batch and semi-continuous cultures. The batch culture was performed to compare the performance of ALPBR and BC in terms of growth rate and the maximum achievable cell density. The semi-continuous system was only carried out in the ALPBR. All bioreactor systems were cultivated with an initial cell concentration of about 1×10^5 cells mL^{-1}.

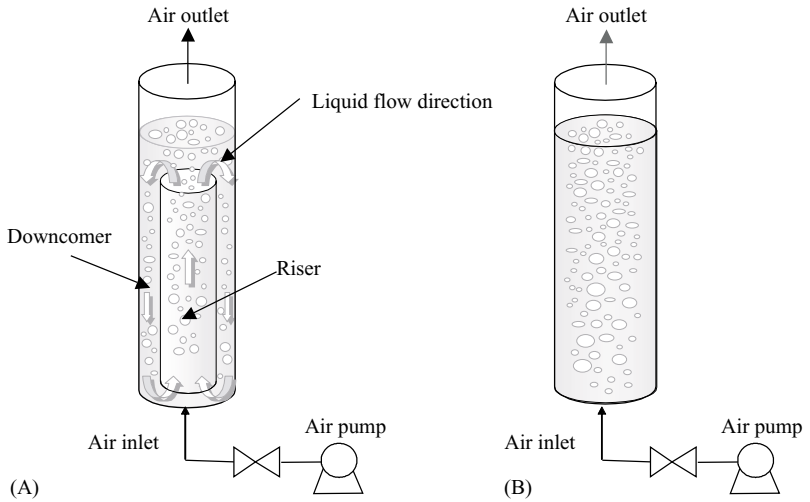

Fig. 1. Schematic diagram for (A) airlift photobioreactor and (B) bubble column.

2.3. Analytical measurements

Cell concentration (N) was measured using the common blood cell count device, haemacytometer. The cell concentrations at two different time periods were employed for the calculation of the growth rate as expressed by

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \quad (1)$$

where μ is the specific growth rate (h^{-1}), N_1 the cell concentration at t_1 (cells h^{-1}), N_2 the cell concentration at t_2 (cells h^{-1}), t_1 the first sampling time (h), and t_2 the second sampling time (h).

The photosynthetic activity of the diatom was measured in terms of photosynthetic oxygen evolution rate (POER). The culture was intermittently sparged with nitrogen gas to reduce the dissolved oxygen level. Subsequently, the rate of increase in dissolved oxygen concentration in the unaerated culture was determined where POER could then be calculated from

$$\text{POER} = \frac{\text{DO}_s \times 60}{CV} \quad (2)$$

where POER is the photosynthetic oxygen evolution rate (mg O_2 mg^{-1} chlorophyll h^{-1}), DO_s the slope of the curve of dissolved oxygen (DO) concentration versus time (mg O_2 min^{-1}), C the chlorophyll concentration (mg chlorophyll mL^{-1}) and V the volume of algal culture (mL).

The chlorophyll concentration was measured according to the standard method detailed in Ref. [9].

3. Results and discussion

3.1. Effect of light intensity

The effect of light intensity was investigated in a series of experiments where the cultures of different cell densities were exposed to the light at different intensities over a short 15 min time interval, and the results are displayed in Fig. 2. The photosynthetic rate was represented by the rate of oxygen released from the culture sample and therefore called photosynthetic oxygen evolution rate (POER). The maximum POER (or equivalent to maximum photosynthetic rate) often took place at the light intensity of around 400 μmol photons m^{-2} s^{-1}.

The suitable cell concentration for the highest POER was about 3×10^6 cells mL^{-1}. It seemed that an increase in cell density resulted in a more active cell but with cell concentration above 3×10^6 cells mL^{-1}, the activity of cell dropped which could be attributed to the self-shading effect. The effect of light intensity on growth may be explained by the damage/repair mechanism of the photosystem II (PS II) D1 protein [4,10–13]. In brief, these statements indicated that the over-saturation of light caused damages to the PS II D1 protein that carried the binding sites for the electron carrier. The extent of the damage was a function of light intensity. However, a simultaneous repair-mechanism existed which produced new D1 molecules to replace damaged ones. The rate of this repair mechanism was believed to be independent of light. In other words, at low light intensity, all damaged D1 protein molecules were replaced almost immediately, and the net damage to the photosynthetic was negligible. At high light intensity, on the other hand, although repair occurred simultaneously with the damage, it occurred at a lower rate, and this led to an apparently lower photosynthetic rate and also the associated growth rate.

3.2. Effect of nutrients and Vitamin B_{12} in F/2 medium

Generally, the standard F/2 medium was used for the cultivation of the diatom but it was not especially designed for the growth of *C.calcitrans*. This investigation therefore attempted to identify nutritional factors that controlled the growth of the culture of *C.calcitrans* by making modification to this standard medium. This was achieved by alter-

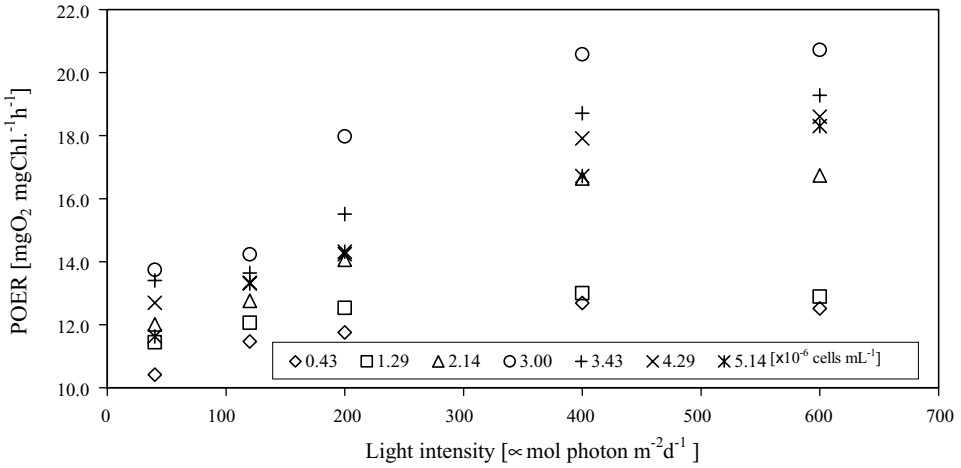

Fig. 2. Effect of light intensity on photosynthesis oxygen evolution rate (POER).

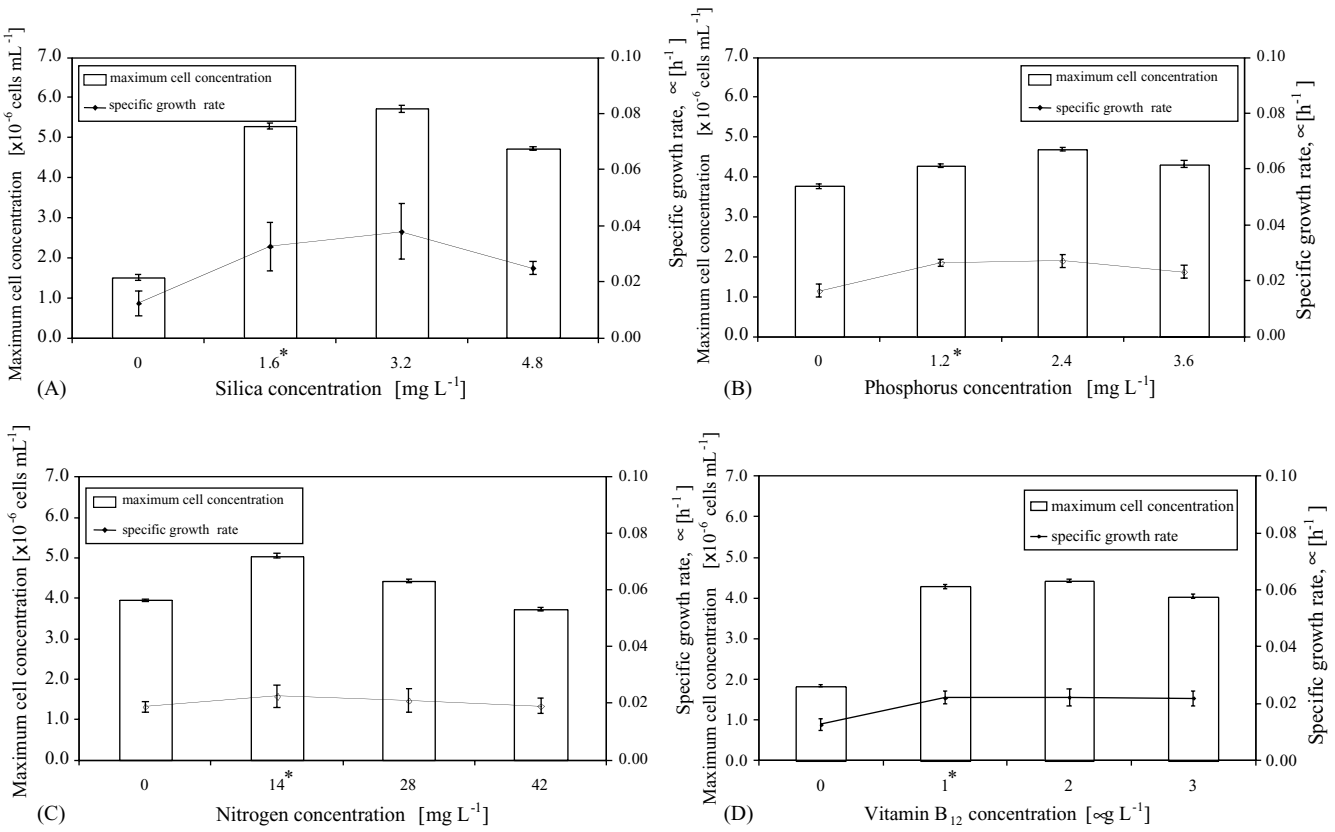

Fig. 3. Effect of nutrient concentration on maximum cell concentration and specific growth rate: (A) silica concentration; (B) phosphorus concentration; (C) nitrogen concentration; (D) Vitamin B_{12} concentration.

ing the concentration of each of the nutrient while maintaining the concentration of other components. Specifically, the experiments were started with a cell concentration of 5×10^5 cells mL^{-1} in a modified F/2 medium where the composition of silica (as sodium metasilicate), phosphorus (as phosphate), nitrogen (as nitrate), and Vitamin B_{12} were varied.

Fig. 3A illustrates that the addition of silica (as sodium metasilicate) at 3.2 mg L^{-1} resulted in the highest growth rate. This value was twice as much as that recommended in the standard F/2 medium of 1.6 mg L^{-1}. The absence of silica, Si = 0 mg L^{-1}, caused a declining cell concentration where the maximum cell concentration fell below initial concentration. It was accepted that diatom could not survive with an inadequate supply of silica because silica was not only needed in the cell wall formation, but it was also required for deoxyribonucleic acid (DNA) synthesis.

The suitable phosphorus concentration for the diatom growth was observed to be around 2.4 mg L^{-1} (Fig. 3B). This concentration was also two times higher than that recommended in the standard F/2 medium. The most important role of phosphorus was in energy transfers through energy carrying agents, e.g., adenosine triphosphate (ATP), NADPH, etc. Therefore excessive or insufficient supply of phosphorus could have negative impact on cell growth. When external phosphate concentrations were high, the ability of cells in the assimilation of phosphorus compound was repressed and the growth was inhibited. On the other hand, an inadequate level of external phosphorus reduced the cell capacity in authorizing ATP and other energy compounds which also led to a limited cell growth.

As demonstrated in Fig. 3C, nitrogen concentration as stated in the standard F/2 medium (14 mg L^{-1}) was most suitable for the growth of *C.calcitrans*. Concentrations above and below this optimal 14 mg L^{-1} led to a slightly lower growth rate. This could be explained by considering the effect of nitrogen on cellular metabolism where both nitrogen starvation and overdose led to a dramatic decrease in the efficiency of energy transfer from harvesting complexes to photo system II (PS II) reaction center [12].

Fig. 3D indicates that Vitamin B_{12} was essential for growth but only a tiny amount would suffice the growth of the cell. In this case, even the smallest amount used in the medium (1 μg L^{-1}) was adequate for the growth. Note that the optimal amount of Vitamin B_{12} still could not be determined from this experiment and it could be that a lower dose than 1 μg L^{-1} could be adequate for an efficient growth of the diatom.

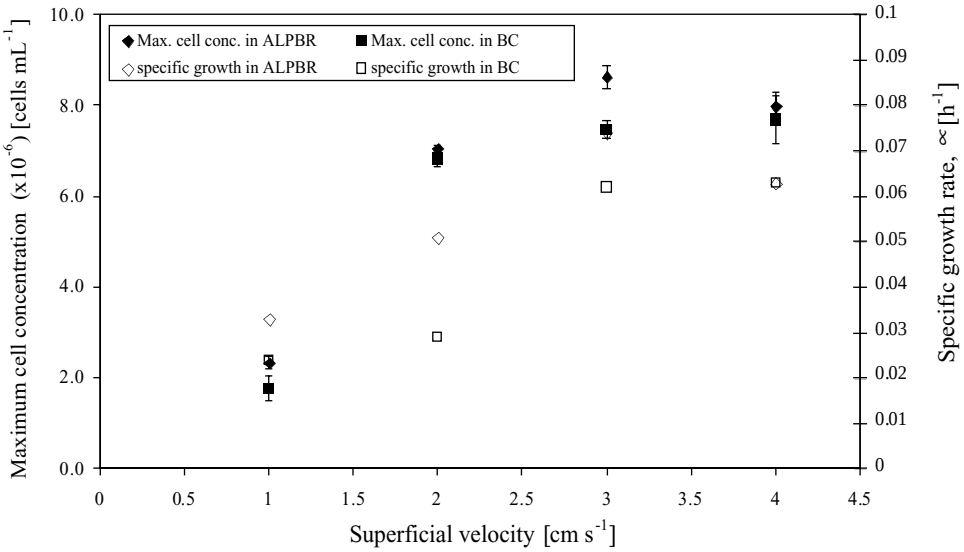

Fig. 4. Cell concentration time profile in airlift photobioreactor and bubble column.

3.3. Cultivation in bioreactors

Fig. 4 demonstrates the growth curves of *C.calcitrans* in the two bioreactors. Note that for these large-scale units, the light intensity at 400 μmol photon m^{-2} s^{-1} could not be supplied due to experimental limitation and the maximum achievable light intensity for these systems was only 200 μmol photon m^{-2} s^{-1}. The performance of the ALPBR was superior to that of the BC both in terms of maximum cell density and growth rate, i.e., maximum growth rate and maximum cell concentration were 7.41×10^{-2} h^{-1} and 8.88×10^{6} cells mL^{-1} for the ALPBR, respectively, and 6.3×10^{-2} h^{-1} and 7.68×10^{6} cells mL^{-1} for the BC. The difference in the performance of the two bioreactors must be derived from the difference in their behavior. In the BC, the aeration only superimposed random movement with no net movement of the liquid [7]. Since cells were not properly recirculated in the BC, some cells were exposed to high light density at the region adjacent to the wall of the column where the light source was located. Those cells in the middle of the column were only exposed to low light density and resulting in an ineffective photosynthesis and low growth rate when compared to those obtained from the ALPBR. The configuration of the ALPBR with riser and downcomer caused uneven densities of fluid in the two sections and induced a certain pattern of liquid movement, i.e., liquid moved up in the riser and down in the downcomer. The well-defined flow pattern in the ALPBR meant that the diatom in the riser would, after a certain time period, flow to the downcomer where the light was applied. Hence, most diatoms were exposed to more even light intensity. In addition, the liquid movement in the ALPBR prevented an accumulation

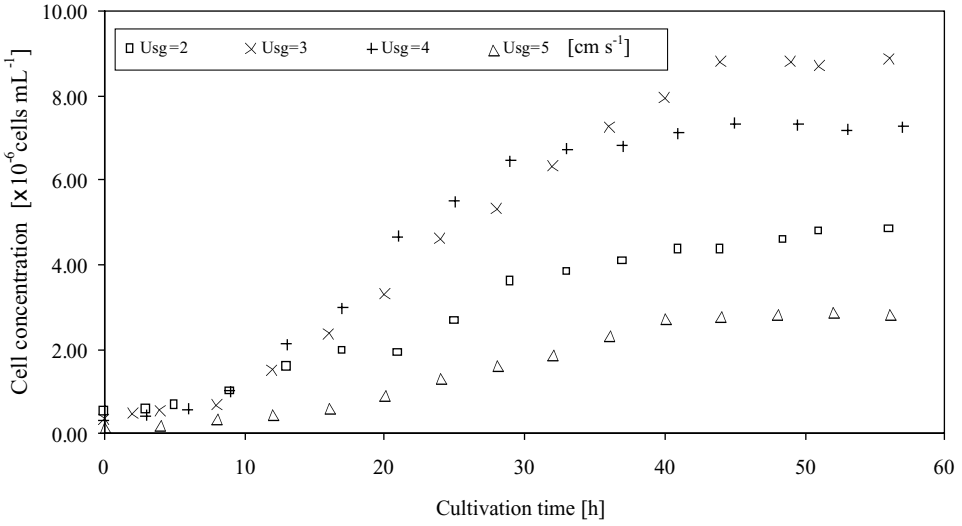

Fig. 5. Effect of superficial gas velocity on the growth of *C.calcitrans.*

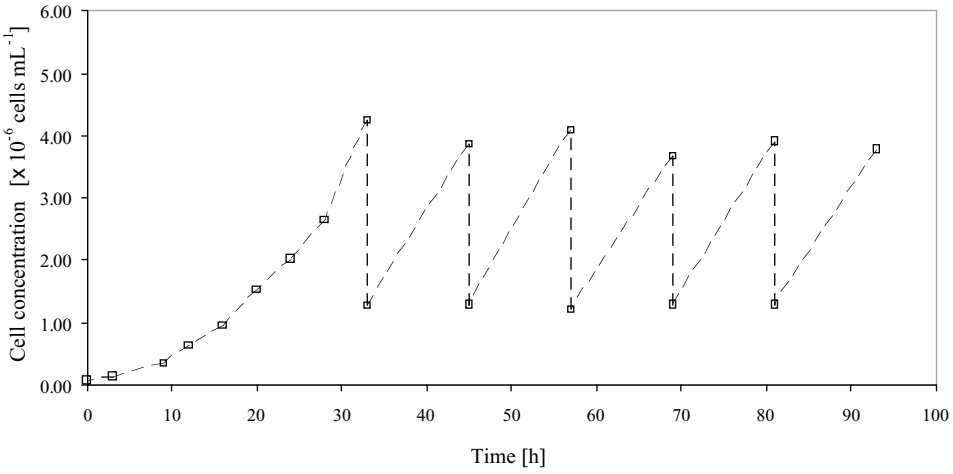

Fig. 6. Semi-continuous cultivation of *C.calcitrans* in ALBR at $U_{sg} = 3\,cm\,s^{-1}$.

of cells at the bottom of the column. At high cell density, it was likely that cell precipitation caused an uneven cell density along the length of the column. This accumulation of cells might cause starvation, death, and easy contamination of the whole culture, which reduced the overall growth rate of the algal culture. This problem was not found in the ALPBR as the liquid movement facilitated cell circulation even at high cell density. Hence, there would be less cell accumulation at the bottom of the column when compared to the BC.

It could then be concluded at this point that algal cells in the ALPBR could utilize light source more effectively than cells in the BC resulting in a higher growth rate. This finding agreed well with that of Merchuk et al. [7] who indicated that the growth of the red microalga *Porphyridium* sp. in the ALPBR was much better than that obtained from the cultivation in the BC.

It is interesting to note that the operation in the larger scale system such as 17 L ALPBR could provide a relatively high growth rate ($7.41 \times 10^{-2}\,h^{-1}$) and maximum cell density (8.88×10^{6} cells mL^{-1}) in comparison with the cultivation in a small 2.5 L glass bubble column (maximum growth rate of $3.8 \times 10^{-2}\,h^{-1}$ and maximum cell density of 5.8×10^{6} cells mL^{-1}), when operated at the same range of light intensity (400–600 μmol photons $m^{-2}\,day^{-1}$) and superficial gas velocity (3–4 $cm\,s^{-1}$). This demonstrated that the design of the cultivation system could provide a suitable operating condition for the cells and a better performance of the system was obtained.

3.4. Effect of aeration rate

The effect of aeration rates on algal mass production in the ALPBR is presented in Fig. 5. Increasing aeration rate (measured in terms of superficial velocity or U_{sg}) in the range of 2–5 $cm\,s^{-1}$ was found to have influence on the growth rate of the algae. The maximum cell growth rate ($7.41 \times 10^{-2}\,h^{-1}$) occurred at the aeration rate of 3 $cm\,s^{-1}$ above which the growth rate dropped.

At a lower range of the aeration (U_{sg} = 2–3 $cm\,s^{-1}$), an increase in aeration rate greatly induced mixing, liquid circulation rate and also the mass transfer between gas and liquid phases in the system [14,15]. The better mixing could potentially lead to a more efficient utilization of nutrients by the diatom. A higher mass transfer might also facilitate the removal of metabolic gases such as oxygen, preventing the accumulation of these gases, which might adversely affect the growth rate [16].

At a high aeration rate (U_{sg} = 4–5 $cm\,s^{-1}$), the growth rate of *C.calcitrans* declined. High aeration rates led to a system with more gas bubbles along the length of the downcomer. This was because the liquid velocity was sufficiently high that relatively large gas bubbles were dragged down into the downcomer. These gas bubbles in the downcomer were somewhat undesirable as it could prevent the passage of light to the center of the bioreactor as the light penetration ability was obstructed and dissipated by the swarm of gas bubbles. Our preliminary experiment indicated that as much as 40% of light intensity could be suppressed in the riser (or in the draft tube) with the presence of gas bubbles in the downcomer. As a result, the diatom was subject to a lower light intensity and a lower growth rate was observed.

3.5. Semi-continuous production

From the growth curve of the batch culture of *C.calcitrans* illustrated in Fig. 5, the maximum cell concentration at superficial velocity of 3 $cm\,s^{-1}$ (the most suitable condition for the growth) was found to be approximately $(7–8) \times 10^{6}$ cells mL^{-1} after 48 h of operation. A lag phase was observed during the initial period of the culture (the first 9 h) followed by an exponential growth during the next 35 h. This was then followed by a stationary phase where the cell concentration reached a maximum of 8.88×10^{6} cells mL^{-1}.

Hence, for a semi-continuous operation, the initial inoculation with the initial cell concentration of 1×10^5 cells mL^{-1} was left growing for 33 h to ensure that cells were in the most active period. After that the culture was harvested at about 50–70% of the culture. This was to control the cell concentration for the next run at approximately 1.2×10^6 cells mL^{-1}.

The results in Fig. 6 illustrated that *C.calcitrans* was able to maintain the exponential growth rate with the selected harvesting condition. The specific growth rate was approximately 9.65×10^{-2} h^{-1} which indicating that cells could adjust well to the growth conditions in the ALPBR. The cell concentration at the harvest period was 4.08×10^6 cells mL^{-1}.

It is interesting to note that the culture systems employed in this work here could achieve a very high productivity. Both cell concentration and growth rate were was relatively high in comparison with the reported results, which only could achieve cell concentration at 2.56×10^6 cells mL^{-1} in 4 days of the cultivation diatom *C.calcitrans* [17]. This was equivalent to 6.4×10^8 cells L^{-1} day^{-1} whereas the cultivation in the ALPBR in this investigation could provide a high productivity of 9.45×10^9 cells L^{-1} day^{-1}.

4. Conclusion

This work shows that, with a slightly modified standard F/2 medium, the growth of *C.calcitrans* could be enhanced by as much as 16%. Parameters that were significant for the growth of this diatom were the composition of the medium particularly silica, nitrogen and phosphorus contents. There existed an optimal light intensity for the cultivation of this diatom and light intensity below or above 400 μmol photons m^{-2} s^{-1} were not found to have positive influence on the diatom growth. Most importantly, this work emphasized the importance of bioreactor design on the mass production of the diatom *C.calcitrans*. The operation of cultivation column in airlift mode was proven to be successful and a high growth rate could be achieved even with a lower light intensity than the optimal. Due to a well-defined flow pattern, which allowed a more effective light utilization of the diatom, the cultivation of *C.calcitrans* in the ALPBR was found to be superior to that in the BC. The optimal aeration rate of 3 cm s^{-1} was found to yield the specific growth rate of 7.41×10^{-2} h^{-1} with a maximum cell concentration of 8.88×10^6 cells mL^{-1} in batch culture system. A long-term semi-continuous operation could be achieved successfully with a maximum specific growth rate (μ) of 9.65×10^{-2} h^{-1}. The harvest of cell should be performed at every 12 h of operation with the cell concentration at the harvest period of approximately 4.08×10^6 cells mL^{-1}.

Acknowledgement

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Appendix A

Standard F/2 (Guillard's) medium compositions [8]

Nutrient	Amount (g)
Solution A: Nitrate and phosphate stock solution (1 L)	
$NaNO_3$	84.15
$Na_2HPO_4 \cdot H_2O$	6.0
$FeCl_3 \cdot 6H_2O$	2.90
$Na_2EDTA \cdot 2H_2O$	10.0
Solution B: Silicate stock solution (1 L)	
$Na_2SiO_3 \cdot 9H_2O$	33.0
Solution C: Trace metal stock solution (1 L)	
$CuSO_4 \cdot 5H_2O$	1.96
$ZnSO_4 \cdot 7H_2O$	4.40
$Ma_2MoO_4 \cdot 2H_2O$	1.26
$MnCl_2 \cdot 4H_2O$	36.0
$CoCl_2 \cdot 6H_2O$	2.0
Solution D: Vitamins stock solution (1 L)	
Vitamin B_1	0.4
Vitamin B_{12}	0.002 mg
Biotin	0.10 mg

To prepare the culture medium for *C.calcitrans*, simply add 2 mL of solutions A and B and 1 mL of solutions C and D in 1 L of fresh seawater.

References

[1] S.Y. Chiou, W.W. Su, Y.C. Su, Optimizing production of polyunsaturated fatty acids in *Machantia polymorpha* cell suspension culture, J. Biotechnol. 85 (2001) 247–257.
[2] I. Liang, Growth response of *Chaetoceros calcitrans* (Bacillariophyceae) in batch culture to a range of initial silica concentrations, Mar. Biol. 5 (1) (1985) 37–41.
[3] Y.K. Lee, C.S. Low, Effect of photobioreactor inclination on the biomass productivity of an outdoor algal culture, Biotechnol. Bioeng. 38 (1991) 995–1000.
[4] E.M. Grima, A.S. Sanchez Perez, F.G. Camacho, J.L. Garcia Sanchez, F.G. Acien Fernandez, D.L. Alonso, Productivity analysis of outdoor chemostat culture in tubular air-lift photobioreactors, J. Appl. Phycol. 8 (4–5) (1996) 369–380.
[5] A. Richmond, N. Zou, Efficient utilisation of high photon irradiance for mass production of photoautotrophic micro-organisms, J. Appl. Phycol. 11 (1999) 123–127.
[6] J.C. Merchuk, N. Ladwa, A. Cameron, M. Bulmer, I. Berzin, A.M. Pickett, Liquid flow mixing in concentric tube air-lift reactors, J. Chem. Technol. Biotechnol. 66 (1996) 174–182.
[7] J.C. Merchuk, M. Ronen, S. Geris, S. Arad, Light/dark cycles in the growth of the red microalga *Porphylidium* sp., Biotechnol. Bioeng. 59 (1998) 705–713.
[8] R.R.L. Guillard, Culture of phytoplankton for feeding marine invertebrates, in: W.L. Smith, M.H. Chanley (Eds.), Culture of Marine Invertebrates Animal, Plenum Press, New York, 1975, pp. 15–41 (for general algae culture techniques).
[9] J. Stein, Handbook of Phycological Methods: Culture Methods and Growth Measurements, Cambridge University Press, London, 1973, p. 448 (for general algal culture).
[10] A. Sukenik, P.G. Falkowski, J. Bennett, Potential enhancement of photosynthetic energy conversion in algal mass culture, Biotechnol. Bioeng. 30 (1987) 970–977.
[11] I.R. Davison, Environmental effects on the algal photosynthesis: temperature, J. Phycol. 27 (1991) 2–8.
[12] J. Vymazal, Algae and Element Cycling in Wetlands, CRC Press Inc., Boca Raton, Florida, 1994.
[13] Y. Huang, G.L. Rorrer, Optimal temperature and photoperiod for the cultivation of *Agardhiella subulata* microplantlets in a bubble-

column photobioreactor, Biotechnol. Bioeng. 79 (2) (2002) 135–145.
[14] J.C. Merchuk, Y. Stein, Local hold-up and liquid velocity in airlift reactors, AIChE J. 27 (3) (1981) 377–388.
[15] M. Gavrilescu, R.Z. Tudose, Modelling of liquid circulation velocity in concentric-tube airlift reactors, J. Chem. Eng. 69 (1998) 85–91.
[16] H.L. Tung, C.C. Tu, Y.Y. Chang, W.T. Wu, Bubble characteristics and mass transfer in an airlift reactor with multiple net draft tubes, Bioprocess Eng. 18 (1998) 323–328.
[17] G.P.B. Samonte, C.C. Espergadera, R.D. Caturao, Economics of microalgae (*Chaetoceros calcitrans*) production using the multi-step method in the Philippines, Aquaculture 112 (1) (1993) 39–45.