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Full Paper

Cultivation options for indoor and outdoor growth of *Chaetoceros gracilis* with airlift photobioreactors

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Abstract: Various configurations and modes of airlift photobioreactors were examined in the cultivation of *Chaetoceros gracilis*. Internal loop and external loop airlifts were cultivated in a batch mode in a controlled indoor environment. The external loop system provided a better performance than the internal loop system due to better light exposure. A continuous operation was conducted in an internal loop airlift photobioreactors-in-series. This was designed to minimise the effect of light blocking due to overgrown cells as the high-density culture was, in this configuration, only limited to the last airlift column in the series. Outdoor large-scale operation was conducted in a flat-panel airlift photobioreactor. Due to uneven light availability, the outdoor culture could not perform as well as the indoor one in terms of growth rate. Among the four systems investigated, the continuous culture in airlift photobioreactors-in-series provided the best performance with the highest cell density of 12.12×10^6 cells mL^{-1}. Cost analysis based on the maximum number of reactors that can be installed in one square metre indicates that the indoor system requires lowest operating cost per unit cultivation area, whereas the outdoor system provides highest profit as a result of the inherited large productivity.

Keywords: *Chaetoceros gracilis*, indoor culture, outdoor culture, airlift photobioreacter

INTRODUCTION

Chaetoceros gracilis is one of the most popular diatoms in Thailand and is used in feeding shrimp larvae. Conventionally, this diatom is cultivated in open pond systems in which an inherent low specific growth rate allows an easy contamination by foreign, faster growing microorganisms.

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Closed bioreactor systems are often proposed as alternatives, where not only the well-defined environment facilitates the control of contaminants, but also the various important environment parameters (light intensity, temperature, etc.) can be manipulated to suit the growth of each individual species. Examples of such closed systems include flat plate/panel [1-3], tubular [4, 5], helical flow [6, 7] and airlift [8]. Airlift photobioreactors attract considerable attention as an alternative bioreactor for microorganisms such as *Tetrahymena thermophila* [9], *Haematococcus pluvialis* [10, 11] and *Chaetoceros calcitrans* [12, 13]. The use of airlift is recommended for the cultivation of algae as it allows a more effective circulation of cells, which enhances light exposure [12, 14] and effectively maintains microalgal suspension with reasonably low energy requirement [15, 16]. In addition, Issarapayup et al. [2] showed that a flat-panel airlift photobioreactor (FPAP) can be easily scaled up by extending the length of reactor without losing algal growth performance.

Outdoor cultivation of microalgae is suitable for large-scale culture and the future development of most microalgal applications will need to have a reliable and economical industrial-scale cultivation process. Sunlight as a light source reduces the operating cost and electricity consumption by as much as 2.5 times when compared with the system with artificial lighting [17, 18]. An outdoor pond may need a circulation cascade to increase light exposure of microalgae [19]. However, outdoor condition is still quite difficult to control, and the productivity of microalgae is typically variable with seasons [17, 19, 20]. The performance of the photobioreactors can still be improved by the design of the set-up and manipulation of the operation of the system. This work demonstrates how different designs of the airlift photobioreactor affect both indoor and outdoor cultivation of *Chaetoceros gracilis*.

MATERIALS AND METHODS

Preparation of Stock Culture

The original inoculum of *C. gracilis* was obtained from the Department of Aquaculture, Faculty of Fisheries, Kasetsart University. The stock culture was prepared by inoculating the diatom in the sterile modified F/2 medium incubated at 121˚C [12]. The culture was then transferred to 250 mL of the medium in a 500-mL flask and inoculated until the cell concentration reached approximately 4 x 10^6 cells mL^{-1}. It was finally scaled up to 1,000 mL and 17 L and then transferred to the large-scale airlift photobioreactors.

Design Options for Indoor Cultivation

Batch culture

Two types of 17-L airlift photobioreactors were employed. They were made from clear acrylic plastic to allow light passage through the column. The internal-loop airlift photobioreactor (IAP) has a draft tube installed centrally within the outer column, which separates the downcomer from the riser (Figure 1(a) and Table 1). The 17-L external-loop airlift photobioreactor (EAP) consists of two vertical tubes operating as the riser and the downcomer, both with the height of 190 cm (Figure 1 (b)). The 5.4-cm-diameter riser is connected to the 10.4-cm-diameter downcomer near the top and the bottom of the system. Batch culture, both in IAP and EAP, was operated in a well-ventilated room where the temperature was maintained at 30±2ºC. The system was sterilised using sodium dichloroisocyanurate. After two days, residual chlorine was neutralised with sodium thiosulphate. An initial cell concentration was prepared at 1 x 10^5 cells mL^{-1} for all experiments. A calibrated flow meter (rotameter) was used to control the volume of gas flow supplied to the system through a porous gas sparger attached to the base of the column, where the superficial gas velocity

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(u_{sg}) was controlled at 3 cm s^{-1}. Fluorescent light bulbs (36 watt) were provided on the outer surface of the column to supply light necessary for photosynthesis. The light intensity at the reactor surface was controlled at approximately 10,000 Lux (135 µmols photon $m^{-2}s^{-1}$). In this experiment, the light source was placed 10 cm away from the surface of the reactor and the intensity was adjusted by adding the shade between the light and the column. The light intensity was measured with a digital light meter (DT-1309, CEM, Shenzhen Everbest Machinery Industry Co. Ltd., China).

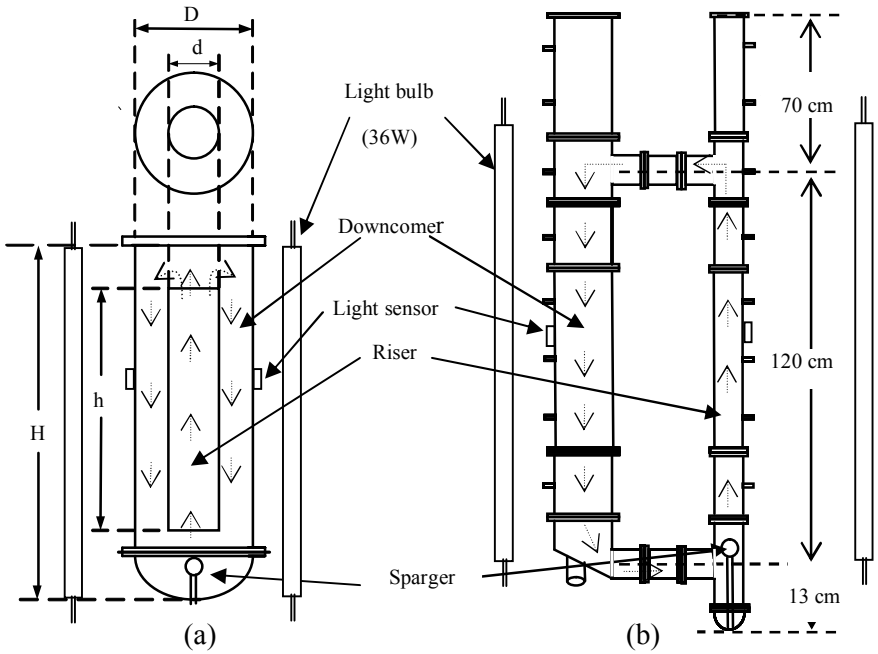

Figure 1. Experimental set-up for the cultivation of *C. gracilis* in airlift photobioreactor: (a) IAP and continuous airlift photobioreactor (CAP); (b) EAP. Arrows indicate flow direction.

Table 1. Dimensions of airlift photobioreactor (IAP and CAP)

Parameter	Dimension (cm)	
	IAP	CAP
Column outside diameter (D)	15	10
Draft tube outside diameter (d)	8	5
Column and draft tube thickness	0.3	0.3
Column height (H)	120	60
Draft tube height (h)	100	40

Continuous culture

The continuous airlift photobioreactor (CAP) with the size of 3 L (dimension given in Table 1) was used with fluorescent light bulbs (18 watt) being on both sides of the column with average light intensity at the centre of the column of approximately 135 µmols photon $m^{-2}s^{-1}$. A few designs of CAP are proposed as illustrated in Figure 2. The first configuration is the single column (System I), which was used as a control experiment. System II is operated with two CAPs connected in series, whereas System III is one with three connected columns. In all configurations, the system was first cultivated as a batch culture with only the first CAP (Column I) operated with an initial

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cell concentration of approx 1 x 10^5 cells mL^{-1}. When the growth of the diatom reached the mid-exponential phase, the columns were then connected in series (two columns for System II and three for System III) and sterilised modified F/2 medium was fed into the first column using a peristaltic pump with the overflow stream to control the total volume at 3 L in each CAP. A medium feed rate was varied as indicated in Table 2. A calibrated flow meter (rotameter) was used to control the air volumetric flow rate supplied to the system through a porous gas sparger at the base of the column, with a superficial gas velocity (u_{sg}) of 3 cm s^{-1}.

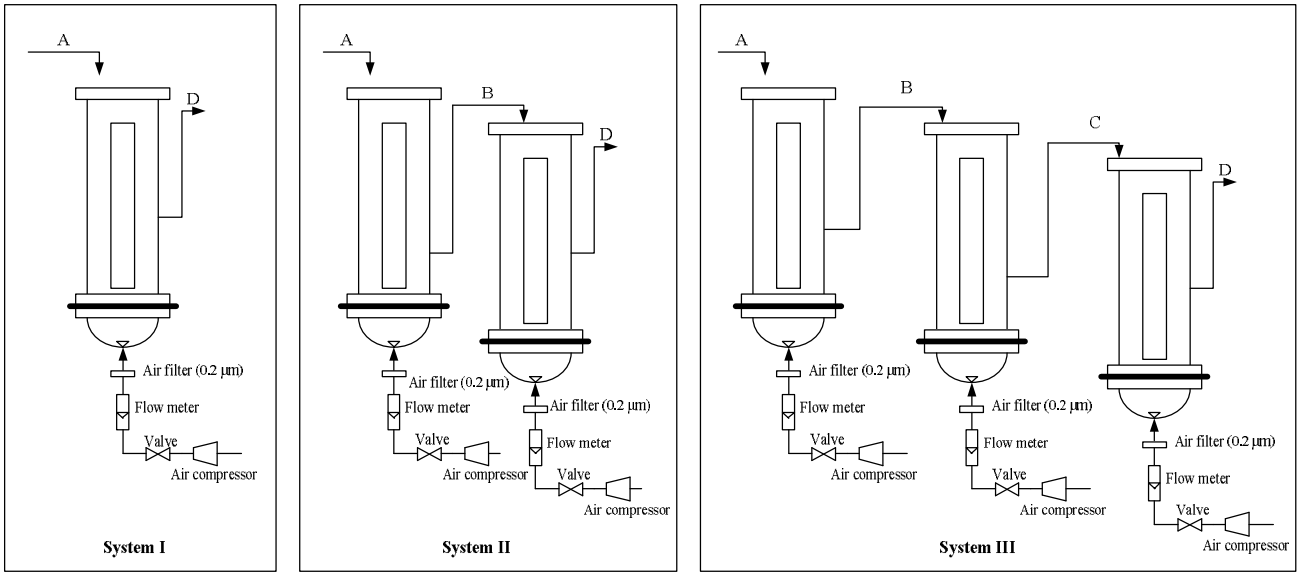

Figure 2. Experimental set-up for the cultivation of *C. gracilis* in CAPs-in-series

Table 2. Operating conditions for CAPs-in-series

Experiment	System	Medium feed rate (mL min^{-1})			
		Stream A	Stream B	Stream C	Stream D
Set 1	I	1	-	-	Overflow
Set 2	I	2	-	-	Overflow
Set 3	I	3	-	-	Overflow
Set 4	II	2	2	-	Overflow
Set 5	II	4	4	-	Overflow
Set 6	II	6	6	-	Overflow
Set 7	III	3	3	3	Overflow
Set 8	III	6	6	6	Overflow
Set 9	III	9	9	9	Overflow

Design Options for Outdoor Cultivation

Flat-panel (100 L) airlift photobioreactors (FPAPs) were set up as an outdoor IAP (Figure 3 and Table 3). Aeration with an overall superficial velocity (u_{sg}) of 3 cm $s^{-1}$was provided through a series of spargers which were installed 6 cm apart at the bottom of the reactor. The light intensity was measured with a light sensor (Vernier Labquest, with data logger), whereas the temperature

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was measured via a thermocouple (IP67, Hanna instrument Inc., with data logger). The operation of these large-scale airlifts was carried out under three climate conditions, i.e. Period I (summer), Period II (rainy season) and Period III (winter).

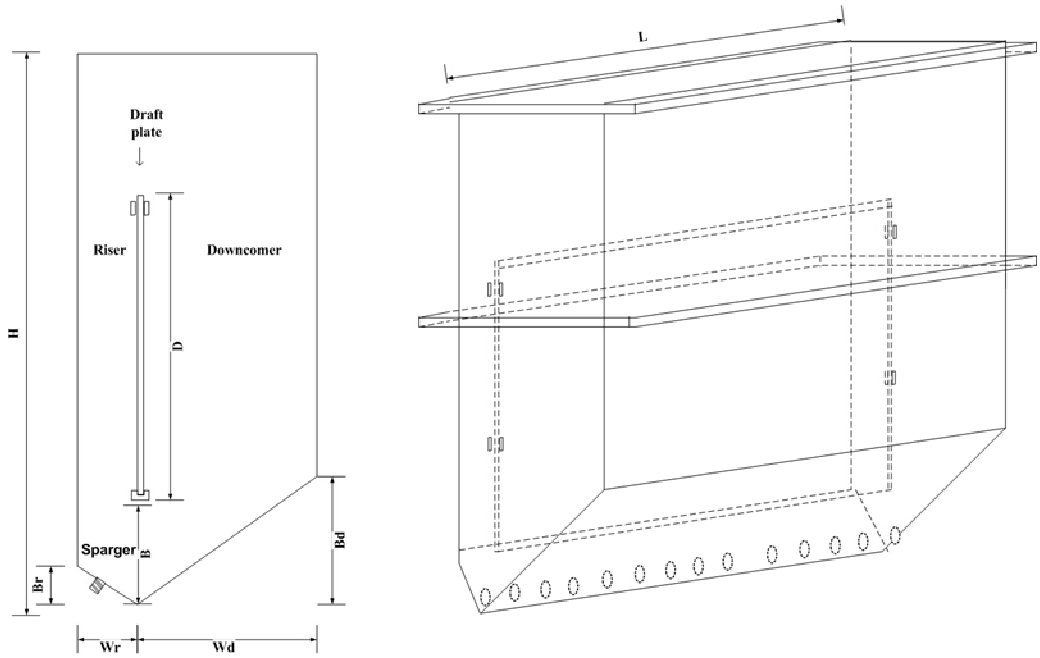

Figure 3. Experimental set-up for the cultivation of *C. gracilis* in flat-panel airlift photobioreactor (FPAP). Arrows indicate flow direction.

Table 3. Dimensions of FPAP

	cm
Reactor height (H)	100
Draft plate height (D)	30
Bottom clearance (B)	9
Riser bottom clearance (Br)	3
Downcomer bottom clearance (Bd)	10
Height of volume	50
Reactor length (L)	120
Riser width (Wr)	5
Downcomer width (Wd)	15

Calculations

The specific growth rate for batch cultivation can be obtained from the slope of plot between the natural logarithm of cell concentration during the exponential phase and cultivation time as follows:

$$\ln (N_2/N_1) = \mu (t_2-t_1) \quad (1)$$

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

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For continuous cultivation, the specific growth rate (μ) is equal to the dilution rate (D) at steady state, calculated from:

$$\mu=\frac{F}{V}\times 60 \quad (2)$$

where F is medium feed rate (mL min^{-1}) and V is volume of system (mL).

The productivity for batch culture is calculated from the overall growth period, which represents the average growth of the culture as follows:

$$P=(\frac{N_f-N_i}{t_f-t_i})\times v\times 1000 \quad (3)$$

where P is productivity (cells h^{-1}), N_f is final cell concentration (cells mL^{-1}) at t_f, N_i is initial cell concentration (cells mL^{-1}) at t_i, and v is volume of system (mL).

For continuous cultivation, the productivity can be calculated from:

$$P=N\times F\times 60 \quad (4)$$

where N is final cell concentration in the last column of CAP-in-series (cells mL^{-1}) and F is medium feed rate (mL min^{-1}).

The average temperature under outdoor cultivation can be calculated from the average of the sum of maximum temperature and minimum temperature during the day. The average surface energy intensity is the integration over time of solar energy irradiating on the surface of one square metre (MJ m^{-2}).

RESULTS AND DISCUSSION

Design Options for Indoor Cultivation

Batch culture: IAP and EAP

Figure 4 displays the growth of *C. gracilis* in both the IAP and EAP operating at u_{sg} of 3 cm s^{-1}. The final cell concentration obtained from the EAP was around 9.82 $\times 10^6$ cells mL^{-1} with a productivity of 1.76 $\times 10^9$ cells $h^{-1}$and a specific growth rate of 9.21 $\times 10^{-2}$ h^{-1} at 94 h, while the IAP gave final cell concentration of 8.34 $\times 10^6$ cells mL^{-1}, a productivity of 1.46 $\times 10^9$cells h^{-1} and a specific growth rate of 8.56 $\times 10^{-2}$ h^{-1} at 96 h. The performance of the EAP was better than that of the IAP, apparently due to a better light distribution throughout the column. As the riser and the downcomer of the EAP are separate columns, they are better exposed to light. Our test experiments demonstrated that the light intensities in the EAP system with and without aeration were not much different (measured at the centre of the riser and downcomer), indicating that there was no light shading effect from the bubbles. On the other hand, the light intensity at the centre of the riser in the IAP decreased about 9% after aeration, which lowered the light availability to the culture. In a normal cylindrical airlift configuration (like IAP), when the system is aerated with adequate gas throughput, more bubbles are dragged down the downcomer of the airlift, causing obstruction to light penetration. In addition, as the alga starts to bloom, dense cells in the downcomer further block light passage to the system, which even lowers the intensity for those cells in the riser section. This is why as much as 20% drop in cell productivity is observed in the IAP when compared with the EAP.

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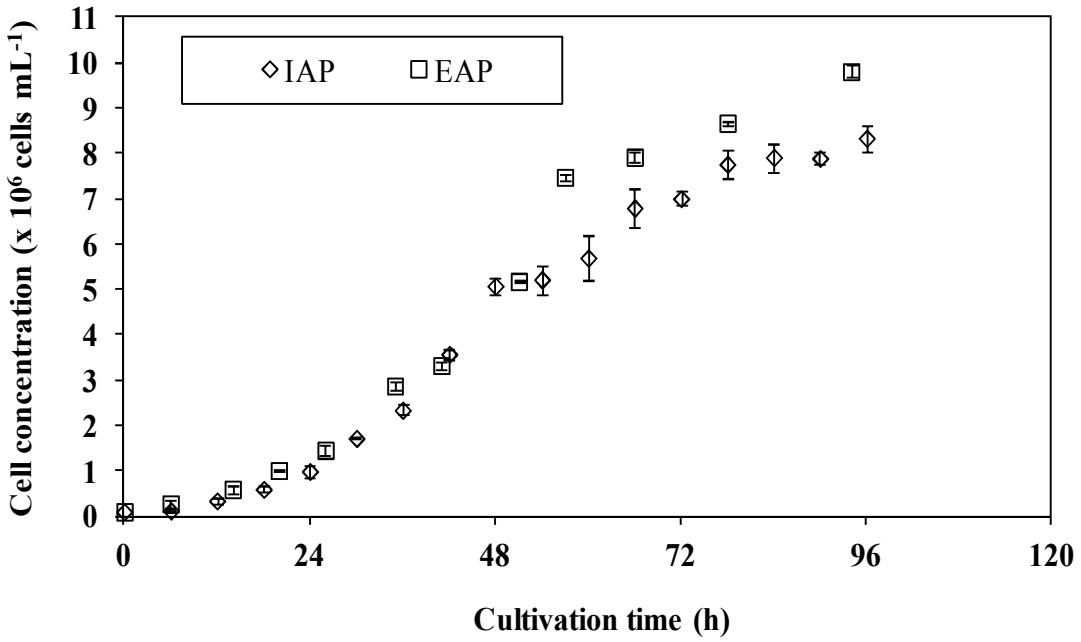

Figure 4. Growth of *C. gracilis* in 17-L IAP and EAP

Continuous culture

Although the IAP provides a lower performance when compared with the EAP, it was employed in the study on continuous culture due to its ease of set-up, maintaining and operating. A CAPs-in-series system is proposed to overcome the self-shading problem in which the over-grown culture obstructs light penetration to the system. In this set-up, cells are allowed to grow in separate compartments connected in series. The first compartment contains the culture at low concentration and therefore is exposed to high light intensity, and only the last compartment contains cells growing at high cell density and is subjected to light obstruction effect. Table 4 shows that an increasing dilution rate from 0.02 to 0.06 leads to a better productivity. Wash-out starts to take place at the dilution rate of 0.08. The productivity is enhanced by installing a series of reactor so that the next reactor is started with culture of higher cell density. However, such configuration in which reactors are installed in series means that the total volume of the system increases and this reduces the dilution rate. In Table 4 the dilution rate of each reactor is provided along with the overall dilution rate of the system where reactors are attached in series. The results indicate that the best productivity can be obtained from a series with 3 reactors, each being operated at a dilution rate of 0.06 (to prevent wash-out in the first column) with an overall dilution rate of 0.02.

Design Options for Outdoor Cultivation

Batch culture

FPAP was employed for outdoor cultivation with no control of light intensity or temperature. Figure 5 displays the profiles of light intensity and temperature during different cultivation periods. The daily maximum light intensity was around 60,000-100,000 Lux whilst the temperature varied between 30-40^{o}C. Table 5 provides the average surface energy intensity and temperature for each season over the one-year period of this experiment. It can be seen that Periods I (summer) and III (winter) were subjected to very similar environmental conditions and both the

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Table 4. Performance of CAPs-in-series systems in the cultivation of *C.gracilis*

Experiment	System	Medium feed rate (mL min^{-1})	$Di^{\dagger}$ (h^{-1})	$Do^{\ddagger}$ (h^{-1})	Final cell concentration (cells mL^{-1})	Productivity (cells h^{1})
Set 1	I	1	0.02	0.02	5.79 x 10^6	0.35 x 10^9
Set 2	I	2	0.04	0.04	4.54 x 10^6	0.55 x 10^9
Set 3	I	3	0.06	0.06	4.00 x10^6	0.72 x 10^9
Set 4	II	2	0.04	0.02	8.10 x 10^6	0.97 x 10^9
Set 5	II	4	0.08	0.04	Wash-out*	Wash-out*
Set 6	II	6	0.12	0.06	Wash-out*	Wash-out*
Set 7	III	3	0.06	0.02	12.12 x 10^6	2.18 x 10^9
Set 8	III	6	0.12	0.04	Wash-out*	Wash-out*
Set 9	III	9	0.18	0.06	Wash-out*	Wash-out*

† Dilution rate of individual reactor (D=medium feed rate/individual volume of reactor)
‡ Overall dilution rate of system (D= medium feed rate /total volume)
* Wash-out from one reactor and transferred to the other in series

temperature and energy intensity were in a similar range. Due to the shading by clouds and rain, Period II (rainy season) exhibited lower culture temperature and energy intensity.

The cultivation was started with an initial cell concentration of 1×10^5 cells mL^{-1} and the average growth of *C. gracilis* in each Period (I, II, III) is illustrated in Figure 6. A maximum cell concentration of 4.50×10^6 cells mL^{-1} and specific productivity of 3.76×10^4 cells mL^{-1} h^{-1} (3.76×10^9 cells h^{-1}) were obtained from 117-h cultivation in Period I. The cultivation in Period II shows a decrease in maximum cell concentration to 3.45×10^6 cells mL^{-1} and specific productivity to 2.36×10^4 cells mL^{-1} h^{-1} (2.36×10^9 cells h^{-1}) which occurred at 142 h. The maximum cell concentration further decreased to 3.05×10^6 cells mL^{-1} with specific productivity of 3.12×10^4 cells mL^{-1} h^{-1} (3.12×10^9 cells h^{-1}) at 95 h when the alga was cultivated in Period III. Note that cell growth ceased after 95 h in Period III. The light exposure duration in Period I (summer) was 1.5 h longer than that in Period III (winter) and this could directly affect the extent of photosynthesis as observed.

Semi-continuous culture

For semi-continuous culture in 100-L FPAPs under the condition as specified in Period I (Figure 5), the maximum cell concentration was found to increase with increase in initial cell concentration as illustrated in Figure 7. The maximum cell concentration of 4.5×10^6 cells mL^{-1} was obtained during the first round of cultivation with initial cell concentration of 0.1×10^6 cells mL^{-1}. This was equivalent to a specific productivity of 3.76×10^4 cells mL^{-1} h^{-1} (2.93×10^9 cells h^{-1}) at 117 h. Then the culture was partially harvested at the seventh day and replenished with fresh medium in a predefined volume such that the initial cell concentration for the second round became 1×10^6 cells mL^{-1}. In this consecutive batch, the maximum cell concentration increased to 7.1×10^6 cells mL^{-1} after 96 h of cultivation, with a specific productivity of 6.50×10^4 cells mL^{-1} h^{-1} (5.59×10^9 cells h^{-1}). The culture was thereafter harvested with the same initial cell concentration for the next batch. The third batch could be harvested after three days at the same maximum cell concentration (7.2×10^6 cells mL^{-1}) but with a much higher specific productivity of 8.86×10^4 cells mL^{-1} h^{-1} (8.86×10^9

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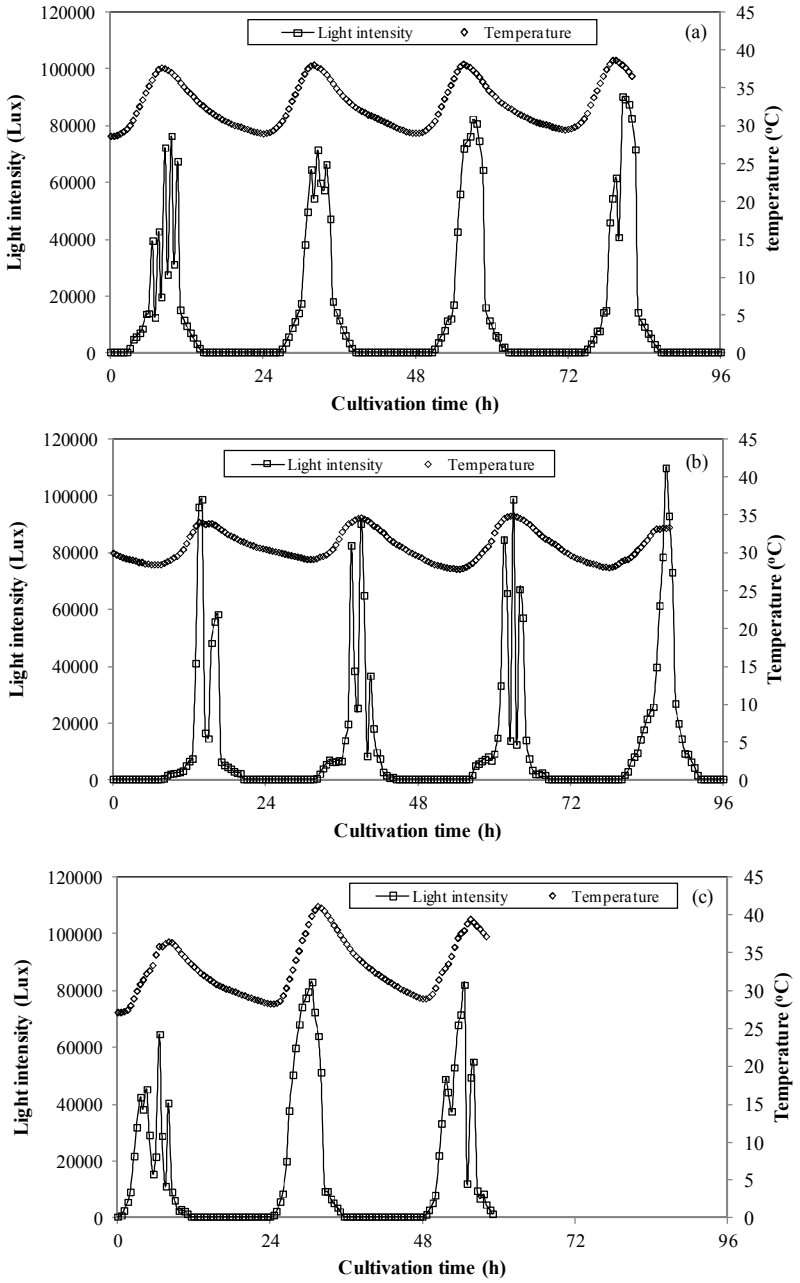

Figure 5. Profile of light intensity and temperature: (a) Period I (summer); (b) Period II (rainy season); (c) Period III (winter). The light intensity after Hour 60 in Period III was not reported due to equipment malfunction.

Table 5. Average surface energy intensity and average temperature during cultivation periods

Period	Average surface energy intensity (kWh m^{-2})	Average temperature (^oC)
Period I (summer)	0.53±0.07	33.7±0.4
Period II (rainy season)	0.40±0.05	31.4±0.5
Period III (winter)	0.50±0.07	33.6±1.6

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cells h^{-1}). This results lead to the conclusion that the culture grows better if started with higher cell density as the low-density culture could be subjected to light inhibition during the initial stage. This result corresponds well to the reported cultivation of *Arthrospira platensis* [21] and *Tetraselmis chuii* [22].

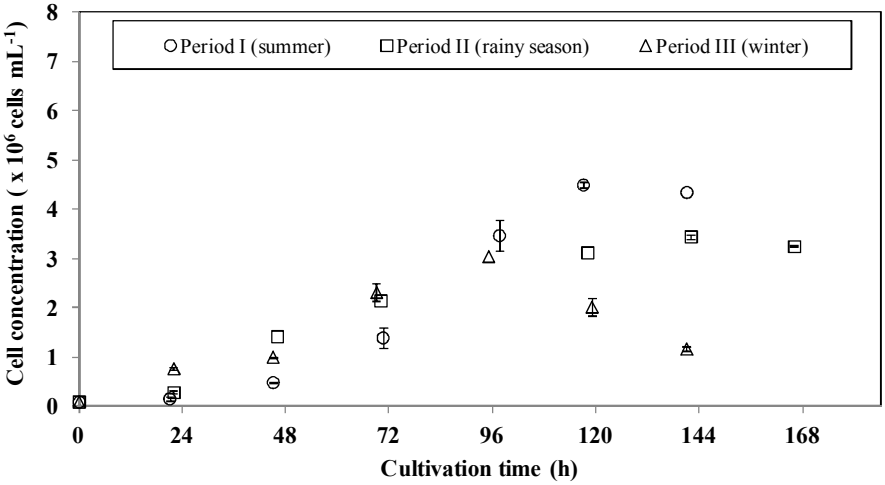

Figure 6. Growth of *C. gracilis* in 100-L FPAP in different seasons

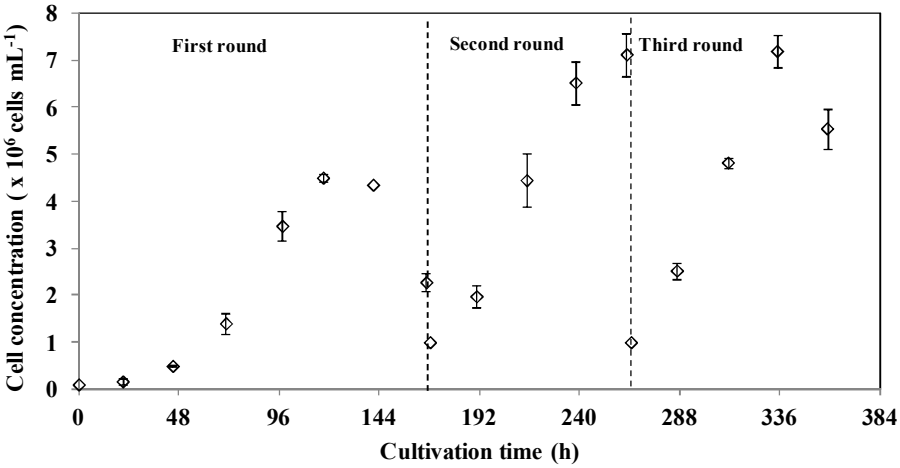

Figure 7. Growth of *C. gracilis* in semi-continuous 100-L FPAP

Economical Analysis

To conduct an economic analysis for the cultivation of *C. gracilis*, it is assumed that the target culture has a cell density of about $5x10^5$ cells mL^{-1} as this is a typical trading value in the Thai market. The cost estimates for *C. gracilis* cultivation using batch culture with different reactor types and sizes are based on the full utilisation of the area of 1.5×1.5 m^2 and the resulting cost distribution is depicted in Table 6. The estimates cover fixed costs and operating costs, i.e. nutrients, electricity and water, but labour is excluded. The fixed and operating costs are found to be around 40% and 60% respectively of the total cost. For the area of 1.5×1.5 m^2, the total number of IAP that can be installed is four whilst that of EAP or FPAP is two reactors. Note that the IAP is smaller and simpler in design so a large number can be fitted in the same area when compared with

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Table 6. Annual costs estimation for cultivation of *C. gracilis* in indoor and outdoor airlift systems

	Unit	EAP (17L, indoor)	IAP (17L, indoor)	FPAP (100L, outdoor)
Initial cell concentration	cells mL^{-1}	100,000	100,000	100,000
Max cell concentration	cells mL^{-1}	9,820,000	8,340,000	3,670,000
Working volume	L $reactor^{-1}$	17	17	100
Cultivation time	Days $batch^{-1}$	4	4	5
Number of cycle (330 days $year^{-1}$)	batches $year^{-1}$	83	83	66
Final cell concentration	cells mL^{-1}	500,000	500,000	500,000
Total product volume	L $batch^{-1}$	334	284	734
Volume of brine water (30 ppt) for dilution	L $batch^{-1}$	317	267	634
Productivity	L $year^{-1}$	27,545	23,394	48,444
Operating costs				
- Nutrient requirements				
Nutrient cost	\$ L^{-1}	0.0052	0.0052	0.0052
Nutrient charge	\$ $year^{-1}$	7.28	7.28	34.24
-Electricity requirements				
1.Lighting	kWh $Batch^{-1}$	10.37	10.37	
2. Compressor	kWh $Batch^{-1}$	1.37	3.20	48.00
Total electricity requirements	kWh $Batch^{-1}$	11.74	13.57	48.00
Electricity charge	\$ kWh^{-1}	0.0938	0.0938	0.0938
Total Electricity Charge	\$ $year^{-1}$	91	105	297
- Water/Brine requirements for medium preparation/dilution				
Volume of brine water (concentration)	L $reactor^{-1}$	67	57	147
Brine water (concentration) charge	\$ L^{-1}	0.0156	0.0156	0.0156
Total brine water (concentration) charge	\$ $year^{-1}$	86.08	73.11	185.63
Tap water charge	\$ m^{-3}	0.3125	0.3125	0.3125
Total tap water Charge	\$ $year^{-1}$	6.89	5.85	12.11
Total brine water (30 ppt) charge	\$ $year^{-1}$	92.96	78.95	163.50
Total operating cost	**\$ $year^{-1}reactor^{-1}$**	**191.04**	**191.17**	**494.74**
Fixed costs				
Land and construction (assume 30% of operating cost)	\$ $year^{-1}$	57	57	148
Reactor cost	\$ $reactor^{-1}$	469	313	531
Reactor life time	year	5	5	5
Reactor charge	\$ $year^{-1}$	94	63	106
Compressor	unist $reactor^{-1}$	0.14	0.33	4
Compressor cost	\$ $unit^{-1}$	81.25	81.25	81.25
Compressor life time	year	10	10	10
Compressor charge	\$ $year^{-1}$	1.16	2.71	33
Total fixed cost	**\$ $year^{-1}reactor^{-1}$**	**152.22**	**122.56**	**287.17**
Total cost	**\$ $year^{-1}reactor^{-1}$**	**343.26**	**313.73**	**781.91**
Number of reactors per 1.5x1.5m^2		2	4	2
Total operating cost	\$ $year^{-1}m^{-2}$	170	340	440
Total fixed cost	\$ $year^{-1}m^{-2}$	135	218	255
Total cost	**\$ $year^{-1}m^{-2}$**	**305**	**558**	**695**
Income (0.47 \$ L^{-1})	**\$ $year^{-1}m^{-2}$**	**11,477**	**19,495**	**20,185**
Profit	**\$ $year^{-1}m^{-2}$**	**11,172**	**18,937**	**19,490**
Gain (profit/total cost)		**37**	**34**	**28**

Note: Electricity and water charges were based on current Thailand rates (2012): 1\$ = 32 THB.

other configurations. Figure 8 shows that the main operating costs are electricity and brine water. For indoor cultivation, the cost of lighting becomes the major cost which contributes to about 42% of the total cost. Electricity is also the major cost for large-scale outdoor cultivation with around 60% of the overall cost, and this is due to the use of compressors to supply aeration. Brine water is used to adjust the cell density after the harvest and this constitutes around 33-48% of the total cost. For large-scale and outdoor cultivation, the total costs are higher than a small-scale one owing to the effect of sizing. The total cost of FPAP (100 L) is around 695 US\$ $year^{-1}m^{-2}$, whereas the small-scale reactors cost about 305-558 US\$ $year^{-1}m^{-2}$. However, the large-scale cultivation provides

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better benefits in terms of return as it exhibits higher productivity provided that it is installed within the same area. This analysis suggests that for high profitability, options like outdoor and large-scale cultivation should be considered, along with the reuse of brine water. However, the EAP provides the best gain, which suggests highest return per unit of investment.

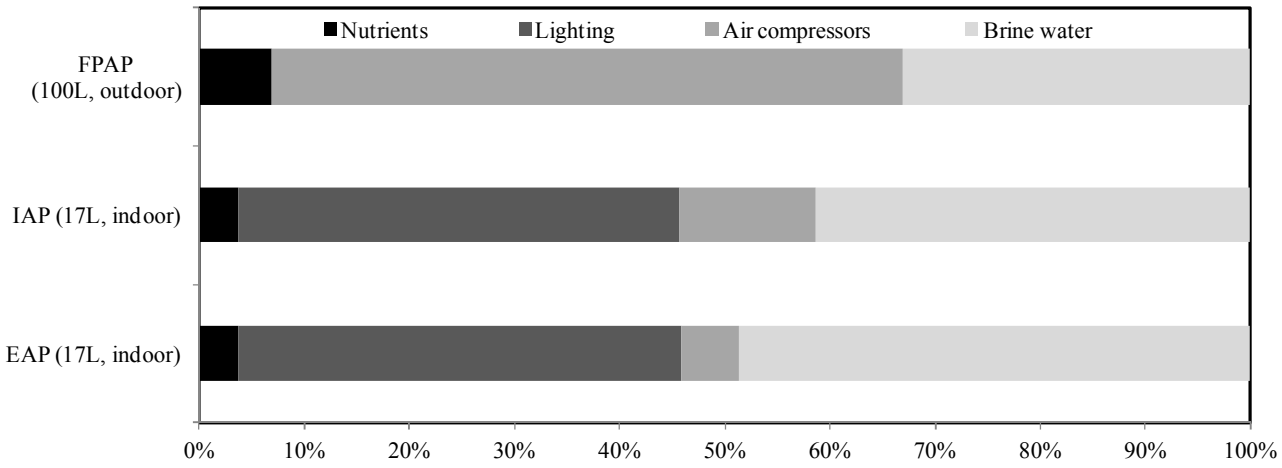

Figure 8. Proportion of operating costs for each airlift photobioreactor

CONCLUSIONS

An airlift photobioreactor has proven effective for the cultivation of *Chaetoceros gracilis*. For indoor cultivation, a good alternative design is achieved through the use of an external-loop airlift system, from which the final cell concentration of 9.82×10^6 cells mL^{-1} from EAP can be obtained with a specific growth rate of 9.21×10^{-2} h^{-1}, which was higher than 8.34×10^6 cells mL^{-1} and 8.56×10^{-2} h^{-1} obtained from IAP. A continuous mode with reactor-in-series configuration provides a higher final cell concentration of 12.12×10^6 cells mL^{-1}. The growth rate in this case is limited by the wash-out condition, which occurs at the individual dilution rate of 0.08 h^{-1}. The outdoor operation, although suffering from uncontrolled environmental conditions, provides satisfactory growth performance. The higher maximum cell specific productivity obtained from indoor culture is counterbalanced by the economy of scale and the ease of operation of the outdoor system.

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