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Specific Light Uptake Rate Can be Served as a Scale-Up Parameter in Photobioreactor Operations

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Abstract Lumostatic operation for cultivation of *Haematococcus pluvialis* was assessed to test the scale-up strategy of photobioreactors. Lumostatic operation is a method of maintaining a proper light condition based on the specific light uptake rate (q_e), by cells. Lumostatic operations were performed in 0.4-, 2-, 10-, and 30-l scale bubble column photobioreactors and the results were compared with cultures illuminated with constant light intensity. Significant differences were observed in the maximal cell concentrations obtained from 0.4-, 2-, 10-, and 30-l scale photobioreactors under constant light intensity, yielding the maximal cell concentrations of 2.8×10^5, 2.2×10^5, 1.5×10^5, and 1.1×10^5 cells/ml, respectively. The maximal cell concentration in a 0.4-l photobioreactor under lumostatic operation was 4.3×10^5 cells/ml. Furthermore, those in 2-, 10-, and 30-l scale photobioreactors were about the same as that in the 0.4-l photobioreactor. The results suggest that lumostatic operation with proper q_e is a good strategy for increasing the cell growth of *Haematococcus pluvialis* compared with a constant supply of light energy. Therefore, lumostatic operation is not only an efficient way to achieve high cell density cultures with minimal power consumption in microalgal cultures but it is also a perfect parameter for the scale-up of photobioreactors.

Key words: Lumostatic operation, specific light uptake (q_e), *Haematococcus pluvialis*, scale-up, bubble column photobioreactors (BCPs)

Industrial microalgal biotechnology shows an extraordinary potential for cultivation of energy crops, such as those used in feed, food, lipid, vitamin, pigment, cosmetic, and pharmaceutical industries, as well as those used in the treatment of waste materials [4, 5, 17]. This biotechnology has not only advanced screening methods, cultivation systems, separation, and purification skills, but has also improved the scale-up process of high-density culturing [4, 5, 17]. The advantages of high cell density cultures include easier harvesting, lower production costs, better prevention of contamination from other bacteria, and easier control of environmental conditions such as temperature, pH, and CO_2 [2, 13]. High cell density cultivation of microalgae in large-scale processes is affected by environmental factors such as light energy, pH, CO_2, and nutrient supply [16]. The geometric change during a scale-up process and the high cell density cultures decrease the light distribution and penetration efficiency in the photobioreactor during the culturing period [21]. Light availability is an essential factor for cell growth in phototrophic cultivation of *Haematococcus pluvialis* [18]. When the photobioreactor size is small or the cell concentration is low, light deficiency and mutual shading effects are not major issues and cells exhibit maximum growth rates [9, 17]. As the photobioreactor size and cell concentration increase, however, low light availability and mutual shading effects increase; this decreases the specific light uptake rate and, as a result, decreases the specific growth rate [3, 5, 15, 19].

Lumostatic operation is a way by which to avoid light deficiency during the cultivation period and to maintain proper light conditions based on the specific light uptake rate (q_e) by cells. As mentioned above, the lumostatic operations have many advantages: first, it can obtain higher productivities and/or higher cell concentrations without damaging the photosynthetic apparatus; second, it can achieve the same amount of biomass with reduced cultivation time and less power consumption; third, it can save the downstream processing costs, such as harvesting and extraction, owing to the higher cell density and the higher product concentration. The aim of this study was to increase the cell concentration in *H. pluvialis* cultures, to enhance the process of scale-up with a lumostatic operation technique in bubble column photobioreactors, and to maintain the light condition during the period of cultivation.

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Table 1. Characteristic values of BCPs.

Bubble column photobioreactor (l)	Total volume (l)	Working volume (l)	Inner diameter (cm)	Column height (cm)
0.4	0.5	0.4	3.5	60
2	2.5	2	8.5	58
10	13	10	11.0	170
30	35	30	17.0	170

MATERIALS AND METHODS

Strain and Culture Conditions

Haematococcus pluvialis UTEX 16 of the Culture Collection of Algae at the University of Texas at Austin was used in this study. The alga was cultivated in Modified Bold's Basal Medium (MBBM), and included 246.5 mg/l of $NaNO_3$, 24.99 mg/l of $CaCl_2 \cdot 2H_2O$, 73.95 mg/l of $MgSO_4 \cdot 7H_2O$, 4.98 mg/l of $FeSO_4 \cdot 7H_2O$, 74.9 mg/l of K_2HPO_4, 175.57 mg/l of KH_2PO_4, 25.13 mg/l of NaCl, 49.68 mg/l of

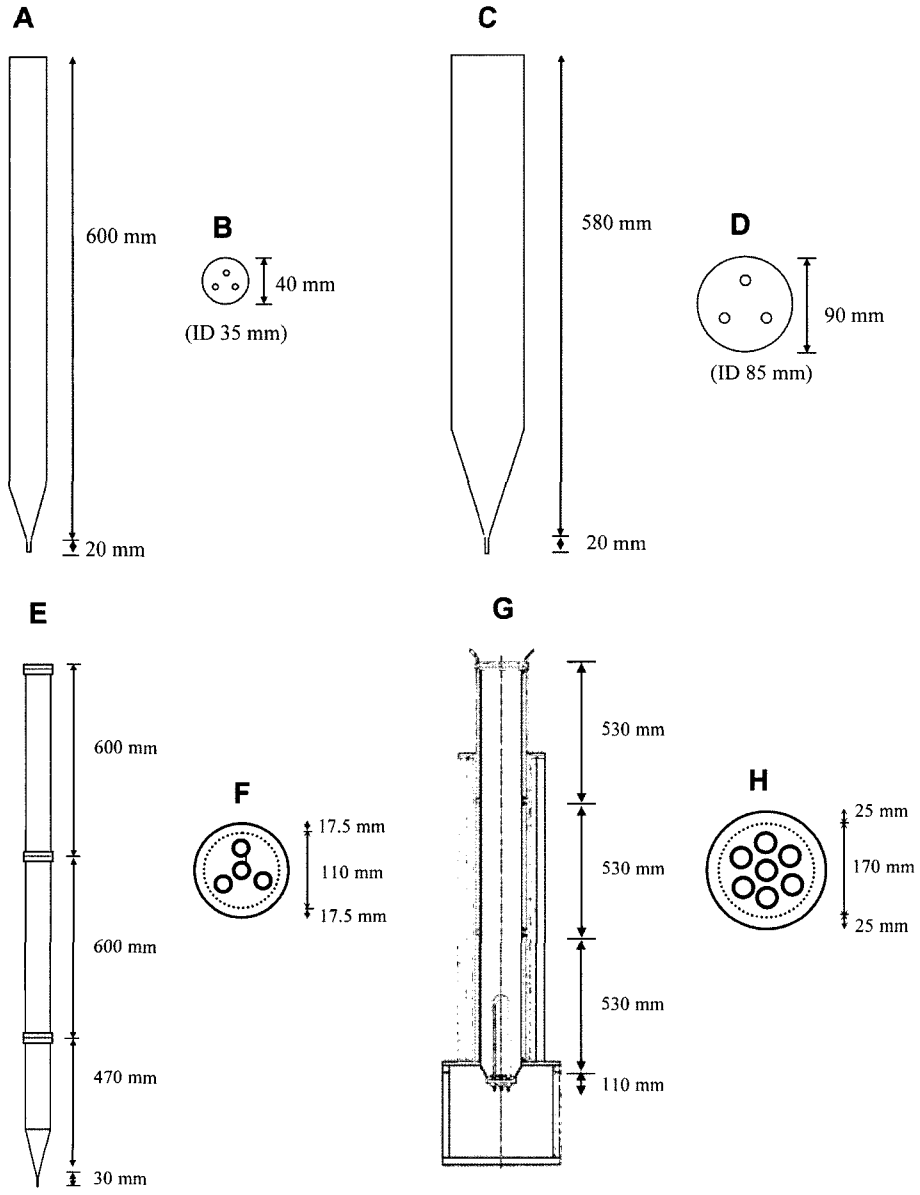

Fig. 1. Schematic diagrams of BCPs: **A**. Side view of 0.4-l BCP; **B**. top view of 0.4-l BCP; **C**. Side view of 2-l BCP; **D**. Top view of 2-l BCP; **E**. Side view of 10-l BCP; **F**. Top view of 10-l BCP; **G**. Side view of 30-l BCP; **H**. Top view of 30-l BCP.

$C_{10}H_{16}N_2O_8$ (EDTA), 1.57 mg/l of $CuSO_4 \cdot 5H_2O$, 1.19 mg/l of $Na_2MoO_4 \cdot 2H_2O$, 11.13 mg/l of H_3BO_3, 1.44 mg/l of $MnCl_2 \cdot 4H_2O$, 8.83 mg/l of $ZnSO_4 \cdot 7H_2O$, 0.49 mg/l of $Co(NO_3)_2 \cdot 6H_2O$, 6.06 mg/l of MoO_3, 30.86 mg/l of KOH, and 0.98 mg/l of H_2SO_4 [8].

A preculture of 100 ml was prepared in a 250-ml Erlenmeyer flask, and the cells were transferred to 2-l BCPs containing MBBM with 0.2 VVM flow rate aeration with 5% CO_2 gas and 95% air under a constant continuous light intensity of 40 $\mu E/m^2/s$. The temperature and pH were kept constant at 25°C and 7.0±0.5, respectively, during the period of cultivation. The amount of nitrogen in the culture was kept constant by the addition of $NaNO_3$ during cultivation [6, 7].

Design of Bubble Column Photobioreactor (BCP)

To investigate the process scale-up, four bubble column photobioreactors (BCPs) of 0.4, 2, 10, and 30 l were designed and constructed. All of the BCPs were constructed from Pyrex glass. The characteristic values of the BCPs used are shown in Table 1. The BCPs of 0.4 and 2 l were cylindrical and had stoppers consisting of three ports for sampling, gas outlet, and nitrogen control (Fig. 1). The cylinder of 10-l BCP was composed of three parts: one part was the same as the cylinder of 2-l BCP and the other two parts were two cylinders at 600 mm high and a head cap containing four ports for sampling, gas outlet, nitrogen control, and inoculation. The 30-l BCP was composed of three cylinders with 170 mm diameter and 530 mm high and a head cap containing seven ports for the sampling, gas outlet, nitrogen control, inoculation, pH sensor, temperature sensor, and condenser port. The H/D (height/diameter) ratios of 0.4-, 10-, and 30-l BCPs were between 10 and 17. The inner diameter of the 2-l BCP was 40 mm greater than that of 0.4 l, but the height of the two BCPs was about the same as shown in Table 1. The heights of the 10-l and 30-l BCPs were the same with 1,700 mm (Fig. 1E through 1H and Table 1), but the bottom designs of the two BCPs were different to facilitate the injection of mixed gas into the BCPs [5].

Measurement of Light Source

Light intensity was measured by an LI-COR quantum sensor (LI-190SA, LI-COR, Lincoln, NE, U.S.A.) equipped with a Data Logger (LI-1400, LI-COR). A fluorescent lamp (FL 18D, OSRAM, Korea) was used as a light source in all experiments. The fluorescent lamps were set up in circular frames around the BCPs and the light intensity was adjusted by the number and distance of the fluorescent lamps from the BCPs. The fluorescent lamps supplied the light energy for both the growth of *H. pluvialis* and the induction of astaxanthin.

Lumostatic Operation

Lumostatic operation was studied based on the specific light uptake rate, q_e, as a way to maintain proper light conditions to increase the cell concentration in *H. pluvialis* culture. The q_e by the cell concentration was calculated with the following equation [5, 19], in which E_{in} and E_{out} are the average input and output light energy of vessel surface, respectively.

$$q_e = \frac{(E_{in} - E_{out})A}{V \cdot C}$$

A and V are the surface areas of the column vessel and the volume of the culture, respectively. C indicates the cell concentration. E_{in} and E_{out} were determined by averaging the value of light intensity measured at 8 points (every $\pi/4$ radian) in the internal and the external of vessel surface. E_{in} was adjusted by changing the number and distance of fluorescent lamps from the surface of each photobioreactor, which was measured inner wall of the empty cylinder. E_{out} was measured from the outside of the BCP cylinder during the culture [5, 19].

Analytical Methods

The cell concentration and average cell size were measured using a Coulter Counter (Model Z2, Beckman Coulter, Inc., Fullerton, CA, U.S.A.). The data from the Coulter counter were adapted by AccuComp software and exported to Microsoft Excel worksheets in order to calculate the cell concentrations and cell sizes. The concentration of nitrate was analyzed by a spectrophotometer (Model HP8453B, Hewlett Packard, Waldbronn, Germany). The concentration of nitrate was measured after the removal of cells by centrifugation at 3,000 rpm for 10 min according to the standard method [1]. Astaxanthin concentration was analyzed by a spectrophotometer (Model HP8453B, Hewlett Packard, Waldbronn, Germany).

RESULTS AND DISCUSSION

Process Scale-Up with Constant Surface Light Intensity

Four BCPs with working volumes of 0.4, 2, 10, and 30 l were used to investigate the scaling-up process of the photobioreactors. A constant surface light intensity of 40 $\mu E/m^2/s$ was supplied for illumination to all four BCPs (Fig. 2A). Supplying the constant surface light intensity is the most common way to culture microalgal cells indoors. Since the nitrogen limitation induces heterocyst formation (thus astaxanthin accumulation), fed-batch cultivation was performed in order to prevent the culture from nutrient deficiency and to keep the cells in vegetative form.

As shown in Fig. 2B, the smaller the BCP volumes, the faster the initial growth rate, which resulted in the higher final cell concentration. The highest cell concentration in the 0.4-, 2-, 10-, and 30-l BCPs were 2.8×10^5, 2.2×10^5, 1.5×10^5, and 1.1×10^5 cells/ml, respectively. During the 4-week culture, the cell concentration increased 10–25-folds and thus the amount of light received per cell would be

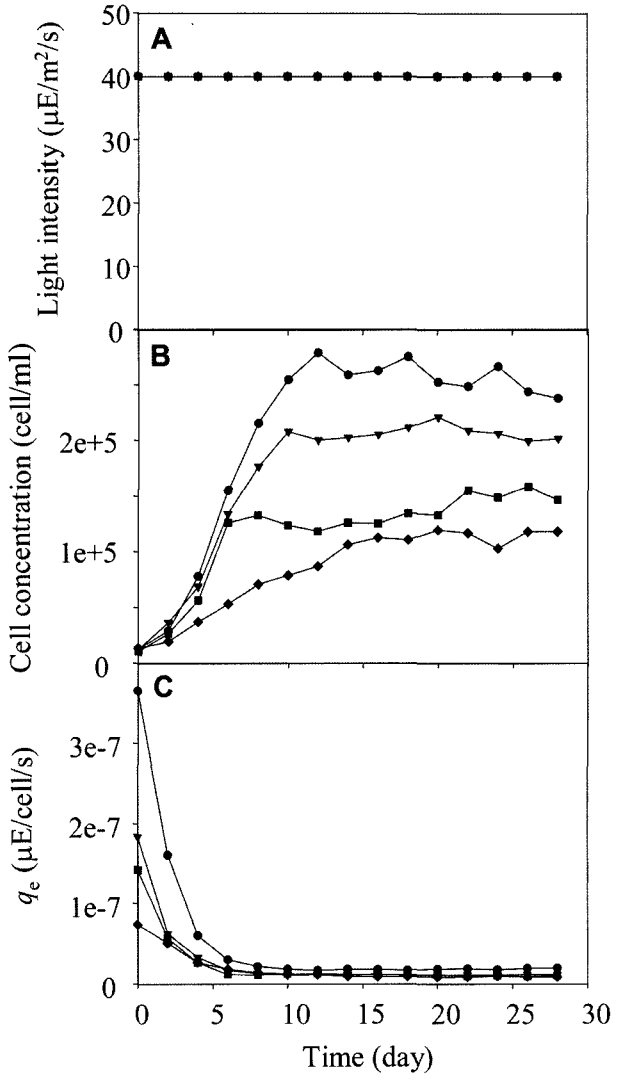

Fig. 2. Supply of constant light intensity in differently-scaled photobioreactors: Light intensity profiles (**A**), cell concentration profiles (**B**), and specific light uptake rate profiles (**C**) of the 0.4-l BCP (●), 2-l BCP (▼), 10-l BCP (■), and 30-l BCP (◆).

decreased accordingly. Thus the specific light uptake rate, q_e, was calculated to explain the light limiting situation (Fig. 2C). The q_e profiles in Fig. 2C clearly showed the difference in the light energy supplied per cell per unit time (in μE/cell/s) in differently scaled BCPs. The specific light uptake rates, or the light energy supplied per cell, decreased dramatically within 5 days of cultures (Fig. 2C). Then, the growth of the cells would be limited by the light. For example, in the 0.4-l BCP, the cell concentration increased from 1.2×10^4 cells/ml to 2.8×10^5 cells/ml, while the q_e decreased from 3.7×10^{-7} μE/cell/s to 1.6×10^{-8} μE/cell/s during the same period (● in Fig. 2C). A similar situation happened in the other BCPs. The initial q_e in the 2-l BCP was about 1.8×10^{-7} μE/cell/s, but the q_e value dropped quickly to less than one tenth of the initial (▼ in Fig. 2C).

The experimental results showed that cells cultivated in 0.4-l BCPs (which had the largest q_e value) grew faster and higher than those in larger-scaled BCPs (2-, 10-, and 30-l BCPs, which had smaller q_e values than that of the 0.4-l BCP).

These data clearly suggested that the larger BCPs quickly entered into the light-limiting condition under the constant surface light intensity, since the specific light uptake rates by the cells were decreased quickly (Fig. 2C) as the cell concentrations increased (Fig. 2B). Consequently, the process of scale-up under constant surface light intensity cannot be a paved road to successful industrial-scale photobioreactors. During this conventional way of scale-up, the cell productivity will be reduced significantly because of the increased diameter of the photobioreactor, and thus the lower light intensity per cell basis. In other words, the

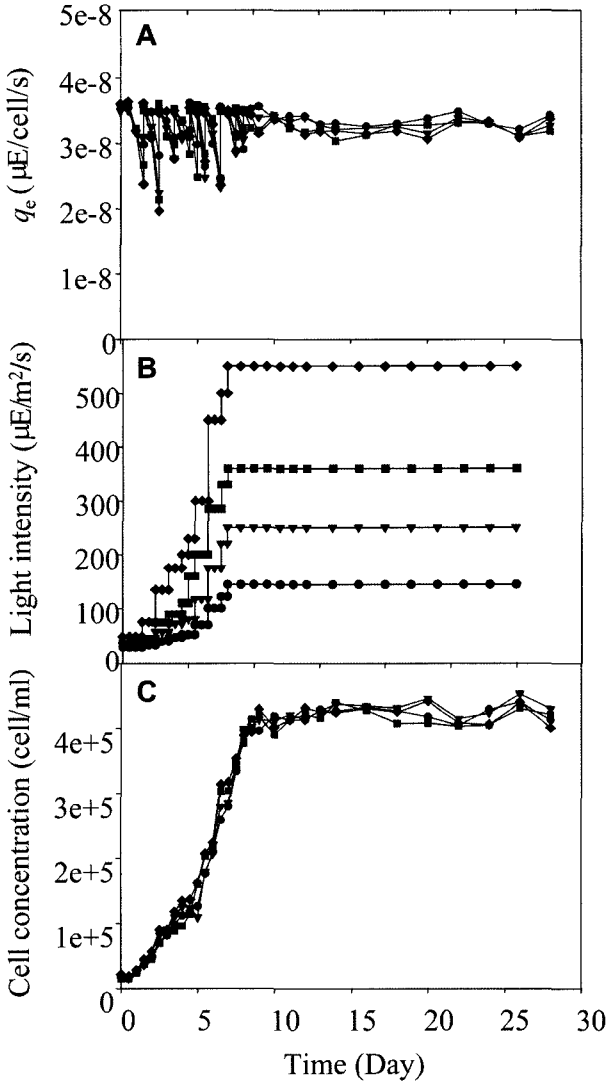

Fig. 3. Lumostatic operation in differently scaled photobioreactors: Specific light uptake rate profiles (**A**), light intensity profiles (**B**), and cell concentration profiles (**C**) of the 0.4-l BCP (●), 2-l BCP (▼), 10-l BCP (■), and 30-l BCP (◆).

volume of the cultures increased three-dimensionally, but the supplied light intensity will be increased only two-dimensionally. Moreover, the efficiency of light energy will be decreased by a mutual shading effect at higher cell densities. In order to achieve a commercial scale of the proposed bioprocess, a new parameter should be introduced.

Scale-Up Process with Lumostatic Parameters

Lumostatic operation was already proved to be a desirable way to achieve high-density cultures by partially overcoming the mutual shading effect [5]. Because of the superior performance of the lumostatic operation, the q_e was chosen as the scale-up parameter for photosynthetic cultures. Lumostatic operation with a q_e of 3.5×10^{-8} μE/cell/s was performed for high cell density cultivation of *H. pluvialis* to minimize mutual shading and to avoid photoinhibition [5, 14]. Again, the same four BCPs with working volumes of 0.4, 2, 10, and 30 l were used to investigate the scale-up process using the q_e.

The specific light uptake rates (Fig. 3A) were calculated every 30 min (longer intervals after 10 h) and the light intensities (Fig. 3B) of the BCPs were adjusted to maintain the q_e of 3.5×10^{-8} μE/cell/s based on cell concentrations. This q_e value was chosen based on the performance from the preliminary experiments with various q_e values (data not shown). The resulting profiles of cell concentrations in differently scaled BCPs are shown in Fig. 3C. As can be seen in Fig. 3C, a dramatic change could be observed in lumostatic operations under constant specific light uptake rate. The highest cell concentration obtained in the 0.4-l BCP under lumostatic operation was 4.4×10^5 cells/ml, which is a 57.7% increase over the highest concentration obtained under constant light intensity of 40 μE/m^2/s (compare in Figs. 2C and 3C). The initial q_e under constant light intensity (3.7×10^{-7} μE/cell/s) was over 10 times higher than that under lumostatic operation. However, the q_e under constant light intensity dropped quickly to 1.6×10^{-8} μE/cell/s, whereas that under lumostic operation maintained at the same level.

Furthermore, the final cell concentrations of all four BCPs were virtually identical regardless of the scale

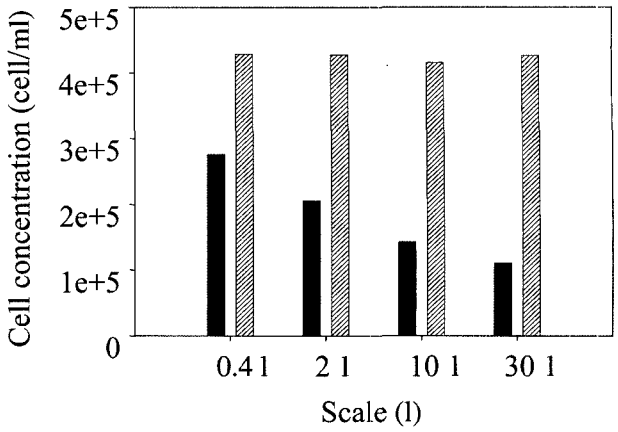

Fig. 4. Comparison of the final cell concentrations under lumostatic operation and under constant light energy.
Symbols: (-■-), supply of constant light intensity; (-▨-), lumostatic operation.

(Fig. 3C). The highest cell concentrations in the 0.4-, 2-, 10-, and 30-l BCPs under lumostatic operation were between 4.4×10^5 and 4.5×10^5 cells/ml. Contrary to the results under constant surface light intensity, the light energy supplied per cell was maintained at the same level (Fig. 3A) as the cell concentration increased. As a result, the initial growth rates of all four BCPs as well as the cell concentration profiles were also identical (Fig. 3C). The scale-up based on the specific light uptake rate, q_e, will give the identical growth curve and the final cell concentration cell growth regardless of the scale of BCPs. It should be noted that the geometric characteristics of the BCPs used in these experiments were not identical (Table 1). The usefulness and the superiority of the specific light uptake rate (q_e) as a scale-up parameter of the photobioreactors was evident from the results reported here.

One more interesting result was observed in larger scale BCPs. The cell concentration in the 0.4-l BCP under lumostatic operation was 160% higher than that under constant light intensity (Fig. 4). The cell concentrations of the 2-, 10-, and 30-l BCPs were improved much more to

Table 2. Experiment results of lumostatic operation and constant light intensity in the BCPs.

Bubble column photobioreactor (l)	Lumostatic operation	Culture period (day)	Max. cell concentration (cells/ml)	Total supplied photons until 10th day (μE)	Max. cell concentration/ total supplied photons until 10th day (cell/mE)
0.4	×	28	2.8×10^5	1.4×10^6	8.0×10
	○		4.4×10^5	2.4×10^6	7.2×10
2	×		2.2×10^5	4.0×10^6	1.1×10^2
	○		4.5×10^5	1.1×10^7	7.8×10^2
10	×		1.5×10^5	1.3×10^7	1.2×10^2
	○		4.4×10^5	5.5×10^7	7.8×10
30	×		1.1×10^5	2.6×10^7	1.3×10^2
	○		4.4×10^5	1.7×10^8	7.6×10

200, 300, and 400%, respectively, by lumostatic operation (Fig. 4). This fact could mean that the scale-up under lumostatic operation is more powerful in larger scale. Furthermore, the surface light intensities of BCPs were not proportionally increased as the volume of BCPs increased (Fig. 3A). For example, the highest surface light intensities for the 0.4- and 30-l BCPs (7.5 times difference) were 145 and 550 $\mu E/m^2/s$ (3.8 times difference), respectively. Since the total light energy per cell was identical in all four BCPs, the light utilization efficiency may be the same if the two BCPs had the same geometric characteristics. However, this means that the light utilization efficiency may be improved during the scale-up process by achieving the same performance with less total light energy supplied. The experimental profiles of cell concentration, light intensity, and total photon are shown in Table 2.

Consequently, maintaining the same specific light uptake rate during the scale-up of photosynthetic cultures is the most effective and efficient strategy to obtain not only high-density cultures but also a successful large-scale photobioreactor with possibly less energy.

This paper demonstrates that lumostatic operation based on the specific light uptake rate (q_e) can be the most effective, economic, and practical strategy to obtain high-density large-scale cultures of photosynthetic organisms. The efficiency of the lumostatic operation based on the specific light uptake rate was confirmed in high cell density cultures of *H. pluvialis* in 0.4-, 2-, 10-, and 30-l BCPs. The cell growth under lumostatic operation was almost identical regardless of the scale, whereas the performance under constant light illumination decreased as the scale of the BCP increased. The cell concentrations of the 0.4-, 2-, 10-, and 30-l BCPs under lumostatic operation were improved to 160, 200, 300, and 400%, respectively, compared with the performance of controls under fixed light intensity.

Lumostatic operations using the specific light uptake rate are not only an efficient way to obtain high cell density cultures, but they are also an economic way to reduce cultivation time and to save power consumption regardless of the scale. The specific light uptake rate is one of the best parameters for the scale-up of photobioreactors.

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