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Enhancing Photon Utilization Efficiency for Astaxanthin Production from *Haematococcus lacustris* Using a Split-Column Photobioreactor

Z-Hun Kim, Hanwool Park, Ho-Sang Lee, and Choul-Gyun Lee*

National Marine Bioenergy R&D Center & Department of Biological Engineering, Inha University, Incheon 22212, Republic of Korea

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**Corresponding author*
Phone: +82-32-860-7518;
Fax: +82-32-873-7518;
E-mail: leecg@inha.ac.kr

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A split-column photobioreactor (SC-PBR), consisting of two bubble columns with different sizes, was developed to enhance the photon utilization efficiency in an astaxanthin production process from *Haematococcus lacustris*. Among the two columns, only the smaller column of SC-PBR was illuminated. Astaxanthin productivities and photon efficiencies of the SC-PBRs were compared with a standard bubble-column PBR (BC-PBR). Astaxanthin productivity of SC-PBR was improved by 28%, and the photon utilization efficiencies were 28–366% higher than the original BC-PBR. The results clearly show that the effective light regime of SC-PBR could enhance the production of astaxanthin.

Keywords: Bubble-column photobioreactor, astaxanthin, *Haematococcus*, liquid circulation, photon efficiency

Astaxanthin, which is one of the most valuable carotenoids, is used for pharmaceutical and nutraceutical applications because of its strong antioxidant potential [1]. A freshwater green alga, *Haematococcus lacustris*, is one of the best astaxanthin producers and capable of undergoing transformation to the aplanospore stage when the cells are exposed to various stressful conditions such as excessive light [3, 5], salt stress [14], pH [22], high temperature [6], and nutrient starvation [19, 20]. However, it is not easy to achieve a high level of astaxanthin production from *H. lacustris* because of its complex morphological and metabolic changes [2, 9].

Many novel photobioreactors (PBRs) have been explored for their ability to produce high levels of astaxanthin from *Haematococcus* [3, 21, 23]. In addition, the cell physiology, including photosynthetic activity and responses to wavelength and intensity of light, has been investigated extensively [7, 10, 17]. Despite these attempts, developments of the PBRs for astaxanthin production have progressed slowly when compared with rapid advances in other types of algal biotechnology [4, 8, 12, 15]. Among factors that need to be considered when designing effective PBRs for astaxanthin production, high light stress has been shown to have the greatest effect on carotenoids accumulation in *H. lacustris* [11, 22]. However, owing to light absorption and mutual shading by cells, photons are only available in an extremely narrow zone close to the culture surface even if light is supplied at high levels [10, 16]. The imbalanced light regime in the culture causes ineffective astaxanthin accumulation, prolonged culture time, and additional power consumption. Thus, PBRs for astaxanthin production should have high photon utilization efficiency and high levels of astaxanthin productivity to enable efficient production of astaxanthin [5].

In the present study, a novel split-column photobioreactor (SC-PBR) was developed to increase the photon efficiency for astaxanthin production from *H. lacustris*. The SC-PBR consists of two connected bubble columns of different sizes with continuous circulation of culture broth from one column to the other. Light was only supplied to the smaller column to enable efficient astaxanthin induction by improving light distribution.

The green microalga, *Haematococcus lacustris*, was purchased from the Culture Collection of Algae at the University of Texas at Austin (USA). Modified Bold's Basal Medium (MBBM) was used as the culture medium [11]. The seed cultures were prepared in 0.25-L Erlenmeyer flasks containing 0.1 L of fresh MBBM in an illuminated shaking incubator, after which the cells were transferred to a 3-L BC-PBR containing 2 L of fresh MBBM under continuous light intensity of 40 μE m^{-2} s^{-1} 0.2 vvm aeration using 5%

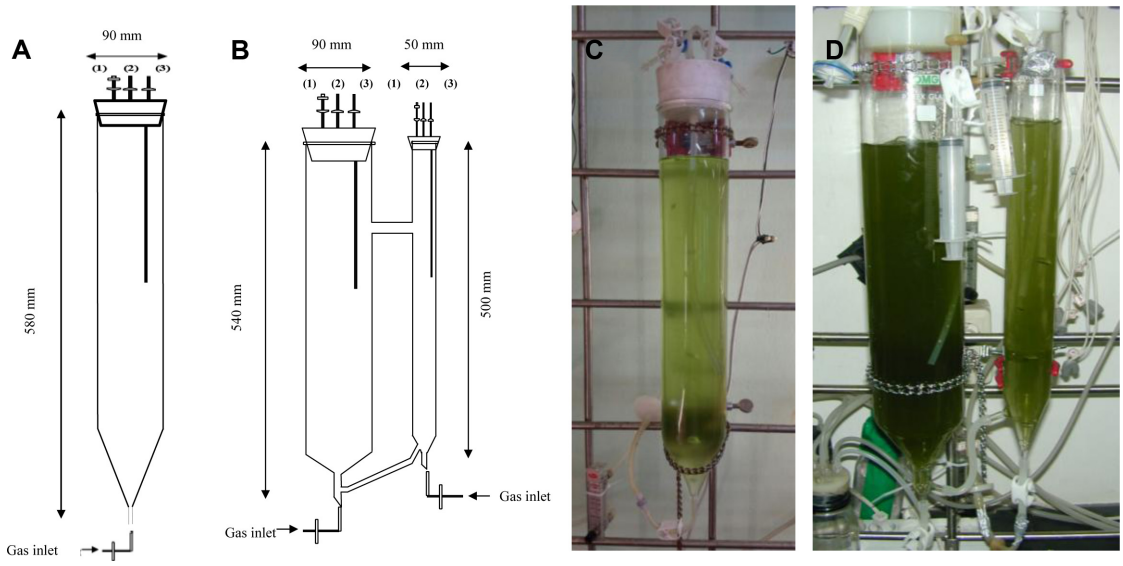

Fig. 1. Schematic diagrams and photographs of a bubble-column photobioreactor and a split-column photobioreactor.
Schematic diagrams of a bubble-column photobioreactor (**A**) and split-column photobioreactor (**B**). Photographs of a bubble-column photobioreactor (**C**) and split-column photobioreactor (**D**). Medium inlet port (1), gas outlet port (2), and sampling port (3).

CO_2 enriched air. Nitrate was replenished when its level fell below 50 mg/ml to maintain vegetative growth. All experiments were inoculated with exponentially growing cells at an initial density of 1×10^5 cell/ml.

BC-PBRs were made of Pyrex glass with a 90 mm outer diameter and a length of 580 mm [12]. In addition, the SC-PBR was composed of two columns with working volumes of 0.5 and 2 L. Columns were connected with opaque silicon tubes to enable circulation of culture broth from the larger column to the smaller column (Fig. 1). The tops of the BC-PBRs and SC-PBRs were plugged with silicon stoppers that each had a medium inlet, gas outlet, and sampling port. The gas inlet was positioned at the end of the tapered bottom and 5% CO_2 enriched air was supplied to each column at 0.2 vvm (Fig. 1A).

The cell concentration and size distribution were measured using a Coulter Counter (Model Z2; Beckman Coulter, Hialeah, FL, USA). The astaxanthin concentration was measured using a spectrophotometer (Hewlett-Packard, Waldbronn, Germany) after extraction with acetone and centrifugation at 450 ×*g* for 10 min using a predetermined calibration curve of synthetic astaxanthin (Sigma-Aldrich, St. Louis, MO, USA) [17]. For illumination of PBRs, 55 W fluorescent lamps were used and light intensities were adjusted by the number and distance of the lamps from the PBRs. The light intensities were measured using a quantum sensor (LI-COR Inc., Lincoln, NE, USA). Light was only supplied to the smaller column of the SC-PBR at 150, 300, and 815 μE m^{-2} s^{-1}, whereas the larger column was wrapped with aluminum foil to block ambient light (Table 1).

H. lacustris was cultivated in SC-PBRs under different light conditions: (i) half light intensity, (ii) same light intensity,

Table 1. Operating conditions for astaxanthin production from *Haematococcus lacustris* using split-column photobioreactors.

Experimental run	Culture vessel	Culture Vol. (L)	Light condition (μE m^{-2} s^{-1})	Daily supplied photons (E/day)	Notes
BC	Bubble-column PBR	2.5	300	2.67	Control culture
SC1	Split-column PBR	2 (L):0.5 (S)[a]	0 (L):150 (S)	0.49	Supplied half of the light intensity of the control only to the smaller column of the SC-PBR
SC2	Split-column PBR	2 (L):0.5 (S)	0 (L):300 (S)	0.98	Supplied the same light intensity as the control only to the smaller column of the SC-PBR
SC3	Split-column PBR	2 (L):0.5 (S)	0 (L):815 (S)	2.67	Supplied the same amount of photons as the control only to the smaller column of the SC-PBR

[a]"L" and "S" stand for the larger column (2 L) and the smaller column (0.5 L) in a spilt-column photobioreactor, respectively.

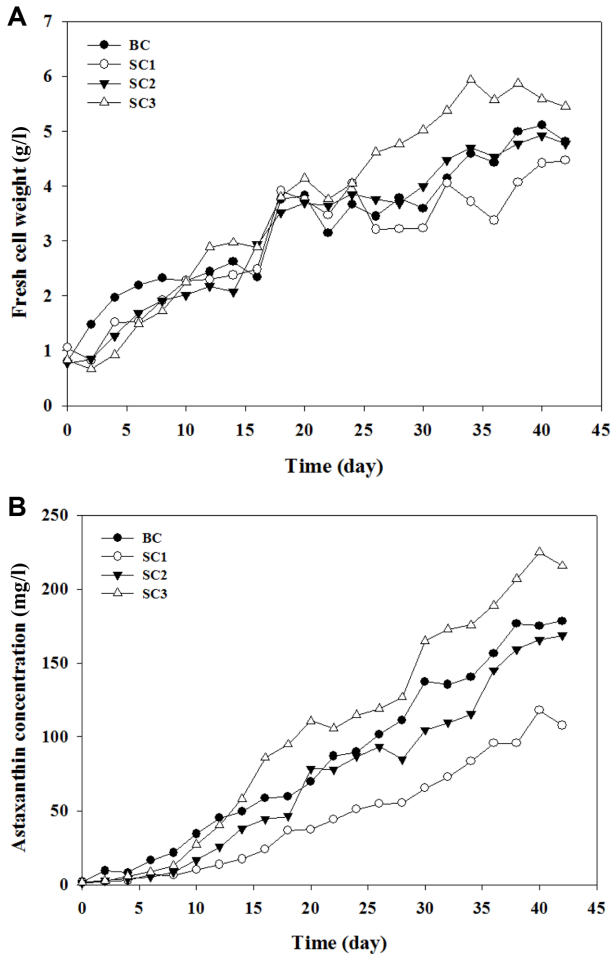

Fig. 2. Time profiles of (**A**) fresh cell weight (g/l) and (**B**) astaxanthin concentration (mg/l) in bubble-column and split-column photobioreactors.

Bubble-column photobioreactor under 300 μE m^{-2} s^{-1} of illumination as a control (●; BC); split-column photobioreactor under 150 μE m^{-2} s^{-1} of illumination only in the smaller column (○; SC1); split-column photobioreactor under 300 μE m^{-2} s^{-1} of illumination only in the smaller column (▼; SC2); split-column photobioreactor under 815 μE m^{-2} s^{-1} of illumination, with the same number of photons as BC, only in the smaller column (△; SC3).

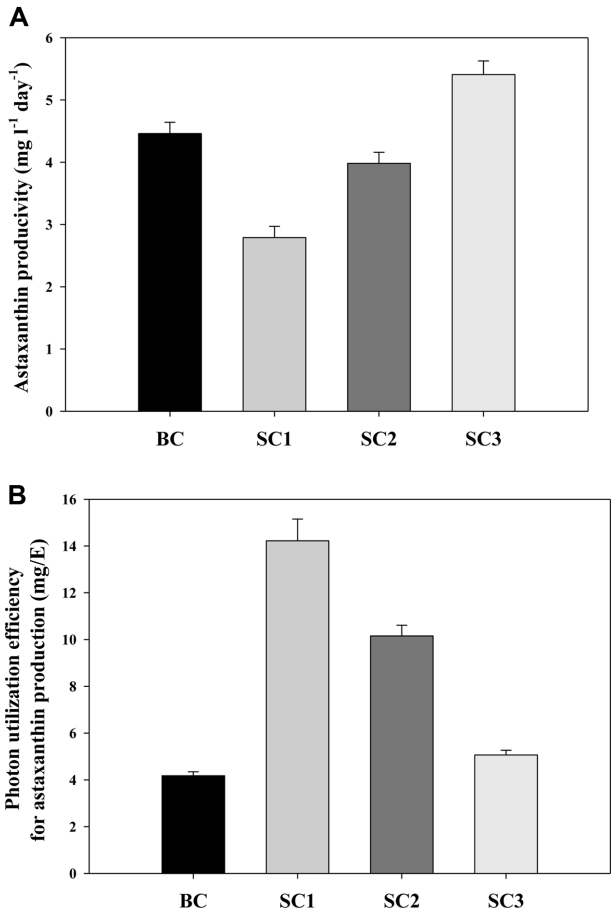

Fig. 3. Comparisons of (**A**) astaxanthin productivity (mg l^{-1} day^{-1}) and (**B**) photon utilization efficiency for astaxanthin production (mg/E) between a bubble-column photobioreactor and split-column photobioreactors.

Astaxanthin productivities and photon utilization efficiencies were calculated based on astaxanthin concentration at day 40 and day 42. Photon utilization efficiency for astaxanthin production was calculated by the following equation: photon utilization efficiency for astaxanthin production = (astaxanthin concentration (mg/l) × culture volume (L)) / (daily supplied photons (E/day) × culture time (day)).

and (iii) same number of photons as the control (Table 1). Fig. 2 shows time profiles of fresh cell weight and astaxanthin concentration of the BC-PBR and SC-PBRs. The cell weight of BC-PBR increased rapidly until day 6, whereas those of the SC-PBRs showed gradual increases because of different light availabilities. However, after 26 days of cultivation, the profiles of cell weights in the SC-PBRs differed distinctively based on light intensity (Fig. 2A). As shown in Fig. 2B, the patterns in astaxanthin accumulation in the SC-PBRs and control differed. While the astaxanthin concentration in the control increased at a steady rate throughout the culture period, the astaxanthin accumulation rates in the SC-PBRs accelerated during the later period (after day 24). These results indicate that photons can be effectively utilized for astaxanthin production in SC-PBR systems by offering an appropriate light regime despite the increased cell density as the culture progressed. In general, light penetration depth in algal culture generally decreases as the culture becomes dense because of mutual shading and light absorbance by cells [16]. Indeed, it was shown

that the light penetration depth in the astaxanthin-producing cultures was much shorter than that in vegetative growth cultures with the same number of cells because of differences in their cell sizes and the change of the dominant pigment from chlorophyll to astaxanthin [18]. Thus, light stress to individual cells would be diminished, and this light regime would be neither effective nor sufficient for inducing carotenoid accumulation. Because the cultures for astaxanthin production are likely to operate with higher initial cell concentration, SC-PBRs could be more efficient at photon utilization for astaxanthin induction. In contrast to the BC-PBR, cells would be subjected to relatively high light stress when passing through the narrow column in a SC-PBR, even at high cell density.

As shown in Fig. 3A, the astaxanthin productivity (5.35 mg l^{-1} day^{-1}) of the culture of SC-PBR (SC3 in Table 1) was 28% higher than that of the control (4.17 mg l^{-1} day^{-1}), even though both reactors were supplied with the same number of photons. It is also worth noting that the productivity of SC-PBR (SC2 in Table 1), which was provided with the same light intensity as the control, showed a similar value (3.97 mg l^{-1} day^{-1}) to the control even though it was only supplied with 37% of the number of photons supplied to the PBR. Moreover, photon yields from the SC-PBRs were 28–366% higher than those of the control (Fig. 3B). Thus, astaxanthin production using SC-PBRs rather than the BC-PBR might have greater photon efficiency and astaxanthin productivity.

In the present study, SC-PBRs were examined to improve astaxanthin productivity by improving the photon utilization efficiency. The performance of the SC-PBRs showed potential for enhanced production of astaxanthin by providing an effective light regime to the PBR. However, the ratio of column sizes, light regime, and cultivation mode still need to be optimized to further improve astaxanthin productivity using the SC-PBR.

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