

High-Density Algal Photobioreactors Using Light-Emitting Diodes

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Lack of high-density algal photobioreactors (PBR) has been a limitation in exploiting the biotechnological potential of algae. Recent developments of highly efficient light-emitting diodes (LED using gallium aluminum arsenide chips) have made the development of a small LED-based PBR possible. We have calculated theoretical values of gas mass transfer requirements and light-intensity requirements to support high-density algal cultures for the 680 nm monochromatic red light from LED as a light source. A prototype PBR has been designed based on these calculations. A cell concentration of more than 2×10^9 cells/mL (more than 6.6% v/v), cell doubling times as low as 12 h, and an oxygen production rate as high as 10 mmol oxygen/L culture/h were achieved using on-line ultrafiltration to periodically provide fresh medium. © 1994 John Wiley & Sons, Inc.

Key words: *Chlorella vulgaris* • light-emitting diodes (LED) • oxygen production • photobioreactor (PBR)

INTRODUCTION

Algal biotechnology is drawing increasing interest due to its potential as a source of valuable pharmaceuticals, pigments, carbohydrates, and other fine chemicals.^{4,7,9,13,39} Currently, its application is being extended to the areas of waste water treatment^{6,10,14} and agriculture.²³ Further, the ability to regenerate oxygen from carbon dioxide is key to the design of an efficiently controlled ecological life-support system.^{21,31} However, lack of suitable photobioreactors (PBR) makes the cost of algally-derived compounds higher than those derived by chemical synthesis,^{3,37} and thus has prevented widespread use of algal cultures.

Important factors in achieving high-density productive algal culture are light, gas exchange, medium supply, and biological limitations.^{17,34,35} To effectively exploit the commercial potential of algae, a cheap, durable, reliable, and highly efficient light source is needed. If the light source has a narrow spectral output that overlaps the photosynthetic absorption spectrum, the emission of light at unusable frequencies would be eliminated and therefore the overall energy conversion improved. One light source that meets these criteria is a light-emitting diode (LED).³⁸ LED are light and small enough to fit into virtually any PBR, they have a very long life-expectancy, and their electrical efficiency is so high that heat generation is minimized. LED

have a half-power band-width of 20–30 nm which can be matched with photosynthetic needs. However, the light intensity of conventional LED is too low to be used in PBR. Recently, double-power double-heterostructure (DDH) gallium aluminum arsenide (GaAlAs) chips which can emit light at much higher intensity and efficiency have been developed.⁴⁰ These DDH GaAlAs LED arrays can emit photon fluxes as high as $900 \mu\text{mol}/\text{m}^2/\text{sec}$,¹ which is sufficient to support photosynthesis in many C_3 plants.⁴⁶

For the past decade, it has been shown that high-density photoautotrophic algal cultures can be established. A number of PBR have been introduced to obtain productive high-density photosynthetic cultures. Most of them were thin panel or tubular PBR,^{22,26,33,36,42,44} but modified stirred-tank^{43,48} or hollow-fiber PBR⁴⁷ were also tested. New-concept PBR with internal illuminations through optical fibers^{5,15,24,27,41} have also been employed. The key design challenges were to maximize surface-to-volume ratio, to provide a more efficient light source, to deliver the light more efficiently to the culture, and to provide a more effective mass transfer of gases. Direct comparison of the performance of these PBR is difficult, but the general performance characteristics can be enumerated. The maximum biomass concentrations achieved were normally in the range of 5–6 g/L.^{22,27,42,43,44,48} Some PBR were able to obtain higher cell concentrations than 10 g/L,^{15,41} with the highest reported cell concentration being 20 g/L.³³ Few studies have reported the oxygen production rate. All the PBR reported used full-spectrum light source, such as sunlight or metal halide lamps. Here we report that red LED can be used in PBR to obtain high cell densities and high oxygen production rates.

PHOTOBIOREACTOR DESIGN CONSIDERATION

The major factors that must be considered in the design of high-density PBR are light delivery and distribution, gas transfer into and out of the reactor, maintenance of medium components in balance, and prevention of accumulation of potentially detrimental secondary metabolites.

Goals

The goal is to achieve maximal biomass production rates using LED. The highest cell density previously obtained in

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our laboratory was 1×10^9 cells/mL ($\approx 3\%$ v/v)¹⁵ by using a 1,000-W xenon lamp as the light source. The oxygen production rate of this PBR system was between 4–6 mmol/L culture/h. The goal is to achieve equal or higher culture density and oxygen production rate using red LED as compared to a full-spectrum light source.

Estimation of the Specific Oxygen Production Rate (SOPR)

Each mol of chlorophyll is reported to produce 50–400 mol of oxygen per h,²⁹ and the per-cell average chlorophyll content is in the range of 0.5–1.0 fmol.²⁵ The product of these numbers will yield a range of expected per-cell oxygen productivity; 25–400 fmol O₂/cell/h.

A simple experiment with a small, illuminated cuvette with a dissolved oxygen (DO) electrode was employed to verify this range. The measured SOPR fell within the estimated range. The SOPR was between 5–200 fmol/cell/h, decreasing with increasing cell concentration as expected due to mutual shading (Table I). Since the measured SOPR was inversely proportional to the cell concentration, the design challenge is to deliver and distribute the light efficiently to minimize the effect of mutual shading. The SOPR will have to be high at high-cell densities if the design of a PBR is to be optimized for oxygen production. A target in situ SOPR is 100 fmol of oxygen per cell per h at a cell concentration of $1 \cdot 10^9$ cells/mL.

The Light Requirement

Enough light to produce 100 fmol of oxygen/cell/h has to be provided. According to the Z-scheme photosynthesis,¹² 8 mol of photons are required to generate 1 mol of oxygen. One mol of 680 nm photons per h is equivalent to about 50 W. As a result, each cell needs to experience 40 pW equivalent of photons to achieve the targeted SOPR of 100 fmol/cell/h.

The desired cell density and the specific illuminated surface area are then used to compute the required light flux. For the 10^9 cells/mL culture and 1.0 cm² illuminated area

per cm³ culture, one needs to provide 40 mW/cm² or more because the maximum photosynthetic efficiency of *Chlorella* has been reported to be in the range of 32% to 54%.^{20,28,32} A closely packed LED array (90 LED on a 2" × 4" printed circuit board, spaced about 0.76 cm apart) could provide a light intensity as high as 25 mW/cm² on each illuminating surface of the PBR.

Light Delivery and Distribution

In addition to the light source, the light delivery and the light distribution inside a PBR are key factors in PBR design. Light should be delivered so that photon loss is minimized, heat generation by the light source eliminated, and potentially harmful wavelengths filtered. By using LED, all of these problems are circumvented. The best way to minimize photon loss is to have internal illumination. The small volume characteristics of LED makes this possible. Furthermore, their high efficiency and sharp spectrum eliminate the need for a cooling system and filter. Therefore, LED represent perhaps the optimal light source for PBR.

Good light distribution will maximize the overall light utilization by minimizing mutual shading as well as photo-inhibition. It should be noted that any light not absorbed or not used in photosynthesis will be converted into thermal energy.

Gas Transfer

Gas transfer is an important issue in PBR design; carbon dioxide needs to be supplied and oxygen generated removed. Carbon dioxide is the carbon source utilized for photosynthesis.³⁰ Depending on the type of species, CO₂ can be absorbed in dissociated (HCO₃⁻ or CO₃⁻²) or undissociated form (CO₂ or H₂CO₃).² In either case, carbon dioxide needs to be dissolved into the culture broth. Oxygen is a product of photosynthesis as well as an inhibitor of algal growth. Thus, the produced oxygen needs to be removed to prevent oxygen from reaching inhibitory level.

The target of SOPR is 100 fmol O₂/cell/h. For 10^9 cells/mL, 100 mmol oxygen needs to be removed per L of culture

Table I. Specific oxygen production rate in units of fmol/cell/h at various light intensities and cell concentrations

		Cell concentration (cells/mL)					
		1.3×10^6	1.5×10^7	2.9×10^7	8.5×10^7	2.5×10^8	4.5×10^8
Approx. relative light intensity	0.17						0.7
	0.25						9.2
	0.42	209					15.7
	0.50		63		7.7	18.1	8.0
	0.67		131				
	0.83	188		127	8.3	29.0	10.2
	1.00		148		9.1		11.2
	FL		81				

Light intensities are expressed as relative intensities to the maximum (the intensity of 2.1 V/device = 1). A 22 W cool-white circline fluorescent light (General Electric Co.), or FL, was used as a full-spectrum control.

per h. To achieve this rate of oxygen transfer, the volumetric mass transfer coefficient ($k_L a$) needs to be larger than 80 h^{-1} . However, SOPR may be reduced as the cell concentration increases due to the light limitation in a high-density culture.

One way to remove 100 mmol of oxygen/L culture/h is by internal sparging. The overall mass transfer rates by sparging were calculated for two different bubble sizes (1 mm and 3 mm in diameter).¹⁸ Both could give the required oxygen transfer ($k_L a$ of about 200 h^{-1} and 100 h^{-1} , respectively, at the aeration rate of 20 m/h). The results from the above calculations, as well as those for typical bioreactors,⁸ are summarized in Table II.

Mixing

Good fluid mixing is important for achieving high cell concentration in a PBR. Good mixing can keep the cells in suspension, eliminate thermal stratification, help nutrient distribution, and improve gas exchange. Furthermore, mixing can reduce the degree of mutual shading and lower the probability of photoinhibition. The last reason is perhaps the most important one for requiring the right extent of mixing to achieve a successful PBR. Proper mixing in the direction perpendicular to flow (parallel to light path) will significantly reduce the mutual shading and thus increase the portion of the cells exposed to the light. This mixing will also move the cells close to the illuminated surface into the center of the PBR, which will give these photon-saturated cells the opportunity to use up all the absorbed light energy for photosynthesis before they are exposed to the light again. This mixing, in turn, will also provide the same opportunity to the cells in the center (that already used up the absorbed photons) to "see" the light. As a result, a proper mixing may significantly improve overall light-utilization efficiency. Sparging will improve overall light-utilization efficiency in two ways: (1) existence of air bubbles will increase light penetration depth, and (2) rising motion of bubbles will induce some degree of mixing tangential to the flow direction.

On-Line Supply of Medium Components

Yet another important parameter in PBR design is to keep all medium components in balance with growth and photosynthetic requirements. Discussion of this topic can be

Table II. Overall mass transfer coefficient under various conditions

System configuration	$k_L a$ (1/h)
Stagnant water (specific surface area of $1 \text{ cm}^2/\text{cm}^3$)	7.56
Prototype PBR (20m/h aeration, 3mm diam. bubble)	110
Prototype PBR (20m/h aeration, 1 mm diam. bubble)	200
Bubble fermentor (no agitation, 20 m/h aeration)	10–30
Conical flasks (shaking at 200 rpm, no aeration) ^a	25–100
CSTR (500 rpm, 20 m/h aeration) ^a	325
CSTR (750 rpm, 20 m/h aeration) ^a	1,000
CSTR (1,680 rpm, 20m/h aeration) ^a	2,650

^aData from ref. 8.

found elsewhere.¹⁶ The on-line replenishment of medium (supply of medium components as well as removal of any potentially harmful secondary metabolites) was achieved either by dialysis or ultrafiltration using Cell-Pharm model BR170 (molecular weight cut-off of 70 kDa, UniSyn Technologies, San Diego, CA). The difference between the two methods is the application of external force for ultrafiltration; the dialysis mode supplies the medium components purely by diffusion.

Ultrafiltration was preferred because it was faster and thus disturbed gas measurement less. By applying vacuum (about 400 mmHg) to the shell side of the ultrafiltration cartridge, replenishment of 200 mL of medium (about three times the volume of medium in the PBR system used) could be achieved in 10 min.

PHOTOBIOREACTOR DESIGN AND CONSTRUCTION

LED were chosen as a light source for all the reasons stated above, such as high electrical energy conversion efficiency, sufficient light intensity, and small volume and weight characteristics (which will make the most efficient internal illumination possible). For the required mass transfer rate, internal sparging was employed.

Black polycarbonate plate (Ain Plastics, Inc., Mount Vernon, NY) and black noreprene tubing (Cole-Parmer Instrument Co., Niles, IL) were chosen as materials used for PBR construction and the tubing material because of their biocompatibility, chemical and mechanical properties, opaqueness, oxygen impermeability, and autoclavability.

From a series of simple preliminary experiments with various geometries, a PBR with a vertical rectangular slab-shaped illumination chamber was found to be suitable. This shape meets all the stated criteria, such as good light distribution with the smallest possible dark volume, proper mixing without damaging cells by shear stress, and minimization of wall growth.

The volumes of the illumination chamber were either 80 cm^3 or 52 cm^3 , depending on the depth of culture (1.55 cm and 1.00 cm, respectively). Both illumination chambers had the same illumination area of about 100 cm^2 (8 in², sum of the two sides). Each illumination chamber had two LED units on each side which consisted of either 24 LED for low-intensity light delivery or 90 LED for high-intensity light delivery. The two LED units were mounted in such a way that the LED on the different sides of the illumination chamber were alternated to have better light distribution inside the illumination chamber. The power to the LED units was increased as the cell concentration increased with time.

Mass transfer was achieved via internal sparging through four 3-mm diameter nozzles (a half-inch apart from each other) located at the bottom of the illumination chamber. At a gas flow rate of 100 mL/min with a liquid circulation rate of 100 mL/min, calculated superficial velocities were 0.42 cm/sec and 0.67 cm/sec, depending on the thickness of the illumination chamber.

The resulting PBR had a gas/liquid phase separation module allowing for gas exit (Fig. 1). Introducing sparging added some complexity to the system because the gas analyzer used had to have a hermetically sealed gas phase to measure the gas consumption/production rate accurately (see Materials and Methods section).

MATERIALS AND METHODS

Cell Line and Culture Medium

C. vulgaris (UTEX 398, recently renamed as *C. kessleri*¹⁹) was obtained from The Culture Collection of Algae at UTEX (Austin, TX) on proteose agar. The N-8 culture medium was used without modification.⁴⁵

LED, Light Measurements, and Power Supplies

Red DDH GaAlAs LED were obtained from Quantum Devices Inc. (Barneveld, WI). These LED have narrow spectral outputs that peak at wavelengths of 680 nm. The LED were powered by simple DC power supplies (GP-105, GoldStar Precision, Cerritos, CA) with constant voltage between 1.7 V and 2.1 V per device, except one point where 2.4 V were applied.

The light intensity of the LED units was measured by a silicon photo cell (Model 0560.0500, Testoterm GmbH & Co., Germany). The light intensities of a high-intensity LED unit were about 3 and 12 mW/cm² when powered at 1.7 and 2.1 V, respectively.

Culture Conditions

Two different types of LED units were constructed: a low-intensity unit with 24 LED in a 2" × 4" printed circuit board and a high-intensity one (a closely mounted LED array which had 90 LED in the same PCB, about 0.76 cm apart). The maximum intensity achieved by two high-intensity units (one on each side of the PBR) was 50 mW/cm² by providing a power of 2.4 V per LED. However, the PBR were normally powered at 2.1 V/device to give a light in-

tensity inside the PBR of 23 mW/cm². All the experiments were performed at a constant temperature of 25°C and initial pH of 6.2, and were done batch-wise with N-8 medium. A peristaltic pump was used to circulate the culture medium and to keep the cells in suspension. Premixed gas with the composition of 15% O₂, 5% CO₂, and balanced with N₂ was introduced through the gas analyzer and refreshed whenever either O₂ or CO₂ concentration had changed more than 0.5% from the premixed concentration.

The seed culture was prepared by 500-mL flasks with 150-mL working volume using an LED unit with 65 of 680 nm LED. The flask was doubly wrapped with extra-heavy-duty aluminum foil to block the ambient lights.

Cell Analysis

Samples were prepared by diluting the cell culture broth with the appropriate amount of isotonic diluent (Fisher Scientific, Pittsburgh, PA). The cell concentration was determined by a microprocessor-controlled electronic particle counter, Coulter Counter model ZM with a Channelizer model C256 (both from Coulter Electronics, Inc., Hialeah, FL) as well as by occasional confirming counts using a hemacytometer. Since the average cell size was highly variable (about 100 fL/cell or μm³/cell while the cells are actively growing and about 30 fL/cell at stationary phase), the total cell volume [= cell concentration (cells/mL) × average cell volume (fL/cell)] was used to calculate the volumetric specific growth rate and thus the doubling time.

Gas Composition Measurement

The gas concentrations were measured by a Micro-Oxymax gas analyzer (Columbus Instruments, Columbus, OH). This system was chosen due to its high sensitivity to detect the small gas composition change by 100 mL of algal culture. To obtain the desirable sensitivity, a slightly pressured closed system in which the air in the gas phase of a test chamber is pumped through the gas sensors and then returned to the test chamber, was necessary. The O₂ and CO₂ gas concentrations in the air in the test chamber were measured periodically, and the changes in the concentrations as well as the volumes of the algal culture and gaseous phase were used to compute gas consumption or production rates. Whenever the concentration of either gas in the closed gas loop deviated more than ±0.5%, the gas phase was refreshed from a gas cylinder with the premixed gas. A reference chamber containing a reference supply of air was used to recalibrate the sensors prior to each measurement. The oxygen sensor operated as an oxygen battery (fuel cell) and the carbon dioxide sensor was a single-beam nondispersive infrared device.

RESULTS AND DISCUSSION

Performance of the Prototype PBR

A schematic diagram of prototype PBR with internal sparging with gas/liquid separator is shown in Figure 1. A typical

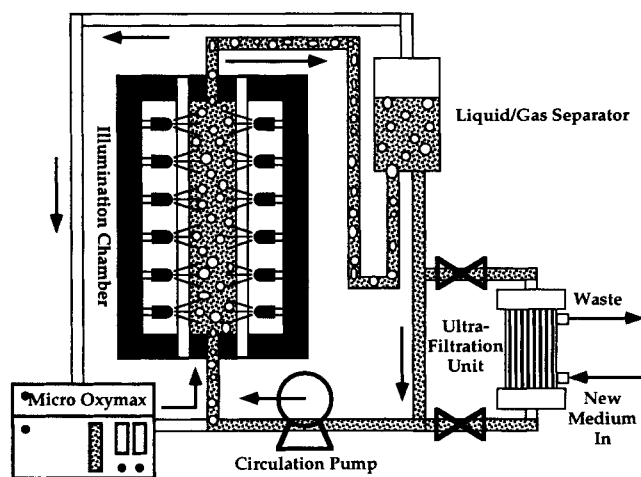


Figure 1. Schematic diagram of the LED-based prototype PBR that uses internal sparging and on-line ultrafiltration.

growth curve in this PBR with low light-intensity units (a 24-LED unit on each side, 1.55 cm apart each other) is shown in Figure 2 (closed symbols). Ultrafiltration (UF) was used to semicontinuously feed the nutrients without removing cells from the PBR system. The first ultrafiltration was performed at 92.3 h and the average cell size immediately increased. This means that cells actively divided. UF was performed every other day starting at 115.5 h, where the average cell size started to increase again. The successive UF did not show the same visible effect as the first two. However, it was necessary to keep the cells alive. The volume of fresh medium used by each UF was 2- to 4-fold of the total PBR volume, depending on cell concentration. The specific growth rate in the logarithmic phase was 0.035 h^{-1} (a doubling time of 20 h), resulting in a maximum cell density at the end of the operation of more than 2% (v/v). However, it took over 350 h to increase the cell concentration from $1 \cdot 10^8$ (at 140 h) to $8 \cdot 10^8$ (closed symbols). This growth was extremely slow because a 100-fold increase in cell number could be achieved in about 100 h following the initial lag phase.

Monitoring the oxygen production rate was challenging. The gas analyzer required a hermetically sealed loop to measure the gas composition, and on-line ultrafiltration was required to support cell concentration greater than $1 \cdot 10^8$ cells/mL. Even though the setup was carefully designed to measure gas concentration, the gas reading (oxygen production rate per L culture, OPR/L) fluctuated whenever the gas loop was disturbed by cell sampling, UF, or to refresh the gases in the hermetically sealed gas loop. The highest oxygen production rate achieved in this configuration (the PBR with low-intensity LED units and 1.55-cm light path) was about 2 mmol/L culture/h at 150 h (dotted line in Fig. 3). The second peak in OPR/L at 330 h was a result of increasing the power to the LED units to 2.4 V. By applying this voltage, the light intensity could be increased about

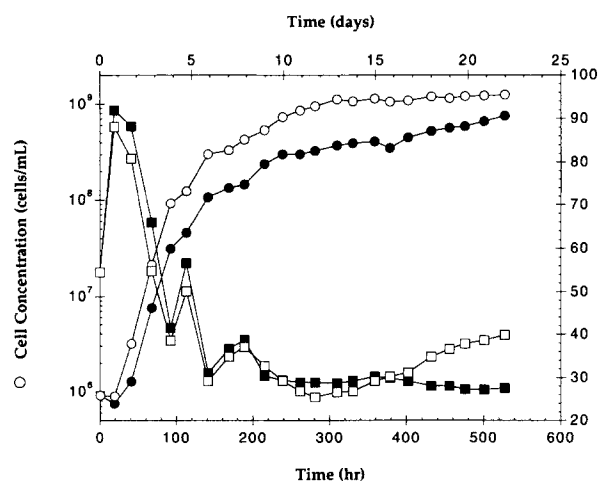


Figure 2. The growth curves (○ and ●, cells/mL) and corresponding average cell sizes (□ and ■, fL/cell) in the two different light intensities. Closed symbols for the low-intensity (48 LED/chamber or 24 LED/illuminating surface) and open markers for high-density LED units (180 LED/chamber = 90 LED each on both sides).

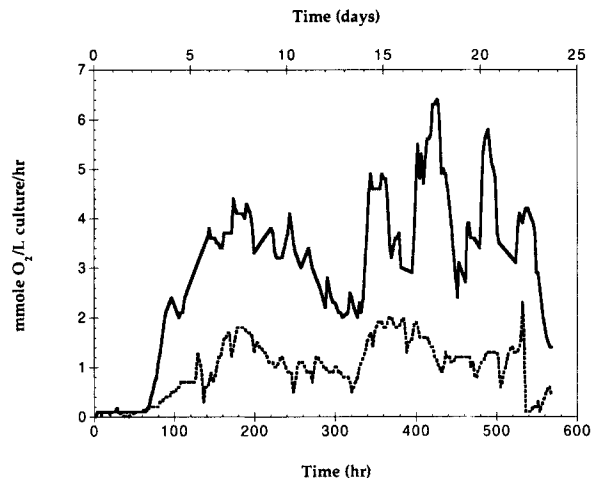


Figure 3. Oxygen production comparison between low-intensity (----) and high-intensity (—) PBR from the same conditions as in Figure 2.

100% (more than 40 mW/cm^2). However, this voltage was not within the normal operating range of the LED used. Therefore at 2.4 V, the LED generated a significant amount of heat and finally some of the individual LED were burned out. All the later experiments used 2.1 V as the maximum power.

These results implied that this prototype PBR was operating in a light-limiting environment. High efficiency in light absorption of algae resulted in uneven accessibility to the incident light or mutual shading. Perfect mixing would overcome this limitation. A high degree of mixing generally introduces a high degree of shear stress, to which algae are sensitive.¹¹ Another way to address the light-limitation was to deliver more photons to overcome mutual shading. High-intensity LED assemblies were constructed by increasing the number of LED per unit (to 90 LED/unit) rather than increasing the power supplied to each unit.

Modifications to Improve the Performance of the PBR

The original low-intensity LED units were replaced by two high-intensity units 1.55 cm apart from each other. The expected change in cell growth was obtained (open symbols in Fig. 2). The higher number of LED could support a cell concentration of $1.2 \cdot 10^9$ cells/mL ($\approx 5\%$ v/v) and a specific growth rate during the exponential phase of 0.046 h^{-1} (a doubling time of 15 h). The 2.5-fold difference in the total biomass (% v/v) between a low-intensity PBR and a high-intensity one was consistently observed in several independent experiments. The difference in the total biomass was smaller than the difference in the light intensity, which was 3.75-fold. Again, once the culture entered the stationary phase, the growth rate dropped significantly and the culture was able to grow to very high cell concentrations only if a series of UF were performed.

The peak oxygen production rate at the end of the logarithmic phase (170 h) was about 4.5 mmol/L culture/h (solid

line in Fig. 3). A higher rate of up to 6 mmol/L culture/h could be obtained by the higher voltage (2.4 V; see previous section) at 430 h. This was a 3-fold increase over the performance obtained by using the original lower-intensity LED units in the PBR. The ratio of improvement was smaller than the ratio of light intensity, suggesting that even at the higher light intensity the PBR eventually became light-limited.

A final design modification was to reduce the cross-section of the PBR to allow the light to penetrate the entire culture volume inside the PBR. The depth of the culture (or the distance between the two illuminating surfaces) was 1.55 cm in all the PBR mentioned before. A PBR with a shorter light path of 1.00 cm and two high-intensity LED units was constructed. Figure 4 shows the growth data from an experiment with the new PBR (open symbols) and a PBR with original thickness with high density (closed symbols). A specific volumetric growth rate of 0.058 h^{-1} (a doubling time of 12 h) in exponential phase and a final cell concentration greater than $2 \times 10^9 \text{ cells/mL}$ (more than 6.6% v/v) were obtained in the PBR with the reduced depth. By reducing the light path by 35%, there was a 2-fold increase in the maximal cell density, and the exponential growth rate was increased by 20%. Thus, a comparable cell concentration to the highest algal cell concentration reported³³ was achieved by monochromatic red lights from LED.

A similar improvement in OPR/L was obtained (solid line in Fig. 5), whose peak production rate was 10 mmol/L culture/h at 105 h. The SOPR at this point was less than 20 fmol/cell/h at a cell density of about $5 \cdot 10^8 \text{ cells/mL}$. This result re-emphasizes the need to distribute the light more evenly to individual cells. Better light distribution could be obtained by either (1) achieving a higher degree of fluid mixing without damaging the cells, or (2) reducing the culture depth.

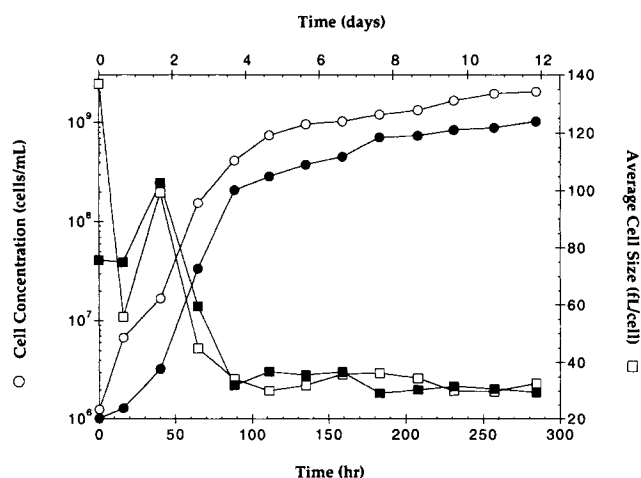


Figure 4. The cell concentration (\circ and \bullet , cells/mL) and the average cell size (\square and \blacksquare , fL/cell) of the original PBR (closed symbols, thickness of the illumination chamber: 1.55 cm) and PBR with thinner illumination chamber (open symbols, thickness: 1.0 cm).

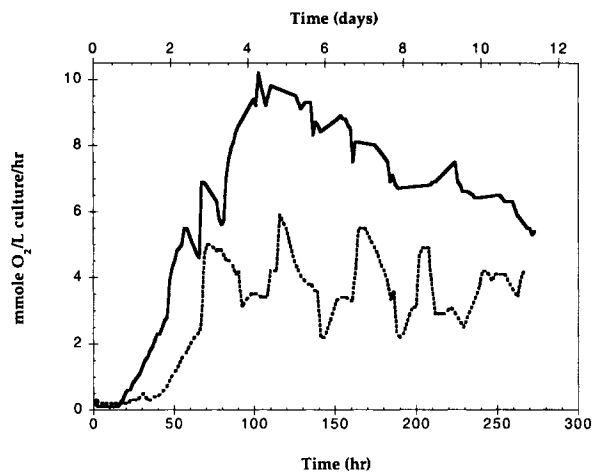


Figure 5. The oxygen production rates in a thicker PBR (---) and a thinner PBR (—), both with high light intensity (180 LED/PBR). Conditions the same as in Figure 4.

CONCLUSION

LED-based photobioreactor systems were designed, constructed, and tested to achieve high-density photoautotrophic algal cultures. These PBR used LED emitting at 680 nm as the sole light source. The LED units were located internally for direct illumination and could emit the photosynthetic photon flux of up to 50 mW/cm^2 . By introducing (1) internal sparging (which improves overall light utilization), (2) on-line ultrafiltration (supplying medium components semicontinuously while removing any secondary metabolites), (3) higher light intensity (increasing the number of LED per illumination area), and (4) a shorter light path, both the highest cell concentration (more than $2 \times 10^9 \text{ cells/mL}$) and the highest total cell volume fraction (more than 6.6% v/v) were achieved. By modifying these four factors, the oxygen production rate in the final PBR was increased almost 5-fold from the initial PBR, giving the maximal oxygen production rate of 10 mmol oxygen/L culture/h. Further challenges are to improve light distribution and continuously perfuse fresh medium of the proper composition to reach the stated goal of a SOPR of 100 fmol/cell/h at a cell concentration of $1 \cdot 10^9 \text{ cells/mL}$.

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