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BIOLOGICAL DETERMINANTS OF PHOTOBIOREACTOR DESIGN

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Abstract:

A novel photobioreactor (PBR) system using light-emitting diodes (LEDs) as a sole light source was constructed and operated with continuous medium perfusion. Direct internal illumination by 680 nm LEDs could deliver as high as 50 mW/cm² of light into the culture medium. Gas transfer by internal sparging had the capacity to transfer 250 mmol O₂/L culture/h. *Nutritional limitations could be overcome by continuous perfusion, supplying the medium components to the culture without increasing osmolarity, while removing potentially inhibitory cellular wastes.* When the PBR operated in a continuous perfusion mode with a perfusion rate of 6 reactor volumes a day (6 VVD), it could support ultra high-density algal cultures up to cell concentrations of 4·10⁹ cells/mL and total cell volume fractions of 9.4% v/v (about 25 g dry weight/L). The oxygen production rate at its peak was 13 to 15 mmol/L culture/h. This performance represents the highest reported cell densities attained in photoautotrophic cultures. Continuous perfusion allowed for long-term stable oxygen production, while oxygen production in batch mode ceased when stationary phase was reached. The results presented suggest that PBR technology can still be significantly improved.

NUTRIENT LIMITATIONS AND METABOLITES SECRETION AT HIGH CELL DENSITIES

Introduction

The history of the commercial use of algal cultures spans about 50 years with applications to waste-water treatment, production of biomass, and production of specialty chemicals. Interest has developed in using algae to fix carbon dioxide to produce oxygen and biomass for life-support in space, and for reducing CO₂ emissions from power plants. Unlike the rapid and significant advances in the biotechnological use of bacteria, yeast, and mammalian cells, algal biotechnology and the development of photoautotrophic cultures has progressed slowly in spite of their recognized utility. Perhaps the most restraining factor is the unavailability of efficient large scale cultivation techniques and the underdeveloped stage of photobioreactor (PBR) technology.

Large-scale open algal cultures, such as lined and unlined ponds, typically result in low cell densities of 0.01 to 0.06% w/v, resulting in expensive harvesting procedures and unfavorable economics in addition to difficulties with species control. High-density photoautotrophic algal cultures were exclusively established by enclosed forms of PBRs such as tubular air-lift types. Most of the PBRs described were able to support the algal cultures up to 5 to 6 g (dry cell weight)/L. A few PBRs were more successful by constructing special lighting systems (10-17 g/L).

The efficient design of bioreactor systems relies on the important principle of balancing the physical rate processes with the biological dynamics that take place. In PBRs the physical rate processes are light delivery, gas mass transfer, and fluid flow and mixing. The biological rate processes are metabolism, photosynthesis, growth and the effects of secondary metabolism. We have reported the performance of a high-density LED-based PBR. The next step is to outline the rates at which nutrients have to be supplied and the waste products removed in order to maintain a viable high density culture in stationary phase.

Materials and Methods

Strain

Chlorella kessleri (UTEX 398, formerly known as *C. vulgaris*) obtained from The Culture Collection of Algae at UTEX (Austin, TX) were cultured in N-8 medium (initial pH of 6.2). The seed culture was prepared by 250 mL flasks with 100 mL working

volume in an illuminated shake incubator (Model G-27, New Brunswick Scientific Co. Inc., Edison, NJ). Inoculum density to the PBRs was about $1 \cdot 10^6$ cell/mL.

Culture Conditions

On-line replenishment of medium was performed by perfusion using hollow fiber cartridges (model BR170, UniSyn Technologies, San Diego, CA). Perfusion rates used were 0.15 mL/min and 0.30 mL/min (3 and 6 times the working volume a day, respectively). Two other PBRs were run parallel to compare the performance increase by continuous perfusion; a PBR with batch mode and another PBR with off-line medium exchange after the cell concentration reached $4 \cdot 10^7$ cell/mL (centrifuged down the culture, resuspended to fresh medium, and returned to the PBR). A peristaltic pump recirculated the culture medium at a rate of 100 mL/min to keep the cells in suspension.

Pre-mixed gas with the composition of 10% CO₂ and balanced with N₂ was introduced through the gas analyzer and refreshed whenever either O₂ or CO₂ concentration had changed more than 1% from the pre-mixed concentration. A gas recycle rate of 100 mL/min through the internal sparger was found to be enough for the required gas transfer rate and sparging also helped mixing as bubbles rose in a random motion inside the PBRs.

Measurements of Culture

A gas analyzer with high sensitivity was used to measure the oxygen production rate (OPR). A Micro-Oxymax gas analyzer (Columbus Instruments, Columbus, OH) was connected to the PBR system through a 500 mL gas container and the entire gas phase was hermetically sealed to obtain the desired sensitivity (Fig. 1). The gas was recirculated continuously by a separate peristaltic pump and the gas composition in the head space of each PBR was measured at 1 h intervals using a multiplexer. A longer interval made the gas concentration difference larger between the readings, which enabled the detection of small changes in gas phase composition. However, obtaining high sensitivity by this method inevitably introduced some fluctuation in the gas readings since any changes in pressure or volume of gas phase by sampling and perfusion affected the OPR calculation.

The cell concentration was determined by Coulter Counter model ZM with a Channelizer model C256 (both from Coulter Electronics, Inc., Hialeah, FL) after proper dilution with isotonic diluent (Fisher Scientific, Pittsburgh, PA). Since the average cell size was highly variable (about 100 fL/cell while the cells are actively growing and about 30 fL/cell at stationary phase), the specific volumetric growth rate (based on total cell volume) was used to calculate the doubling time.

The chlorophyll content of the cells was quantified by using a spectrophotometer (UV-160, Shimadzu, Japan) after diluting to proper concentrations following methanol extraction.

Results and Discussion

Chlorella, inoculated at $1 \cdot 10^6$ cells/mL, was grown to $3 \cdot 10^8$ cells/mL (about 1% v/v) in batch mode (○ in Fig. 2). The culture did not grow after this cell concentration was reached. When medium components were supplied by either continuous perfusion or off-line exchange, the same PBR reached a final algal biomass concentration that was an order of magnitude higher than the maximum concentration obtained in batch mode (□, Δ, and ◇ in Fig. 2). The final cell concentrations in a PBR with an off-line medium exchange rate of 1 volume fresh medium per volume reactor per day (1 VVD) was over 7.7% v/v ($2.2 \cdot 10^9$ cells/mL) at 490 h (Fig. 2, denoted by Δ in all Figs.) while the PBR with perfusion rates of 3 VVD supported cell densities up to 8.2% or $2.9 \cdot 10^9$ cells/mL at the end of the run (Fig. 2, represented by □). The highest final cell concentration was achieved in the PBR with the highest medium perfusion rate (6 VVD). At 20 days after the inoculation, more than 9.4% v/v of total biomass or $4 \cdot 10^9$ cells/mL was recorded in the PBR system with 6 VVD perfusion (Fig. 2 denoted by ◇ in all Figs.). The culture seemed to be affected by another limitation other than substrate or biological limitations, since the linear growth rate began to level off after the culture reached 8% v/v (data not shown). Furthermore, all the cultures in the PBRs with *in situ* medium supply reached the cell concentration of $1 \cdot 10^9$ cells/mL (about 3% v/v) in 5 days. From this observation, it was apparent that certain forms of medium supply and waste removal were critical for *Chlorella* to overcome the limiting cell density in batch mode.

Despite the similar growth patterns in all *in situ* medium-supplied PBRs, there was a significant difference in the quality of the cultures. Chlorophyll content per cell revealed that the medium supply rate of 1 VVD could not maintain a healthy cell population (Fig. 3). Average chlorophyll content in the PBR with 1 VVD was about half of the other two perfused PBRs. Medium exchange with concentrated medium (3X) at 443 h to this 1 VVD PBR doubled the per cell chlorophyll content temporarily the next day (from 0.25 fmol/cell to 0.45 fmol/cell). This confirmed that some medium components were depleted after the culture reached $1 \cdot 10^9$ cells/mL. Per cell chlorophyll content in batch mode dropped significantly just after the cell concentration reached the plateau at about 100 h, which suggested that the culture was not viable. The average cell volume during the logarithmic growth was 3-4 times larger than during stationary phase. Therefore the per

cell chlorophyll content varied between the two growth phases but chlorophyll content per biomass was constant throughout the culture.

The results obtained from OPR measurement were consistent with these findings. *Chlorella* in the batch mode PBR stopped producing oxygen after the culture reached $1 \cdot 10^8$ cells/mL (thick dotted line in Fig. 4). No chlorophyll content, no oxygen production, and no oxygen consumption clearly suggested that the algae in the batch mode PBR were not viable after 100 h. OPR from the other PBRs were stable for a long period. The peak OPR of the PBR with a 6 VVD perfusion rate (thick solid line) was over 15 mmol/L culture/h. All the PBRs with medium supply could sustain high OPRs for extended periods. Oxygen production of the PBR with 3 VVD perfusion (thin dotted line) was between 8 and 5 mmol/L culture/hr for 400 hrs and that of the PBR with 6 VVD was between 10 to 6 mmol/L culture/hr for 300 hrs. However, frequent draining and refilling of the culture (to achieve 1 VVD medium exchange by centrifugation followed by resuspension) caused a large fluctuation in the OPR reading (thin solid line). These results suggest that an optimized photobioreactor can produce oxygen stably over extended periods of time.

Taken together, the medium capacity of N-8 for *Chlorella* culture was about $1 \cdot 10^8$ cells/mL. This concentration could be easily confirmed by the stoichiometric calculations based on the chemical composition of *Chlorella* and the amount of each component of N-8 medium. However, previous results on the effects of osmolarity and the possible accumulation of potentially harmful cell wastes give a significant advantage to algal cultures with perfusion or on-line ultrafiltration over the batch mode cultures with highly concentrated medium.

The data presented here demonstrates that PBR technology may be significantly improved. Advances will most likely come through the improved understanding of the underlying biological dynamics. A proper balancing of biological and physical factors will allow high-density algal cultures in enclosed PBRs to be economically feasible for the production of high-value algally-derived products.

Conclusion

The prototype photobioreactor system was operated in a continuous perfusion mode to achieve high-density photoautotrophic algal cultures by continuously supplying medium components while removing cellular metabolic wastes. This PBR was illuminated by GaAlAs LEDs emitting at 680 nm located internally for direct illumination. When the PBR operated in perfusion mode with a perfusion rate of 6 reactor

volumes a day, the LED-based perfusion PBR could support ultra high-density algal cultures up to the cell concentration of $4 \cdot 10^9$ cells/mL and the total cell volume fraction of 9.4% v/v (about 25 g dry weight/L). The highest oxygen production rate was about 13 to 15 mmol/L culture/h.

Figure Legend

- Figure 1. Schematic diagram of the LED-based perfusion PBR. Two peristaltic pumps were used for gas and liquid recirculation at a rate of 100 mL/min each. On-line perfusion as gas phase refreshment was performed as described in the text.
- Figure 2. Comparison of growth curves in PBRs with different medium supply/removal rate. Growth curves are shown in the units of biomass due to highly variable average cell size. The four PBRs used here were: one batch mode PBR ($\bigcirc$); one PBR with off-line medium exchange (whose medium change by centrifuge and resuspension started after the cell concentration reached $4 \cdot 10^7$ cells/mL, Δ); two PBRs with continuous perfusion with rates of 0.15 mL/min (3 times the total reactor volume a day, $\square$) and 0.30 mL/min ($\diamond$).
- Figure 3. The time profiles of chlorophyll content per cell. The symbols used are as in Figure 2: batch ($\bigcirc$), semi-batch with 1 VVD medium exchange (Δ), and continuous perfusion with 3 VVD ($\square$) and 6 VVD ($\diamond$). The initial high chlorophyll content was due to the higher average cell volume of actively growing cells.
- Figure 4. The measurement of oxygen production rate. The oxygen production in the PBR with batch mode is shown by the thick dotted line. The thin solid and thin dotted lines are from the PBRs with 1 VVD and 3 VVD medium exchange rate, respectively. The thick solid line represents the PBR with 6 VVD medium exchange.

Fig. 1

Fig.2

Fig. 3

Fig 4