# 効率的な大型フォトバイオリアクターの開発

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本研究は光合成細胞を面積が狭い場所でも効率的かつ大量に培養できる立体的なフォトバイオリアクターの開発を目的とした。まず、フォトバイオリアクターの設計およびスケールアップの指標として光供給係数を確立した。この指標は単位体積当りに供給されるエネルギー量と光分布係数の積であり、フォトバイオリアクターの種類や培養する細胞に関係なく、リアクター内の光環境を的確に表わしている事を確認した。この指標を用いて新規な内部照射式フォトバイオリアクターの設計およびスケールアップ法を提案した。効率的な大型フォトバイオリアクターは複数の最適な光環境を有する小規模なリアクター（１ユニット）で構成されると考え、最適なユニットサイズ（最適な光環境を有するユニットの大きさ）は一本の光源が最も効率的に光照射をできる体積と定義した。次に目的のプロセスの最適なユニットサイズを実験的に求め、そのユニットの数を三次元的に増やす事により、１ユニットと同様の光環境を有する大型フォトバイオリアクターの構築が可能になる。以上の方法に基づいて、クロレラ細胞培養の最適なユニットサイズを決定し、光供給係数を維持しながら20L規模のフォトバイオリアクターへスケールアップした。この20Lフォトバイオリアクターを用いてクロレラ細胞による炭酸ガス固定およびユーグレナ細胞によるα－トコフェロール生産を検討した所、１ユニットで培養した場合と同様な結果が得られ、本フォトバイオリアクターの設計およびスケールアップ法の有効性が示された。従って、本方法を用いることにより効率的な立体的大型フォトバイオリアクターの構築が可能であると結論した。

## Introduction

Presently, the world economy depends almost entirely on the use of fossil fuel but the excessive use of fossil oils have resulted in various environmental problems. There has been a 25% increase in atmospheric carbon dioxide within the past 150 years (Barnola *et al.*, 1987) and a probable link between atmospheric carbon dioxide and global temperature has been suggested by several authors (Genthon *et al.*, 1987). Higher plants have been playing a major role (through photosynthesis) in maintaining the balance between the atmospheric oxygen and carbon dioxide concentrations. However, an increase in world population and industrialization have led to considerable deforestation with consequent decrease in the sink for carbon dioxide. Consequently, much attention is now focused on photosynthetic microorganisms, which have higher photosynthetic rates per unit biomass than the higher plants and can be cultivated in a compact space for production of many valuable metabolites.

Currently, open ponds are used for almost all commercial cultivation of photosynthetic cells, but it is difficult to obtain high productivities with the open ponds because both the temperature and light intensities vary throughout the day and around the year. Furthermore, open cultivation ponds require large area of land and are thus not suitable for countries such as Japan where the cost of land is very high.

In this short report, a method of designing and constructing large scale compact (tank type) photobioreactors which can be operated in narrow spaces is described.

**Light Supply Index**

Since light is the most important factor affecting photoautotrophic cultivation, rational design and scale-up of photobioreactors must be based on the light characteristics inside the photobioreactors. The first step in this study was therefore to determine an index for the quantitative evaluation of light conditions inside photobioreactors. Preliminary experiments have shown that neither incident light intensities nor average light intensities was a good index of light supply capacity of a photobioreactor. We therefore proposed the use of light supply coefficient, which is a product of light energy supplied per unit volume and a light distribution coefficient, as an engineering parameter for designing and scaling up of photobioreactors.

*Light energy per unit volume* The unit of light intensity used in this study is $\mu$mol/m$^2$ • s where 1 $\mu$mol = 0.2176 J. The light energy supplied per unit volume is thus given by Eq. 1.

$$\frac{E_t}{V} = \frac{0.2176 I_O S_A}{1000V} \qquad (1)$$

where $I_O$ = incident light intensity ($\mu$mol/m$^2$ • s), $E_t$ = total light energy supplied to the reactor (kJ/s), $S_A$ = illumination surface area (m$^2$), and V = culture broth volume (m$^3$).

*Light distribution coefficient*

This is a measure of homogeneity of light intensity (light distribution) inside photobioreactors. The method for calculating the light distribution coefficient (Kiv) depends on the shape of the photobioreactor as well as on the method of illumination. For an internally illuminated cylindrical photobioreactor, the central part of the photobioreactor is illuminated while the region close to the photobioreactor surface is dark (Fig. 1). By assuming that the light source is either cylindrical or housed in a cylindrical glass tube, the liquid volume in the photobioreactor can be expressed by Eq. 2.

$$V = \pi h \left| \left(\frac{D}{2}\right)^2 - \left(\frac{d}{2}\right)^2 \right| \qquad (2)$$

where D = diameter of the photobioreactor, h = height of the reactor (m) and d = diameter of the light source (glass tube housing the light source) (m). If the photobioreactor is visualized as being composed of two concentric compartments (cylinders) of equal volume (Fig. 1), the entire photobioreactor volume can be expressed in terms of the volume of the inner cylinder, as shown in Eq. 3, and the distance ($D_I$) from the surface of the light source to the surface of the inner cylinder can be calculated from Eq. 4.

$$\pi h \left| \left(\frac{D}{2}\right)^2 - \left(\frac{d}{2}\right)^2 \right| = 2\pi h \left| \left(D_I + \frac{d}{2}\right)^2 - \left(\frac{d}{2}\right)^2 \right| \qquad (3)$$

$$D_I = \frac{-d + \sqrt{d^2 + 2\left\{\left(\frac{D}{2}\right)^2 - \left(\frac{d}{2}\right)^2\right\}}}{2} \qquad (4)$$

Assuming that light absorption and dispersion can be neglected (no cells inside the photobioreactor), the total light energy on the surface of the light source is the same as the total light energy on the surface of the concentric cylinder (diameter = $2D_I + d$). This is represented by Eq. 5 and the relationship between the light intensity on the surface of the inner cylinder (I) and the incident light intensity ($I_O$) is given by Eq. 6.

$$I_O \pi dh = I \pi h (2D_I + d) \qquad (5)$$

$$I = \frac{I_O d}{2D_I + d} \qquad (6)$$

In the presence of suspended cells in the photobioreactor, light attenuation inside the photobioreactor due to light absorption by the cells can be assumed to obey the Lambert-Beer law. Thus, the light intensity (I) at a distance ($D_I$) from the illumination surface is given by Eq. 7.

$$I = I_O \exp(-EXD_I) \qquad (7)$$

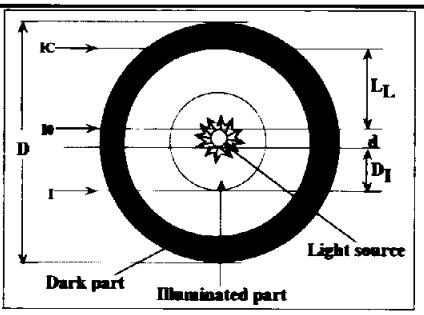


**Fig. 1. Calculation of light distribution coefficient for internally illuminated cylindrical photobioreactor.** A: the light extinction coefficient($m^2$/kg), I: Light intensity($\mu$mol/$m^2$.s), IC: Critical light intensity($\mu$mol/$m^2$.s), I0: Incident light intensity($\mu$mol/$m^2$.s), $L_L$: Distance from the illumination surface to a point where I = I0 (m), X: Cell concentration(kg/$m^3$), d: Diameter of the light source (m), h: Height of the reactor(m), D: Diameter of the reactor (m), $D_I$: distance from the surface of the light source to the surface of the inner cylinder(m)

Combining Eqs. 6 and 7 yields Eq. 8. When 50% of the entire volume (only the inner compartment) receives enough light for photosynthetic growth, the light intensity on the surface of the inner cylinder, $I = I_C$, $D_I = L_L$, and X = Kiv. Thus, for an internally illuminated cylindrical photobioreactor, Kiv can be calculated from Eq. 9. Equations for calculation of Kiv for externally illuminated cylindrical and cuboidal photobioreactors have been presented in our previous paper (Ogbonna *et al.* 1995).

$$I = \frac{I_O d}{2D_I + d} \exp(-EXD_I) \qquad (8)$$

$$Kiv = \frac{\ln[I_0 d / \{ I_C(2L_L + d) \}]}{EL_L} \qquad (9)$$

**Effect of Light supply coefficient on the linear growth rate of the cells** Irrespective of the cell type, photobioreactor type, and size, there was a linear relationship between the linear growth rates and the light supply coefficient (Et/V · Kiv) (Ogbonna *et al.*, 1995). This coefficient is, therefore, a good index of light supply efficiency of various types of photobioreactors and can be used for design and scale-up of photobioreactors. It was therefore adapted as a scale-up parameter in this study.

**A new method for photobioreactor design and scale up** It was assumed that data obtained with a small-scale photobioreactor can be reproduced for a large-scale photobioreactor if both photobioreactors have the same light supply coefficient. As a method of constructing a large photobioreactor having the same light supply coefficient as a smaller one, a new concept in photobioreactor design and scale-up was proposed. The photobioreactor is conceptualized as consisting of units. One unit consists of a reactor volume (space) which is illuminated from the center by a single light source. An optimum unit size for a process (a unit volume which is efficiently illuminated by the single light source, and thus has an optimum light supply coefficient) is experimentally determined, and a larger reactor with the same light supply coefficient can be constructed by simply increasing the number of units in three dimensions (Ogbonna *et al.*, 1996).

**Determination of optimum unit size** The first step in constructing an efficient large scale photobioreactor is to experimentally determine the optimum unit size for the target process. The optimum unit size can be determined using the experimental set up shown in Fig. 2. The incident light intensity is kept constant and the light supply coefficient is varied by using vessels of various diameters. The optimum unit diameter is then determined from the plot of the light supply coefficient (with the corresponding vessel diameter) against the linear growth rates as shown in Fig. 2 for *Chlorella* cells. The linear growth rate increased with increase in the light supply coefficient (corresponding to decrease in the vessel diameters). On the other hand, the yield coefficient increased with increasing unit diameter. Thus, with a given light intensity, the optimum unit size depends on both the type of cell and the process economy. If light represents a significant percentage of the total production cost, then greater importance should be attached to efficiency of light utilization and the unit size giving the highest yield coefficient should be selected. However, if the cost of light is relatively cheap (for example, if solar energy is used), then the design criterion should be to obtain the highest productivity. In this case, a high light supply coefficient is desirable, provided that the light intensity is not too high as to cause photoinhibition. In most cases, a compromise is made between the productivity and yield coefficient.

**Construction of a 20 L prototype reactor** A prototype photobioreactor, consisting of eight units, was constructed with Pyrex transparent glass as schematically shown in Fig. 3. The diameter of each unit was 0.075 m and each unit was equipped with a centrally fixed glass

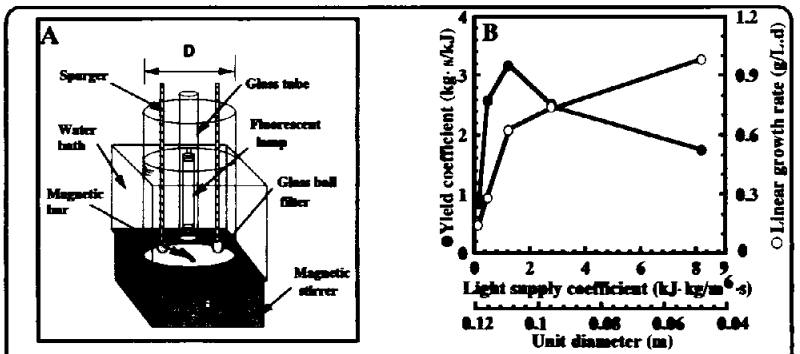


Fig. 2. Determination of the unit optimum diameter for construction of a large scale photobioreactor. *Chlorella* was used in this experiment. A: experimental set-up, B: experimental results.

tube into which the light source was inserted. The illuminating system was either 20 W or 40 W fluorescent lamps. Since the lamps were not mechanically fixed and could easily be replaced, only the reactor was heat-sterilized (where necessary) and after cooling, the lamps were inserted, thus making it possible to cultivate under sterile conditions. A modified impeller was installed for mixing. This impeller had very low shear stress but good mixing capacity (Ogbonna and Tanaka, 1997). Aeration was done through a ring sparger with 12 holes. The diameter of each hole was 0.5 mm. The glass housing units served as baffle plates in breaking the gas bubbles, thus increasing the $k_L a$. The photograph of the 20 L photobioreactor is shown in Fig. 4.

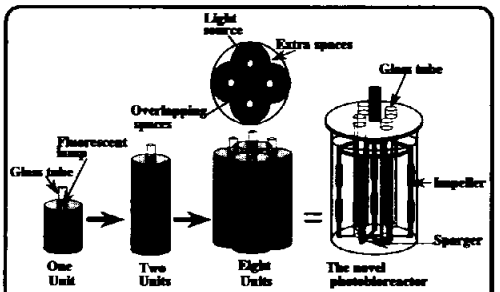


Fig. 3 Construction of an eight unit-photobioreactor with a total volume of 20.0 L

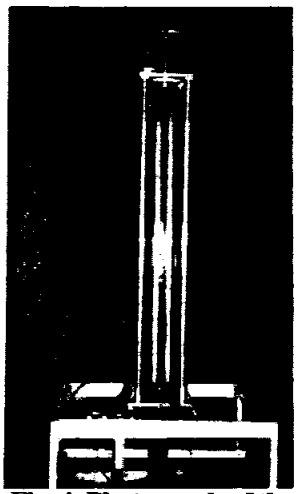

Fig. 4. Photograph of the 20 L photobioreactor

**Cultivation in the novel photobioreactor** *Chlorella pyrenoidosa* C-212 and *Euglena gracilis* Z strain were cultivated in the novel photobioreactor. The media and cultivation conditions for *Chlorella* were as described previously (Ogbonna *et al.*, 1996). A modified Hutner medium was used for cultivation of *Euglena gracilis* and cultivation conditions were as described for *Chlorella*.

The effects of scaling-up the reactor on carbon dioxide fixation by *Chlorella pyrenoidosa*

is shown in Table 1. By using the method proposed in this study, one unit reactor (2.5 L) was successfully scaled up to eight units (20 L) while maintaining the light supply coefficient constant at 1.6 kJ • $kg/m^6$ • s. Consequently, the linear growth rate, final cell concentration, and thus the volumetric $CO_2$ fixation rates were the same in all the reactors. The final cell concentrations and the linear growth rates (productivity) obtained in this study are high when compared to the values reported for other photoautotrophic algae cultures. The daily carbon dioxide fixation rate increased linearly with the reactor volume, an indication of successful scale-up. The photosynthetic efficiency based on the total light intensity was about 6.9% in each reactor, which is high in comparison with other systems.

A comparison of α-tocopherol production by *Euglena gracilis* Z in 2.5 L and 20 L photobioreactors is shown in Table 2. As in the case of *Chlorella*, both the linear growth rates and the volumetric α-tocopherol productivity were almost the same in both reactors. *Chlorella* was used for determination of the optimum unit size and as a result, the light supply coefficient in the photobioreactors was higher than the optimum value for *Euglena gracilis* cells. The linear growth rates were thus relatively low, but the final α-tocopherol contents of the cells (1600 μg/g) were higher than the values obtained at the optimum light intensity for cell growth. Preliminary experiments with this cell have shown that the optimum light intensity for α-tocopherol production is higher than that for cell growth.

The above results have shown that the proposed light supply coefficient is a good parameter for photosynthetic process scale-up. In other words, by keeping this coefficient constant, results obtained in a small reactor can be reproduced in a large-scale reactor.

**Table 1 Effect of photobioreactor scale on cell growth and carbon dioxide fixation by *Chlorella pyrenoidosa*.**

| Reactor volume (L) | Linear growth rate (g/L.d) | Final cell conc. (g/L) | Volumetric $CO_2$ fixation rate (g-$CO_2$/L.d) | $CO_2$ fixation rate per reactor (g-$CO_2$/d) | Photosynthetic efficiency (%)[a] |
|---|---|---|---|---|---|
| 2.5 | 0.62 | 6.9 | 1.11 | 2.78 | 6.94 |
| 5.0 | 0.61 | 7.0 | 1.10 | 5.5 | 6.83 |
| 20.0 | 0.62 | 6.8 | 1.11 | 22.2 | 6.93 |

a: Calculated based on the total light energy supplied and assuming cell calorific value of 24kJ/g-cell.

**Table 2 Scale up of α-tocopherol production by *Euglena gracilis* strain Z**

| Reactor volume (L) | Linear growth rate (g/L.d) | Final cell conc. (g/L) | Volumetric α-tocopherol productivity (μg/L.d) | α-tocopherol productivity (μg/d) | Yield per supplied energy (μg/kJ) |
|---|---|---|---|---|---|
| 2.5 | 0.076 | 1.46 | 179 | 358 | 0.84 |
| 20.0 | 0.075 | 1.5 | 187 | 3180 | 0.87 |


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