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A Novel Internally Illuminated Stirred Tank Photobioreactor for Large-Scale Cultivation of Photosynthetic Cells

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A new concept in photobioreactor design with reactor scale-up as a primary design criterion is proposed for the development of large-scale stirred tank photobioreactors. A photobioreactor is considered as consisting of units, with each unit being composed of a light source (or light distributing object) and its surroundings. The light supply coefficient of each unit depends on its size and the light intensity of the light source. At a given light intensity, the optimum unit size which gives the desired light supply coefficient for the target process is experimentally determined. A large photobioreactor with a desired light supply coefficient is obtained by increasing the number of units. A prototype photobioreactor, consisting of 4 units, was constructed for the cultivation of *Chlorella pyrenoidosa*. Each unit was equipped with a centrally fixed glass tube into which the light source was inserted. The illuminating system consisted of either 4-W fluorescent or halogen lamps with controllable light intensity. By changing the light intensity, it is possible to use the photobioreactor for the cultivation of various cells with different optima light supply coefficients. Mixing was achieved by means of an impeller, designed in such a way that while rotating it does not touch the glass tubes, which also serve as baffle plates. Although the hydrodynamic stress generated by the impeller was low, a high degree of mixing was achieved even at low rotation speeds. Since the light distributing objects were not mechanically fixed to the reactor, and were separated from the broth by the glass tubes, the reactor could be sterilized by autoclaving and the light distributing objects inserted to the glass tubes after cooling. The photobioreactor was equipped with a ring sparger for aeration. When *C. pyrenoidosa* was cultivated in the new photobioreactor at low and moderately high light supply coefficients, both the linear growth rates and the final cell concentrations increased linearly with the light supply coefficient of the reactor. A comparison of the results obtained in this new photobioreactor with those of the commercially available photobioreactors with either external or internal illumination showed that the cell yield from the supplied light energy was highest in the new photobioreactor.

[**Key words:** stirred tank photobioreactor, scale-up, internal illumination, closed system]

A variety of valuable pharmaceuticals, pigments, vitamins, proteins, carbohydrates, and other fine chemicals can be derived from algae and other photosynthetic cells (1, 2). Consequently, the practical application of photosynthetic cell culture for the production of useful metabolites is under intensive investigation. Algae cultivation is also being exploited in other areas such as waste water treatment (3–5), atmosphere control through CO_2 fixation (6), and in the development of closed environment life support systems (CELSS) (7, 8).

Currently, open ponds are used for almost all commercial production of algae. However, it is difficult to obtain high levels of productivity in the open ponds because of diurnal and seasonal variation in both the temperature and light intensity. Effective exploitation of the commercial potential of algae, therefore, requires the development of efficient and cost-effective photobioreactors. In this regard, closed photobioreactors, in which complete control of the cultivation conditions is possible, are preferred to open cultivation ponds.

Various closed photobioreactors have been proposed for microalgae cultivation, the most common being vertical or horizontal tubular (9–11), helical (serpentine) (12) and inclined or horizontal thin-panel (13, 14) types. The main design criterion in these photobioreactors is to supply light efficiently by maximizing the illumination surface-to-volume ratio. As a result, the tubes are often very narrow, while the panels are very thin. However, even with these types of photobioreactors, the algal productivities are still less than one-tenth of the productivities of conventional yeast or bacterial fermentors. Weissman *et al.* (15) noted that in order to achieve algal volumetric productivities similar to those of conventional fermentors, very thin reactors (less than 0.1 mm in thickness) would be needed. Lee and Palsson have developed a high-density algal photobioreactor with light-emitting diodes (16, 17). While such a reactor may be promising for CELSS, it is not suitable for mass culture.

Utilization of solar energy for mass algae production has been thought to require the use of photobioreactors with large exposed illumination surfaces, such as open ponds, horizontal or inclined tubular, and panel photobioreactors (11, 13, 14). However, with advances in solar energy technology (18), efficient utilization of solar energy is possible even with photobioreactors having exposed surface-to-volume ratios as low as those of the conventional bacteria and yeast bioreactors. However, an appropriate method for harvesting the solar energy and distributing the light inside the photobioreactor is required. In this regard, photobioreactors with internal optical fibers (18, 19) for light distribution have been reported. However, these optical fibers are currently very expensive, and many technical problems remain to be solved before they can be efficiently used in photobioreactors. Internal illumination seems to be the only practical means of constructing efficient, large-scale stirred tank photobioreactors but although many small-scale internally illuminated photobioreactors have been

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reported (12, 19, 20), neither quantitative evaluation of the light conditions in the reactors nor the design criteria and scale-up method were discussed.

Our present research is aimed at developing an efficient large-scale stirred tank photobioreactor. The relative significance of the various growth phases during batch cultivation of photosynthetic cells was first investigated (21) and a light supply coefficient was proposed as an engineering parameter for the design and quantitative evaluation of light conditions inside photobioreactors (22). In the present paper, the design of an internally illuminated stirred tank photobioreactor which has an optimum light supply coefficient is described, and a method of maintaining the light supply coefficient constant during scale-up is proposed.

MATERIALS AND METHODS

Design criteria

Reactor scale-up considerations A number of photobioreactors that work well on a small scale have been proposed, but most of them are extremely difficult to scale up. Light supply is the most important engineering challenge in photobioreactor design and construction since successful scale-up can only be achieved if the optimum light conditions are maintained in a large reactor. In order to keep the light conditions inside the photobioreactor constant during scale-up, a new concept in photobioreactor design is proposed. The photobioreactor is conceptualized as consisting of a number of units. One unit consists of a reactor volume (space) with a single light distributing object. At a constant light intensity, the light supply coefficient (a product of the light energy per unit volume and a light distribution coefficient) decreases with increasing unit size (22). At a constant light intensity, therefore, there is an optimum unit size for a given cell and process. The optimum unit size for a process is first determined experimentally, and the photobioreactor is then scaled up by increasing the number of these units. In this way, the optimum light supply coefficient of the reactor remains constant during scale-up.

Light source Utilization of solar energy is very desirable since it is abundant and free. At present, solar energy utilization in outdoor open ponds seems to be the only means by which cheap algae-derived products can be produced commercially. However, due to diurnal and seasonal changes in the solar light intensity, high volumetric productivity cannot be achieved if solar radiation is used directly for reactor illumination. Nevertheless, it can be efficiently utilized by capturing, concentrating, and distributing it within the reactor. For maximum productivity, it should be supplemented with an artificial light source so that an optimal amount of light can be supplied continuously to a photobioreactor during a process. It is thus necessary to design a photobioreactor which can utilize any source of light. In other words, connecting the light source to the bioreactor should be easy and simple so that the light source can easily be changed, even during the cultivation.

Light supply coefficient of the photobioreactor The optimal light supply coefficient depends on the algal strain and process, which means that each cell type and process ideally requires a different photobioreactor. However, for economic reasons it is desirable to use the same photobioreactor for a number of processes. This constraint demands that it should be possible to change the light supply coefficient of the photobioreactor to suit the process. The light supply coefficient of a photobioreactor is a function of the size of each unit and the light intensity. It is technically easier to change the light intensity than the size of the unit. Depending on the required range of the light supply coefficient, the size of each unit is fixed, and by changing the light intensity (by using a light source with controllable light intensity), the reactor can be used for various processes. In this way, the light supply coefficient of the photobioreactor can be changed even during the cultivation, keeping it low at initial stage when the cell concentration is still low, and increasing it as the cultivation progresses.

Mixing Mixing is very important in photobioreactors since it helps to keep the cells in suspension, distributes both the nutrients and the generated heat within the photobioreactor, improves mass transfer in the reactor, and facilitates the movement of cells in and out of the illuminated part of the photobioreactor, thus decreasing light shading and lowering the probability of photoinhibition. However, since the growth rates of most photosynthetic cells are very low, only a very low degree of mixing is required to achieve most of the above objectives (15, 23). Furthermore, if the light distribution within the reactor is minimal, there is little advantage in moving the cells close to and away from the light source (23). On the other hand, since mobile and filamentous algae are very fragile and sensitive to shear stress, it is desirable to keep the hydrodynamic stress as low as possible.

Sterilization Although the risk of contamination by heterotrophic microorganisms is low (since there is no organic carbon source in the medium), sterilization is important in order to avoid contamination by other photoautotrophs.

Design goals On the basis of the above considerations, we aimed to design and construct a tank-type photobioreactor which could be efficiently scaled up by maintaining a constant light supply coefficient. The reactor should be sterilizable, and should possess good mixing properties but low hydrodynamic stress. It should be possible to use both solar and artificial light sources for illumination and the light supply coefficient should be controllable so that the same photobioreactor can be used for cultivation of various cells with different optimal light supply coefficients.

Microorganism and growth conditions *Chlorella*

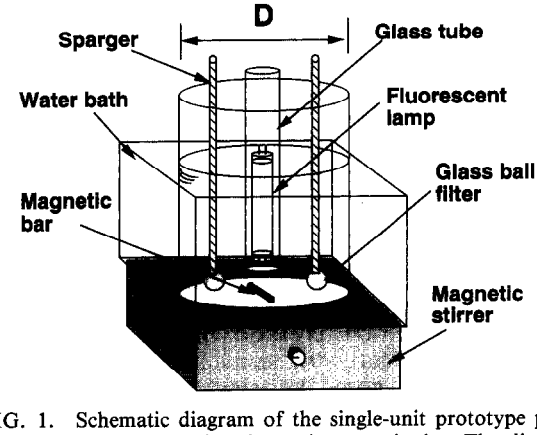

FIG. 1. Schematic diagram of the single-unit prototype photobioreactor used to determine the optimum unit size. The diameters (D) of the units used were 4.5, 5.5, 6.5, 8.5, and 11.5 cm.

pyrenoidosa C-212 from the algal collection of the Institute of Applied Microbiology, University of Tokyo, Japan was used in this study. The medium composition and cultivation conditions were as described previously (23). Air containing 5% CO_2 was used for aeration during cultivation in various types of photobioreactors. The aeration rate was 0.6 vvm and the cultivation temperature was 36°C.

Determination of optimum unit size Prototype photobioreactor units with various diameters (Fig. 1) were used to determine the optimum unit size for the new photobioreactor. A glass tube with an external diameter of 2.4 cm was fixed in the center of the unit, and a 4-W daylight fluorescent lamp was inserted into the glass tube for illumination. The light intensity at the surface of the glass tube was 163 μmol/m^2·s. The photobioreactor unit was immersed in a water bath for temperature control. Mixing was achieved by sparging 5% CO_2 in air through two glass ball filters at a rate of 0.6 vvm and by means of magnetic stirrers.

Comparison of cell growth and yield in various photobioreactors The growth and yield of *C. pyrenoidosa* from the supplied light energy in the new photobioreactor were compared with those obtained in a commercially available cylindrical photobioreactor uniformly illuminated over the entire surface by eighteen 10-W fluorescent lamps arranged vertically around the reactor, and a cylindrical photobioreactor internally illuminated by a cold light pipe (Shibata Hario Co. Ltd., Tokyo). Detailed descriptions of these two photobioreactors were given previously (21). The cultivation conditions were as described above.

Light supply coefficient of photobioreactors The light supply coefficient of a photobioreactor is defined as the product of the light energy supplied per unit volume (E_t/V) and the light distribution coefficient (K_{iv}) (22). E_t/V was calculated from Eq. 1, while the K_{iv} values for the externally and internally illuminated cylindrical photobioreactors were calculated from Eqs. 2 and 3, respectively.

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

$$K_{iv}=\frac{\ln [I_O D/\{I_C(D-2L_L)\}]}{EL_L} \quad (2)$$

$$K_{iv}=\frac{\ln [I_O d/\{I_C(2L_L+d)\}]}{EL_L} \quad (3)$$

Here, E_t=the total light energy supplied (kJ/s), V=the culture broth volume (m^3), I_O=the incident light intensity (μmol/m^2·s), S_A=the illumination surface area (m^2), K_{iv}=the light distribution coefficient (kg/m^3), D=diameter of the reactor (m), I_C=the critical light intensity below which no photosynthetic growth can occur (μmol/m^2·s), L_L=the distance between the illumination surface and the point where the light intensity (I)=I_C (m), E=the light extinction coefficient (m^2/kg), and d=the diameter of the glass tube housing the fluorescent lamp (m). A detailed explanation of the above equations and their derivation was given previously (22).

Analytical methods The light intensities, linear growth rates, and cell concentrations were determined as described previously (21–23). The yield coefficient ($Y_{X/E}$) was calculated from Eq. 4, where X_{final}=the final cell concentration (kg/m^3).

$$Y_{X/E}=\frac{X_{final}}{E_t/V} \quad (4)$$

RESULTS AND DISCUSSION

Optimum unit size The optimum unit size is defined as the diameter of the unit which gives the desired light supply coefficient. It has been shown that when light is the only limiting factor, the specific growth rate during the exponential growth phase (μ) is not a good growth index during batch cultivation of algae. On the other hand, a good relationship between the linear growth rate and the final cell concentration was found during the cultivation of both *Chlorella* and *Spirulina* cells in various types and sizes of photobioreactors (21). The linear growth rate was therefore used as the growth parameter in this study. The relationship between the unit diameters with their corresponding light supply coefficients and the linear growth rate during the cultivation of *Chlorella* is shown in Fig. 2. The linear growth rate increased linearly with decreasing unit diameter (increasing light supply coefficient), but there was inflection at high light supply coefficients.

The linear growth rates obtained in the prototype photobioreactor units were relatively low compared with those obtained with other photobioreactors having the same light supply coefficients (22). Magnetic stirring and gas sparging were insufficient to achieve the required degree of mixing in the prototype photobioreactor units. Cell sedimentation and adhesion to the reactor walls were observed during the cultivation. Nevertheless, since the aim of the experiments was to estimate the optimum unit size from the relationship between the unit diameter and the cell growth rate, it was considered that the absolute values of the linear growth rates were less important than their relative values. As shown in Table 1, the maximum yield coefficient was observed when the diameter of the photobioreactor unit was 0.065 m. It is known that at high light intensities, the light utilization efficiency decreases due to photoinhibition and/or energy loss in

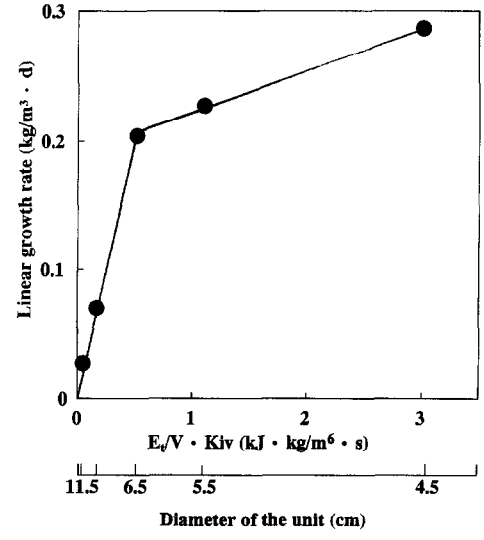

FIG. 2. Effect of photobioreactor unit diameters corresponding to various light supply coefficients on the linear growth rate of *C. pyrenoidosa*. The light intensity in each unit was 163 μmol/m^2·s while the various light supply coefficients were obtained by changing the diameter of the unit as shown in the figure.

TABLE 1. Cultivation of *C. pyrenoidosa* in internally illuminated cylindrical photobioreactors of various diameters for determination of the optimum unit diameter

Diameter(D) (m)	V^a (m^3)	$I_o{}^b$ (μmol/m^2·s)	E_t/V^c (kJ/m^3·s)	$K_{iv}{}^d$ (kg/m^3)	$E_t/V \cdot K_{iv}{}^e$ (kJ·kg/m^6·s)	K^f (kg/m^3·d)	$X_{final}{}^g$ (kg/m^3)	$Y_{X/E}{}^h$ (kg·s/kJ)
0.045	0.00022	163	1.370	2.20	3.014	0.286	2.26	1.65
0.055	0.00037	163	0.817	1.35	1.103	0.227	1.78	2.33
0.065	0.00055	163	0.550	0.938	0.516	0.204	1.61	2.93
0.085	0.001	163	0.303	0.547	0.166	0.071	0.55	1.82
0.115	0.0019	163	0.160	0.308	0.049	0.028	0.22	1.38

[a] Working volume; [b] incident light intensity; [c] light energy per unit volume (calculated from Eq. 1 with adjustment for the non-illuminated bottom zone); [d] light distribution coefficient (calculated from Eq. 3); [e] light supply coefficient; [f] linear growth rate; [g] final cell concentration; [h] yield coefficient (calculated from Eq. 4).

the form of heat. This is a possible reason for the decreased yield coefficient at very high light supply coefficients. On the other hand, at low light supply coefficients, the illuminated volume fraction of the unit is low, and a large portion of the unit is in the dark. This would lead to increased maintenance energy and thus a decrease in cell growth and yield (23).

Since it is desirable to use the same photobioreactor for various processes, the optimum unit size determined for a single process should only serve as a reference. The actual unit size of the photobioreactor can be either smaller or larger than the optimum size, depending on the range of the light supply coefficient at which the photobioreactor will be operated.

Photobioreactor construction The above results showed that in the case of *Chlorella*, a 0.065-m diameter unit is the optimum size for a photobioreactor if 4-W fluorescent lamps are to be used for illumination (I=163 μmol/m^2·s). However, in the case of a cylindrical geometry, scaling up the reactor from 1 unit to 4 units, for example, would result in a reactor whose total volume would be higher than four times the volume of a single unit, due to the extra spaces (Fig. 3). Consequently, the light supply coefficient of the photobioreactor would be lower than that in a single unit. A constant light supply coefficient can be achieved by using either a smaller unit size (Fig. 3A) or employing an overlapping arrangement (Fig. 3B) where the area of the overlapping spaces is equal to the area of the extra spaces.

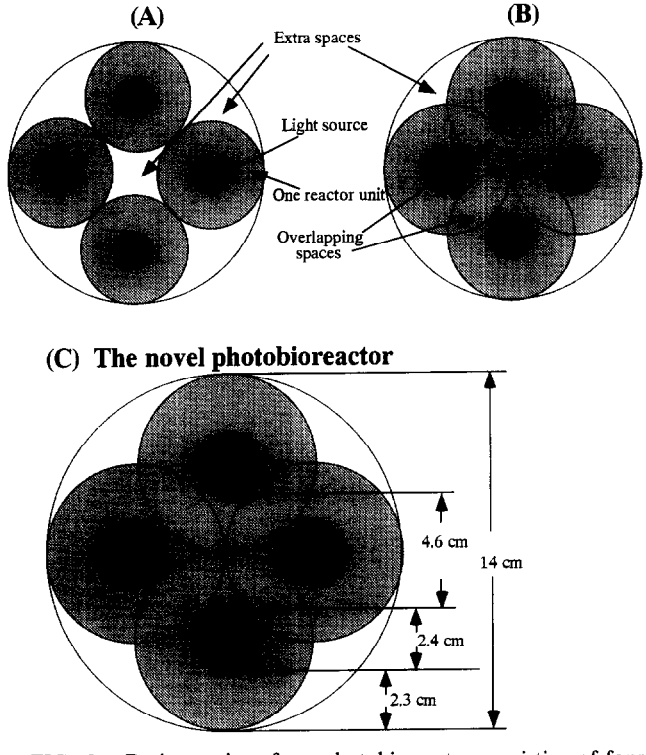

FIG. 3. Design options for a photobioreactor consisting of four units. A photobioreactor with the desired light supply coefficient can be constructed by using unit sizes which are smaller than the optimum size (A) or by using the optimum unit size but having an overlapping arrangement whereby the overlapping area is equal to the extra space area (B). The dimensions of the prototype reactor are also shown (C).

In the overlapping arrangement, the light intensity in the overlapping spaces would increase due to light from two adjacent units, while the light intensity in the extra spaces would be low since they are further away from the light sources. It is thus necessary to use unit sizes (distance between light sources) and light intensities that give a high light distribution coefficient (minimal heterogeneity in light intensity). However, the effect of this heterogeneity in light intensity can be minimized by good mixing.

The reactor can be scaled up by increasing the number of units in three dimensions. In order to maintain a constant light supply coefficient during scale-up, it is necessary to arrange the units in such a way that the area of the overlapping spaces is approximately equal to the area of the extra spaces. Depending on the size of the reactor (number of units), different unit arrangements can be used to achieve this, but it is easiest if 4^n units (n= integer) are used as the base, and the desired reactor size is then obtained by increasing the height (increasing the number of units in a vertical direction). For example, to construct a reactor comprising of 20 units, 4 units (n= 1) can be used as the base (Fig. 3B) and the height increased 5 times, or 16 units (n=2) can be used as the base and the height increased 1.25 times.

In this study, the overlapping arrangement was employed and a photobioreactor comprised of 4 units was constructed using Pyrex transparent glass, 0.5 cm in thickness (Figs. 4 and 5). The diameter of each unit was 0.07 m, which is slightly larger than the optimum value of 0.065 m. Consequently, the light supply coefficient of the photobioreactor when 4-W fluorescent lamps were used for illumination was lower than the optimum value of 0.516 kJ·kg/m^6·s (Tables 1 and 2). However, the optimum light supply coefficient can easily be achieved by increasing the light intensity in each unit. Transparent glass tubes were used as housings for the fluorescent lamps, so the reactor could be illuminated simply by inserting the fluorescent lamps into the tubes. A gap is provided between the bottom of the tubes and the bottom of the reactor to allow for the rotation of the impeller. At high cell concentrations, the light intensity in this region was negligibly small and it was thus consi-

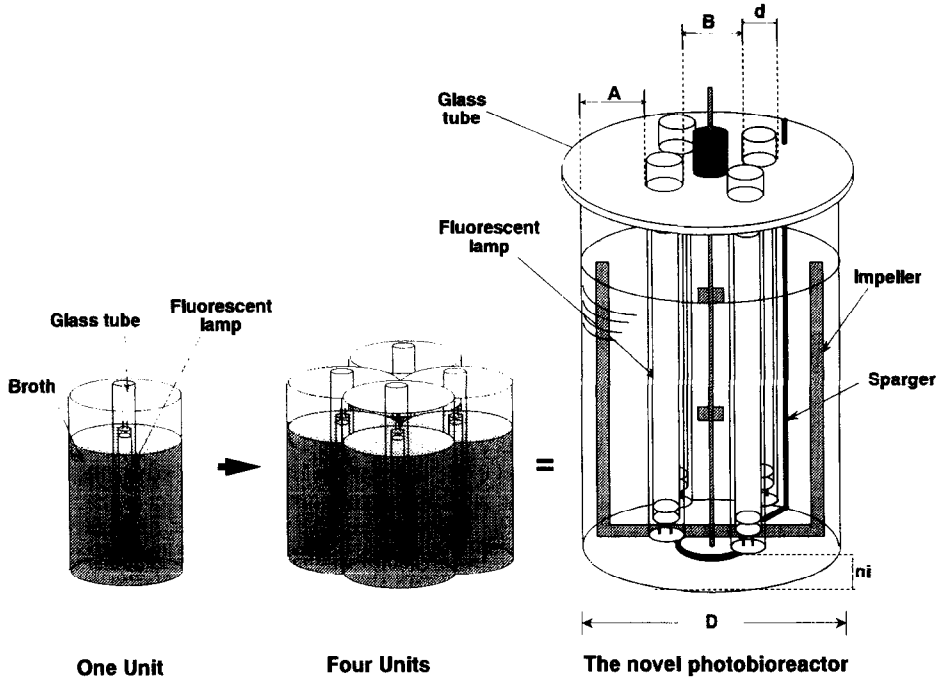

FIG. 4. Schematic diagram of the new photobioreactor. The diameter of the reactor (D) is 14 cm while the diameter of the glass tube housing the fluorescent lamp (d) is 2.4 cm. The distance from the reactor wall to the glass tubes (A) is 2.3 cm and between opposite glass tubes (B) is 4.6 cm. The non-illuminated bottom zone (the space between the bottoms of the glass tubes and the reactor) is indicated by ni.

dered to be unilluminated (Fig. 4). The internal diameter of the photobioreactor was 0.14 m and the height 0.24 m. The distances between the opposite and adjacent light sources were 0.046 and 0.026 m, respectively.

A paddle impeller (24, 25) similar to the one reported by Suzuki *et al.* (26) was used for mixing. It is shaped in such a way to ensure that it does not touch the glass tubes during rotation. This impeller has very low shear stress (25) but a very high mixing capacity. Aeration was done through a ring sparger (5.5 cm in diameter) with 4 holes (each 1 mm in diameter). The glass housing units serve as baffle plates to break up the gas bubbles, thus increasing the k_La. The k_La of the reactor at an aeration rate (with air) of 0.6 vvm and agitation speed of 250 rpm was 100 h^{-1}. This was enough to prevent cell sedimentation, achieve a sufficient rate of CO_2 transfer to the culture, and prevent O_2 build-up within the reactor (16). Since the light distributing objects are not mechanically fixed (Fig. 5), the same reactor can be used for efficient cultivation of various cells by using a light source with a controllable light intensity or by simply replacing the light source with another one giving the desired light intensity. The reactor is first sterilized and the light distributors are inserted after cooling, thus making it possible to cultivate cells under sterile conditions.

Cultivation in the new photobioreactor With 4-W fluorescent lamps, the light supply coefficient of the new photobioreactor was 0.374 kJ·kg/m^6·s which, from the data presented in Fig. 2, would result in a linear growth rate of 0.15 kg/m^3·d. As shown in Fig. 6, during the cultivation of *C. pyrenoidosa* in this photobioreactor, a final cell concentration of 1.37 g/*l* was obtained with a linear growth rate of 0.164 kg/m^3·d. The linear growth rate obtained with this 4-units photobioreactor

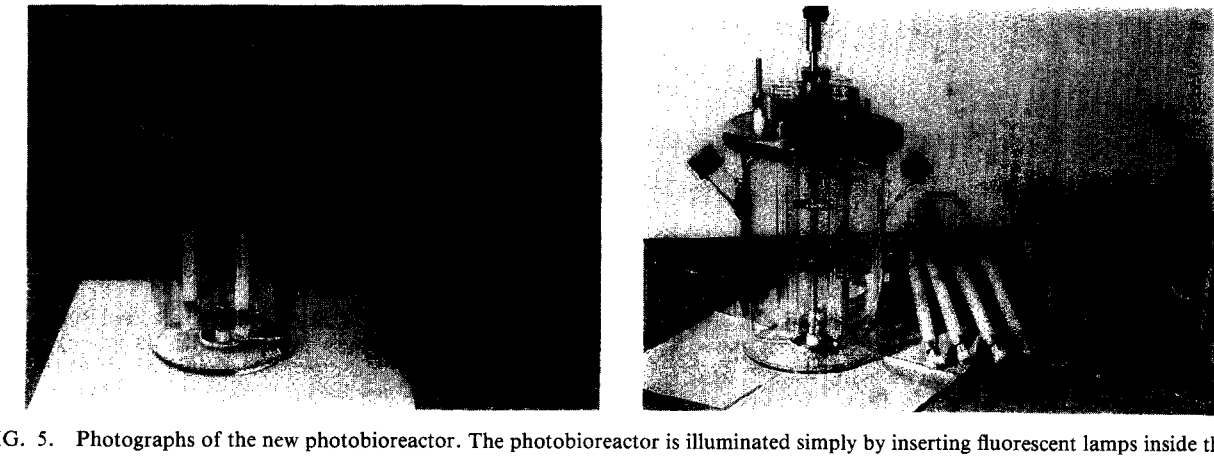

FIG. 5. Photographs of the new photobioreactor. The photobioreactor is illuminated simply by inserting fluorescent lamps inside the glass tubes.

TABLE 2. Comparison of the performance of the novel photobioreactor with those of externally and internally illuminated cylindrical photobioreactors during cultivation of *C. pyrenoidosa*

	V[a] (m^3)	I_o[b] ($\mu mol/m^2 \cdot s$)	E_t/V[c] ($kJ/m^3 \cdot s$)	K_{iv}[d] (kg/m^3)	$E_t/V \cdot K_{iv}$[e] ($kJ \cdot kg/m^6 \cdot s$)	K[f] ($kg/m^3 \cdot d$)	X_{final}[g] (kg/m^3)	$Y_{X/E}$[h] ($kg \cdot s/kJ$)
Novel	0.0026	163	0.465	0.805	0.374	0.164	1.37	2.95
Novel	0.0028	205	0.778	0.889	0.692	0.281	2.20	2.82
External	0.003	203	1.178	0.745	0.878	0.353	2.78	2.36
Internal	0.002	205	0.421	0.708	0.298	0.133	1.08	2.57

[a] Working volume; [b] incident light intensity; [c] light energy per unit volume (calculated from Eq. 1 with adjustment for the non-illuminated bottom zone); [d] light distribution coefficient (calculated from Eq. 2 or 3); [e] light supply coefficient; [f] linear growth rate; [g] final cell concentration; [h] yield coefficient.

was higher than that predicted from the results of the cultivation in single units (Fig. 2) due to better mixing conditions in the 4-units reactor. In contrast to the single units, there was neither cell sedimentation nor cell adhesion to the walls during the cultivation in the 4-units reactor. This shows that by using the method proposed here, a reactor can be scaled up from 1 to 4 units without decreasing the energy utilization efficiency of the photobioreactor.

Comparison with other photobioreactors The performance of the new photobioreactor was compared with those of representative commercially available externally and internally illuminated photobioreactors (Table 2). Although the light energy supplied per unit volume in the externally illuminated photobioreactor ($E_t/V=1.178\ kJ/m^3 \cdot s$) was almost three times that of the new reactor operated at a light supply coefficient of $0.374\ kJ \cdot kg/m^6 \cdot s$ ($E_t/V=0.465\ kJ/m^3 \cdot s$), the light distribution was more homogeneous in the new photobioreactor. Consequently, the yield coefficient (as calculated from Eq. 4) in the new photobioreactor was 25% and 15% higher than those obtained in the externally and internally illuminated photobioreactors, respectively. Using a halogen lighting system with controllable light intensity, it was possible to operate the new photobioreactor at different light supply coefficients. When the light supply coefficient was increased to $0.692\ kJ \cdot kg/m^6 \cdot s$, both the linear growth rate (K) and the final cell concentration (X_{final}) increased in proportion to the light supply coefficient (Table 2), but as expected, the cell yield from the supplied light energy decreased. The light supply coefficients of the commercially available reactors used in this study could not be varied. Nevertheless, the major disadvantage of the externally illuminated reactor is its low light distribution coefficient, and variation in the light intensity is not an effective method of solving this problem.

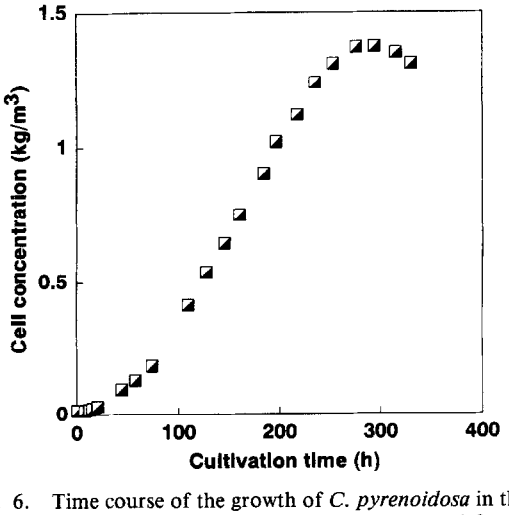

FIG. 6. Time course of the growth of *C. pyrenoidosa* in the new photobioreactor. Four-watt fluorescent lamps were used for illumination, giving a light supply coefficient of $0.374\ kJ \cdot kg/m^6 \cdot s$.

The most important advantage of the novel photobioreactor described here is its scale-up potential. By increasing the number of the units in three dimensions, a large-scale stirred tank photobioreactor with the desired light supply coefficient can be constructed. The scale-up of this photobioreactor will be presented in our next paper.

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