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Glutamate Production from CO_2 by Marine Cyanobacterium *Synechococcus* sp. Using a Novel Biosolar Reactor Employing Light-Diffusing Optical Fibers

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ABSTRACT

A photobioreactor was constructed in the form of a Perspex column 900 mm tall with an internal diameter of 70 mm. The reactor volume was 1.8 L and the light source consisted of a metal-halide lamp to reproduce sunlight. Light was distributed through the culture using a new type of optical fiber that diffuses light out through its surface, perpendicular to the fiber axis. A cluster of 661 light-diffusing optical fibers (LDOFs) pass from the light source through the reactor column (60-cm culture depth) and are connected to a mirror at the top of the reactor. This biosolar reactor has been used for the production of glutamate from CO_2 by the marine cyanobacterium *Synechococcus* sp. NKBG040607. We present here details of the construction of the biosolar reactor and characterization of its properties. The effect of light intensity on glutamate production was measured. Carbon dioxide-to-glutamate conversion ratios were determined at different cell densities: the maximum conversion ratio (28%) was achieved at a cell density of

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3×10^8 cells/mL. A comparison of glutamate production using the LDOF biosolar reactor described here with production by batch culture using free or immobilized cells showed that use of an optical-fiber biosolar reactor increased glutamate-production efficiency 6.75-fold. We conclude that as a result of its high surface-to-volume ratio (692/m) increased photoproduction of useful compounds may be achieved. Such a system is generally applicable to all aspects of photobiotechnology.

Index Entries: Glutamate; marine cyanobacteria; *Synechococcus;* biosolar reactor; optical fiber.

INTRODUCTION

In consequence of the availability of sunlight as a cheap and plentiful energy source, photosynthesis is becoming increasingly important as a means of producing industrially valuable compounds using photobioreactors. In addition, CO_2 fixation as an end result of such processes is an extremely desirable side effect in a time when increasing global CO_2 concentrations are threatening the atmospheric and biological stability of the planet. At present, conventional agriculture has extremely low photosynthetic efficiency, with only about 0.2% of the total incident solar energy captured (*1*); under optimum conditions, some photobioreactors allow up to 18% of the incident energy to be stored (*2*). For a photobioreactor to be effective, it should be closed to the atmosphere to allow complete control of conditions. Furthermore, for maximum utilization of solar energy, maximum surface:volume ratio is required. Numerous attempts have been made to increase this ratio, for example, the use of thin glass tubes containing the culture (*3*), the use of a plastic-cone insert for light redistribution (*4*), and the use of rigid, flat panels (*5*). However, these methods are unsuitable for industrial scale-up, when the surface:volume ratio becomes critical.

We describe here the construction and use of a column-type photobioreactor employing a cluster of specially constructed optical fibers that pass through the cell culture, supplying light to it. This is possible because the optical fibers used differ from conventional fibers in that light passing through them is diffused out through their surface, perpendicular to the axis of the fiber. Light is thus transferred through the culture from a metal-halide lamp, which simulates sunlight and is below the reactor. As a result of many fibers passing through and supplying light to the cell culture, the surface-area:volume ratio is high (692/m) when compared to more conventional glass-tube reactors (129/m) (*3*).

The majority of biotechnological applications that use photobioreactors involve the production of biomass as a raw material. However, the use of photosynthetic organisms to continuously convert carbon dioxide into valuable compounds that may be easily extracted from the culture is of growing importance. This work shows how the LDOF biosolar reactor

may be used in such a process. The cyanobacterium *Synechococcus* sp. NKBG040607 secretes large quantities of glutamate (*6*) and was used for the continuous production of glutamate from CO_2. Glutamate production was also carried out using immobilized cells as well as batch cultures.

This paper presents details of the construction of a new type of photobioreactor that employs light-diffusing optical fibers (LDOFs) to distribute light through the culture. This system has a high surface area:volume ratio, greater energy efficiency, and improved scale-up properties, making it an attractive bioreactor for all processes involving growth of photosynthesis microorganisms.

MATERIALS AND METHODS

Strain and Culture Conditions

The glutamate-producing cyanobacterium *Synechococcus* sp. NKBG040607 was used in this study. This strain was isolated in our laboratory from the coastal water of Japan. Cells were grown at 27°C, aerobically, in BG11 medium (*7*) supplemented with 3% NaCl under illumination of 50 $\mu E/m^2/s$ using cool white fluorescent light at the surface.

Immobilization of Marine Cyanobacterium

One liter of cyanobacterial cell culture was centrifuged at 8000*g* for 10 min and washed twice with Tes buffer (0.1*M* Tes, 3% NaCl, pH 8.0). The cells were resuspended in 50 mL of Tes buffer. The cell suspension was then mixed with 50 mL of 4% sodium alginate solution and dropped into 100 m*M* calcium chloride solution using a syringe and needle. The immobilized cyanobacterial cells were washed with Tes buffer containing 3% NaCl. The immobilized cell particles were spherical, with a diameter of about 2–3 mm.

Analytical Procedure

Glutamate excretion from free or immobilized cells was measured using a glutamate dehydrogenase assay, as described elsewhere (*8*). Dissolved CO_2 was determined using a modified pH electrode (DKK Ltd., Tokyo, Japan) containing sodium bicarbonate, (10 m*M*) and a silver chloride reference electrode in an ABS polymer housing. Sodium bicarbonate was used to provide a standard curve of CO_2 concentration.

Apparatus for Measurement of Diffusion Efficiency and Light Intensity Distribution

A Minolta photometer (model T-1M) was used for measuring light emitted from the surface of the LDOFs during determination of the light intensity distribution at the LDOF surface. LDOFs were placed on a black

surface. The photometer was placed in contact with the LDOFs and light intensity measured. An integrating sphere (Optel Ltd., Tokyo, Japan) was used to determine the quantity of diffused light and output light of single LDOFs during measurement of diffusing efficiency. Input light was measured by using an integrating sphere to determine the intensity of the light being emitted from one end of a nondiffusing optical fiber. In this case, input light is equal to output light. Output light (the light passing through the fiber) was also measured using an integrating sphere. In this case, the end of the LDOF fiber was placed just inside the integrating sphere so that the output light could be measured directly. The integrating sphere used to determine the quantity of diffused light has a conversion coefficient *K*, measured by a calibration curve that converts lux to lumens. The conversion coefficient *K* of the sphere was 0.010059526, where measured intensity $\times K$ = luminous flux.

RESULTS

Construction of LDOF Biosolar Reactor

Figure 1 is a schematic diagram showing construction of the LDOF biosolar reactor. The light source consists of a metal-halide lamp that simulates sunlight. Light enters the bottom of the reactor and is transmitted up through the optical fibers, which are drawn in Fig. 1 as vertical lines within the vertical column reactor area. As light is transmitted along each of 661 fibers, it is diffused into the surrounding culture as a result of the internal surface of the fiber, which is striated, rather than smooth as in a conventional optical fiber. A fuller description of the mechanism of light diffusion from these fibers will be presented elsewhere (Oyama et al., manuscript in preparation). LDOFs consisted of a polymethylmethacrylate (PMMA) core and a sheath of fluororesin. Small-scale production methods of preparation were used to prepare fibers 1.5 m in length. These were prepared from conventional PMMA optical fibers and processed using a cool air/infrared treatment/cool air/heat shock process to creat internal striations. Fibers are 1 mm in diameter and are arranged on 2-mm centers. These fibers are flexible and durable. The fiber cluster provides a 60-cm long light-diffusing section for photosynthetic growth, resulting in a surface area:volume ratio of 692/m. When light reaches the top of the reactor, it is reflected back down the fiber by a mirror plate placed at the end of the LDOFs. Gas exchange occurs in the central column, air being pumped in from the bottom at 3.9 vvm (aeration rate/volume). A peristaltic pump circulates the culture as shown. The working culture volume is 1.8 L. Cells were inoculated through the top of the reactor into the extra capillary space and agitated by aeration, resulting in homogeneous growth. After use, the reactor is cleaned by removing the central

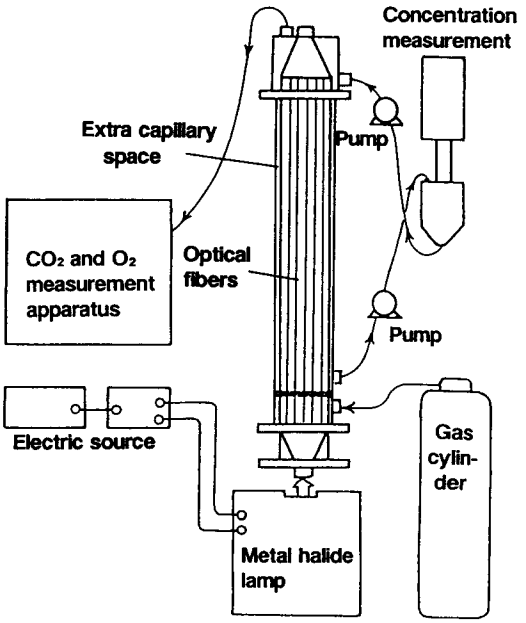

Fig. 1. Schematic diagram of a biosolar reactor using LDOF and the support system. The central column is the biosolar reactor, including LDOFs inside. Vertical lines indicate the fibers. The area from the top end of the fiber to the border point of aeration space is the culture space (1.8 L). The light source consists of a metal-halide lamp. The culture is circulated by pumps, and cell concentration (absorbance) is measured in the course of circulating. CO_2 and O_2 change is measured by the measurement apparatus connected to the biosolar reactor.

fiber cluster and rinsing the apparatus with water. Hydrogen peroxide (3–6%) was used for sterilization. The LDOFs have been used for more than two months.

Diffusing Efficiency of LDOFs

The diffusing efficiency of a LDOF is defined as the ratio of total measured diffused light to the light consumed (input light – output light), where output light is the quantity of light that passes directly through the fiber. Results are shown in Table 1. The quantity of diffused light and of output light was measured on single fibers, using an integrating sphere.

Light Intensity Distribution at the LDOF Surface

The amount of light diffusing from the surface of optical fibers along the length of the culture area was determined using a linear array of 19 LDOFs placed on a black surface. Light energy was passed along the fibers via a terminal fiber bundle. At the other end the fibers were col-

Table 1
Diffusing Efficiency of Optical Fibers

Light	Luminous flux (Lm)	Diffusing efficiency (%)
Consumed light*	75.5	
Input light	146.8	100
Output light	71.3	
Diffused light	75.6*	

*Consumed light was calculated as input light-output light. Efficiency of 19 optical fibers was measured and averaged.

lected and joined to a mirror in exactly the same way as in the bioreactor. Light being emitted from the surface of the fibers was measured using a light meter at various distances from the point corresponding to the base of the culture area. Light measurements at each distance were taken at three points across the surface of the fiber array and averaged. The results are shown in Fig. 2. The light-intensity distribution was also determined at three other illumination intensities, and the diffusing intensity profiles were found to have the same characteristics (data not shown).

Light Intensity Variation and CO_2-to-Glutamate Conversion Ratio

A culture of *Synechococcus* sp. NKBG040607 was prepared in a simple column reactor that did not contain LDOFs and that was illuminated from above. A submersible photosensor was used to measure the light intensity at various culture depths. The light intensity of the surface was 1000 $\mu E/m^2/s$. Light intensities in the culture were measured at two cell densities. The light intensity sharply decreased with increasing depth for both cell densities. In addition, light intensities in the culture were measured when the illuminating intensity at the surface was only 20 μ E/m^2/s. The results are shown in Fig. 3.

The ratios of conversion of CO_2 to glutamate were calculated and correlated with cell density, as shown in Table 2. CO_2 was converted to glutamate as follows:

$$5CO_2 \longrightarrow \text{Calvin cycle} \longrightarrow \text{TCA cycle} \longrightarrow \alpha\text{-ketoglutarate} \xrightarrow[NH_4^+]{} \text{glutamate}$$

The conversion ratio is 100% when fivefold moles of decreased CO_2 is equal to produced glutamate moles. Glutamate production was measured after 24 h of cultivation. CO_2 concentration was calculated before and after cell cultivation, and decreased CO_2 was estimated. A cell density of 3×10^8 cells/mL gave the greatest glutamate production ratio, 28%.

The effect on glutamate production of varying the light intensity at the LDOF surface was also investigated (Fig. 4). A 7- to 10-d culture of cyanobacteria (late log to early stationary phase) was used as an inoculum and

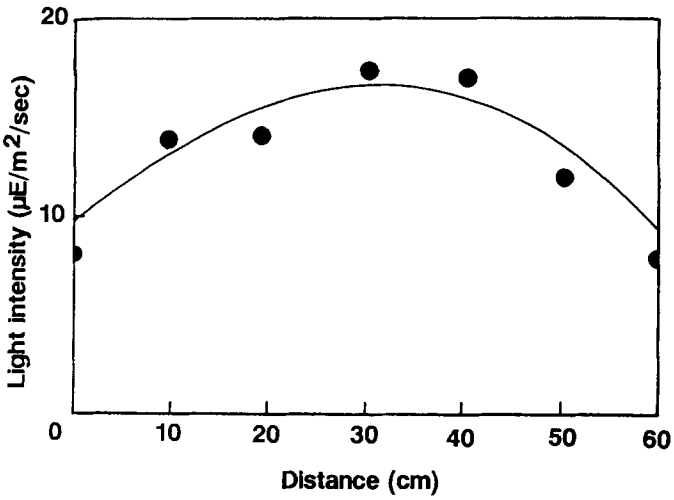

Fig. 2. Light-intensity distribution at the surface of LDOFs. Light intensity is measured using a photometer placed in contact with the LDOFs (*see* Material and Methods section). Light is emitted from the surface of the fiber throughout its length. Distance is measured from the point at which light is introduced to the point of contact of the photometer. Input light intensity: 33,400 $\mu E/m^2/s$.

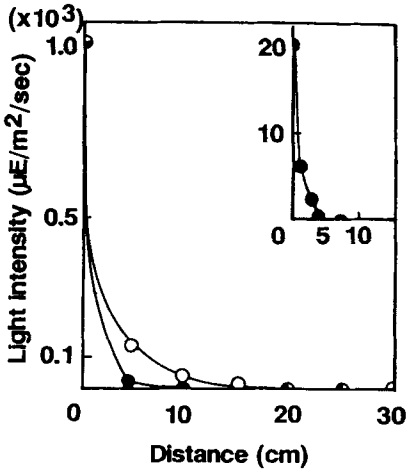

Fig. 3. The distribution of light intensity as a function of distance from the surface at various cell concentrations. Light intensity is measured by a submersible photosensor. The surface light intensity is 1000 μ E/m²/s. The light intensities are measured at each depth and at two cell densities. The small figure included also shows the decrease curve increasing the depth when the surface light intensity is 20 μ E/m²/s. (●, 2.3×10^8 cells/mL, ○ 7.9×10^7 cells/mL).

the light intensity gradually increased from low to high level. Glutamate production was estimated at five different intensities. The same culture was used for all readings. Figure 4 shows maximum glutamate production at 20 $\mu E/m^2/s$. At higher light intensities photobleaching and cell death resulted in loss of glutamate production.

Table 2
Conversion Ratio of CO_2 to Glutamate at Various Cell Densities

Cell density ($\times 10^8$ cells/mL)	Glutamate production (mg/g dry wt cells/d)	Conversion ratio of CO_2 to glutamate (%)
3.0	15.4	28
5.5	10.9	21
14.3	1.4	25
66.3	1.0	16

It is 100% conversion ratio when 5 mol of decreased CO_2 is equal to produced glutamate moles.

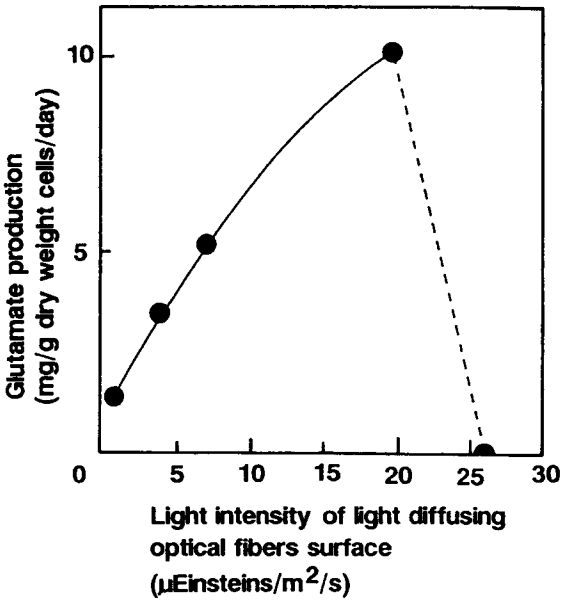

Fig. 4. The effect of light intensity on glutamate production by a biosolar reactor using LDOFs. The light intensity of LDOFs is calculated by the incident light intensity. The light was gradually increased from a low to a high level using the same cell culture. The inoculated culture had been cultured for 7–10 d previously.

Comparison of Different Glutamate Production Methods

Table 3 shows glutamate production rates for three different production methods. Batch culture was carried out in a 1-L flask that contained 700 mL of culture. The cells were illuminated from below. The immobilized system consisted of cells in 100 mL of sodium alginate beads that were incubated in 200 mL of medium in a 500-mL flask. The energy efficiency of each system (glutamate/μE) is shown.

Table 3
Comparison of Different Glutamate Production Methods

Method	Light intensity* (μEinstein/m^2/s)	Incident luminous flux (Lm)	Glutamate production ($\times 10^{-4}$ μmol/mL/h)	Energy efficiency ($\times 10^{-5}$ μmol/μE)
Batch culture[a]	150	107	1.1	1.0
Batch culture[b] immobilized cells	150	71	1.3	0.8
Biosolar reactor light diffusing optical fiber (LDOF)	7	692	15	5.4

[a] A 1 L flask containing 700 mL of culture (base area of flash = 143 cm^2).
[b] 100 mL of immobilized cell particles in 200 mL of medium in a 500 mL flask (base area of flask = 95 cm^2).
* Light intensity was measured at the base of the flask and at the surface of the fiber for the LDOF. Input light intensity to light receiving bundle (surface area = 6 cm^2); 2.2×10^4 μE/m^2/s. Total surface area of fibers = 12450 cm^2.

DISCUSSION

The LDOF bioreactor described here is a new type of bioreactor. Its main advantage is the presence of a high surface-area:volume ratio enabling more energy-efficient photosynthetic conversions to be obtained. Optical fibers have been used previously as part of a biosolar reactor (*9*). However, in this case, conventional fibers were used to transmit light from a collection device to the culture area. Light dispersion was carried out using etched fibers that did not provide uniform illumination. The LDOF bioreactor, on the other hand, combines the functions of transmission and dispersion of light, allowing uniform illumination and more-efficient energy transfer.

The calculated diffusing efficiency of the LDOFs approaches 100%, so no energy is lost from the system as heat, as shown in Table 1. The culture at the center of the biosolar reactor (that is, equidistant from the top and bottom of the reactor area) experiences the highest light intensity. This phenomenon was found to be independent of incident light intensity, and probably occurs as a result of the internal geometries of the LDOFs.

The ability of light to penetrate into cultures of *Synechococcus* was found to be very poor, especially at high cell densities (Fig. 3). Light penetration into cells immobilized on sodium alginate was found to be extremely poor, as was light penetration into liquid cultures at low light intensities.

This means that, in order to obtain efficient light transfer to all cells in a culture, each cell should be as close to the light-providing surface as possible. This situation is readily achieved in the LDOF biosolar reactor, where the optical fibers in the LDOF cluster are arranged in 2-mm centers.

Ideally, a photobiotechnological process should result in maximum conversion of CO_2 to useful product that may be readily removed from the medium. The system described here approaches this ideal. Theoretically, 5 mol of CO_2 are converted through Calvin and TCA cycles to 1 mol of glutamate. CO_2-conversion ratios of just under 30% were attained, resulting in production of glutamate, which was secreted into the medium, facilitating purification of the product. Energy efficiency (amount of glutamate per light energy input) was also obtained. The efficiency of glutamate production (μmol glutamate/μE) was found to be highest for the LDOF bioreactor. This efficiency was 5.4 times greater than that obtained when using batch culture and 6.75 times greater than that obtained when using immobilized cells. In these experiments, the flask size did not so effect glutamate production (data not shown). Incident luminous flux and cell concentration are the important factors. Incident luminous flux was calculated from Fig. 3. The higher glutamate production in batch culture using free and immobilized cells is shown. If incident light intensity or cell concentration increases, then the light intensity in the culture decreases very rapidly with culture depth, so higher glutamate production is not obtained. On the other hand, in the case of the biosolar reactor, it is possible to obtain higher glutamate production, because the culture depth is always 1 mm as a result of the design of the LDOF bundle. Hence, incident light intensity will give increased glutamate production (Fig. 4). Thus, it is clear that the biosolar reactor is advantageous to glutamate production.

Future work is currently being directed toward continuous removal of valuable products from LDOF biosolar reactors as well as toward optimization, design improvement, and scale-up of this type of reactor for use in production of commodities of higher value, such as antibiotics and pharmaceuticals. The concomitant removal of CO_2 during product synthesis will become increasingly important, especially if such systems are adopted on an industrial scale.

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