0273-2289/92/3435—0449$02.00

CO_2 Removal by High-Density Culture of a Marine Cyanobacterium *Synechococcus* sp. Using an Improved Photobioreactor Employing Light-Diffusing Optical Fibers

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ABSTRACT

A light diffusing optical fiber (LDOF) photobioreactor with an improved gas input system has been used for the high-density culture of a marine cyanobacterium *Synechococcus* sp. Optimum conditions for CO_2 removal and biomass production were investigated. Maximum CO_2 removal of 4.44 g/L/d was achieved using an initial cell concentration of 6.8 g/L. The biomass yield was 0.97 g/L for a 12-culture time. Continuous cultures, in which medium was filtered using a ceramic membrane module, showed enhanced growth, with a final cell concentration of 11.2 g/L. These results demonstrate the potential of LDOF photobioreactor units for CO_2 removal and biomass production using marine cyanobacteria.

Index Entries: Photobioreactor; marine cyanobacterium; *Synechococcus*; CO_2 removal; optical fiber.

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INTRODUCTION

Increasing attention has recently been paid to the biotechnological potential of converting solar energy and CO_2 into biomass and industrially valuable compounds using marine cyanobacteria (*1–3*). The three major environmental variables that control growth of marine cyanobacteria are solar energy, temperature, and nutrient availability. Very little applied research has been carried out with cyanobacteria despite their potential significance to biotechnology. Although their potential as biofertilizers and their ability to excrete amino acids are well known (*4*), few commercial-scale processes have been attempted. This is mainly because of the cost of biomass production using conventional reactors and the difficulty in identifying suitable high-value products for industrial-scale production.

For efficient solar energy utilization by cyanobacterial cultures, a maximum surface area to volume ratio is desired for efficient light absorption. We have designed a novel illumination system based on light-diffusing optical fibers and have developed a column-type photobioreactor that employs these fibers for light distribution. Efficient energy distribution is achieved as a result of a bundle of fibers passing through and supplying light to the cell culture. The surface area to volume ratio is high (692 mm^{-1}) when compared to more conventional reactors. We have previously reported glutamate production by the marine cyanobacterium *Synechococcus* sp. NKBG 040607 using a prototype LDOF photobioreactor (*2*).

In this work, biomass production by a marine cyanobacterium *Synechococcus* sp. using the LDOF photobioreactor has been carried out. This cyanobacterium produces biologically active compounds that promote plant regeneration in artificial seeds (*5*). High-density culture of *Synechococcus* sp. NKBG 042902 in both batch culture and continuous culture was performed. CO_2 removal and biomass production under several culture conditions are presented.

MATERIALS AND METHODS

Strain

A marine cyanobacterium *Synechococcus* sp. NKBG 042902 was used in this study. This strain was isolated from the coastal waters of Japan and purified in our laboratory. Growth and characteristics of this strain have been described previously (*5,6*).

Batch Culture

Cells were precultured in modified BG11 (*7*) medium unless otherwise stated. This medium was BG11 based and contained in addition (per liter): 10 g NaCl, 5 g $NaNO_3$, and 100 mg K_2HPO_4. Unmodified BG11 medium

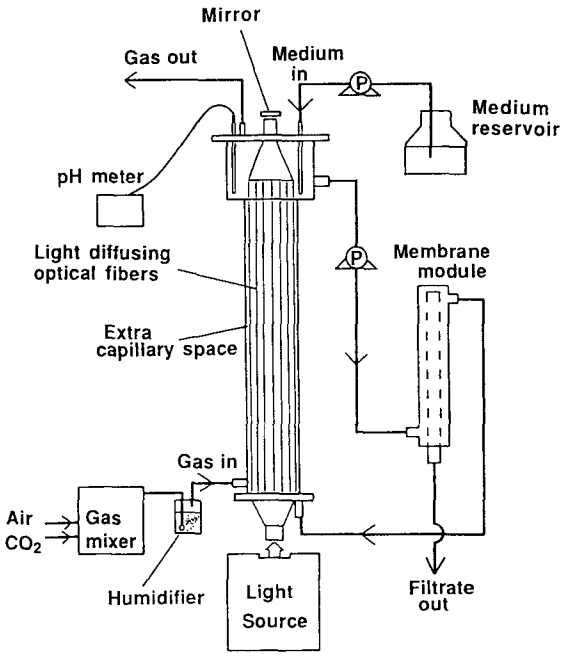

Fig. 1. Schematic diagram of the LDOF photobioreactor system showing the ceramic membrane filtration module used for removing medium. The central column of the reactor, which contains the LDOF bundle, has a culture vol of 2.5 L. Vertical lines represent fibers (661 fibers in total). P: pump unit.

was used as described previously (2). Surface illumination of the culture vessel was 50 $\mu E\ m^{-2}\ s^{-1}$. After harvesting by centrifugation, cells were resuspended in fresh medium and cultured in the LDOF photobioreactor (STI Japan, Tokyo). A gas nozzle unit for improved gas/liquid mixing was employed. The light source unit (STI, Tokyo, Japan) of the photobioreactor was constructed from a 400-W metal halide lamp. The delivered light was in the visible region of the spectrum between 380–700 nm, with little infrared and reduced ultraviolet radiation. These improvements to the lighting system reduced photodamage and overheating of cultures.

The reactor was operated at a culture vol of 2.5 L. An air/CO_2 mixture was used for aeration at a flow rate of 800 mL/min 0.32 vvm). (vol of reactor·gas flow rate/min). Different CO_2 concentrations were obtained by changing the air/CO_2 ratio using a gas mixer (Kofloc Flow Instruments, Kyoto, Japan). The culture was circulated using a roller pump at a flow rate of 200 mL/min (Masterflex PA-71B, Cole-Parmer Instrument Co., IL).

Filtration Culture

Continuous culture was carried out by filtering medium using a ceramic membrane module installed in the medium recirculation line (Fig. 1) (*8*). The membrane is a tubular external pressure type ceramic membrane with

a pore size of 0.1 μm (outside diameter 10 mm; inside diameter 7 mm; length 205 mm; Kubota Co., Osaka, Japan). Culture was circulated at 500 mL/min. The medium was filtered and removed at a rate of 70 mL/h. Fresh medium was continuously supplied by a peristaltic pump under constant volume conditions. Other culture conditions were as described for batch culture.

Analysis

CO_2 concentrations in the supply and exhaust gases were measured by gas chromatography and used to calculate CO_2 removal. Biomass yield was determined by the increase in cell dry wt.

RESULTS AND DISCUSSION

CO_2 Removal in Batch Culture

Two media were used to determine CO_2 and biomass yield of cells grown at 20 μE m^{-2} s^{-1}: BG11 and modified BG11, which contained increased nitrate and phosphate, and reduced sodium chloride (*see* Materials and Methods). The results are shown in Table 1. The CO_2 removal and the CO_2 to biomass conversion ratio increased significantly when modified BG11 medium was used. The effect of light intensity at the fiber surface on CO_2 removal was also examined. CO_2 removal gradually increased by approx fourfold when the light intensity at the fiber surface was increased from 2.5 μE m^{-2} s^{-1} to 20 μE m^{-2} s^{-1} (Fig. 2).

The effect of CO_2 concentration of the input gas on growth and CO_2 removal was examined (Table 2). The light intensity at the fiber surface was set at 20 μE m^{-2} s^{-1}, at which CO_2 removal was maximum. CO_2 removal more than doubled when the CO_2 concentration was increased from 0.03 to 0.55%. However, when the input gas CO_2 concentration was increased from 0.55 to 1.10%, only a slight increase occurred.

Biomass Yield in Batch Culture

The effect of light intensity at the fiber surface on biomass yield was examined. Biomass yield increased by 4.2-fold when the surface light intensity was increased from 2.5 μE m^{-2} s^{-1} to 20 μE m^{-2} s^{-1} (Fig. 3). The biomass yield increased by 2.5-fold when the CO_2 concentration of the input gas was increased from 0.03 to 0.55% (Table 2).

The effect of initial cell concentration on CO_2 removal and biomass yield was examined (Table 3). Light intensity at the fiber surface and CO_2 concentration in the input gas were set at 20 μE m^{-2} s^{-1} and 0.55%, respectively. The biomass yield and removal CO_2 were examined when the initial algal cell concentration was increased from 1.4 g/L to 6.8 g/L.

Table 1
CO_2 Removal and Biomass Yield Using Different Media*

Medium	CO_2 removal, g/L	Initial cell conc., g/L	Final cell conc., g/L	Biomass yield, g/L	Conversion ratio from CO_2 to biomass, %
BG11	0.38	1.70	1.86	0.16	80
Modified BG11	1.06	1.40	1.92	0.52	90

*Cells were cultured for 12 h. The working vol was 2.5 L. Light intensity at the light diffusing optical fiber surface: 20 μE m^{-2} s^{-1}. Aeration: 0.5% CO_2, 800 mL/min. When all of the CO_2 removed is converted to biomass, the conversion ratio is 100%. The carbon content of the biomass is taken as 50%.

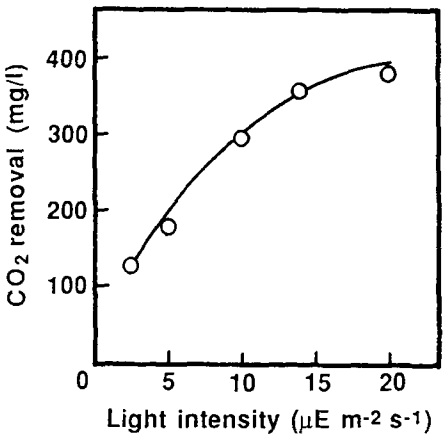

Fig. 2. Effect of light intensity on CO_2 removal. Light intensity as the LDOF surface is calculated from the incident light intensity as previously described (*4*). Initial cell concentration was 1.70 g/L. A CO_2/air mixture with a CO_2 concentration of 0.50% was supplied to the reactor. Cells were cultured for 12 h in BG11 medium.

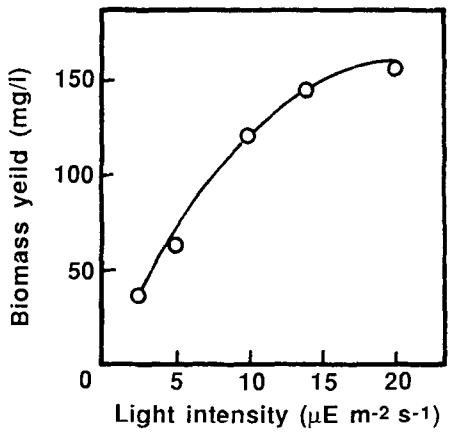

Fig. 3. Effect of light intensity on biomass yield. Light intensity as the LDOF surface is calculated from the incident light intensity as previously described (*4*). Initial cell concentration was 1.70 g/L. A CO_2/air mixture with a CO_2 concentration of 0.50% was supplied to the reactor. Cells were cultured for 12 h in BG11 medium.

As the initial cell concentration increased, the maximum specific growth rate decreased. However, the final biomass yield increased (Table 3). The biomass yield and CO_2 removal after a 12-h culture period were optimum when an initial cell concentration of 6.8 g/L was used.

Growth by Filtration Culture

With recent improvements in membrane-separation technology, bioreactors combined with membranes are increasingly being used in biomass

Table 2
Effect of CO_2 Concentration of the Input Gas on Growth and CO_2 Removal*

CO_2 conc. of input gas, %	CO_2 conc. of output gas, %	CO_2 removal, g/L	Initial cell conc., g/L	Final cell conc., g/L	Biomass yield, g/L	Conversion ratio from CO_2 to biomass, %
0.03	0.00	0.34	0.39	0.56	0.17	91
0.55	0.37	0.85	0.33	0.76	0.43	93
1.10	0.93	0.79	0.45	0.85	0.40	93

*Cells were cultured for 12 h. The working vol was 2.5 L. Light intensity at the light diffusing optical fiber surface: 20 $\mu E\ m^{-2}\ s^{-1}$. The gas flow rate was 2 L/min; CO_2 concentration of the input gas was 0.03%. The flow rate was 800 mL/min when the CO_2 concentrations were 0.55 and 1.10%. When all of the CO_2 removed is converted to biomass, the conversion ratio is 100%. The carbon content of the biomass is taken as 50%.

Table 3
Comparison CO_2 Removal and Biomass Yield
at Various Initial Cell Concentrations*

Initial cell conc., g/L	Final cell conc., g/L	Biomass yield, g/L	CO_2 removal, g/L	Conversion ratio from CO_2 to biomass, %
1.40	1.92	0.52	1.06	90
2.40	3.00	0.60	1.52	73
5.50	6.28	0.78	1.98	72
6.76	7.76	1.00	2.22	82

*Cells were cultured for 12 h. The working vol was 2.5 L. Light intensity at the optical fiber surface: 20 $\mu E\ m^{-2}\ s^{-1}$. Aeration: 0.55%, 800 mL/min. When all of the CO_2 removed is converted to biomass, the conversion ratio is 100%. The carbon content of the biomass is taken as 50%.

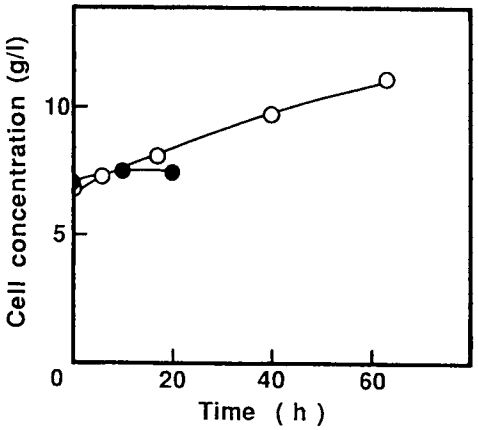

Fig. 4. Cell growth during filtration culture (—○—) and batch culture (—●—). Light intensity at the LDOF surface is 20 $\mu E\ m^{-2}\ s^{-1}$. The inlet gas contained 0.55% CO_2. During batch culture cell growth stopped after 15 h; the experiment was terminated after 63 h.

production processes. Additional technological improvements, such as constant cell density and product level monitoring, have been reported for many fermentation processes (*9–11*). However, most reactors that use membrane separation technology still suffer from a number of drawbacks (*12,13*), such as large permeability power requirement, large size, and complexity, which leads to increased cost. The external pressure ceramic membrane module used in this study is a simple and compact system that requires little power for successful medium filtration. Continuous filtration culture was carried out using the ceramic membrane module in order to maintain cell growth at high cell concentrations by continuously removing spent medium and resupplying fresh medium.

Cell growth during filtration culture and batch culture is shown in Fig. 4. When the medium was not filtered, cell growth stopped after 15 h.

However, when filtration was carried out at a flow rate of 70 mL/h and culture medium was exchanged, cell growth continued to increase for 63 h (the duration of the experiment), and the final cell concentration reached 11.2 g/L.

In this article, we have reported the construction of a new LDOF photobioreactor that has been used for the high-density culture of a marine cyanobacterium *Synechococcus* sp. An optimum CO_2 removal rate of 4.44 g/L/d was obtained, and an optimum biomass yield of 0.97 g/L was achieved for a 12-h culture time. Continuous culture, using a ceramic membrane module for medium filtration, led to sustained cyanobacterial growth to give high final cell densities. Bacterial fouling of the optical fibers was not found to be a problem.

The cyanobacterium used in this study produces high-value plant growth regulatory compounds used in the production of artificial seeds. The importance of this product in the field of plant biotechnology has led us to begin construction of a 30-L LDOF photobioreactor for large-scale biomass production. Such reactors could also have important applications in the field of controlled environment life-support systems (CELSS) currently being developed for CO_2 removal, waste recycling, and food production in space. The feasibility of large-scale CO_2 removal from power station and cement kiln exhaust gases is also currently under investigation. Thus, such reactors may be used to help solve environmental problems, as well as being used for industrial production of useful chemicals.

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