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An integrated system of solar light, artificial light and organic carbon supply for cyclic photoautotrophic-heterotrophic cultivation of photosynthetic cells under day–night cycles

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Abstract

An integrated system of solar light, artificial light and organic carbon supply was developed for cyclic photoautotrophic-heterotrophic cultivation of photosynthetic cells. The energy source for the culture is automatically switched to solar light energy (when the weather is sunny), to artificial light energy (during the cloudy period of the day) or to organic carbon source (at night). Thus minimum amount of artificial light is used while ensuring continuous light supply to the culture during the photoautotrophic phase. The α-tocopherol productivity by *Euglena gracilis* in this system was more than 5 times higher than the value obtained in pure photoautotrophic culture under the same experimental conditions.

Introduction

Although many photosynthetic cells have been extensively studied for production of various metabolites, only a very few of them are commercially cultivated. This is mainly due to the problems associated with the conventional cultivation systems and, to the low growth rates of these strains under photoautotrophic condition. For example, although sterilization is a major part of commercial biological processes using heterotrophic microorganisms, cultivation of photosynthetic cells is still done in open cultivation ponds where sterilization is almost impossible. Thus only the strains that grow under extreme selective conditions, such as high pH or high salinity, can be cultivated in such systems. Furthermore, due to rapid light attenuation in photosynthetic cell cultures, light distribution inside the current commercial systems is usually heterogeneous with inhibitory light intensity at the surface and little or no light at the bottom of the culture. Another major problem with the conventional systems utilizing solar light as the sole energy source is the diurnal changes in the light intensity and day–night cycles. Prolonged bad weather can lead to complete process failure while it has been reported that a high percentage of the biomass produced during the day are lost through respiration at night (Grobbelaar & Soeder 1985, Vonshak & Richmond 1988, Ogbonna & Tanaka 1996, Torzillo *et al.* 1991a,b). This problem can be overcome by using artificial light to supply light continuously to the culture. However, due to the very high cost of artificial light, only very few products can be commercially produced using only artificial light.

It is thus obvious that commercial cultivation of the numerous potentially useful strains requires novel cultivation systems that overcome the problems of the current systems. We have already developed a sterilizable tank type photobioreactor with homogeneous light distribution (Ogbonna *et al.* 1996) and have also developed an integrated solar and artificial light illumination system for continuous light supply to the photobioreactor (Ogbonna *et al.* 1999a). Aside from photoautotrophic growth, many photosynthetic cells can also grow

heterotrophically using various organic carbons or mixotrophically utilizing both light energy and organic carbon sources. Exploitation of heterotrophic metabolism in such photosynthetic cells is a promising method of developing commercial production systems for photosynthetic cells with heterotrophic metabolism. Examples of such systems include sequential heterotrophic-photoautotrophic (Takeyama *et al.* 1997, Ogbonna *et al.* 1999b), and cyclic photoautotrophic-heterotrophic (Ogbonna & Tanaka 1996, 1998) cultivation systems.

In this study, an integrated system of solar light, artificial light and organic carbon source supply was developed for cyclic photoautotrophic-heterotrophic cultivation of photosynthetic cells and its application for α-tocopherol production by *Euglena gracilis* was investigated.

Materials and methods

Integrated solar light, artificial light and organic carbon supply system

The integrated solar light, artificial light and organic carbon supply system is shown in Figure 1. The device used for collecting solar light and distributing it inside the reactor comprised two solar light collectors each with 12 fresnel lenses of 105 mm in diameter. The total illumination surface area for each solar light collector was 864 cm^2. Each solar light collector was covered with a transparent acryl dome that protects the lenses from dust and rain. The solar light collected by each lens was transmitted through an optical fiber (1 mm diam). The light collection device was equipped with light tracking sensor so that the lenses rotate with the position of the sun. About 38% of the collected photosynthetic active radiation were transmitted into the reactor and the rest were lost at the joints between the lenses and the optical fibers, and between the optical fibers and the light radiators. The solar light collection device is commercially available (Laforet Engineering and Information Service Co., Tokyo, Japan) under a trade name of Himawari. Six optical fibers (from six fresnel lenses) were made into a bundle and connected to a light radiator. Thus there were two light radiators per solar light collector and thus two solar light collectors for the 4-unit photobioreactor. The light radiators were cylindrically shaped and made of vertically etched transparent glass or quartz. The light intensity is almost uniform throughout the illumination surface of the light radiators.

A metal halide lamp was used as the artificial light source. Parabolic mirrors were used to produce parallel rays from the lamp which were then filtered and transmitted through the optical fibers to the light radiators inside the photobioreactor. The same light radiators were used for both the solar light and the metal halide lamp. The solar light collection, artificial light illumination and ethanol feeding systems were connected to a control box. When only the artificial light illumination system was used, the maximum light intensity on the surface of each light radiator was 380 μmol m^{-2} s^{-1} while on a very bright day, the solar light intensity on the surface of each light radiator was usually less than 450 μmol m^{-2} s^{-1}. During the day, a light intensity sensor monitors the solar light intensity and the artificial light is automatically switched on when the solar light intensity decreases below 50 μmol m^{-2} s^{-1} and off when the solar light intensity increases above the set value. The solar light intensity at which the artificial light source is switched on can be set as desired but the value was set at 50 μmol m^{-2} s^{-1} to minimize the usage of artificial light and also to avoid rapid on/off switch of the system. Setting the minimum light intensity at this value ensures that light intensity higher than 50 μmol m^{-2} s^{-1} is supplied to the reactor regardless of the prevailing weather condition during the day. At night, the peristaltic pump is turned on and ethanol is fed to the reactor at a predetermined rate.

Microorganism and media composition

Euglena gracilis IAM E-6 (strain Z), obtained from the algal collection of the Institute of Applied Microbiology, University of Tokyo, Japan, was used in this study. A modified Hutner medium described previously (Ogbonna *et al.* 1998) was used except that glucose was omitted and filter-sterilized ethanol was added only during the heterotrophic phase.

Cultivation condition

The stock culture (8 ml) was inoculated into 80 ml of the medium in a 100-ml Roux flask and cultivated under continuous illumination at 30 °C for 24 h. Seven daylight-fluorescent lamps for plant growth experiments (8FL-40-s-PG, National Electric Co. Ltd., Tokyo), arranged in parallel on a vertical plane, were used as the light source. The light intensity at the surface of the flask was 100 μmol m^{-2} s^{-1}. Aeration and mixing were achieved by sparging air enriched with

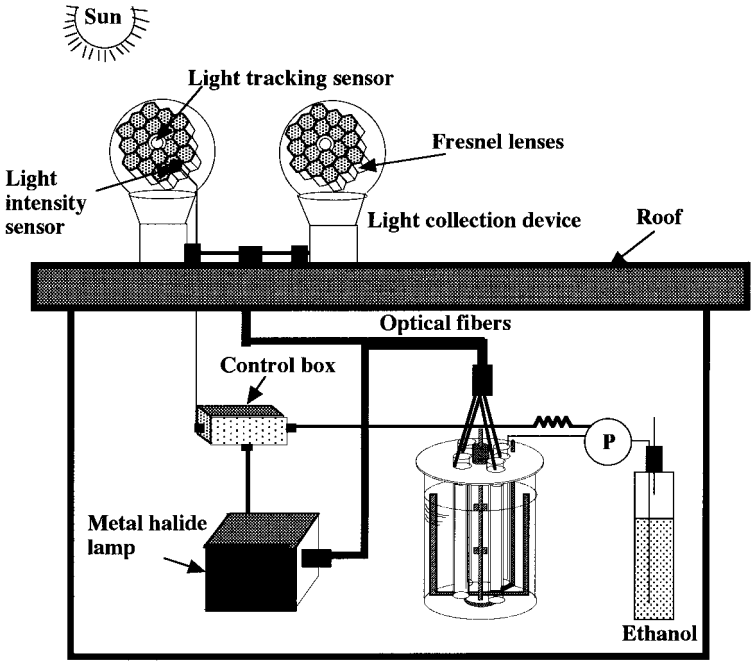

Fig. 1. An integrated system of solar light, artificial light and organic carbon supply for cyclic photoautotrophic-heterotrophic cultivation of photosynthetic cells.

5% CO_2 through a glass-ball filter, which was inserted to the bottom of the Roux flask, at 0.3 vvm.

A 4-unit internally illuminated photobioreactor was used for the cyclic photoautotrophic-heterotrophic cultivation. The concept and methods of constructing the photobioreactor have been described in our previous papers (Ogbonna *et al.* 1996, 1999a). The photobioreactor is sterilizable and equipped with a paddle type of impeller for efficient mixing and mass transfer. The working volume was 3 l and it was inoculated with 200 ml of the pre-culture broth. The agitation speed, aeration rate (with 5% CO_2 in air) and cultivation temperature were 120 rpm, 0.3 vvm and 30 °C, respectively. With the integrated system, the energy source changed automatically to solar light during the sunny period of the day, to artificial light during the cloudy period of the day and to ethanol at night. The rate of ethanol feeding was estimated from the predetermined specific ethanol uptake rate by the cells and the cell concentration at sunset. A specific ethanol uptake rate of 0.05 ml g cell^{-1} h^{-1} was used throughout the cultivation. For the cyclic photoautotropohic-heterotrophic cultivation using only the solar light for the photoautotrophic phase, the artificial light source was disconnected from the system and all other cultivation conditions were as described above. The same photobioreactor was used for photoautotrophic cultivation, using the integrated solar and artificial light illumination system. The experimental conditions were the same as described above except that ethanol was not added to the culture at night. All the experiments were conducted between June and August 1999 in the Agricultural Research Center, University of Tsukuba, Tsukuba City, Japan.

Analytical methods

Cell dry wt determinations were made according to the method described in our previous paper (Ogbonna & Tanaka 1996). Estimation of cell concentration at sunset was done by measuring the optical density (O.D.) at 680 nm (Spectronic 20A, Shimadzu Scientific Instruments, Japan). The O.D. values were converted to dry cell concentrations using predetermined calibration curves. The light intensities were measured by an analogue photometer (LI-185B, Licor, Nebraska, USA). The light intensity on the surface of the light radiators was taken at time intervals during the cultivation and the daily average values were plotted in Figures 2 and 3. The ethanol concentrations were determined by liquid chromatography (Nihon Bunko Model 860-CO). The α-tocopherol content of the cells

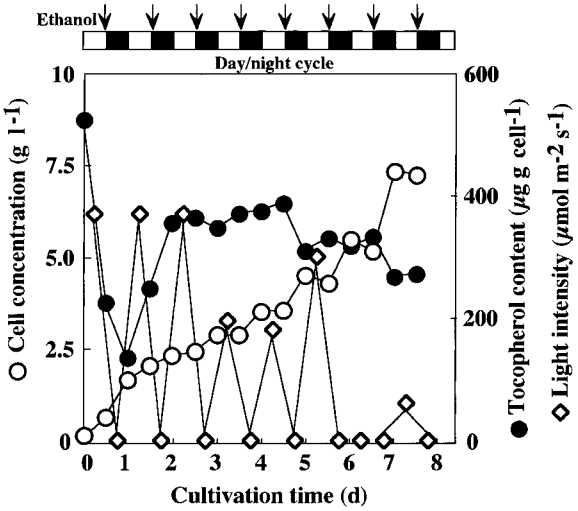

Fig. 2. Changes in cell concentration, cellular α-tocopherol contents and light intensity during cyclic photoautotrophic-heterotrophic cultivation of *Euglena gracilis*. Only solar light was used for the photoautotrophic phase while ethanol was used for the heterotrophic phase. The arrows indicate the start of the heterotrophic phase.

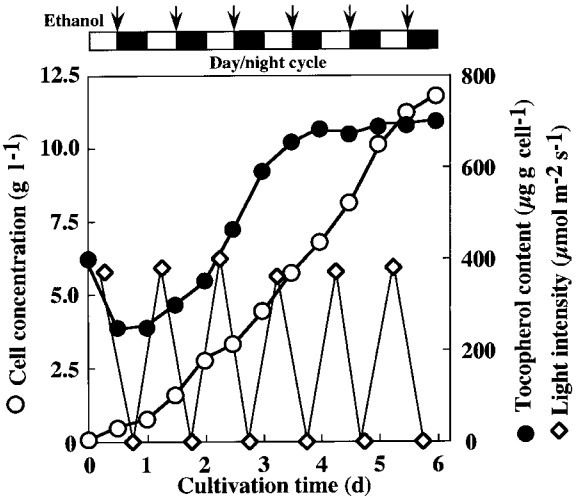

Fig. 3. Cyclic photoautotrophic-heterotrophic cultivation of *Euglena gracilis,* using an integrated system of solar and artificial light for the photoautotrophic phase and ethanol for the heterotrophic phase. The arrows indicate the start of the heterotrophic phase.

was determined from chloroform-methanol (1:2 v/v) extract as described by Shigeoka *et al.* (1986).

Results

The time courses of cell growth, cellular α-tocopherol contents and light intensity on the surface of the light radiators during the cyclic photoautotrophic-heterotrophic cultivation using only solar light for the photoautotrophic phase and ethanol for the heterotrophic phase are shown in Figure 2. Continuous cell growth was maintained only during the first 3 days. From the 4th day of the culture, the weather became cloudy so that the light intensity during the day was too low to support photoautotrophic cell growth. Thus the cells grew only heterotrophically during the night and even decreased during the photoautotrophic phase. The final cell concentration was 7.5 g l^{-1}. The α-tocopherol content of the cells decreased on the first day but increased on the second day and remained more or less stable from the second to the fourth day. From the fifth day, it decreased again due to cloudy weather.

By using the integrated system, the culture was illuminated by solar light during the sunny period of the day and the artificial light was automatically switched on during the cloudy period of the day while ethanol was added to the culture at night for the heterotrophic phase. Thus, light supply to the reactor was very stable throughout the culture. Consequently, as shown in Figure 3, the growth rate was almost the same during the heterotrophic and photoautotrophic phases. In comparison with the system employing only solar light during the day (Figure 2), high cell growth rate was still observed during the photoautotrophic phase even at high cell concentration (during the latter part of the culture). As a result of this, a very high cell concentration of 12 g l^{-1} was obtained in 6 days. The intracellular α-tocopherol concentration also decreased during the first day but started to increase from the second day and stabilized at about 700 μg g cell^{-1}.

Discussion

Development of photosynthetic cell cultivation systems where light is either substituted or supplemented by organic carbon sources can be used to solve some of the problems with the conventional photoautotrophic cultures. It has been reported that cyclic photoautotrophic-heterotrophic cultivation can be used to completely avoid night biomass loss. By adding carbon source to the culture at night, continuous cell growth was achieved under light/dark cycles and the growth rate was even higher than the value obtained under continuous illumination (Ogbonna & Tanaka 1998). However, the intracellular contents of many metabolites depend on the bal-

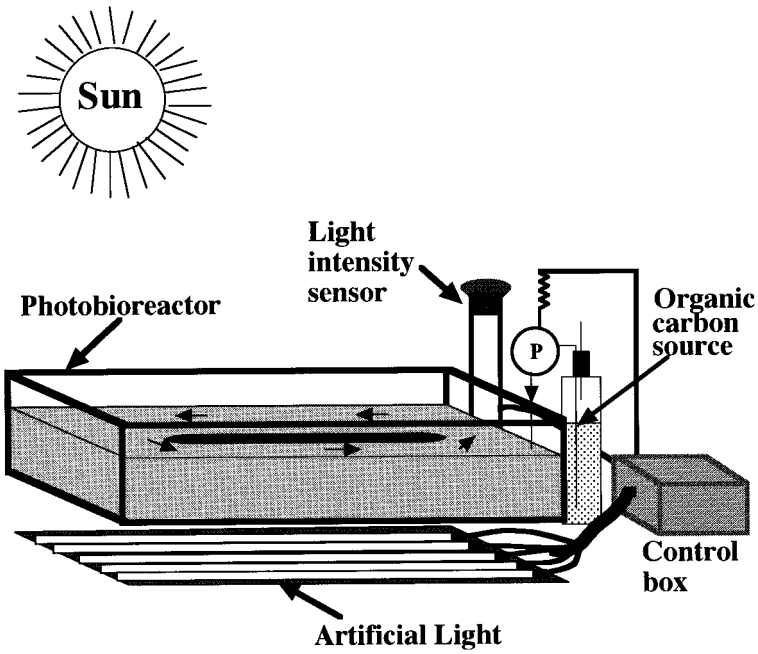

Fig. 4. A proposed low cost system for cyclic photoautotrophic-heterotrophic cultivation of photosynthetic cells, using solar and artificial light for continuous illumination during the photoautotrophic phase.

ance between the photoautotrophic and heterotrophic metabolic phases. The optimal balance between the photoautotrophic and heterotrophic phases depends on the cell strain and the product. As shown in Figure 2, when only solar light was used for the cyclic photoautotrophic-heterotrophic culture, the α-tocopherol content of the cells decreased when the weather became very cloudy. A higher cell concentration could be obtained by prolonging the cultivation time regardless of the weather condition. However, without adequate sunlight during the photoautotrophic phase, prolonging the cultivation would only result in a high biomass concentration with very low intracellular α-tocopherol contents. Thus, to maintain a stable cyclic photoautotrophic-heterotrophic culture with high intracellular α-tocopherol contents, each heterotrophic growth phase should be followed by a photoautotrophic phase under sufficient light intensity. By using the integrated solar light, artificial light and organic carbon source, the duration of the photoautotrophic and heterotrophic phases can be manipulated as desired. Thus, this system has very high potential for production of various useful metabolites using various strains of photosynthetic cells.

Estimation of the production costs based on the energy (light energy and organic carbon) input for cell growth and product synthesis is not reliable because the relative amount of artificial light used depends on the weather condition. On sunny days, the artificial light may not be used at all while on rainy or cloudy days, the artificial light becomes the major source of energy during the photoautotrophic phase. With the same reactor and the same integrated solar and artificial light system, the α-tocopherol productivity obtained in pure photoautotrophic culture was only about 11 μg l^{-1} h^{-1} with a final α-tocopherol concentration of 1.58 mg l^{-1} due to low cell growth rate and night biomass loss. However, by adding ethanol to the culture at night, the productivity increased to 58.3 μg l^{-1} h^{-1} with an α-tocopherol concentration of 8.2 mg l^{-1}. The total amount of ethanol added during the whole 6 days of cultivation was only 17.6 ml 98% ethanol. This increase in α-tocopherol productivity cannot be explained by α-tocopherol synthesis from the supplied ethanol. Ethanol stimulates cell growth and the increased biomass concentration improves light utilization efficiency and thus more tocopherol synthesis. The productivity obtained with this system compares well with the value obtained by sequential heterotrophic-photoautotrophic cultivation (61.9 μg l^{-1} h^{-1}) (Ogbonna *et al.* 1999b) but in the later system, artificial light was used for continuous illumination during the photoautotrophic phase. A major advantage of the system developed in this study is

that artificial light is used only when the solar light intensity decreases below a desired level.

The present system employing solar light collection device and optical fibers is too expensive for commercial production of most metabolites. The current market prices for most algal products do not justify the use of this type of system for their commercial production because of the high investment costs. This system was used only to demonstrate the feasibility of using the three energy sources – solar light, artificial light and organic carbon source for cultivation of photosynthetic cells. Commercial application of this concept requires modification of the system so that the solar light can be used directly. An example of such modification is shown in Figure 4. Either closed horizontal panels or tubular photobioreactors can be used. The solar light illumination is from the top while artificial light source is located below so that it does not shade the culture from solar light.

An anticipated major problem with this system at a large scale is contamination by fast-growing heterotrophs. This problem can be reduced by maintaining a high cell concentration, using selective organic carbon source that are not easily metabolized by potential contaminants, controlling the feed rate of the organic carbon source, adding selective antibiotics intermittently or using a closed system that can be maintained under strict sterile condition. The cyclic photoautotrophic-heterotrophic cultivation system can be operated at much higher cell concentrations than the current commercial systems so that relatively smaller and sterilizable photobioreactors can be used for commercial production.

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