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# Production of Ultrafine Calcite Particles by Coccolithophorid Algae Grown in a Biosolar Reactor Supplied with Sunlight

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## ABSTRACT

Ultrafine calcite particle production by coccolithophorid algae using a biosolar reactor system was carried out. Solar light was collected by Fresnel lenses and transmitted to a bundle of light diffusing optical fibers (LDOFs) that distributed light through the algal culture. The irradiance spectrum of light emitted from the LDOF surface was the same as that of solar light. *Emiliania huxleyi* was cultured for 8 d, and 43 mg/L of calcite were produced. These results demonstrate the potential of the biosolar reactor system for $CO_2$ fixation, and ultrafine calcite particle production using coccolithophorid algae and sunlight.

**Index Entries:** Coccolith; calcite; ultrafine particle; biosolar-reactor; solar collector.

## INTRODUCTION

Microalgae use solar energy to convert $CO_2$ to organic cellular components. However, organic compounds decompose, and $CO_2$ is released back into the atmosphere. When photosynthetic $CO_2$ fixation occurs, the

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relative amount of $CO_2$ in the atmosphere decreases. However, this does not irreversibly lower the atmospheric $CO_2$ content. If $CO_2$ is mineralized in the form of carbonate, it is fixed permanently. Calcareous algae, which produce $CaCO_3$, play an important role in $CO_2$ absorption from the atmosphere (*1*). Calcareous algae are capable of $CO_2$ fixation by both photosynthesis and calcification. Thus, calcareous algae are expected to be good candidates for efficient biological $CO_2$ removal technology.

Coccolithophorid algae are unicellular marine calcareous phototrophs that produce coccoliths and form calcified scales around the cells. The form of coccoliths produced by each species is distinctive. These particles are biocompatible composite materials suitable for a wide range of technically and medically important applications. Most studies on coccolithophorid algae have been concerned with the mechanism of coccolith formation, and the relationship between photosynthesis and calcification (*2–5*). Several papers describe the physiology of calcite mineralization, and characterize the relationship between cell cycle and a calcite formation (*6–8*). However, they do not include information about calcite yield and calcite synthesis rate. On the other hand, the experiments described here represent a biotechnological study that provides calcite yields and production rates from the perspective of applied biology rather than physiology. This is the first description of calcite production for biotechnological application. Also presented is the first information on calcite yields by *Emiliania huxleyi* and *Pleurochrysis carterae*.

The authors have carried out calcite ultrafine particle production from $CO_2$ using a biosolar reactor system. The authors have constructed a culture system consisting of a closed photobioreactor unit containing a bundle of light diffusing optical fibers (LDOFs), which has supplied light to the algae (*9,10*), the light collecting system (*11,12*), and light transmission apparatus that have supplied light to the LDOFs. The high-density culture of a marine cyanobacterium using this photobioreactor has previously been reported (*9,10*). The light collector, called the "Himawari" (*11,12*), employs Fresnel lenses (*11*). The chromatic aberration of these lenses can remove much of the infrared and UV light. The collected light was transmitted to LDOFs using fiberoptic cables and distributed throughout the reactor. Therefore, efficient utilization of solar energy has been achieved. This article describes the production of calcite ultrafine particles employing the biosolar reactor system supplied with sunlight.

## MATERIALS AND METHODS

### Strains and Culture Conditions

Coccolithophorid algae *Emiliania huxleyi* 92D and *Pleurochrysis carterae* 136 were obtained from E. W. de Jong (University of Leiden). Cells were grown in Eppley's medium (*13*) containing 50.5 mg $KNO_3$, 8.7 mg

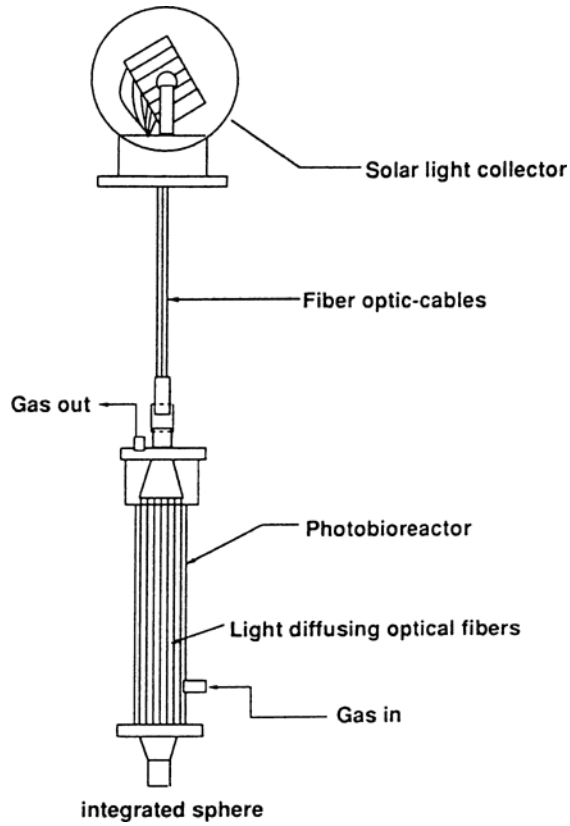


Fig. 1. Schematic diagram of a biosolar reactor system. The solar collector is composed of 36 hexagonal Fresnel lenses. The collected sunlight was transmitted to the photobioreactor using the PCS fiberoptic cables. The photobioreactor has a culture volume of 2.2 L. Vertical lines represent fibers (661 fibers in total).

$K_2HPO_4$, 1 mL trace elements, and 1 mL vitamin solution in 900 mL sea water and 100 mL distilled water. The trace element solution contained 19.6 mg $CuSO_45H_2O$, 44.0 mg $ZnSO_4$ $7H_2O$, 20.0 mg $CoCl_26H_2O$, 360 mg $MnCl_24H_2O$, 12.6 mg $Na_2MoO_4 \cdot 2H_2O$, and 10 g Fe-EDTA in 1 L distilled water. The vitamin solution contained 200 mg thiamine, 1 mg biotin, and 0.2 mg cobamide. Cells were inoculated into 1 L Eppley's medium in flat glass bottles and bubbled with air at a flow rate of 300 mL $min^{-1}$ under continuous illumination from fluorescent lamps (FL20SD-SDL, Toshiba Electric Co., Ltd., Tokyo, Japan). *E. huxleyi* and *P. carterae* were cultivated at optimum light intensities of 110 $\mu E\ m^{-2}\ s-1$ and 140 $\mu E\ m^{-2}\ s^{-1}$, respectively.

## Construction of a Biosolar Reactor System

Figure 1 shows a schematic diagram of the biosolar reactor system that was constructed from a solar collector to collect and concentrate sunlight, an optic fiber cable to transmit collected light, and a photobioreactor

employing LDOFs. A solar collector, Himawari XD-100/36, is composed of 36 hexagonal Fresnel lenses ($\phi$ 105 mm) and has 2577 $cm^2$ of light receiving area. Each Fresnel lens concentrates sunlight by a factor of 10,000. Almost all the UV light was eliminated because of chromatic aberration of the Fresnel lenses. Infrared light is cut by 60% (*11*). The collected light was transmitted to the photobioreactor using 36 large core polymer clad silica (PCS) optical fibers (1.0 mm in diameter, 25 mm in length; Asahi Glass Co., Ltd., Tokyo, Japan). These fibers were made up of specially developed fluorosilicon cladding and a pure silicon core. The ends of the PCS fibers were bundled up and connected with the bundled end of LDOFs (Fig. 1). Remaining infrared radiation in the transmitted sunlight was removed by an optical filter (type CS4-104, Corning, NY).

A photobioreactor employing LDOFs (STI, Tokyo, Japan) (*8–9*) was operated at a culture volume of 2.2 L and at temperature of 22°C. Air was supplied at a flow rate of 500 mL/min. The $CO_2$ concentration was in the range of 360–400 ppm. The pH of the culture suspension was monitored continuously.

## Light Intensity and Spectral Analysis of Collected Sunlight Emitted from the LDOF

The light intensity at the LDOF surface, which indicated quantum photon flux ($\mu E\ m^{-2}\ s^{-1}$), was determined as follows. Luminous flux of light emitted through the LDOFs was measured using an integrating sphere (Optel Co., Ltd., Tokyo, Japan). The integrating sphere has a respective conversion coefficient, K, that is measured by a calibration curve that converts lux (lx) to lumen (lm). The conversion coefficient K was 0.003686925 where the intensity (measured) × K = luminous flux. A quantum meter (LI-185B, LI-COR, Inc., Nebraska) and a photometer (T-1M, Minolta Co., Ltd., Tokyo, Japan) were used for measuring diffused light from the surface of the LDOFs. Light measurements were taken at ten points and averaged. The luminous flux of the diffused light was calculated from the averaged light intensity and total surface area of the LDOFs (1.2453 $m^2$). As a result, 80% of the total luminous flux entering the LDOF bundle passed into the fibers, and 20% was lost. Forty-one percent of the total luminous flux diffused out from the fiber surface, and 39% was emitted through the end of the LDOF. Therefore, the quantity of light passing through the LDOFs is approximately equal to light diffused from the LDOF surface. The luminous flux of emitted light was monitored using an integrating sphere. The light intensity of the LDOF surface (lux) was calculated from the luminous flux and the LDOF surface area, and converted to quantum flux density ($\mu E\ m^{-2}\ s^{-1}$) by means of the conversion coefficient $1.5 \times 10^{-2}\ \mu E\ m^{-2}\ s^{-1}$/ lx measured by calibration curve, which converted lux (lx) to quantum flux density ($\mu E\ m^{-2}\ s^{-1}$). The light spectrum was measured using a multipurpose spectroradiometer MSR-7000 (Optical Science Co., Ltd., Tokyo, Japan).

Table 1
Comparison of Calcite Content and Specific Growth Rate Between *Emiliania huxleyi* and *Pleurochrysis carterae*

| | Calcite content[a] %, weight | Maximum specific growth rate, $h^{-1}$ |
|---|---|---|
| *E. huxleyi* | 37 | 0.058 |
| *P. carterae* | 18 | 0.019 |

[a]Cells of late log phase were used in the measurement of coccolith content. Cells were cultured at 20°C under continuous light. Light intensity: *P carterae* 140 $\mu E\ m^{-2}\ s^{-1}$ *E. huxleyi* 110 $\mu E\ m^{-2}\ s^{-1}$.

### Measurement of Calcite Production

The calcite production was determined as follows. The culture suspension was collected two times in a day. The suspension (10 mL) was suction filtered through a glass-fiber membrane filter (GF/C, Whatman, England, UK), and 50 m*M* $NaHCO_3$ were run through the filter to wash the cells. Then 10 mL 1*N* HCl were run through to dissolve $CaCO_3$ coccoliths. The amount of calcium in the resulting solution was measured by modified o-CPC colorimetric analysis. The amount of calcite in the culture suspension was calculated.

## RESULTS

### Comparison of Calcite Content

Calcite content and maximum specific growth rate of *E. huxleyi* and *P. carterae* were examined (Table 1). The maximum specific growth rate of *E. huxleyi* was 1.5-fold that of *P. carterae*. The calcite content of *E. huxleyi* was twofold that of *P. carterae*. Therefore, *E. huxleyi* was employed for biosolar reactor experiments.

### Collected Sunlight Emitted from the LDOF

Transmitted solar light was filtered to remove the infrared radiation and passed into the LDOFs. The irradiance spectrum of transmitted solar light, which had been filtered, was very similar to that of light emitted from the LDOFs surface (Fig. 2). The biosolar reactor system was run for 8 d. The irradiance profile is shown in Fig. 3. During the experimental period, 5 d were sunny, and 3 d were cloudy. The time for which light diffused from the LDOF surfaces was 7–10 h/d. Maximum light intensity was 8.2 $\mu E\ m^{-2}\ s^{-1}$ on the first day.

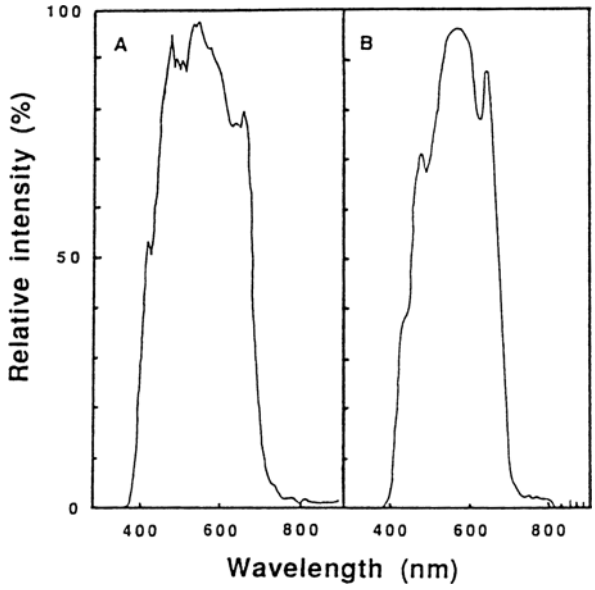


Fig. 2. Spectral irradiance of collected sunlight (A) and diffused sunlight emitted from the surface of the LDOFs (B). Collected sunlight was filtered to cut infrared radiation.

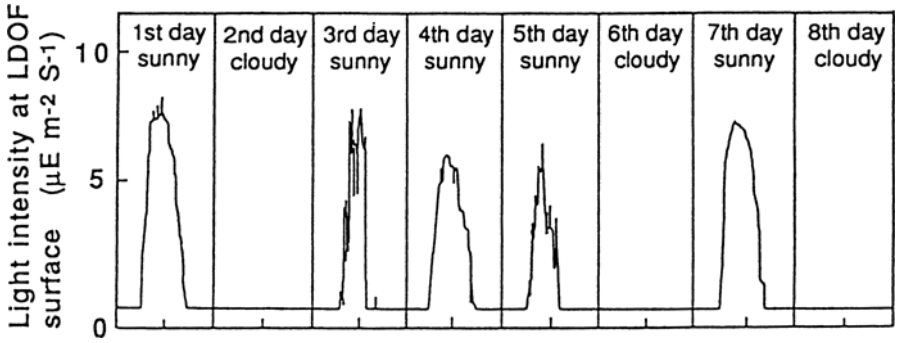


Fig. 3. Daily light intensity levels measured at the fiber surface over an 8-d culture period.

## Calcite Production Using Sunlight

Calcite production by *E. huxleyi* is shown in Fig. 4. Two hundred milliliters of late exponential *E. huxleyi* cell suspension ($4 \times 10^7$ cells/mL) were used to inoculate the bioxolar reactor. Calcite production was monitored for 8 d, after which the culture reached stationary phase. Calcite production took place during periods of illumination and occurred in a stepwise fashion on sunny days. During the third, fourth, fifth, and seventh days, the calcite concentration increased by approx 10 mg/L. The final calcite concentration reached 43 mg/L on the eighth day. Reduction in cell numbers did not occur on cloudy days (data not shown).

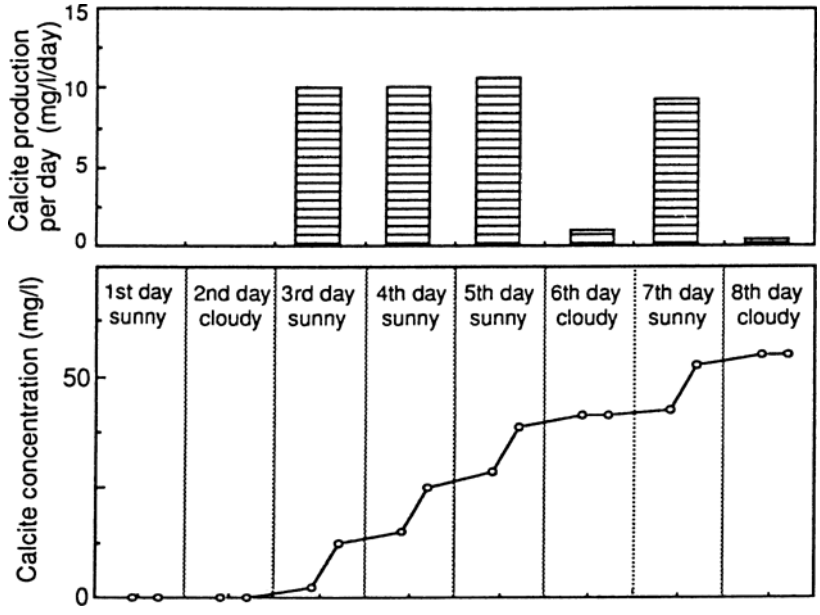


Fig. 4. Time-course of calcite production by *E. huxleyi* using a biosolar reactor system. Air was supplied to the culture at a flow rate of 500 mL/min. The $CO_2$ concentration of supplied air was in the range of 360–400 ppm. The temperature of a culture suspension was maintained at 22°C.

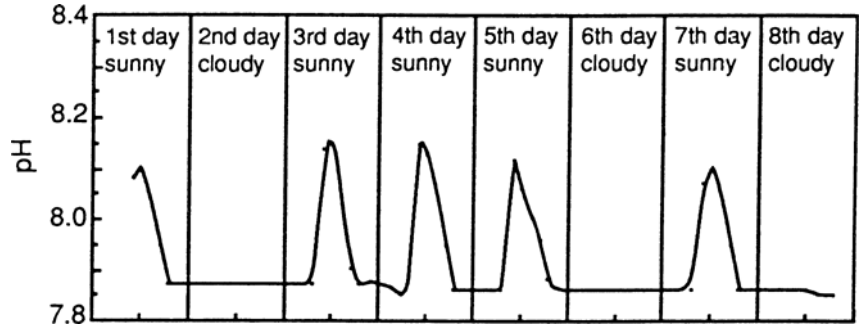


Fig. 5. pH profile of the *E. huxleyi* culture suspension over an 8-d experiment.

The pH of the culture was monitored during calcite deposition (Fig. 5). During periods of sunny weather, the pH increased from a base level of 7.9 up to 8.1. The pH increased in a stepwise fashion on each day.

## DISCUSSION

One aim of algal biotechnology is solar energy conversion. A solar conversion system, in which culture conditions can be optimized, has

been constructed. This system has been used to grow the coccolith-producing marine alga *E. huxleyi*, which contains up to 37% (dry wt.) of calcite.

The three major components required for solar conversion are: an algal bioreactor, a solar collector, and light energy transmission apparatus. Initially, an efficient algal photobioreactor was developed for optimum solar energy utilization, which had a large surface-area-to-volume ratio (692 $m^{-1}$) (*9*). High-density culture of cyanobacteria using the LDOF photobioreactor and an artificial light source has previously been reported (*10*). In order to reduce the electric power required for such a process, a system that collects solar light and supplies it to the photobioreactor is required. A novel solar collector "Himawari" was used in this research. The solar collector is composed of Fresnel lenses, which concentrate sunlight by a factor of 10,000. Maximum light output of 5000 lm was achieved in the case of direct outdoor illumination of 72,000 lx. Collected solar light was transmitted to the reactor using PCS optical fiber cables. The main advantage of this system is that the light transmitted to the algal culture has greatly reduced UV and infrared radiation. Thus, UV-induced cell damage and inhibition of photosynthesis are minimized. In addition, overheating of the reactor in sunny weather is prevented. For this article, batch culture of the coccolithophorid alga *E. huxleyi* was carried out using the biosolar reactor system over a period of 8 d (March 31–April 7, 1992). The optimum light intensity at the LDOF surface for calcite production was 7 $\mu E\ m^{-2}\ s^{-1}$ when artificial light from a Xenon lamp was used (data not shown). In this experiment, the maximum light intensity at the LDOF surface achieved was 8.2 $\mu E\ m^{-1}\ s^{-1}$. Daily sunlight was 7–10 h. The optimum light conditions for calcite production and cell growth of *E. huxleyi* are a 16-h light and 8-h light-dark cycle (data not shown). In spite of the shorter light period, cell growth and calcite production occurred. The overall calcite yield was 43 mg/L. The biosolar reactor therefore provides favorable light distribution and minimal mechanical damage, conditions in which *E. huxleyi* can thrive.

Coccolithophorid algae fix $CO_2$ in the form of inorganic $CaCO_3$. Algal calcification has been reported to increase $CO_2$ emission from the ocean (*15*). The Deffeyes composition diagram (*15*) shows that during $CaCO_3$ formation in oceanic environments, the pH of sea water decreases. $CO_2$ is then emitted from sea water depending on the equilibrium between carbonate species. In the case of coccolithophorid algae, calcification occurs concomitantly with photosynthesis, and a pH drop does not occur. Therefore, there is no enhanced $CO_2$ emission. In batch culture of *E. huxleyi* using the biosolar reactor system, pH of the culture suspension increased during calcite formation as a result of photosynthesis comparing Figs. 3, 4, and 5. Production of calcite ultrafine particles during algal calcification may therefore provide an effective way to reduce $CO_2$ emission caused by burning fossil fuels. In addition, calcite ultrafine particles are a valuable biotechnological resource for high-technology applications.

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