EFFECT OF CO₂ CONCENTRATION ON GROWTH AND CARBON FIXATION RATE OF *PLEUROCHRYSIS CARTERAE*

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Introduction

In considering the accumulation of carbon dioxide in the global atmosphere, the contribution of ocean microalgae is important. The algae fix carbon and transfer it to the bottom of the ocean through sedimentation. Most of the carbon balances so far proposed ignore the contribution of living creatures. In explaining the missing sink of carbon, it is necessary to consider the microalgal contribution.

Behavior of calcareous algae must be investigated in order to take account of the biological contribution to the global carbonic circulation since largest amounts of accumulants on the bottom of the ocean are calcium carbonate originated from cocolithophores of calcareous algae. We have investigated carbon fixation by *Pleurochrysis carterae*, particularly its growth and carbon-fixation characteristics. Thus, this report describes the behavior of the calcareous alga affected by nutrient concentration in the medium and the scheme of proliferation due to photosynthesis.

1. Experimental

1.1 Materials

The alga studied was *Pleurochrysis* aff. *carterae* CCAP 961/2 (syn. ; *Cricosphaera* aff. *carterae*). A GUI medium¹⁾ was used to preserve the culture. The cell culture was transferred to a new solution of medium every 20 days. Seawater from the ocean was filtered with a membrane with 0.45 mm-diameter pores. Some experiments were carried out with a modified GUI medium where concentration of NaNO₃ was limited to 20 percent of that in the GUI medium.

1.2 Experimental apparatus

The experimental apparatus is shown in Fig. 1. Air with a known CO₂ concentration was humidified and sparged into the medium from a glass-ball filter at 100 cm³/min. The reaction vessel was 84 mm in diameter and its

volume was ca. 450 cm³. The pH was adjusted by 0.2 N HCl or NaOH. Four 20 W white fluorescent lights were installed on both sides of the vessel so that the light intensity was 8000 lux on its outer surface. Irradiation with light alternated with a period of darkness every 12 hours. The whole apparatus was set in a thermostat chamber at 291 K.

1.3 Analysis

Cell concentration was determined with a Burkert-Turk hemacytometer after adding a drop of 25% glutaraldehyde aqueous solution to the medium. To determine calcium content in the cells, the cells were collected with a membrane filter from Milipore, 0.22 μm pore diameter, then washed with a Ca-free solution. Calcium carbonate was dissolved by contact with 1N HCl solution for 16 h. The mass of Ca was determined with an atomic absorption analyzer, Hitachi 170-10 model.

Total and inorganic carbon content (TC and IC, respectively) in the algal cells were determined with a TOC analyzer, Shimadzu TOC-500 model, after centrifuging the medium to obtain the cells, followed by washing with filtered seawater. IC was also estimated with a calibration curve for IC and Ca content, which was analyzed using an atomic absorption spectrophotometer, Hitachi 170-10 model. Organic carbon content (OC) was determined by (TC - IC). Errors in the analysis were less than 10 %.

CO₂ concentration in the gas phase was determined with gas chromatography using a Porapak Q. CO₂ concentration in the outlet gas from the incubator fluctuated within 10 %. A photograph of the cells was taken with a scanning electron microscope, JSM-840 from Jasco, Tokyo, at 5000-times magnification after sputtering with Pt ion using a JFC-1100.

1.4 Culture procedure

Here, we studied semi-continuous culture for CO₂ concentrations of 3.5×10^2 ppm, 7.0×10^2 ppm and 2.0×10^3 ppm in the gas phase, and determined carbon fixation rates into the organic and inorganic compounds. Cells which had been cultured in GUI medium for 20 days were transferred and cultured in the modified GUI medium for 7 days. Then, the cells were subjected to semi-continuous

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Fig. 1 Schematic diagram of the experimental apparatus

runs with the modified GUI medium. The medium was exchanged once everyday at exchange ratios of 0.310, 0.405, 0.511, 0.629. The specific growth (reproduction) rate μ was calculated by the equation (1)².

$$\mu = -\ln(1 - E) / t \quad (1)$$

where t is 1 day.

The semi-continuous culture continued until the cell concentration in the medium reached steady state. The pH of the medium were adjusted to 8.1 at 3.5×10^2 ppm- CO_2 , 7.8 at 7.0×10^2 ppm- CO_2 and 7.4 at 2.0×10^3 ppm- CO_2 , respectively. These pH values are the equilibrium values in seawater for the corresponding atmospheric CO_2 concentrations.

2. Results and Discussion

2.1 Specific growth (reproduction) rate

The specific growth (reproduction) rate was determined from the semi-continuous culture under steady state conditions. In Fig. 2, the specific growth rate is plotted as a function of nitrogen content in the cell, Q . The latter was obtained from nitrogen concentration in the feed medium divided by the cell concentration in the medium. This relation was obtained assuming that nitrogen is the limiting nutrient. In this case, the fed nitrogen is approximated to be taken totally into the cells, because more than 98 % of the fed nitrogen was consumed by the cells.

Figure 2 shows that the specific growth rate μ can be expressed by the Droop equation, Eq. (2), as a function of intracellular nitrate concentration.

$$\mu = \mu_m (1 - Q_0 / Q) \quad (2)$$

Here, μ_m denotes the maximum specific growth rate. Q and Q_0 are nitrate content in the cell and the minimum nitrate requirement in the cells, respectively. The same correlation was found to hold for the gas phase CO_2 concentration of 3.5×10^2 and 2.0×10^3 ppm. In this analysis identical values for Q_0 and μ_m , which were obtained in the previous study²⁾, were assigned for both cases assuming that the cells had the same physiological properties.

2.2 Effect of CO_2 concentration on carbon fixation rate

The carbon fixation rate was investigated in relation to nitrate concentration in the medium, S . The relation

Fig. 2 Relation between specific growth rate and nitrate content in a cell

Fig. 3 Effect of nitrate concentration in culture media on carbon fixation rate into organic compounds

between S and the specific growth rate of the alga was formulated as a Monod-type equation in the previous study²⁾. The organic carbon content decreased with increasing nitrate concentration in the medium until $S = 17$ mg/L. Above this S value, the organic carbon content was constant. There was no difference in the organic carbon content for variations of CO_2 concentration in the gas.

The inorganic carbon content did not change with S for CO_2 concentrations of 3.5×10^2 and 7.0×10^2 ppm. However, it became lower for 2.0×10^3 ppm, particularly at high nitrate concentrations in the medium. As a result of those dependencies on CO_2 concentration, the ratio of inorganic carbon to organic carbon increased with S for the cases of 3.5×10^2 and 7.0×10^2 ppm. However, for the case of 2.0×10^3 ppm, the ratio decreased because inorganic carbon fixation decreased. The value was about 0.05 and we can see that there is 20 times more organic carbon than inorganic carbon.

The carbon fixation rate into organic compounds per cell is plotted against S in Fig. 3. The rate increased with increasing S . No difference due to the gas phase CO_2 concentration was observed.

A similar relation for the carbon fixation rate into

Fig. 4 Effect of nitrate concentration in culture media on carbon fixation rate into inorganic compounds

inorganic compounds per cell is shown in Fig. 4. It increased with the increase of S for 3.5×10^2 and 7.0×10^2 ppm. However, it was not affected by S in the case of 2.0×10^3 ppm. This may be caused by the fact that the alga could uptake larger amounts of CO_2 directly from the medium at 2.0×10^3 ppm, compared to 3.5×10^2 ppm, as a substrate for photosynthesis instead of utilizing CO_2 evolved with calcium carbonate formation of Eq. (3)³⁾.

The effect of low pH at 2.0×10^3 ppm of CO_2 on the carbon fixation rate is the subject of future study.

The number of coccoliths per cell is shown in Table 1. Although the numbers may include considerable counting errors, we can see that the number of coccoliths is low at 2.0×10^3 ppm of gas phase CO_2 concentration and at high specific growth rate.

2.3 Estimation of carbon fixation rate by calcareous algae in the ocean

Here, we will discuss in general terms the fixation of CO_2 by calcareous algae in the ocean. In the ocean, other species of calcareous algae such as *Emiliania* sp. also exist, in numbers similar to *Pleurochrysis* sp. We assume they behave in the same way regarding carbon fixation. The depth of the ocean surface where the sunlight reaches is said to be about 100 m^2 . In this region the average concentration of calcareous algae is estimated at about $5 \times 10^3 \text{ cell/L}$. The average nitrate concentration in the open sea is about $1.5 \times 10^{-5} \text{ g/L}$. From these data and our experimental results mentioned above, the total carbon content per cell is calculated to be $8 \times 10^{-11} \text{ g/cell}$ and the inorganic carbon content is calculated at $5 \times 10^{-12} \text{ g/cell}$ for a CO_2 concentration of 3.5×10^2 and 7.0×10^2 ppm. This means total carbon fixation by calcareous algae is about $4 \times 10^9 \text{ Mg/y}$ and carbon fixation into inorganic compounds is $2.3 \times 10^8 \text{ Mg/y}$. For the higher CO_2 concentration at 2.0×10^3 ppm, carbon fixation into inorganic compounds decreased to about half of the above. These estimations²⁾ roughly agree with the order of the missing sink as total carbon, $(10-20) \times 10^8 \text{ Mg/y}$.

There are many discussions regarding the contribution of calcareous algae to the global environment. This

Table 1 Number of coccoliths per a cell

Specific growth rate [d ⁻¹]	Number of coccoliths [cell ⁻¹]	
	350 ppm	2000 ppm
0.310	151	112
0.405	147	146
0.511	121	48

problem is similar to that regarding the contribution of coral reefs. Probably more works should be done to make the discussion clearer on such items as the quantitative analysis of oceanic circulation, the effect of temperature distribution and the light intensity profile in the ocean.

Conclusion

The behavior of *Pleurochrysis carterae* was investigated with regard to organic and inorganic carbon fixation at different CO_2 concentrations in the air. The specific growth rate was expressed by the Droop equation and the effect of CO_2 concentration in the air was not observed. Organic carbon content in the cell was not affected by CO_2 concentration, but inorganic carbon content decreased at high nitrate concentration in a culture medium and at a CO_2 concentration of 2.0×10^3 ppm. Thus, the ratio of inorganic to organic carbon content in the cells became small at high nitrate concentration and at high CO_2 concentration.

The carbon fixation rate of organic and inorganic compounds increased with an increase of nitrate in the medium but at CO_2 concentrations of 2.0×10^3 ppm in the air, the carbon fixation rate into inorganic compounds did not change with nitrate concentration. The number of coccoliths was small at high specific growth rate and at 2.0×10^3 ppm CO_2 .

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Nomenclature

E	= medium exchange ratio	[-]
Q	= nitrate content in the cell	[g/cell]
Q_0	= minimum cell quota of nitrogen	[g/cell]
S	= nitrate concentration in culture medium	[mg/L]
t	= medium exchange interval	[d]
μ	= specific growth rate	[d ⁻¹]
μ_m	= maximum specific growth rate	[d ⁻¹]

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