定濁度連続培養によるChaetoceros debilisの日周期性(2)

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Diurnal Periodicity of the Marine Diatom *Chaetoceros debilis* in Turbidometric Continuous Culture

II. Chlorophyll *a*, Carbon, and Adenosine Triphosphate in the Cell[1),2)]

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Abstract

Diurnal periodicity of chlorophyll *a*, carbon, and adenosine triphosphate (ATP) in *Chaetoceros debilis* cultured with the turbidometric continuous culture apparatus was observed at the interval of two hours throughout four days period. The fluctuations of these components were discussed with sample water volume, one cell, and cell volume basis. In company with the growth stage of cells in the day, different trends were observed with these respective units.

The amounts of these components per cell volume indicated their tendency to increase in the light or alternatively to decrease in dark period. Contrary, an adverse pattern was obtained in the quantities per one cell. The diurnal periodicity was thus well coincided with the process of growth and division of cells. Among three components, chlorophyll *a* revealed the most remarkable diurnal periodicity.

Diurnal periodicity of phytoplankton, from ecological and physiological viewpoints, has been described in a considerable number of papers. Many different results were obtained especially on the diurnal periodicity of chlorophyll through laboratory and field observations. However, only a few studies have been carried out using continuous culture of phytoplankton. PAASCHE (1967, 1968) reported the results from experiments of the diurnal periodicity of cell division and chlorophyll content by employing a semi-continuous culture.

UNO (1971) has found the diurnal periodicity of chlorophyll, carbon, and photosynthetic activities, in diatoms *Phaeodactylum tricornutum* and *Skeletonema costatum*, cultured with a turbidostat. Using *Chaetoceros debilis* as material, the authors re-examined the diurnal periodicity of cellular components such as chlorophyll *a*, phaeopigments, carbon, nitrogen, and adenosine triphosphate by utilizing an improved turbidostat, which has more similarity to natural condition as compared with the previous apparatus.

Materials and Methods

The culture apparatus used in the present study was a turbidometric type which was improved from the previous one (UNO 1971). The details of construction and the environmental con-

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[2)] 定濁度連続培養による *Chaetoceros debilis* の日周期性. II. 細胞中のクロロフィル *a*, 炭素およびアデノシン三燐酸

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ditions for culturing diatom were described in the preceding paper (UENO & UNO 1980). Both light-dark cycle and change of light intensity could be set up by controlling the time processor of the apparatus.

Samples for chemical analyses were pipetted from the outlet neck of the chamber at every two hours interval throughout four days. Particulate samples were then filtered through Whatman GF/C glass fiber filters (25 mm in diameter), and stored in a deep freezer in the dark prior to analyses. Samples of 50–100 ml were filtered for particulate carbon and nitrogen, 20 ml for ATP, and 10–20 ml for chlorophyll *a* and phaeopigments.

Particulate carbon and nitrogen were measured with a CHN analyzer (Yanagimoto CHN Corder MT-2). The glass fiber filters only for carbon and nitrogen analyses were preliminary ignitioned at 450°C for more than one hour by electric furnace. Furthermore, the filter and adsorption blanks were deducted to correct errors that might be introduced during the course of filtering (UNO 1976). ATP was extracted from particulate samples collected on the filter paper, which was then put into 5 ml of 0.3% tris-buffer solution and heated at 100°C for 5 minutes. After cooling at room temperature, 0.5 ml of firefly lantern extract was added to the same volume of sample solution. Finally, the fluorescence was measured by means of an ATP Photometer (SAI Technology, Model-2000). Chlorophyll *a* and phaeopigments were extracted in 90% acetone and determined with a fluorometer (Hitachi UV-VIS 139 Spectrophotometer with fluorometry attachment) following the method of NISHIZAWA et al. (1971), which was modified from YENTSCH & MENZEL (1963).

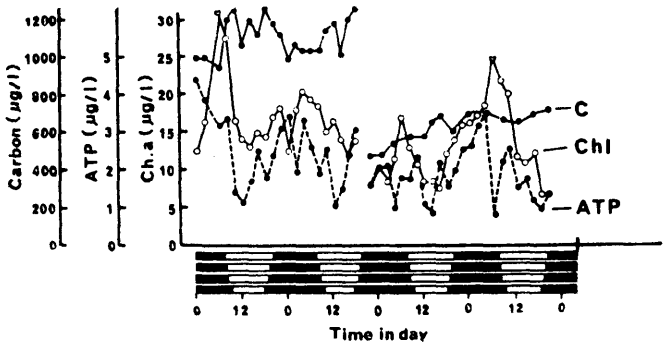

Fig. 1. Diurnal changes of chlorophyll *a*, carbon, and ATP per water volume observed from *Chaetoceros debilis* population cultured with a turbidometric continuous culture apparatus at 15°C under illumination of 6000–24000 lx during light periods.

Results

The diurnal variations of carbon, ATP, and chlorophyll *a* concentration per water volume are shown in Fig. 1. At the third dark period of this experiment, the culturing organisms were diluted to about 1/2 in terms of particulate carbon because of the unexpected breakage of the monitor lamp of turbidity system. However, since the culturing conditions excluding cell density and illumination were always constant, these ratios would presumably be scarcely

influenced by the trouble. The ratios of carbon, ATP, and chlorophyll *a* per one cell and per cell volume are shown in Fig. 2.

Carbon concentration per water volume varied within the range of ±15%. The range of variation was however smaller than that of ATP and chlorophyll *a* (Fig. 1). While carbon content in one cell revealed the diurnal periodicity. The higher values were noticed at the light period and the lower values at the end of dark. The range of variation extended up to

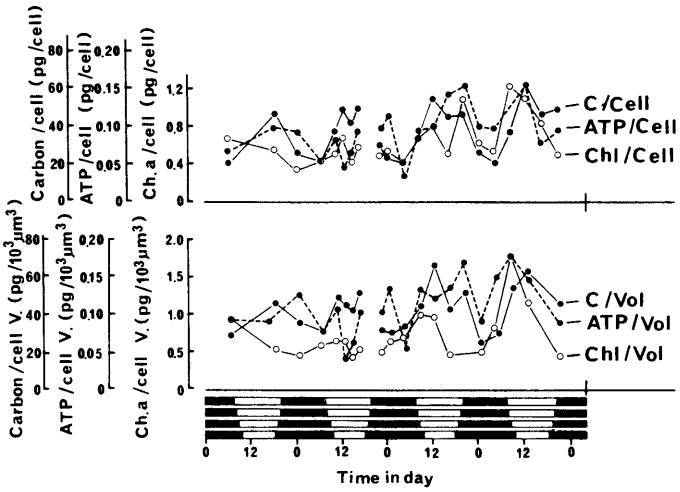

Fig. 2. Diurnal changes of chlorophyll *a*, carbon, and ATP per one cell (top), and per cell volume (bottom) of cultured *Chaetoceros debilis* in a turbidometric continuous culture apparatus.

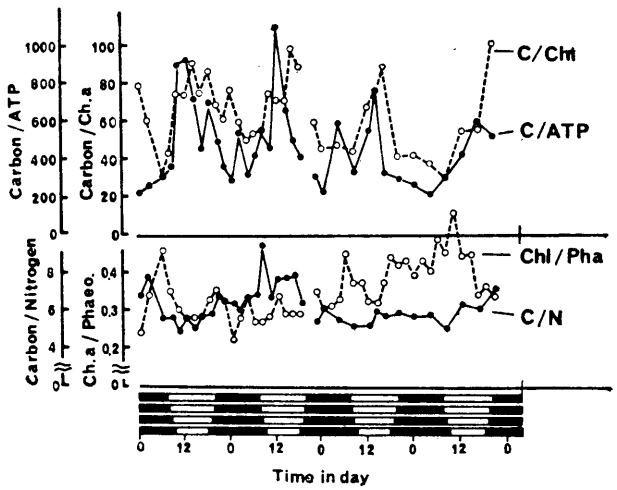

Fig. 3. Diurnal changes of C/Chl *a*, C/ATP (top) and Chl *a*/Pha, C/N (bottom) ratios observed from *Chaetoceros debilis* cultured with a turbidometric continuous culture apparatus.

about 50 %, and its minimal value was found afterward from that of carbon per cell volume (Fig. 2).

Chlorophyll *a* concentration per water volume varied conspicuously and indicated a distinct diurnal periodicity, the maximum at the end of dark period and minimum at the end of light. The increase was therefore noticed in the dark and the decrease in the light periods. The variations of chlorophyll *a* content per one cell and per cell volume were smaller than that of chlorophyll *a* per water volume, although the pattern of periodicity in chlorophyll *a* per one cell did not corresponded to that of per water volume and per cell volume. The minimal value was at the end of light in chlorophyll *a* per water volume. On the other hand, the lower values were obtained at the end of dark in chlorophyll *a* per one cell, and at the begining of dark per cell volume.

The periodicity of ATP concentration per water volume was not so obvious as was observed in chlorophyll *a*. However, in general, the lower values were obtained in the light period and the higher values were obtained in the dark period. ATP content per one cell and per cell volume did not show the periodicity clearly, but the increase was found at the latter half of light period in certain occasion.

Fig. 3 shows the ratios of carbon to chlorophyll *a*, carbon to ATP, carbon to nitrogen, and chlorophyll *a* to phaeopigments. The C/Chl ratio varied within a wide range, from 30 to 100. Its decrease in the dark and increase in the light periods has depended on the change of chlorophyll *a*. The C/ATP ratio also fluctuated considerably within a range from 200 to 1100, and its maximum value was always obtained at the midst of light period. The C/N and Chl/Pha ratios did not show any diurnal periodicity. The C/N ratio varied from 5 to 9, and was more stable in the latter two days of experiments when the Chl/Pha ratio was moderately high.

Discussion

Some contradictory results have been reported concerning the diurnal periodicity of chlorophyll in phytoplankton cells. In their laboratory experiments, EPPLEY et al. (1967) observed that chlorophyll concentration in *Ditylum* increased in the light period only. On the contrary, PAASCHE (1968) observed an adverse result after using the identical species as the experimental material. UNO (1971) found the maximum value of chlorophyll *a* in the dark period with *Phaeodactylum*, which is not agreeable with the result obtained with the same species by PALMER et al. (1974). While the experimental results on *Skeletonema* obtained by JØRGENSEN (1966) and by HONJO & HANAOKA (1969) coincided each other. Furthermore, EPPLEY et al. (1972) reported that large-volume culture on shipboard did not indicate any daily periodicity.

By the field experiments, many different patterns of variation have already been reported (RYTHER et al. 1958, ALEXANDER & CORCORAN 1963, GOERING et al. 1964, ENDO 1967, etc.), though about half of them suggested on obvious daily periodicity.

The differences in the patterns were presumably based on the species, culturing method, light intensity, lighting period, and many other undetectable factors. Actually, GLOOSCHENKO

et al. (1972) pointed out that diel chlorophyll *a* fluctuations changed inversely with different light intensities.

The amount of chlorophyll *a* is being expressed in different ways, viz. chlorophyll *a* per volume of water sample, per one cell, and per cell volume. In the case of synchronized culture, the definition is especially important. SOURNIA (1974) mentioned that experimental results should be expressed as pigment per cell instead of pigment per seawater volume. He has also concluded that chlorophyll content of phytoplankton is subjected to the diel periodicity. In addition to this remark, we propose that chlorophyll content of phytoplankton cells is also influenced by light intensity of their environment, since UNO (1974) has reported the variability of chlorophyll *a* content of *Skeletonema* with changing light intensity.

Comparing the variation of chlorophyll *a*, carbon, and ATP in this paper with that of cell growth and division in previous paper, the periodical pattern was not so clear. The primary results as cell size or cell number by microscopic counting or measuring included only a few errors which would be founded by sampling or reading. On the contrary, more bigger errors which included analytical errors were added to the secondary results as cellular contents per one cell or per cell volume. Further, the analyses of carbon and ATP was inferior to that of chlorophyll *a* in its accuracy and sensitivity.

Among carbon, ATP, and chlorophyll *a* per sample water valume (Fig. 1), the diurnal periodicity was most distinctive in chlorophyll *a*, whereas it was not so evident in ATP, and unrecognizable in carbon. However these patterns were altered with different expressions, i.e. per one cell or per cell volume. The diurnal periodicities of carbon and ATP became clear by using these expressions. The diurnal periodicity of carbon was especially influenced by the periodicity of cell density or cell volume. For the continuously growing cells in the culturing chamber, the three ways of expression, viz. the amount of the carbon per water volume, are respectively useful. These units for chlorophyll *a* and ATP are also meaningful.

In discussing the present results, the diurnal periodicity in growth and division of cells, which has been described in our previous paper (UENO & UNO 1980), should be quoted. Our results indicated that the mean cell volume clearly increased in the light, while it distinctly decreased in the dark periods. Thus, periodical change of cell volume revealed entirely the inverse pattern of the periodicity of cell density. The greater part of cells in the culture enlarged in the light and divided in the dark periods. During the growth of cultured phytoplankton, the rise of turbidity was almost always recognized in the light periods. Increase of cell volume seriously affected in ascending turbidity. On the whole, carbon, ATP, and chlorophyll *a* per one cell all increased in the light period, which reveals the storing activity of cell contents in the light phase through the expansion process to provide for cell division. On the other hand, these components per cell volume generally decreased in the light periods because of the enlargement of cells and appeared as if the contents had already increased in the dark period due to cell division.

These tendencies of the periodicity of contents per one cell and per cell volume indicated an inverse pattern to the variation of cell density and cell volume, respectively. Therefore,

it appears that the difference in the expression between per one cell and cell volume is very significant. In a healthy culture, cell density and cell volume are always variable. Consequently, it is very difficult to determine the amount of cellular substances in culturing organism or phytoplankton biomass. We, however, noticed that the contents per cell volume is most suitable depending on the uniformity of total substances in culture, while the contents per one cell is more convenient to work.

UNO (1971) previously obtained the different results from the information of the present investigation concerning the patterns of the diurnal periodicity of chlorophyll *a* in *Skeletonema* and *Phaeodactylum* cultured in the old type turbidostat on the basis of per sample water volume. The periodicity of chlorophyll *a* per one cell observed in the present study coincided quite well with the findings on *Ditylum* reported by EPPLEY et al. (1967).

In the field experiments, there were many different results on the periodicity of chlorophyll. It is rather difficult to get precise picture in the fields because of the instability of water, mixture of different species, short term fluctuations of light intensity as previously pointed out by GALLEGOS et al. (1980). The results from YENTSCH & RYTHER (1957) and SHIMADA (1958), using per water volume, however have indicated good agreement with the present findings.

C/Chl and C/ATP ratios showed the distinct diurnal periodicities. These variations were not caused by the change of the amount of carbon, but influenced seriously by the fluctuations of chlorophyll *a* and ATP. Mean values of C/Chl and C/ATP ratios, about 60 and 400 respectively, seemed to exist within a reasonable range.

HOLM-HANSEN (1973) described that C/ATP ratio of living plankton is always constant: 286. However, C/ATP ratio measured in this study was variable. The greater part of data obtained was higher than this value, despite the cultured phytoplankton were ordinarily contained with a higher percentage of living cells and did not include non living organic carbon.

C/N ratio did not show any diurnal periodicity, though its pattern of fluctuation was inverse to that of Chl/Pha ratio. Both phenomena seemed to be reasonable.

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