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Preliminary Report on the Photoperiodic Acclimation for Growth of *Chlorella* Cells in Synchronized Culture*

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Abstract

The growth of *Chlorella* cells cultured in synchronization system was studied in order to maintain the strain with a stable life cycle for practical use as food of marine rotifer.

Two experiments, Exp. I and Exp. II, were carried out using a marine type of *Chlorella* sp. acclimated to 16L–8D photoperiod for 15- and 180-days, respectively. Water temperature was controlled constantly at 23.0°C. A Coulter Counter with a 30 μ aperture tube and a size distribution analyzer was used for the measurements of the cell number and diameter.

Increase in cell number is indeed diurnally periodic, with a doubling of number occurring between 11 p.m. and 2 a.m. in Exp. I, and between 2 a.m. and 4 a.m. in Exp. II. Cell divisions were found mainly at midnight in Exp. I and at 3 a.m. in Exp. II. It was considered that the cells in Exp. I were still persisted the solar photoperiod. Consequently, to maintain the strain with a stable life cycle, more than 15 days are necessary to acclimate to a new photoperiod.

Introduction

The growth of culturing *Chlorella* cells involves two separate but interrelated processes, that is, the growth of an individual organism and the growth of a population. Our knowledge on the former process is scanty (HIRATA, 1972; MORIMURA, 1965), though the later process of planktonic population in culture has been known in considerable detail (COOK, 1961; COOK and JAMES, 1960; SWEENEY and HASTINGS, 1958). For practical cultivation of algae used as food for marine rotifer (HIRATA, 1974) or prawn larvae (HIRATA, MORI and WATANABE, 1975), it is necessary to know their life cycle based on the individual growth. A preliminary experiment on the photoperiodic acclimation in the growth of *Chlorella* cells was carried out in order to maintain the strain with a stable life cycle.

Materials and Methods

The material used in the experiments was one of the Yashima Algal Strains, *Chlorella*

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Table 1. Composition of Clewat-32* (per cent).

$FeCl_3 \cdot 6H_2O$	0.385 (as Fe)
$ZnCl_2$	0.166 (as Zn)
$MnCl_2 \cdot 4H_2O$	0.776 (as Mn)
$CoCl_2 \cdot 6H_2O$	0.017 (as Co)
$CuSO_4 \cdot 5H_2O$	0.007 (as Cu)
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.632 (as Mo)
H_3BO_3	2.470 (as B)
$(HOOCCH_2)_2NCH_2N(CH_2COOH)_2$**	Proper quantity

* Modified from SUTO (1959), and manufactured by Teikoku Chemical Industry Co., Osaka.

** EDTA (Ethylene-diamine-tetraacetic acid).

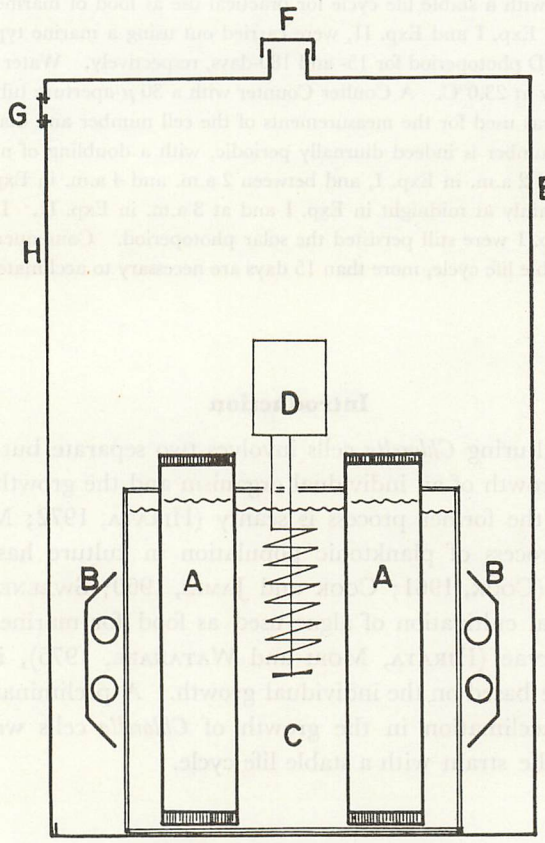

Fig. 1. Schematic view of the apparatus for synchronized culture. A; culturing column (12 cm in diameter and 45 cm in height), B; doubling 40 watts white beam flourescent lamps, C; temperature-controlled bath (45 cm $\times$ 45 cm $\times$ 100 cm), D; Lauda-Thermostat model E12 ($\pm$0.01°C), E; small dark room (65 cm $\times$ 100 cm $\times$ 135 cm), F; ventilator, G; hinge, H; observation window.

sp.* which has been precultured in natural seawater near the window of our laboratory with solar photoperiod during the last 4 years. In such natural condition, photoperiod was changed to 16L–8D, switched-on at 8 a.m. and switched-off at midnight, in a dark room from December 14, 1974. Two experiments, Exp. I and Exp. II, were done using the *Chlorella* cells acclimated for 15- and 180-days with the same treatment, respectively. A Coulter Counter model ZB with a 30 μ aperture tube and a size distribution analyzer model P–64 was used for the measurement of the cell number and diameter. The materials were inoculated in the medium containing 2×10^{-3} moles of KNO_3 and 0.15×10^{-3} moles of KH_2PO_4 (HIRATA, 1974). Besides these nutrients, 30 mg/l of Clewat-32 (Teikoku Chemical Industry Co.) and 10 mg/l of Clewat-Ca were added as inorganic micronutrients (see Table 1). Special caution was taken to ensure a population density below 4×10^6 cells per ml, since a lower density without any dilution gave a clearer and more accurate records by the distribution analyzer.

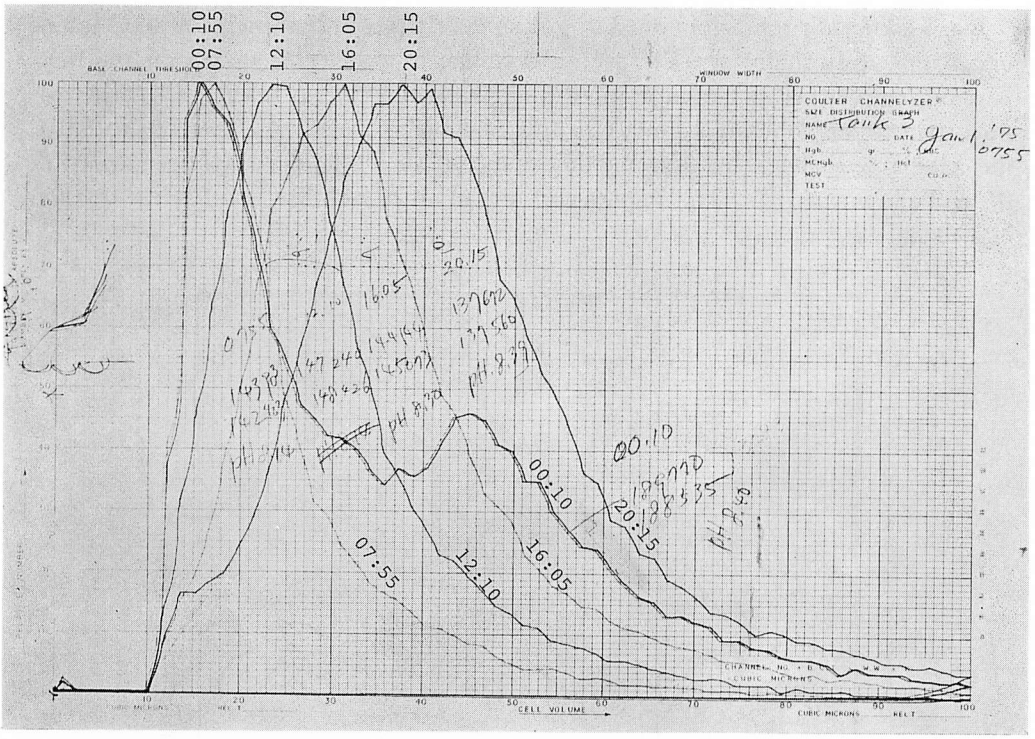

Fig. 2. An example of recordings by the size distribution analyzer model P–64 accompanied with the Coulter Counter model ZB. Records in this figure were obtained from Exp. I. Typed numbers indicate the observation time of day.

* According to TSUKADA, KAWAHARA and TAKEDA (1974), a strain of *Chlorella* isolated from salt-farm along Yashima Bay was identified as *Chlorella saccharophila* (KRÜGER) MIGULA var. *saccharophila* by Dr. Yuichi YONEDA.

A 5-liter transparent column was placed in a temperature-controlled bath at 23.0°C, and was illuminated from both sides by white beam flourescent lamps as shown in Fig. 1. The light intensity was 2,350 to 2,550 lux at the center of the column. Air pump of a vibration type, 3 l/min. capacity, provided air to the medium and was interlocked to the lamps with a time-switch. Consequently, no aeration was supplied during the dark period.

Results and Discussion

An example of the recordings obtained in Exp. I by the size distribution analyzer accompanied with the Coulter Counter is presented in Fig. 2. From this figure, it is easier to understand that the mode of the diameter distribution is getting greater during light period, from 07: 55 in the morning to 20: 15 in the evening. At 00: 10 in the night time, however, number of mother cells was less than that of the daughter cells. This means that the cell divisions occurred around midnight.

Fig. 3 shows the results obtained from Exp. I. It is seen that increase in cell number is indeed diurnally periodic, with a doubling of number occurring for the most part during 3- to 6-hour span around the light termination at midnight. The total time span of the population burst was about 6 hours. During the time of this burst, the cell diameter decreased from the time of light onset. The burst was terminated at the end of light period. This implies that almost all of the cells complete the division in a 24-hour cycle.

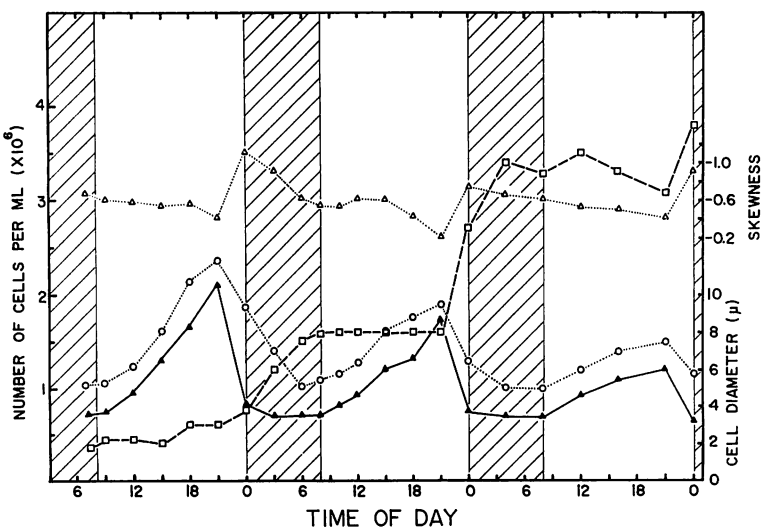

Fig. 3. Results obtained from Exp. I. *Chlorella* cells used were acclimated for 15 days in 16L–8D photoperiod. □----□ shows number of cells per ml, ○·····○ shows mean diameter of the cells, ▲——▲ shows mode of the size distibution, △·····△ shows skewness of the distribution mode.

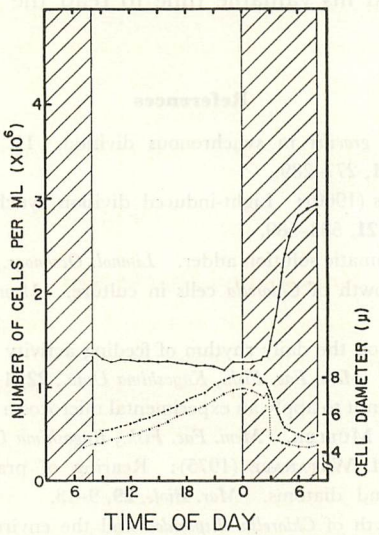

Fig. 4. Results obtained from Exp. II. The materials were acclimated for 180 days in 6L–8D photoperiod. ●——● shows number of cells per ml, ● — ● shows mean diameter of the cells, ●·····● shows mode of the size distribution.

Fig. 4 is the results of the growth of the cells observed in Exp. II. No cell division occurred before the time of light termination. It can be seen from this figure that the increase in cell number occurred at about the second hour of dark period, and was terminated three hours before onset of the subsequent light period. Individual growth curve assured the synchronized division at the time between 2 a.m. and 5 a.m.

After completion of the Exp. I, the cells were still persisted the solar photoperiod. Therefore, to maintain the strain with a stable life cycle, more than 15 days are necessary to acclimate to a new photoperiod.

HIROKAWA (1975) examined the population growth of *Chlorella ellipsoidea* under the condition of both 15L–9D photoperiod and continuous light. His results showed that the growth rate per illumination in the former condition was 1.4 times higher than that of the later one. He emphasized on the effects of periodic illumination, but he has no examination on the photoperiodic acclimation. In the present experiments, an idea of a periodic acclimation or a persistent rhythm (HIRATA, 1973) on the growth of *Chlorella* cells was presented here. In order to know more details on this matter, it is necessary to make a longer-term examination. An instrument like an automatic solution adder (HIRATA, 1963) could be applied to develope this problem.

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