Effects of Salinity and Temperature on the Growth of the Marine Phytoplankton Chlorella saccharophila

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Effects of Salinity and Temperature on the Growth of the Marine Phytoplankton *Chlorella saccharophila*

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Abstract

Effects of salinity and temperature on the population growth of the marine phytoplankton, *Chlorella saccharophila*, were examined in order to find out the optimum levels of these factors affecting its growth.

Salinity experiments were conducted at 14 levels from 0 to 60‰ under 22 to 25°C, with a 12L: 12D photoperiod. The *Chlorella* population grew well at salinities between 15 and 35‰, and the best growth was found at 25‰. The algae grew slowly under the salinities of 5, 45, 50, and 55‰, but exhibited almost no growth in 0 and 60‰.

In the case of the temperature experiments, 7 levels, with 5°C intervals, between 14 to 32°C were prepared under a salinity of 25‰ and a photoperiod of 12L: 12D. The algae grew well at 17, 20, and 23°C; but grew slowly at 26, 29, 32 and 14°C. The best growth was found around 20~23°C.

Introduction

Since the Yashima Station, Seto Inland Sea Farming Fisheries Association (1964a & b) reported on the collection of marine *Chorella* sp. and on the culture medium for them, the marine *Chlorella* has become one of the most common phytoplankton in the field of maricultural sciences. YONETA identified it with *Chlorella saccharophila* var. *saccharophila* (TSUKADA *et al*, 1974). Recently it can be utilized directly or indirectly as food for the rearing of not only rotifers but also harpacticoid copepods, scallop, oysters, prawn, crabs, and porgy, (HIRATA, 1977, 1979, 1980; KADOWAKI *et al*, 1980; KOGA, 1976; TAKAHASHI *et al*, 1976; UKAWA, 1968). Many aquatic botanists, however, are still concentrating their studies on the physiology of fresh-water *Chlorella*, but only a few studies on marine *Chlorella* have been reported (HIRATA, 1975; HIRATA and MURAKOSHI, 1977; TSUKADA *et al*, 1974).

The growth of *Chlorella*, as well as other algae, is affected by many environmental factors. Salinity and temperature are obviously very important environmental factors to be considered in the culture of the marine phytoplankton, *Chlorella saccharophila*. The purpose of this study was to determine the optimum levels of such environ-

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mental factors affecting the growth of this species.

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Materials and Methods

The species used in these experiments was a marine type of unicellular green algae, *Chlorella saccharophila* var. *saccharophila* Yashima strain. It was cultured in natural seawater on the roof garden of the laboratory building, using the Yashima culture medium (SETO INLAND SEA FARMING FISHERIES ASSOCIATION, 1964a; HIRATA, 1980).

Salinity Experiments

The 13 levels of salinity examined were as follows: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60‰. Lower salinity waters were made by dilution of filtered seawater with distilled water, while higher salinity waters were prepared by addition of sodium chloride.

The media were enriched with 6 mg of Ca-superphosphate, 4 mg urea, and 40 mg $(NH_4)_2SO_4$, as nutrients per 500 ml. Besides these nutrients, 12 mg of Clewat-32 was added as inorganic micro-nutrients (HIRATA, 1980).

The culture experiments were carried out in 500 ml glass erlenmyer flasks. Each flask contained 400 ml of medium, inoculated with 0.5×10^6 *Chlorella* cells per ml. Replicate flasks were placed at random in a rectangular water bath illuminated by white beam fluorescent lamp from above. The light intensity was 7500~8500 lux at the center of the water bath. Photoperiodicity was controlled at 12 hours light on and 12 hours light off (12L: 12D). Care was taken to change the positions of the flasks in the water bath to insure equal amount of illumination received.

Temperature Experiments

The seven degrees of temperature used in this experiment were as follows: 14, 17, 20, 23, 26, 29, and 32°C. The *Chlorella* used in this experiment were transferred from the previous experiment, after being pre-cultured for one week each. The salinity of the medium was adjusted to be 25‰ each. The culture water was enriched with 4 mg urea, 8 mg KH_2PO_4, 80 mg $(NH_4)_2SO_4$, and 80 mg KNO_3. Additionally, 12 mg of Clewat-32 were fertilized in each flask as micro-nutrients.

The *Chlorella* were cultured in 500 ml erlenmyer flasks as same as previous experiment. flask contained 400 ml of medium inoculated with 0.1×10^6 cells/ml. Replicate flasks were prepared to insure accuracy of results. The flasks were placed in a water bath, where the water temperature was regulated by using a Unibath model KU-2. The flasks were illuminated by white beam fluorescent lamps (40 w×6) from both side with a light intensity of 4500 lux at the center of the water bath. Periodicity of the light was maintained at 12L:12D.

Observations and Data Analysis

The *Chlorella* density in each experiment was determined daily with the use of a hemocytometer. A digital pH meter YEW 8011–1000 was used to measure the pH and water temperature in the medium. In the case of the temperature experiments, the water temperature was also recorded by a thermo-recorder TBX-24 throughout the experiments.

Each experiment was carried out in nine series. The average of every trials to be reported as three series experiments. Statistical analysis of data was done as parallel experiments, by using the Completely Randomized Design Methods (STEEL TORRIE, 1960; ZAR, 1974) in both maximum and total density. The relative growth rate (k) were calculated by the following formula:

$$k = \frac{\log_{10} N/N_0}{t - t_0} \times 3.22$$

in which: N is number of cells at time t, and N_0 is cell concentration at time t_0.

Results and Discussion

Salinity Experiments

The growth curves of the *Chlorella* populations under each salinity condition are illustrated in Fig. 1. The highest population density during a ten-day culture period are plotted as relative growth rates (k) in Fig. 2.

Under salinities of 15~35‰, the density of *Chlorella* increased rapidly from the third day after inoculation. Statistical analyses among those salinity conditions showed no significant difference. The highest density, about 50×10^6 cell/ml, was found at the salinity of 25‰ on the tenth day. On the contrary, the algae grew slowly under the salinities of 5, 10, 45, 50, and 55‰, but exhibited almost no growth of 0 and 60‰.

The best growth rate, $k=0.51$, of the *Chlorella* population was obtained at the salinity of 25‰. This was then followed by 20‰ ($k=0.50$), 30‰ ($k=0.49$), 15 or 35‰ ($k=0.48$), and 10 or 40‰ ($k=0.45$).

The growth of *Chlorella* linearly decreased with increasing salinities from 40 to 60‰ (Fig. 2). This may suggest that the *Chlorella* was affected by the overloading of the osmoregularoty mechanism, therefore, affecting the growth processes of photosynthesis and respiration. According to NAKANISHI and MONSHI (1965) both photosynthesis and respiration decrease with increasing salinity, but salinity affects photosynthesis more intensively than respiration. OGATA and MATSUI (1965) found that the maximum photosynthesis rate occurred in normal seawater. Photosynthesis depression occurred both in diluted and concentrated seawater.

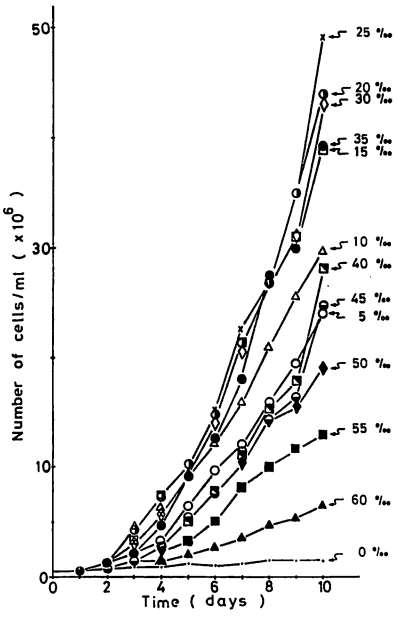

Fig. 1. Population growth of the marine phytoplankton, *Chlorella saccharophila*, cultured under different salinity conditions.

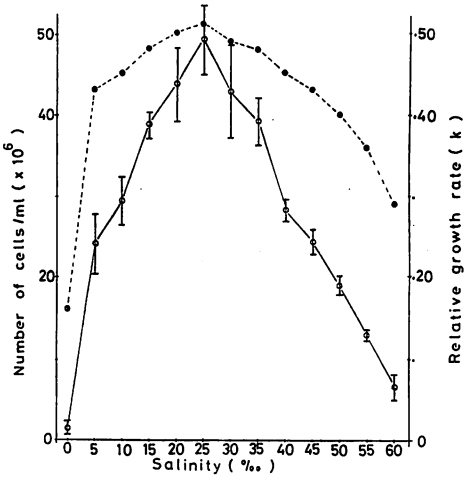

Fig. 2. Highest population density of *Chlorella saccharophila* during 10 days culture under different salinity conditions. O——O: Number of cells, ●---●: Relative growth rate (k).

Temperature Experiments

The growth curves of the *Chlorella* population in each temperature treatment are shown in Fig. 3. Maximum density of the population during the 24-day culture period and relative growth rates are plotted in Fig. 4.

From the fourth day, cell number increased smoothly in the cases of 17, 20, and 23°C. Maximum population densities in these groups was about 100×10^6 cells/ml, and observed on the 14th or 15th day after inoculation. Statistical analysis shows insignificant difference among these groups.

On the other hand, the highest growth rate was obtained at a temperature of 20°C (k=0.47). It was then followed by 23°C (k=0.42), 17°C (k=0.41), 26°C (k=0.37), 29°C (k=0.36), 32°C (k=0.32), and the lowest at 14°C (k=0.30). Therefore, it may be concluded that the optimum temperature for population growth of *Chlorella* is between 20 and 23°C, from the results obtained in this experiment. This conclusion is based on the fact that the *Chlorella* strain used was taken from the earlier experiment (salinity), where the temperature of the water bath was maintained from 20 to 23°C.

It is interesting to note that the population density of *Chlorella* at the temperature of 14°C increased slowly, but smoothly throughout the whole culture period of 24

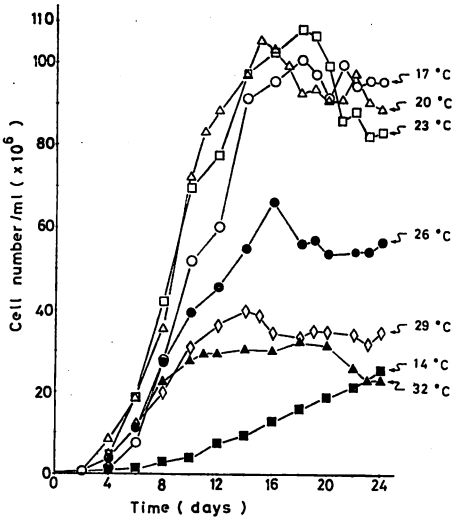

Fig. 3. Population growth of the marine phytoplankton, *Chlorella saccharophila*, cultured under different temperature conditions.

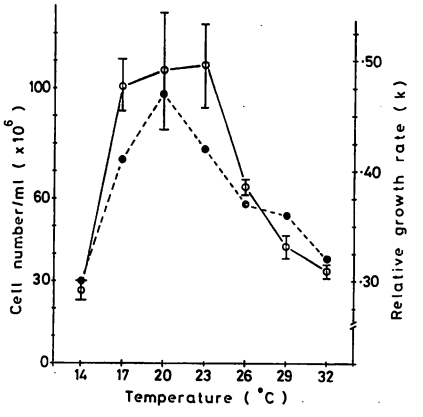

Fig. 4. Maximum population density of *Chlorella saccharophila* during 24 days culture under different temperature conditions. O—O: Number of cells, ●---●: Relative growth rate (*k*).

days. The population density at 14°C increased gradually with increasing age of culture. This may suggest that *Chlorella* cells gradually adapted to the lower temperature.

According to GESSNER (1971), the degree of temperature tolerance of algae depend on (1) the speed of temperature change, both with regard to the extreme values attained and the re-establishment of normal temperature, (2) the physicochemical condition before, during and after exposure to extreme temperatures. Immediately following the rises in temperature, however, the amount of dissolved oxygen decreases, causing secondary modifications in the respiratory rate. Change in temperature can further lead to alternation in the dissociation ratio of carbonic acid, which can subsequently affect photosynthesis. Therefore, temperature adaptation must also be considered in analysis of the data.

References

GESSNER, F. 1970: Temperature — Plants. Mar. Ecol., **1** (2), (O. KINNE ed.), Wiley Interscience, N. Y., 363–406.

HIRATA, H. 1975: Preliminary report on photoperiodic acclimation for growth of *Chlorella* cells in synchronized culture. *Mem. Fac. Fish., Kagoshima Univ.*, **24**, 1–6.

————, 1977: Zooplankton cultivation and prawn seed production in an artificial ecosystem. *Helgolander wiss. Meere.*, **30**, 230–242.

————, 1979: Rotifer culture in Japan. *Europ. Mar. Soc.*, **4**, 361–375.

———, 1980: Culture methods of the marine rotifer, *Brachionus plicatilis*. *Min. Rev. Data File Fish Res.*, **1**, 27–46.

———, M. MURAKOSHI, 1977: Effects of aeration volume on the growth of marine *Chlorella* in culture. *Mem. Fac. Fish., Kagoshima Univ.*, **26**, 15–22.

KADOWAKI, S., T. MAKAZONO, S. IOKU, T. KASEDO, and H. HIRATA, 1980: Seed production of the scallop *Chlamys nobilis* (REEVE) — II. Mixture diet of marine yeast and *Chlorella* sp. for the veliger larvae. *Mem. Fac. Fish., Kagoshima Univ.*, **29**, 209–215.

NAKANISHI, M. and M. MONSHI, 1965: Effect of variation in salinity on photosynthesis of phytoplankton growing in estuaries. *J. Fac. Sci., Tokyo Univ.*, (Sec. III), **9** (2), 19–42.

OGATA, E. and T. MATSUI, 1965: Photosynthesis in several marine plants of Japan as affected by salinity, drying and pH, with attention to their growth habitat. *Bot. Mar.*, **8**, 199–217.

SETO INLAND SEA FARMING FISHERIES ASSOCIATION, 1964a: Cultivation of live food organisms in the Yashima Station — I. Fertilizers for marine phytoplankton culture. *Saibai-Gyogyo News*, **3**, 4.

———, 1964b: Cultivation of live food organisms in the Yashima Station — II. Collections of single-celled green algae and its culture. *Saibai-Gyogyo News*, **4**, 4.

STEEL, R. G. D., and J. H. TORRIE, 1960: Principles and procedures of statistic. McGraw-Hill Book Co. Inc., New York, 481 pp.

TSUKADA, O., T. KAWAHARA, and H. TAKADA, 1974: Good growth of *Chlorella saccharophila* on the basis of dry weight, under NaCl hypertonic condition. *Bull. Japan. Soc. Sci. Fish.*, **40**, 1007–1013.

UKAWA, M. 1968: Rearing of red seabream, *Pagrus major*, in the green water. *Saibai-Gyogyo*, **4**, 10–21.

ZAR, J. H. 1974: Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs., N. J., 620 pp.