DIFFERENTIAL GROWTH RATES OF MICRO-ALGAE IN VARIOUS CULTURE MEDIA

C. P. GOPINATHAN
Central Marine Fisheries Research Institute, Research Centre, Tuticorin.

ABSTRACT

Growth and multiplication of three algae, *Isochrysis galbana*, *Tetraselmis chuii* and *Nitzschia closterium*, belonging to three different algal classes, was studied in the laboratory, using four culture media. The algae yielding different differential growth rates in the media, the experiment proved that no available culture medium is uniformly effective in all the cases, unless supplemented with either trace elements or vitamins, according to the specific requirement of the alga.

INTRODUCTION

For culturing micro-algae, different culture media have been in use depending on the organisms to be cultured. Though Erd-Schreiber's (Schreiber 1925) and Miquel's (Miquel 1892) media were found effective for culturing the diatoms and other nannoplankters initially, several other media came into existence, incorporating trace metals, vitamins and several organic and inorganic salts. Among these, 'f' (Guillard and Ryther 1962), PM (Pantastico 1977), TMRL (Tung Kong Marine Research Lab., Tahiti) and Conway or Walne's (Walne 1974) media were found effective for the maintenance of stock culture as well as for mass-culturing various micro-algae.

Although most algae are photo-autotropic and can grow in purely inorganic media, many require organic compounds, the requirement of which may be either absolute or stimulatory. Whereas most of the algae can be successfully cultured in a synthetic inorganic medium with ease, a few genera require organic compounds for their rapid growth when the cultures are supplemented with soil extracts, yeast extracts or other organic salts. Pringsheim (1946) used soil extracts successfully for a variety of algae. The role of trace metals in regulating phytoplankton growth was studied by Huntsman and Sunda (1978). However, the techniques of culturing different algae involve a clear understanding of their nutritional requirements. In this account, the results of an experimental study of the effect of four culture media on micro-algae of different classes and, thus, of different nutritional requirements under controlled conditions are presented.

Material and Methods

Three species of micro-algae, namely *Isochrysis galbana* (Haptophyceae), *Tetraselmis chuii* (Prasinophyceae) and *Nitzschia closterium* (Bacillariophyceae), were isolated by serial dilution culture method (Sournia 1978), and maintained in the algal-culture room under controlled conditions of temperature (25°C) and light (1 k lux). These cultures were maintained in culture flasks having 32-34 ‰ salinity and in which modified Miquel's media added. In order to study the viability, growth rate, and differentiation in various culture media, four media, 'f', PM, TMRL and Walne's medium, were prepared and tested, experimenting with the micro-algae.

Four 1-litre conical flasks with the four different culture media in standard proportions were used for each micro-algae and an initial inoculum of 5 ml, having 19 x 10^5 cells|ml algae, was added to each flask. After thus inoculating the media with the algae, growth of the latter was estimated by cell counts every day at 11 a.m. For counting, a one-ml sample was removed from each culture and kept in a stoppered tube with a few drops of iodine, which killed and stained the cells. The sample was shaken vigorously to break up the dividing cells to some extent, and the cells were counted with the aid of a haemocytometer. The algae inoculated in a limited volume of the medium generally proliferated in a characteritic pattern, consisting of lag, exponential, stationary and declining phases. The growth rate was noted for 9 days, by which time the algae were found to have entered into the declining and death phases.

The experiment was repeated for a second time when also the same results were noted.

Results

Growth of Isochrysis galbana (Fig. 1)

The growth rate of *Isochrysis galbana* expressed in cell counts in the different media is illustrated in Fig. 1. The first-day counts showed that the culture was in lag phase, but from the second day onwards, there was a gradual increase in the multiplication of cells in all the flasks. In TMRL, though there was initially a slow growth, about 9.2 x 10^5 cells|ml were noted on the 8th day. The maximum growth was observed in Walne's medium, followed by the media TMRL, 'f' and PM in that order. In the Walne's medium, although fluctuations of growth were noted in the middle, the rate of growth reached the peak on the 8th day, with 3.5 x 10^6 cells|ml. All the cultures entered the declining phase on the 9th day. Under optimum conditions, the relative growth constant 'k' in the exponential phase of growth was 0.05 per hour, corresponding to a generation time of 14 h in the Walne's medium.

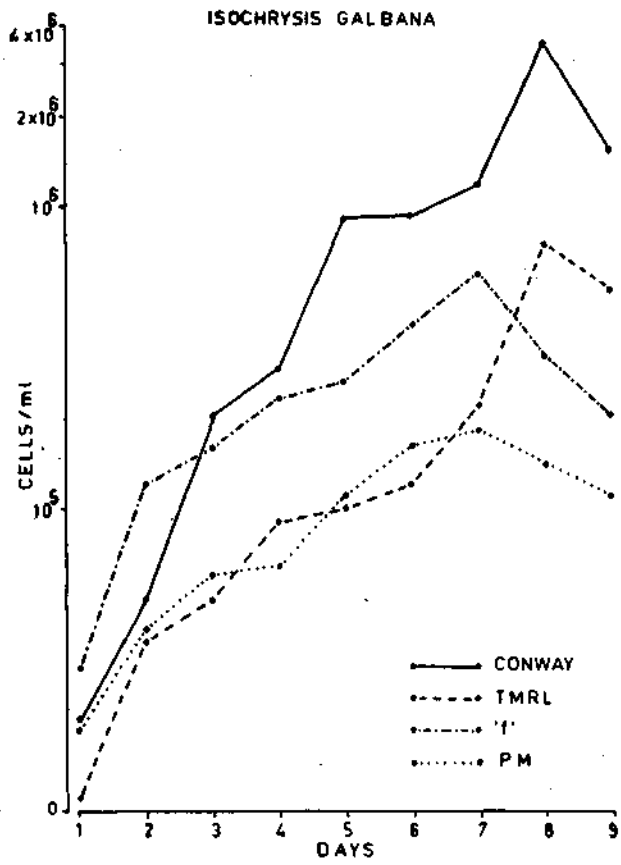

FIG. 1. Growth of *Isochrysis galbana* in 4 culture media.

Growth of Tetraselmis chuii (Fig. 2)

For this green flagellate also, 4 flasks with the four culture media were used as in the previous experiment, and 5 ml of initial inoculum, of 19 x 10^5 cells/ml, of *T. chuii* was introduced in each flask and the cell counts taken daily. Initially all the media showed gradual growth rate and, from the 3rd day onwards, TMRL, 'f' and Walne's media had significant increase in total number of cells. A peak density of 1.0-1.8 million cells/ml was noted in 'f' and TMRL media on 6-7th day, but only 0.7 million cells/ml were observed in Walne's medium on the 7th day. In PM, the cells reached a peak (1.7 million cells/ml) on the 4th day, but immediately declined. From the 8th day onwards, the cells grown in all the media were found to be in the declining phase. The relative growth constant 'k' was found to be 0.033/h, corresponding to a generation time of 21 h in Walne's medium. However, the generation time was found to be 29 h in TMRL, 'f' and PM in its exponential phase of growth.

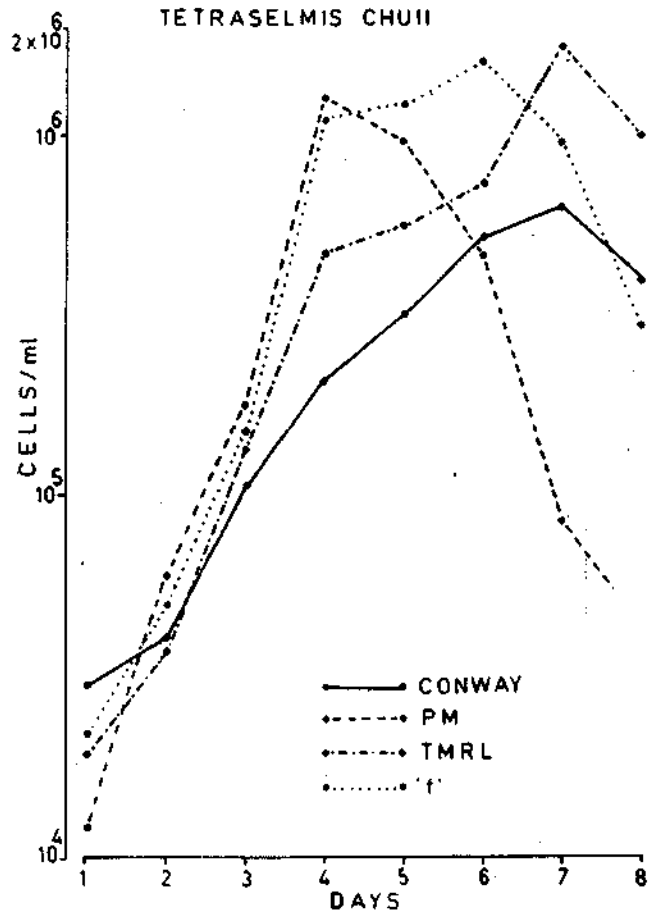

FIG. 2. Growth of *Tetraselmis chuii* in 4 culture media.

Growth of Nitzschia closterium (Fig. 3)

From an initial inoculum of 12 million cells|ml of this pennate diatom culture, 5 ml each was introduced in 4 flasks containing the four culture media. Even from the 2nd day onwards a significant rate of growth was noted in the flsaks containing PM, Walne's and 'f', in the order of intensity of cell multiplication. But in TMRL there was slow growth rate. On the 8th day peak densities, of 13.5 million cells|ml in PM, 11 million cells|ml in Walne's and 10 million cells|ml in 'f' medium were noticed. Even up to the 9th day the diatom was found to thrive well in all the media. The relative growth constant 'f' in the exponential phase of growth of this diatom was 0.035|h (generation time 20 h) in PM, whereas in 'f' the generation time was 22 h and in Walne's and TMRL 23 h. On 9th day all the cultures showed signs of declining phase.

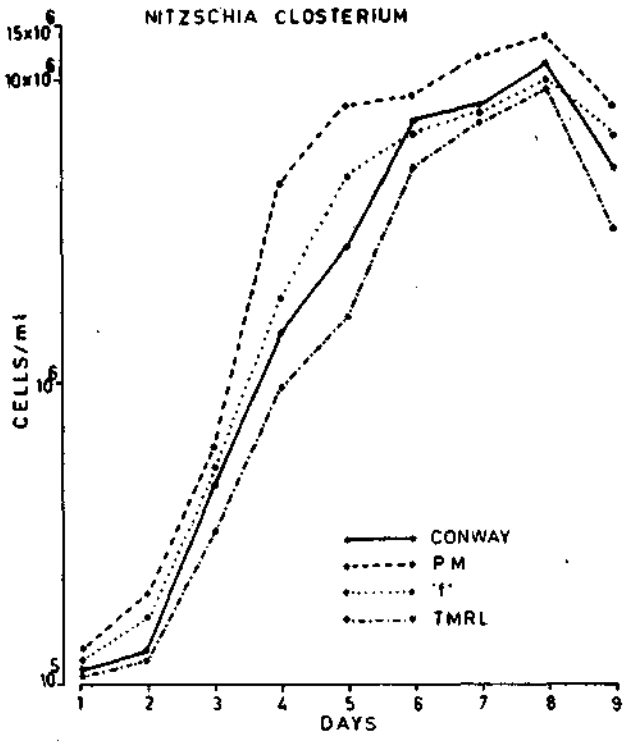

FIG. 3. Growth of *Nitzschia closterium* in 4 culture media.

Discussion

The experiment has revealed that certain nutrients in appropriate quantities are required in the culture media for the algae to multiply. Since the algae belong to different algal classes, namely Haptophyceae, Parsinophyceae (Chlorophyceae) and Bacillariophyceae, the requirements of nutrients may naturally vary. Another factor to be reckoned is that among the culture media the constituents also vary; for example, trace metals and vitamins are lacking in TMRL medium. Nevertheless, it is in TMRL that the green flagellate *Tetraselmis chuii* had maximum growth. Similarly, the diatom *Nitzschia* closterium had maximum growth in PM, which too is devoid of trace metals. On the other hand, the flagellate *Isochrysis galbana* grew comparatively better in Walne's medium, which has both the trace metals and the vitamins. It had already been proved (Provasoli 1958, Kain and Fogg 1960) that *Isochrysis* require both the vitamins Cobalamine (B12) and Thiamine (B1) for rapid growth and multiplication.

Subba Rao (1981) has shown that varied levels of trace metals have varied effects on micro-algae during division of cells, and that addition of chelating agents like EDTA would increase the availability of trace metals to the

algae. According to Huntsman and Sunda (1978) the cells are evolved with mechanisms of adaptation such as to substitute one metal for another, to shift to alternative pathways and to develop resistant forms in unfavourable trace-metal conditions. As a result, the species vary widely in their metal tolerance and requirements and also briefly limit the rates of photosynthesis.

Nitzchia closterium showing maximum growth in PM is quite in accordance with the silicate requirement needed for the diatoms normally to grow. It has been known that the thickness of the frustule would vary with silica concentration, and possibly the rate of growth, making it dfficult to predict the cells that could be obtained from a given silica concentration. Besides, this experiment has indicated that *Nitzchia closterium* requires also the vitamins B_1 and B_{12} for its normal growth and multiplication, because the diatom grew very well in PM but not so in TMRL, which lacked the vitamins. The experiment also showed that *Nitzchia*, and so also *Tetraselmis*, may not require trace metals, since both these organisms throve well in TMRL and PM, and, therefore, the 'f' and Walne's, supposed to be best among all these media, may not be effective for them.

According to Ketchum (1939), the nutrient requirements of algae may be absolute, normal, minimum or optimum. Although many media have been devised for culturing different algae, the experiment proves that no single medium can be said to be the best one. A clear understanding of the nutritional requirement of members of various classes of micro-algae is therefore a prerequisite for determining the technique of culturing and maintaining the algae for a long period.

Acknowledgements

The author is grateful to Dr. P. S. B. R. James, Director, Central Marine Fisheries Research Institute, for encouragement. His sincere thanks are also due to Dr. P. V. Ramachandran Nair, Head of Fishery Environment and Management Division, for his guidance and critically going through the manuscript and offering suggestions and to Dr. K. Satyanarayana Rao, for correcting the typescript.

References

Guillard, R. R. L. and J. H. Ryther. 1962. Studies on marine planktonic diatoms. 1. *Cyclotella nana* Hustedt and *Detonulla confervaceae* (Cleve) Grunow. *Canadian J. Microbiol.*, 8: 229-239.

Huntsman, S. A. and W. G. Sunda. 1978. The role of trace metals in regulating phytoplankton growth with emphasis on Fe, Mn and Cu. In: The Physiological Ecology of Phytoplankton, 8: 285-315.

Kain, J. M. and G. E. Fogg. 1960. Studies on the growth of marine phytoplankton. III. *Prorocentrum micans* Ehrenberg. *J. mar. biol. Ass. U.K.*, 39: 33-50.

KETCHUM, B. H. 1939. The development and restoration of deficiencies in the phosphorus and nitrogen composition of unicellular plants. *J. Cellular. Comp. Physiol.*, 13: 362-373.

MIQUEL, P. 1892. De la culture artificielle des Diatomees. *C. R. Akad. Sci. Paris.*, 44: 1-17.

PANTASTICJ, J. B. 1977. Techniques of the mass culture of diatoms. *SEAFDEC Tech. Rep.*, 4: 1-17.

PRINGSHEIM, E. G. 1949. Pure cultures of algae. Their preparation and maintenance. Cambridge Univ. Press, Cambridge, 119 pp.

PROVASOLI, L. 1958. Growth factors in unicellular marine algae. *Ann. Rev. Microbiol.*, 12: 279-308.

SCHREIBER, E. 1925. Die Reinkultur von marinen phytoplankton and deren Bedeutung fur die Enfor schung der production fahigkeit des Meerwassers. *Wiss. Meers. N. F.*, 4: 1-10.

SOURNIA, A. (ED.) 1978. Phytoplankton manual. *Monogr. Oceanogr. Methods, UNESCO*, 6: 1-337.

SUBBA RAO, D. V. 1981. Growth response of marine phytoplankton to selected concentration of trace metals. *Bot. Mar.*, 24: 369-379.

WALNE, P. R. 1974. Culture of bivalve molluscs. 50 years experience at Conway. *Fishing News* (Books) *Ltd.*, 1-173.