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SOME OBSERVATIONS ON THE GROWTH OF CHLORELLA SALINA

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Abstract

Growth of *Chlorella salina* was studied in different salinities (10, 20, 30 and 40 ppt), PH (6.0 - 10.0) and dilutions (100-400 ml). Maximum growth was observed at 40 ppt after 35 days, at PH 10 after 51 days and at 300 ml after 34 days of inoculation. The experiments were conducted for 60 days under controlled conditions. At PH 8, 9 and 10, *C. salina* shows two growth peaks, one after 16-24 days and the other after 42-54 days of inoculation whereas at PH 6 and 7, it records one growth peak after 16-19 days of inoculation. This shows that *C. salina* can adjust to a wide range of salinity and PH.

Introduction

Production of micro-algae is an integral part of aquaculture since these organisms form the basic of the food chain in many aquaculture operation. Inspite of all the effort to replace live micro algae by inert feeds, aquaculturists are still dependant on the production and the use of micro algae as their live food for commercially important fish, molluscs, crustaceans during atleast part of their life cycles (Laing, 1987). Species of *Chlorella* has been widely used in the marine fish hatcheries in view of transferring the essential fatty acids and other dietary components from algae, through zooplankton to fish larvas (Watanabe *et al.* 1978, 1983; James *et al.*, 1986, 1988) so it is very important to know the growth of *Chlorella* and different conditions of salinity, PH and volume of culture media to assess the optimum condition for better yield. Kain and Fogg, 1960 have studied the growth constant of free marine phytoplankton under controlled conditions of light, temperature, salinities and nutrients. Gopinathan (1984) observed the growth constant of some nannoplankters. It is believed that the growth constant and generation time will depend upon the environmental conditions of the algal cells. Joseph and Nair (1975) have studied the growth kinetics of three species of estuarine phytoplankters in a culture system. Nair (1974) has reviewed the growth kinetics of several species of phytoplankters from natural marine environment. Ammini Joseph and Nair (1984) have studied the growth kinetics of few selected species of nannoplankters in culture system.

The present experiment was aimed at getting an idea of optimum salinity and PH requirements for the growth of C.salina under controlled conditions.

Materials and Methods

Pure culture of *C. salina* with a known number of cells/ml were inoculated in 500 ml Erelweyer's flask in Conway and Walne's medium. The cultures were kept under flourescent light of 1000 lux and a temperature range from 23-26°C for 60 days. The photoperiod was maintained at 16:8 hours of light and dark cycle. Regular observations were taken on the growth of *Chlorella* (No. of cells/ml) by an improved Neurober Haemocytometer as the method of counting erythrocytes (clinical diagnosis by laboratory examinations, D. Appleton Century Co., New York, Kolmer). The following number of cells are inoculated for each experiment. In dilution tests, 14890 cells/ml were inoculated in 100, 200, 300 and 400 ml of culture media. For salinity test 120500 cells/ml were inoculated in 400 ml of Walne's medium at different salinities such as 10, 20, 30 and 40 ppt and for PH test 166500 cells/ml were inoculated in 300 ml of culture medium at different PH such as 6, 7, 8, 9 and 10.

Results and Discussions

The *Chlorella* cells inoculated in 10, 200, 300 and 400 ml of culture medium exhibited different trends in their growth. The maximum number of cells/ml were observed in 300 ml of medium after 34 days of inoculation. In 100 and 200 ml of medium the peak growth rate was observed on 40th day. But as the dilution rate increased, the rate of growth also increased (Fig.1). The generation time of *chlorella* varied in different volumes of culture medium. The doubling time of growth was observed till 3rd day os inoculation after which the growth rate was slow. The growth constant found in different volumes of culture media were as follows:

Volume of Culture medium (ml)	Growth Constant (tg)
100	23.17
200	15.80
300	13.37
400	17.46

Fig. 1 Growth of *Chlorella*
(in different volume of culture media)

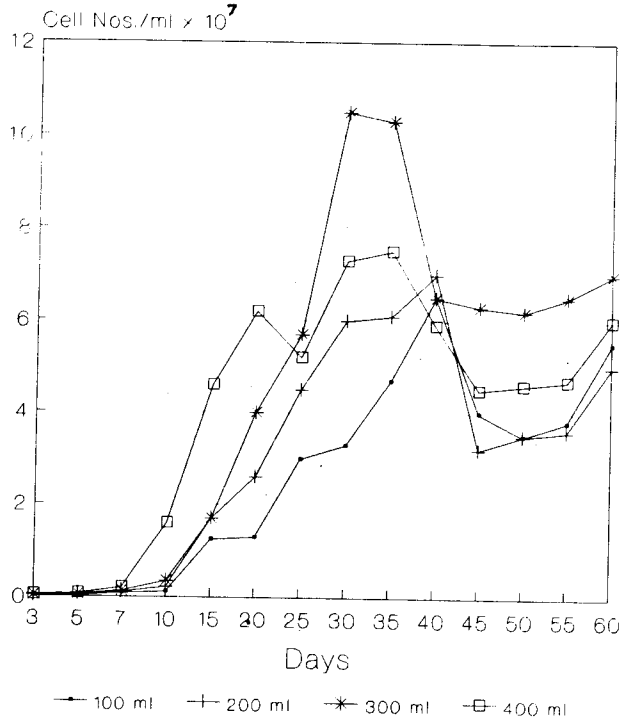


Fig. 2 Growth of *Chlorella*
(in different salinities)

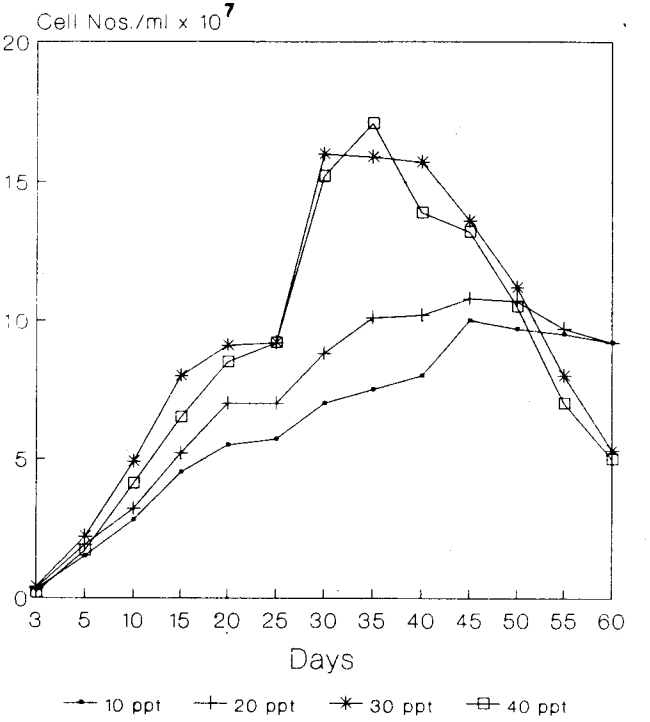
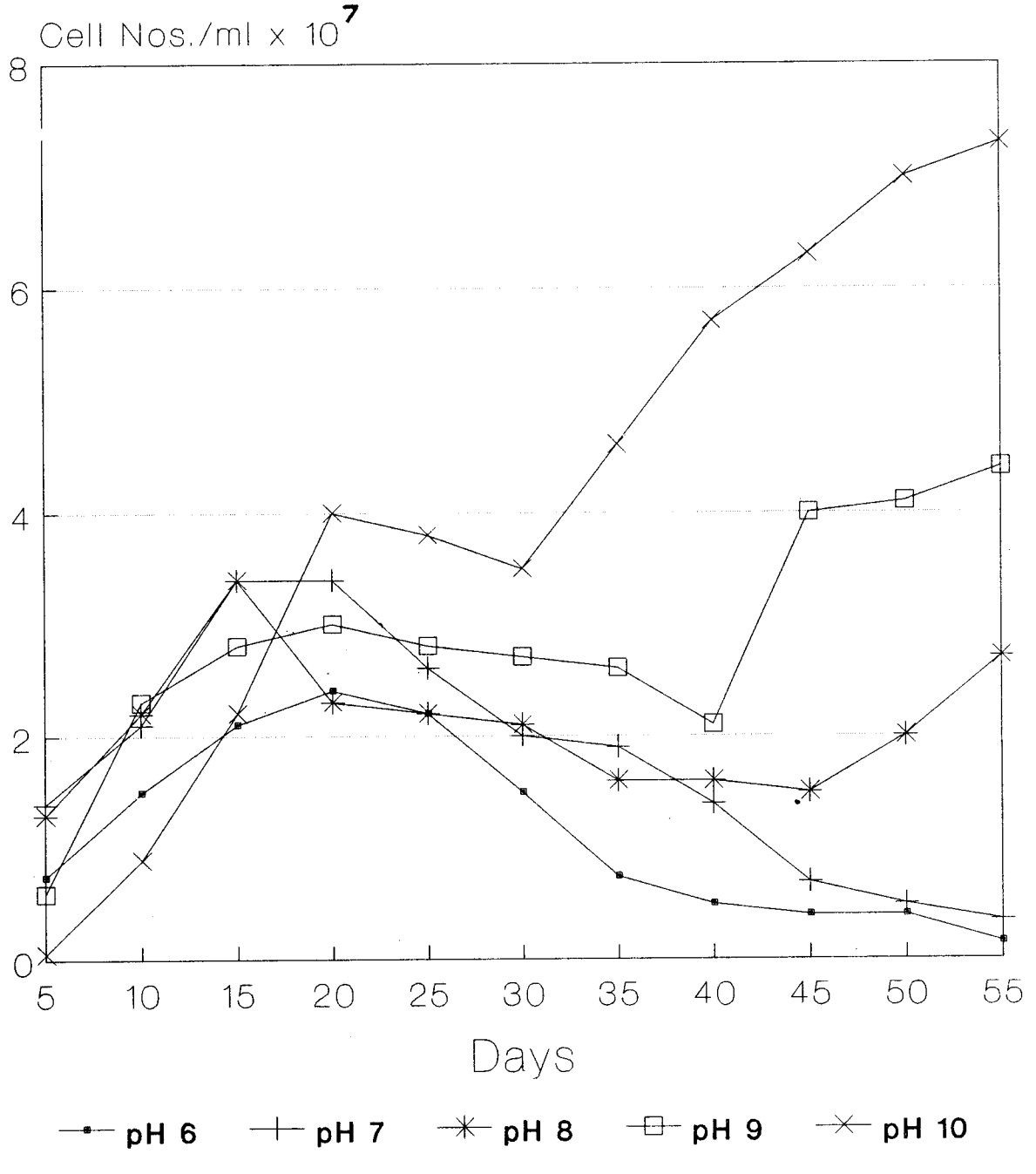


Fig. 3 Growth of *Chlorella* (in different pH)



Among different salinities, *Chlorella salina* showed maximum growth in 40 ppt after 35 days of inoculation. in case of 10 and 20 ppt the peak value was observed on 43 days after inoculation and in 30 ppt the peak observed after 32 days of inoculation. But in all the treatments, there was a steady decline in the growth after the peak period (Fig.2). *C. salina*, as its name implies, shows of wide range of tolerance to salinity and exhibited optimum growth at 30 ppt in the present experiment. Growth constant found in different salinity were as follows :

Salinities (ppt)	Growth constant (tg)
10	21.73
20	21.09
30	20.34
40	21.85

The generation time was found to be less in 30 ppt salinity compared to others.

Among different PH, it was observed that at PH 6 and 7 the growth retarded after 16-19 days of inoculation whereas in PH 8, 9 and 10 there were two growth peaks the first peak observed from 16-19 days after inoculation and the second observed from 42-54 days after inoculation (Fig.3). In PH 10 the initial phase of growth was very slow but increased after 9-13 days of inoculation. The growth constant found in different PH were as follows:

Treatment (PH)	Growth constant (tg)
6.0	22.89
7.0	18.77
8.0	19.23
9.0	22.72
10.0	72.92

The above data suggest that 30 ppt and 7 are the optimum salinity and PH respectively, required for the best growth of *Chlorella* with lease generation time. Further, the dilution experiment indicates that growth is maximum when a single cell of Chlorella was inoculated in 0.02 ml of culture medium.

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References

Ammini Joseph and Nair, P.V.R. 1984. Culture and growth kinetics of some selected nannoplankters. CMFRI Sp. Publ.,19:106-109.

Gopinathan, C.P. 1984. Growth characteristics of some nannoplankters. J. Mar. biol. Ass. India, 26(1&2) : 89-94.

James, C.M., Abu-Rezeq, T., Dias, P.A. and Sulman, A.E., 1986. Production dynamics and nutritional quality of the rotifer *Brachionus plicatilis* under different feed regime. Kuwait Institute for Scientific Research Report No. KISR 2183: 1-30.

James, C.M. and Abu-Rezeq, T.S. 1988. Effect of different cell densities of *Chlorella* capsulata and a marine *chlorella* sp. for feeding rotifer *Brachionus plicatilis*. Aquaculture 69:43-56.

Joseph, K.J. and P.V.R. Nair, 1975. Growth characteristics of certain estuarine phytoplankters. Bull. Dept. Mar. Sci., Univ. Cochin., 7(1) : 151-159.

Kain, J.M. and G.F. Fogg, 1960. Studies on the growth of marine phytoplankton III. Prorocentron Micans Ehrenberg. J. Mar. biol. Ass. U.K., 39 : 33-50.

Laing, J.,1987. The use of artificial diets in rearing bivalve spat. Aquaculture 65: 243-249.

Watanabe, T., Arakawa, T., Kitajinia, C. and Fujita, S. 1978. Nutritional evolution of proteins of living feeds used in seed production of fish. Bull. Jpn. Soc. Sci. Fish. 44 : 985-988.

Watanabe, T., Kitajinia, C., Arakawa, T., Fukusho, K. and Fujita, S. 1978. nutritional quality of rotifer, *Brachionus plicatilis*, as a living feed from the view point of essential fatty acids for fish. Bull. Jap. Soc. Sci. Fish. 44 : 1109-1114.

Watanabe, T., Katajinia, C. and Fujita, S. 1983. Nutritional values of live organisms used in Japan for non-propagation of fish. A review 34 : 115.

Nair, P.V.R. 1974. Studies on primary productivity of the Indian Seas. Ph.D. thesis, University of Cochin.