J. mar. biol. Ass. India, 1982, 24 (1 & 2): 141-146

GROWTH KINETICS AND PHOTOSYNTHETIC CHARACTERISTICS OF A CHRYSOPHYCEAN AND A HAPTOPHYCEAN FLAGELLATE*

AMMINI JOSEPH** AND P. V. RAMACHANDRAN NAIR

Central Marine Fisheries Research Institute, Cochin-682 031

ABSTRACT

The growth kinetics of two nanoplankton flagellates newly isolated from the Cochin area have been described. The parameters measured are the rate of cell doubling, chlorophyll and carotenoids, C^{14} uptake and oxygen exchange. The typical growth curve of the species extends through the exponential growth phase (2nd to 4th day of inoculation), then gradually declines and continues to be stationary for two weeks before decline sets in.

INTRODUCTION

THE PATTERN of growth of several species of phytoplankton cultures, the rate of which is measurable in terms of cell multiplication, pigment concentration or physiological activity have been discussed by Strickland (1960) and Fogg (1975). The relative growth rates of different species vary and hence for the effective utilisation of the cultures, the growth kinetics of each, under the specific culture conditions, has to be defined. The two species discussed in this paper are new isolates and have not been the subject of previous study.

The authors are indebted to Dr. E. G. Silas, Sub-Project Co-ordinator, CAS in Mariculture and Director, Central Marine Fisheries Research Institute and to ICAR/UNDP for offering the Senior Research Fellowship to the first author.

MATERIAL AND METHODS

The nanoplankters used in the present study were two tropical flagellates - *Chromulina freiburgensis* Doflein (Chrysophyceae) and a local isolate of *Isochrysis galbana* Parke (Haptophyceae). These golden yellow monads were isolated by the first author from the coastal waters of Cochin (9° 58'N, 76° 15'E) in 1981 and was maintained in the laboratory of the Central Marine Fisheries Research Institute, Cochin, India.

Sterilised culture medium (Miquel's medium supplemented with trace elements) as taken in three litre Haffkine flasks plugged with non-absorbent cotton. The medium was prepared in filtered sea water of salinity 34‰. These were inoculated with small volumes of healthy cultures and exposed to illumination from fluorescent lamps on a light-dark regime of 10:14 hours at ambient temperature (31 ± 2°C). The light intensity was about 20,000 lux. The cultures were not aerated.

Aliquots were withdrawn at two day intervals for a period of thirty days to measure the growth and activity of the flagellates. The cell numbers were counted on a haemocytometer after fixation in Lugol's iodine. The growth rate (K) and the mean generation time (tg) were calculated from the cell counts as per the equations of Eppley and Strickland (1968).

Samples of cultures were filtered through Millipore HA filters and the chlorophyll and

*This formed a part of Ph. D. Thesis, University of Cochin, Cochin.

**Present address: School of Environmental Studies, University of Cochin, Cochin-682 016, India.

carotenoid pigments were determined by a PYE-UNICAM Spectrophotometer following the method of Richards and Thompson (1952) applying the revised equations of Parsons and Strickland (1963). The rate of photosynthetic production of the flagellates was determined by the C^{14} technique (Steemann Nielsen, 1952) and the light and dark bottle technique of Gaarder and Gran (1927). All the analyses were carried out in replicate.

a density of 1.4×10^6 cells/ml during the period of growth. The cell multiplication was gradual during the forty eight hours following inoculation and was followed by rapid proliferation between the second and fourth day. The rate of growth declined from the fourth to twelfth day and subsequently the culture attained stationary phase. The cell number remained stationary till the 30th day (Fig. 1 a). The highest growth rate recorded for the species

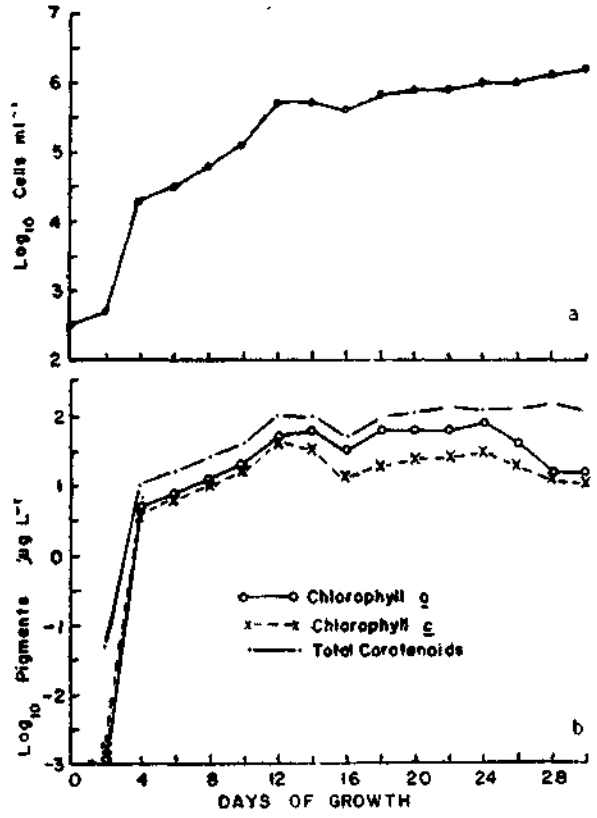

Fig. 1. *Chromulina freiburgensis* in batch culture: a. cell number and b. pigment content.

RESULTS

Fig. 1 & 2 give the change in cell numbers, pigment content and photosynthetic characteristics of *Chromulina freiburgensis* during the period of culture. From an initial concentration of 350 cells/ml, the culture developed to was 2.66 divisions per day corresponding to a generation time of 9 hours.

The amount of the pigments increased exponentially during the second to fourth day, followed by gradual rise till twelfth day and later remained more or less constant (Fig. 1 b).

However, the amount of pigments per unit number of cells was highest during the first week of culture (Table 1). The maximum value for chlorophyll *a*, *c* and carotenoids was 0.245, 0.189 and 0.526μg/10^6 cells. The proportion of carotenoids was quite high in the final phase of culture (Table 1).

The photosynthetic activity as represented by C^{14} uptake increased upto sixteen days and then declined (Fig. 2 b). The highest production was observed to be 60 μgC/10^6 cells/hr on the fourth day.

The culture of the strain of *Isochrysis galbana* showed similar pattern of growth as the former

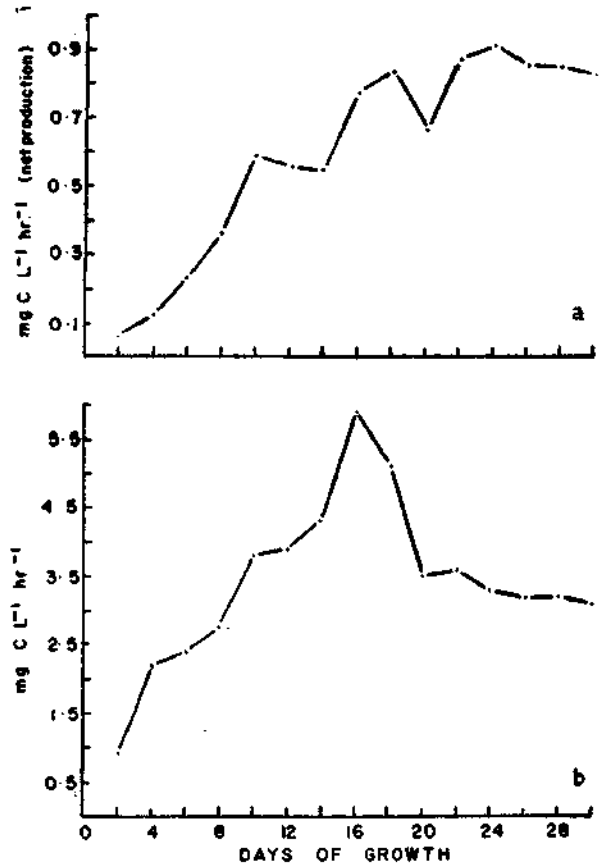

Fig. 2. Rate of photosynthesis of *Chromulina freiburgensis* : a. measured by oxygen method and b. Carbon-14 technique.

The rate of production, indicated by the 'oxygen method', increased from the initiation of the culture upto 10 days of growth with a maximum production rate of 120μgC/10^6cells/hr (gross production) and 40 μgC/10^6 cells/hr (net production). After the tenth day, the rate of production decreased to 10 μgC/10^6 cells/hr. The trend observed is represented in Fig. 2 a.

species, but with decreased growth rate. After four days of inoculation, the culture developed a density of 1.95×10^5 cells/ml from and initial 350 cells/ml. During this period a maximum division rate 1.48 doublings/day for a generation time of 16 hours was achieved. After the fourth day, the rate of growth decreased, the culture gradually attained stationary phase (Fig. 3 a).

TABLE 1. *Pigment content of* Chromulina freiburgensis *(μg/10⁶ cells) grown as batch culture*

Age of culture (days)	Chlorophyll *a*	Chlorophyll *c*	Total carotenoids	Carotenoids/ chlorophyll *a*
2	0.198	0.150	0.405	2.05
4	0.245	0.189	0.526	2.15
6	0.236	0.178	0.453	1.92
8	0.214	0.159	0.364	1.70
10	0.182	0.134	0.312	1.71
12	0.112	0.074	0.200	1.79
14	0.110	0.058	0.182	1.65
16	0.083	0.034	0.138	1.66
18	0.085	0.030	0.143	1.68
20	0.080	0.036	0.152	1.90
22	0.078	0.030	0.148	1.90
24	0.069	0.028	0.143	2.07
26	0.040	0.018	0.125	3.13
28	0.012	0.010	0.128	10.31
30	0.010	0.008	0.105	10.46

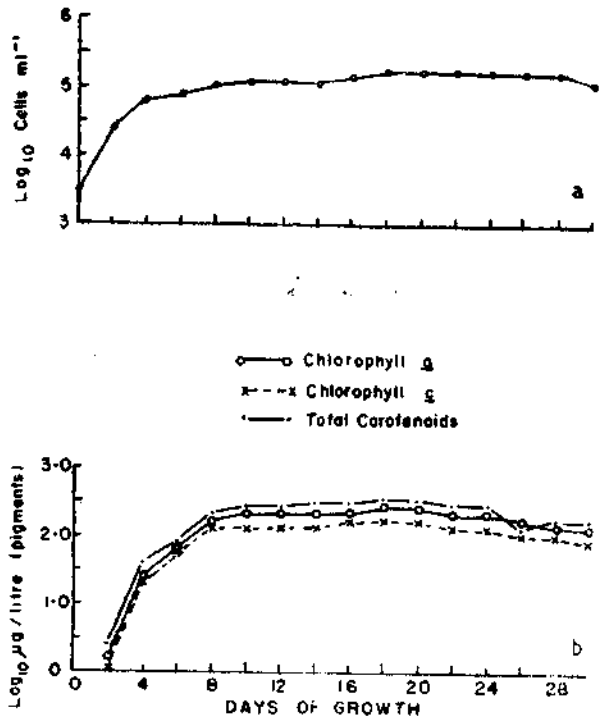

Fig. 3. *Isochrysis galbana* in batch culture: a. cell number and b. pigment content.

The maximum density attained by the culture was 6.9×10^5 cells/ml.

The pigment content of the culture showed a pattern of variation similar to the cell counts (Fig. 3 b). Per unit number of cells, the amount of pigments was high from the eighth to twelfth day of inoculation with the maximum amounts of 0.584, 0.418 and 0.662 μg/10^6 cells of chlorophyll *a*, *c* and carotenoids respectively (Table 2). As in *C. freiburgensis* the carotenoids were found

TABLE 2. *Pigment content of* Isochrysis galbana *grown as batch culture* ($\mu g/10^6$ *cells*)

Age of culture (days)	Chlorophlly *a*	Chlorophyll *c*	Total carotenoids	Carotenoids/ Chlorophyll *a*
2	0.068	0.042	0.100	1.47
4	0.142	0.105	0.195	1.38
6	0.265	0.202	0.364	1.37
8	0.584	0.418	0.662	1.13
10	0.502	0.380	0.612	1.22
12	0.468	0.330	0.594	1.27
14	0.430	0.316	0.570	1.33
16	0.424	0.304	0.553	1.30
18	0.420	0.300	0.550	1.31
20	0.405	0.253	0.468	1.16
22	0.365	0.230	0.460	1.26
24	0.318	0.192	0.390	1.23
26	0.226	0.165	0.280	1.24
28	0.228	0.152	0.264	1.16
30	0.108	0.101	0.256	2.30

to increase from the thirtieth day after inoculation.

The photosynthetic activity of *I. galbana* in the culture also showed exponential, stationary and declining phases as indicated by the oxygen and C^{14} measurements (Fig. 4 a, b). The maximum rate of production observed was $40\mu gC/10^6$ cells/hr (gross production) and

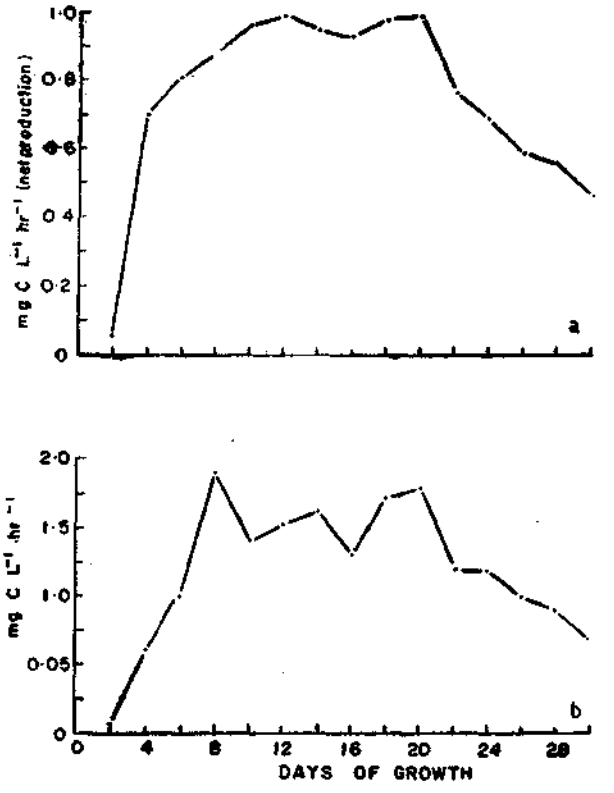

Fig. 4. Rate of photosynthesis of *Isochrysis galbana* : a. measured by oxygen method and b. Carbon- 14 technique.

12 μgC/10^6 cells/hr (net production) measured by the oxygen technique and 16 μgC/10^6 cells/hr according to the C^{14} measurements.

The motility of the flagellates decreased with ageing of the culture. The non-motile cells in the stationary phase settled down forming a thin layer at the bottom of the culture flask. The colour of the ageing cultures also changed from golden yellow to an orange-red tint.

Discussion

The pattern of growth exhibited by the present species is very similar to the 'growth curves' described earlier (Fogg, 1975). The present results, though are similar to the previous investigations, the growth rate of *I. galbana* is found to be quite high compared to the strain obtained in temperate regions for which a generation time of 30.2 hrs has been reported (Kain and Fogg, 1960). The difference in the rate of growth of the two species - *C. freiburgensis* and *I. galbana* is attributed to the specificity of each species.

The pigment content of the flagellates was high during the exponential phase of the culture probably due to availability of sufficient nutrients and high photosynthetic activity. However, the amount of chlorophyll *a* decreased after a week of inoculation. Similar results have been observed in batch growth of cultures by previous workers (Morris and Glover, 1974; Vijayaraghavan *et al.*, 1975). The relative proportion of carotenoids increased in the aged cultures resulting in the change of colour of the culture from golden yellow to orange red. Accumulation of carotenoids in ageing cultures has been frequently encountered (Fogg, 1975). The possible reason for this as suggested by Droop (1954) is the depletion of nitrogen or phosphorus.

The rate of photosynthetic production in both the species was maximum during the exponential phase coinciding with a high concentration of chlorophyll and carotenoids. The reduction in the rate of production of the ageing cultures may be attributed to the low chlorophyll *a* content and nutrient depletion which is bound to occur in batch cultures. Moreover, many cells in the old cultures were in the non-motile resting stage when metabolic activity is bound to be low.

References

Droop, M. R. 1954. Conditioning governing haematochrome formation and loss in the alga *Hemato coccus pluvialis* Flolow. *Arch. Mikrobiol.*, **20**: 391-397.

Eppley, R. W. and J. D. H. Strickland 1968. Kinetics of marine phytoplankton growth. In: M. R. Droop and E. J. Ferguson Wood (Ed.) *Advances in Microbiology of the Sea*. Academic Press, London, **1**: 23-62.

Fogg, G. E. 1975. *Algal cultures and phytoplankton Ecology* (2nd ed). University of Wisconsin Press, London, 175 pp.

Gaarder, T. and H. H. Gran 1927. Investigations of the production of plankton in the Oslo Fjord. *Rapp. Proc. Verb. Cons. Expl. Mer.*, **42**: 1-48.

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.

Morris, I. and H. E. Glover 1974. Questions on the mechanism of temperate adaptation in marine phytoplankton. *Mar. Biol.*, **24**: 147-154.

Parsons, T. R. and J. D. H. Strickland 1963. Discussion of spectrophotometric determination of marine plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. *J. Mar. Res.*, **21**: 155-163.

Richards, F. A. and T. G. Thompson 1952. The estimation of characterisation of plankton populations by pigment analysis. II. A spectrophotometric method for the estimation of plankton pigments. *Ibid.*, **11**: 156-172.

Steemann Nielsen, E. 1952. The use of radio-active carbon (^{14}C) for measuring organic production in the sea. *J. Cons. int. Explor. Mer.*, **18**: 117-140.

Vijayaraghavan, S., K. J. Joseph, V. K. Balachandran and V. Chandrika 1975. Production of dissolved carbohydrates (DCHO) in three unialgal cultures. *J. mar. biol. Ass. India*, **17** (1): 206-212.