STUDIES ON THE GROWTH OF MARINE PHYTOPLANKTON

II. *ISOCHRYSIS GALBANA* PARKE

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(Text-figs. 1–8)

A good deal is known about the growth in culture of *Phaeodactylum tricornutum* Bohlin (*Nitzschia closterium* W. Sm. forma *minutissima* Allen & Nelson) (see Spencer, 1954; Harvey, 1955; Provasoli, McLaughlin & Droop, 1957). This organism, however, is a somewhat aberrant member of the Chrysophyta, and other marine and brackish-water representatives of the group, e.g. *Syracosphaera carterae* Braarud & Fagerland (Braarud & Fagerland, 1946; Provasoli, McLaughlin & Pintner, 1954), *Prymnesium parvum* Carter (Reich & Kahn, 1954; Droop, 1954), and certain other species (Droop, 1954, 1955 *a*, *b*) have been studied much less intensively from this point of view. It has therefore seemed worth while making a general study, similar to that reported in the first paper of this series for the diatom *Asterionella japonica* Cleve & Müller ex Gran, of the growth requirements of a representative of the Chrysophyceae. *Isochrysis galbana* Parke, a flagellate of some importance as a food organism of the oyster, has been selected for this purpose. Johnston (1955) has used unialgal cultures of this species in studies of dissolved organic matter in sea water and, since the investigation being described was carried out, Droop (1957) has reported that it has a requirement for cobalamin.

MATERIALS AND METHODS

The culture of *Isochrysis* used was isolated by Dr M. W. Parke from Port Erin fish ponds in 1938 (Parke, 1949). It was obtained from the Plymouth collection shortly after it had been partly freed of bacteria by phototaxis. It was not purified further.

The methods used for the culture and study of this organism were almost the same as those used for *Asterionella* (see Kain & Fogg, 1958). In stagnant culture a glass bead was included in each boiling tube to help in the removal of cells adhering to the bottom. Media were autoclaved at 15 lb. for less than 1 min instead of for 15 min. The media used were identical to those used for *Asterionella*, omitting sodium silicate and the buffer. They are denoted AO

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(similar to AK for *Asterionella*), AQ_N (similar to AR_N) and AQ_A (similar to AR_A). AO and AQ_N, containing natural sea water and soil extract in the case of AO, presumably contained cobalamin though it cannot be certain that the amount of this was always sufficient to maintain the optimum growth of *Isochrysis*. Vitamins were sometimes added to AQ_A.
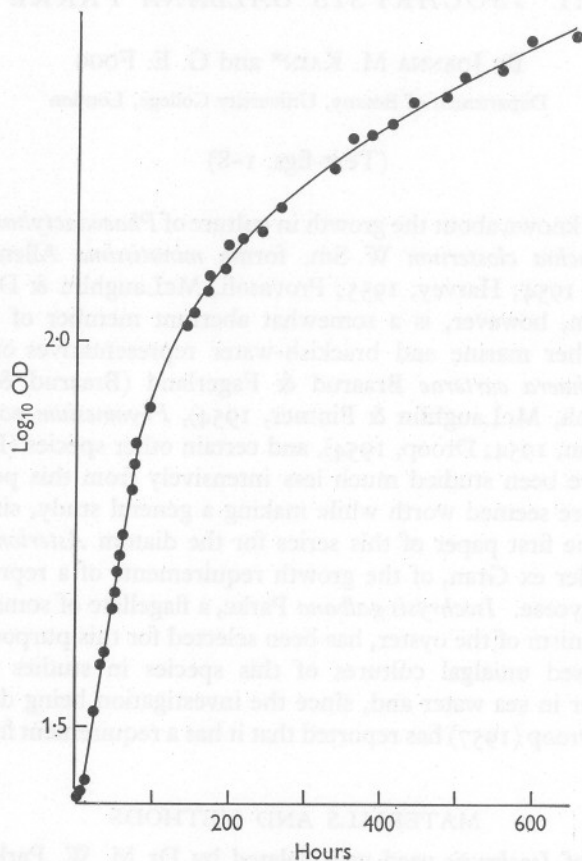

Fig. 1. The mean growth curve, determined by optical density readings, of two cultures of *Isochrysis*.

EXPERIMENTS

Growth

An example of the growth curve of *Isochrysis* in stagnant Erdschreiber medium is shown in Fig. 1. There was normally a lag period of up to 24 h after which growth was exponential, with a relative growth constant (defined as $(\log_e N_t - \log_e N_0)/t$ in days) of $k = 0.25$ to 0.55. After 100–150 h growth was limited by the availability of carbon dioxide but continued slowly for up to 500 h, giving final populations of the order of 10,000 cells/mm^3. During the

growth of *Isochrysis* in stagnant culture the pH of the medium rose to a higher level than for *Asterionella*, pH 9 being exceeded after 400 h.

Nitrogen supply

Isochrysis was grown in a series of concentrations of potassium nitrate in the artificial sea water medium (AQ_A), from an inoculum that had been washed in nitrate-free artificial sea water. Cell counts were made after 700 h and the cell crop in each culture calculated by subtracting the cell concentration in the controls to which no nitrate had been added from the cell concentration in the culture itself. This is shown plotted against the initial nitrate concentration in Fig. 2, which also gives the quantity of nitrate added to the medium divided by the crop obtained from it. The two lowest concentrations were evidently limiting and in these the mean nitrogen content was 0·0507 $\mu\mu$g-atom N/cell (0·7 × 10^{-9} mg N/cell).

Phosphorus supply

An analogous experiment on the phosphorus requirement was made with a series of concentrations of dipotassium hydrogen phosphate. The results are shown in Fig. 3. The mean quantity of phosphorus in the cells in the lowest three concentrations, where it was limiting, was 0·000972 $\mu\mu$g-atom P/cell (0·03 × 10^{-9} mg P/cell).

Salinity

The relative growth constant of *Isochrysis* in natural sea water was reduced to 0·27 at 10‰ S but showed no statistically significant deviation from a mean value of 0·36 over the range 15–40‰ S.

Artificial sea water

In medium AQ_A, composed principally of the major constituents of sea water and the trace elements with EDTA as used by Provasoli *et al.* (1957), the growth of *Isochrysis* was normal only when soil extract was present. This may be at least partly explained by its requirement for cobalamin. Unpublished work by Miss S. Arregger has shown that *Isochrysis* grows well in a synthetic medium, S 50 of Droop (1958), in which 0·25 g/l. of glycylglycine seems to be an important component.

Temperature

The relative growth constants at different temperatures in medium AO, expressed as percentages of the mean of those of control cultures at 25° C, are plotted in Fig. 4. Between 20° and 25° C was optimal; at 30° C no growth
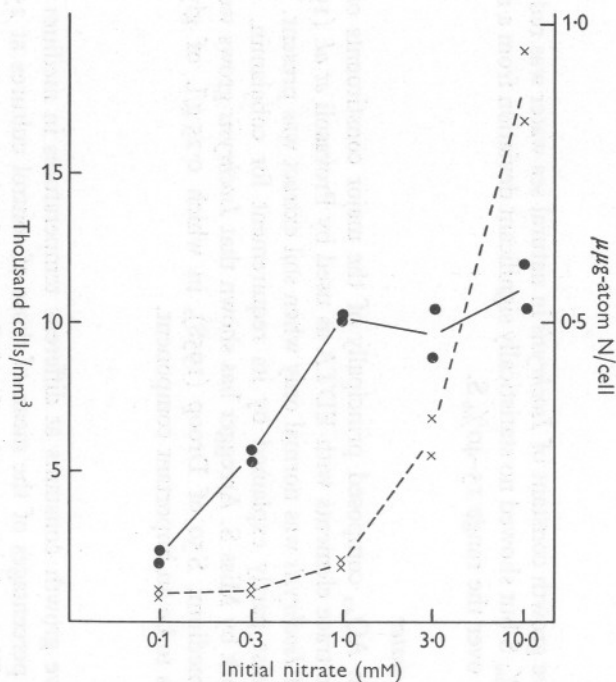
Fig. 2. The cell crop and nitrogen per cell of *Isochrysis* in relation to concentration of potassium nitrate. −●—●−, cell crop; −−×−−−×−−, nitrogen/cell.

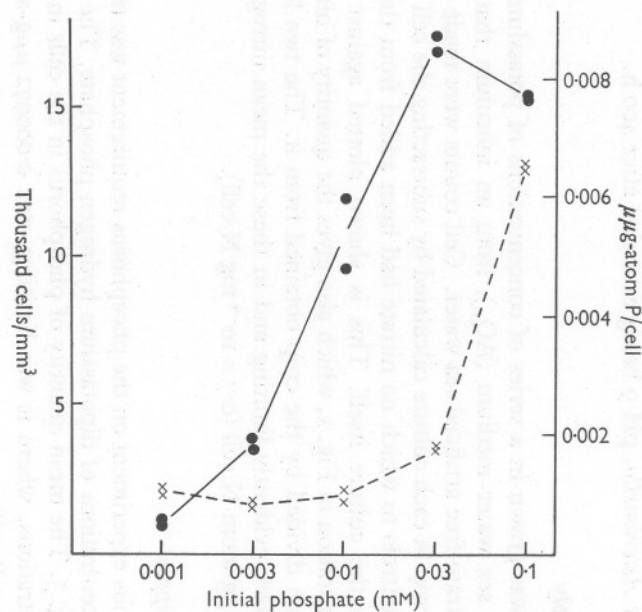Fig. 3. The cell crop and phosphorus per cell of *Isochrysis* in relation to concentration of dipotassium hydrogen phosphate. −●—●−, cell crop; −−×−−−×−−, phosphorus/cell.

took place, but exposure for a week to this temperature was not lethal as cultures returned to 20° C were able to grow.

Light

The results of two experiments to determine the relative growth constants at various light intensities in medium AQ_N are shown in Fig. 5. Saturation was reached at about 1500 lux, an intensity of the same order as that saturating
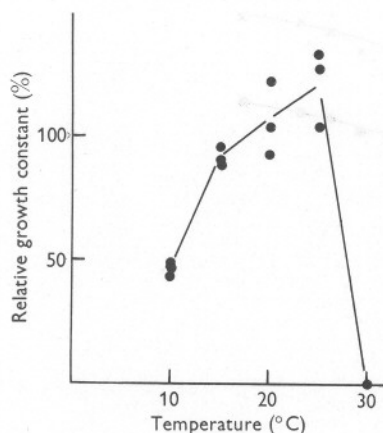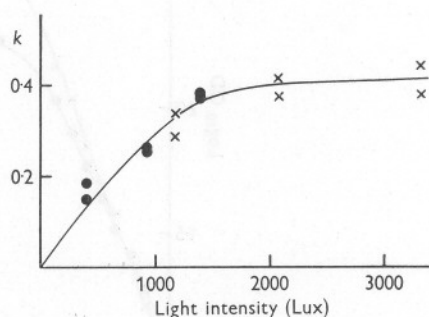

Fig. 4

Fig. 5

Fig. 4. The relative growth constant, as % of controls, of *Isochrysis* at different temperatures. (See text for further explanation.)

Fig. 5. The relative growth constant (k) of *Isochrysis* in different intensities of fluorescent light. ●, Expt. 1; ×, Expt. 2.

for the growth of *Chlorella pyrenoidosa* (Myers, 1953); there was no inhibition at 3000 lux. In both experiments the optical density per cell at the end of the exponential phase was considerably lower at higher than at lower light intensities.

Aeration

The mean growth curves of triplicate aerated and stagnant cultures in medium AQ_A are shown in Fig. 6. The mean relative growth constant was higher under aerated (0·526 ± 0·025) than under stagnant (0·446 ± 0·026) conditions, but the difference between the values was scarcely statistically significant at the 5% level. It is clear, however, that growth at the initial rate was continued to a much higher cell concentration (about 14,000 cells/ mm^3) under aerated than under stagnant conditions (about 6000 cells/mm^3). A final population of 24,000 cells/mm^3 was attained in aerated culture on occasion.

Hydrogen-ion concentration

In determining the optimum pH for the growth of *Isochrysis* it was not possible to use the buffer tris(hydroxymethyl)aminomethane at a really effective concentration as this was inhibitory (see below). Instead 1·65 mM of tris was used and daily adjustments made to the pH of the medium (AQ_A), by means of the dummy tube method previously described (Kain & Fogg,
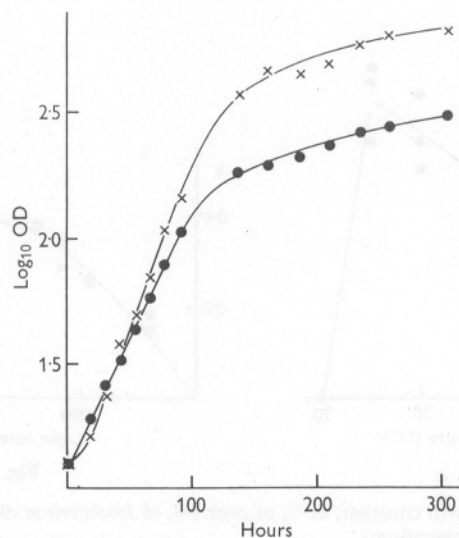

Fig. 6. The growth curves, determined from optical density readings, of *Isochrysis* in stagnant and aerated culture. –●—●–, stagnant; – – ×– – –×– –, aerated.
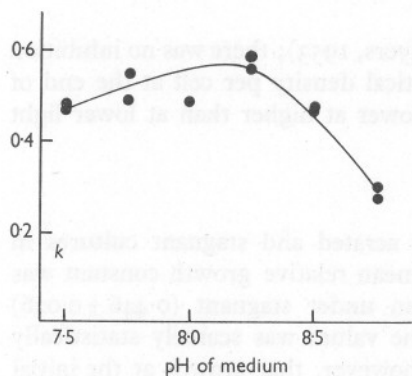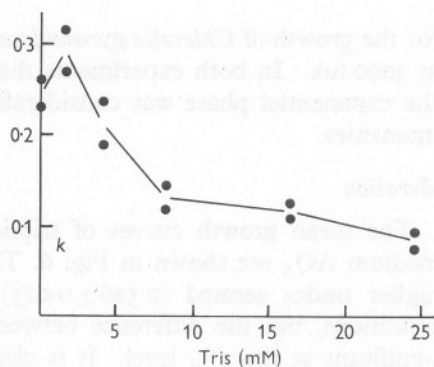

Fig. 7. The relative growth constant (*k*) of *Isochrysis* in media adjusted to various pH's.

Fig. 8. The relative growth constant (*k*) of *Isochrysis* in relation to concentration of the buffer tris.

1958). The maximum variation observed was 0·3 pH units at pH 7·5. It was mostly about 0·1 pH unit. The pH of each experimental tube was determined at the end of the exponential phase and found not to differ by more than 0·12 pH units from that of the corresponding dummy tube. The relative growth constants are shown in Fig. 7. There was clear inhibition at pH 8·75 only.

The relative growth constants in a range of tris concentrations are shown in Fig. 8. There was a marked inhibition at 8·25 mM and above. The difference between the k values for 1·65 mM and for the controls is not statistically significant. In a further experiment using triplicate cultures k values of 0·446 ± 0·026 and 0·446 ± 0·017 were obtained for control and 1·65 mM tris cultures respectively. It therefore appears that low concentrations of tris have no appreciable stimulatory effect on *Isochrysis*. The marked inhibition of the growth of *Isochrysis* in 8·25 mM of tris shows that this organism differs from those tried by Provasoli *et al.* (1957) which were all tolerant of 0·1 % (8·25 mM) of the buffer.

These results will be discussed together with those for other species on a later occasion.

SUMMARY

The flagellate *Isochrysis galbana* has been grown in unialgal culture under controlled conditions and its growth has been measured by means of optical density determinations and cell counts.

The relative growth constant has been found to be 0·55 $\log_e$ units per day under optimum conditions. Waters with salinities from 15 to 40‰ S supported rapid growth. The optimum temperature was 20° to 25° C and the optimum light intensity from 1500 to at least 3000 lux. The rate of growth was inhibited at pH 8·75 and above. The buffer tris(hydroxymethyl)aminomethane had an inhibitory effect at concentrations of 8 mM and above. Final yields of the order of 24,000 cells/mm³ could be obtained in cultures aerated by bubbling air. The minimum nitrogen requirement per cell was 0·0507 $\mu\mu$g-atom N/cell and the minimum phosphorus 0·000972 $\mu\mu$g-atom P/cell.

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