

## UPTAKE OF GLYCOLLATE BY *SKELETONEMA COSTATUM* (Grev.) Cleve IN BACTERIZED CULTURE

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**Abstract:** Glycollic acid, supplied at a concentration of  $1 \text{ mg l}^{-1}$ , increased the relative growth rate of *Skeletonema costatum* (Grev.) Cleve growing in bacterized culture at limiting light intensities. There was little or no such effect at intensities approaching saturation. The presence in the medium of alumina, an adsorbent for glycollate, prolonged the lag phase, the cells remaining viable for up to 5 days. Uptake of glycollate was not appreciably affected by the bicarbonate concentration of the medium. After 3 h, 80-92% of the glycollate carbon assimilated was found in the alcohol and benzene insoluble fraction of the cells. This is in agreement with the supposition that glycollate carbon is assimilated directly by the diatom rather than after degradation by bacteria to carbon dioxide.

### INTRODUCTION

That glycollic acid is an important extracellular product of photosynthesis by some algae, both in culture and in the natural environment, is well-documented (Tolbert, 1974; Fogg, 1975). This substance is a likely intermediate in the flow of energy from algae to bacteria (Fogg, 1966) and several bacterial strains which can utilize this substrate for growth have been isolated from a natural lake population (Wright, 1970). The question arises whether glycollate excreted by algae during photosynthesis is irretrievably lost to the algae, or whether it may, if present in the environment, be re-assimilated by them. It is, therefore, interesting from an ecological point of view to investigate whether a neritic marine diatom such as *Skeletonema costatum*, known to excrete glycollic acid (Hellebust, 1965; Ignatiades & Fogg, 1973) can utilize glycollate either heterotrophically or by photoassimilation, and to determine the effects of factors such as light intensity and bicarbonate concentration, which may influence such uptake.

Our investigations suggest that *S. costatum* behaves rather differently towards glycollic acid when in axenic culture than when in bacterized culture. Since any results obtained with bacterized cultures are presumably of greater ecological relevance we report them in this paper, leaving the investigations with axenic cultures, which are still in progress, to a later publication.

### METHODS AND MATERIALS

Cultures of the alga *Skeletonema costatum* (Grev.) Cleve were started from an

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axenic inoculum provided by Dr M. R. Droop, Oban. The alga was grown in an enriched sea-water medium (medium 'f', Guillard & Ryther, 1962) at 3000 lux and 20 °C. Cultures were aerated by bubbling air through the medium at a rate of 4 l/h. These batch cultures were unialgal but not axenic. The alga was used for experiments when in the early exponential phase of growth at a cell concentration of about  $10^4$  cells  $\text{ml}^{-1}$ . At this stage careful examination of samples under the phase contrast microscope showed few bacterial cells and aseptic techniques were used throughout to avoid further contamination.

*The growth of alga in the presence and absence of supplied glycollate*

Experiments were made at light intensities of 600, 1000, and 4000 lux. Algal inocula to give a final concentration of about 200 cells  $\text{ml}^{-1}$  were introduced into 50 ml of medium 'f' in 125 ml cotton wool-plugged glass bottles. Socks made of black nylon gauze cut down light intensity by the required amounts. These cultures were not bubbled with air. Cultures were grown in triplicate at each level of 0 mg  $\text{l}^{-1}$  and 1 mg  $\text{l}^{-1}$  added Na-glycollate at each of the 3 light intensities. Growth was estimated by cell counts made in duplicate on each culture at 24 h-intervals using a Palmer-Maloney counting chamber (Palmer & Maloney, 1954).

*Determinations of the effect of added bicarbonate on glycollate uptake*

5 ml of alga ( $4.3 \times 10^5$  cells  $\text{ml}^{-1}$ ) grown at 3000 lux with aeration by bubbling were diluted to 500 ml with artificial sea water without added bicarbonate; the sea water had been enriched with the nutrients used for medium 'f' and the pH adjusted to 7.9. For the experiments at 600 lux the alga was allowed to adapt to this light intensity for 2 h prior to experimentation.

Non-radioactive bicarbonate was added to aliquots of the suspension to give final concentrations of 4.9 mM, 9.8 mM, and 19.6 mM. 1 ml Na- $1^{14}\text{C}$ -glycollate (0.145 mg  $\text{l}^{-1}$ ) was added to each aliquot and the alga incubated for 3 h. Duplicate samples were taken at each bicarbonate concentration. The concentration of bicarbonate present in the samples, before the addition of bicarbonate, was calculated following the procedure of Strickland & Parsons (1968) and was 0.001 mM.

After incubation, the alga was filtered off, the filters washed with 25 ml sea water and the radioactivity measured in a liquid scintillation counter. All counts were corrected for adsorption on cell surfaces, membrane filters, and background.

*The distribution of  $^{14}\text{C}$  from glycollate into the alcohol soluble, benzene soluble, and alcohol insoluble fractions*

The method was adapted from that of Brown, Fager & Gaffron (1948). 1  $\mu\text{C}$  (0.145 mg  $\text{l}^{-1}$ ) Na- $1^{14}\text{C}$ -glycollate was added to each 50 ml aliquot of alga sampled at the third day of growth ( $10^4$  cells  $\text{ml}^{-1}$ ) in batch culture. Following a 3-h incubation at 3,000 lux, *i.e.*, the light intensity used for growing the cultures, the alga was

filtered on to 0.45  $\mu\text{m}$  Millipore filters to remove excess radioactive glycollate, washed with 50 ml filtered sea water, and the alga and filter extracted in 10 ml of boiling 80 % ethanol for 4 min. The suspension was then centrifuged for 20 min at 40,000  $g$ . The pellet was removed and again extracted with 10 ml of boiling 80 % ethanol for 4 min. Following the second centrifugation the radioactivity of the pellet fraction – which included disintegrated material from the filter – was counted. This fraction was colourless and powdery and contained the protein-polysaccharide part of the cells.

The two 10 ml portions of ethanol extract were combined and evaporated in a current of air. The residue was extracted successively with small quantities of benzene and the washings combined and made up to a volume of 10 ml. This alcohol-soluble–benzene-soluble fraction, was yellowish brown in colour and contained the lipid and pigment material of the cells. That part of the residue left was soluble in 80 % ethanol and the final volume of this, the alcohol-soluble–benzene-insoluble fraction, was also made up to 10 ml.

In each experiment two 50 ml of aliquots of algae were killed with 3 % formaldehyde, each centrifuged at 40,000  $g$  and the pellets re-suspended in 50 ml filtered sea water. 1  $\mu\text{C}$  radioactive glycollate was added to each aliquot of killed cells and the suspension shaken vigorously for 2 min. The alga was then filtered, the filters washed with 50 ml sea water and extracted in the same way as the experimental samples. The radioactivities of the three fractions were used to correct the values for the living alga reported below.

## RESULTS AND DISCUSSION

It is evident from Fig. 1 that glycollate has effects on the growth of *S. costatum* similar to those reported for a freshwater *Chlorella* (Sen & Fogg, 1966) and the marine species *Ditylum brightwellii* (Paredes, 1967–68a), *Micromonas squamata* (Paredes, 1967–68b), and *Nitzschia closterium* (Tokuda, 1968). There is a lag phase in *Skeletonema costatum* cultures at all three light intensities and at 600 lux it seems to be shorter in the presence of glycollate but this effect is not statistically significant. If, however, Sen & Fogg (1966) are correct in suggesting that the lag phase is mainly required for the establishment of an equilibrium concentration of glycollate in the external medium, then the presence in the medium of an adsorbent for glycollate might be expected to prolong this phase indefinitely. To test this, alumina, material used to adsorb glycollate in the analytical method of Shah & Fogg (1973), was added to medium which was then inoculated with *Skeletonema*. Cell counts were made at intervals after carefully stirring the cultures. The effect of the alumina was to prolong the lag phase up to 5 days, after which the diatom was no longer viable. Up to 5 days, however, when the alga was carefully decanted into fresh medium with 1  $\text{mg l}^{-1}$  glycollate, growth was resumed (Fig. 2). Glycollate is not the only substance adsorbed on alumina. Moed (1971), for example, used it to adsorb the yellow organic

acids in fresh waters. Our experiment does not, therefore, unequivocally show that glycollate is implicated but at least the results are in agreement with Sen and Fogg's (1966) hypothesis.

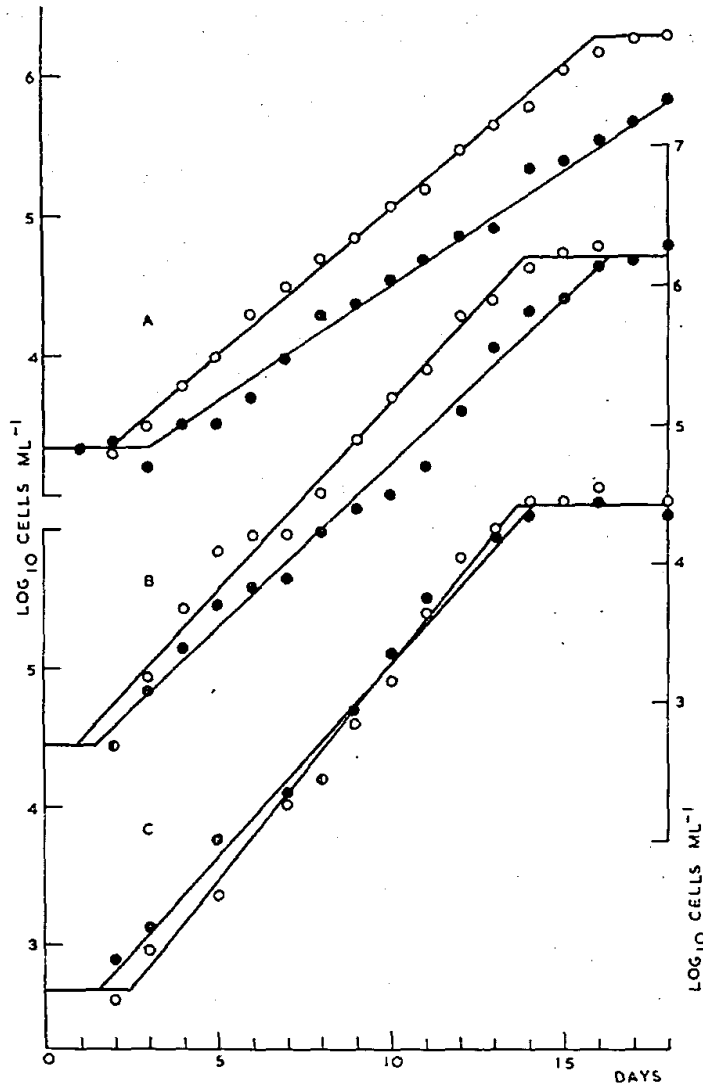


Fig. 1. Growth curves of *Skeletonema costatum* at different light intensities in the presence (○) and absence (●) of  $1 \text{ mg l}^{-1}$  sodium glycollate:  $\log_{10}$  cell numbers  $\text{ml}^{-1}$  plotted against age of culture in days: A, 600 lux; B, 1000 lux; C, 4000 lux, all with continuous illumination at  $20^\circ \text{C}$ .

The effects of two other adsorbents, magnesium oxide and ferric hydroxide, were also tested but even after one day of exposure to these substances the alga was not viable.

During the exponential phase of growth the relative growth constant increases in the expected manner with increasing light intensity (Table I). At the lower light intensities the relative growth constant is higher in the presence of glycollate and this effect is

proportionally greater (22 %) at 600 lux as compared with 1000 lux (14 %). This is similar to the findings of Sen & Fogg (1966) with *Chlorella*.

The final yield of *Skeletonema costatum* in these cultures was similar, irrespective of light intensity or the presence or absence of glycollate (Fig. 1).

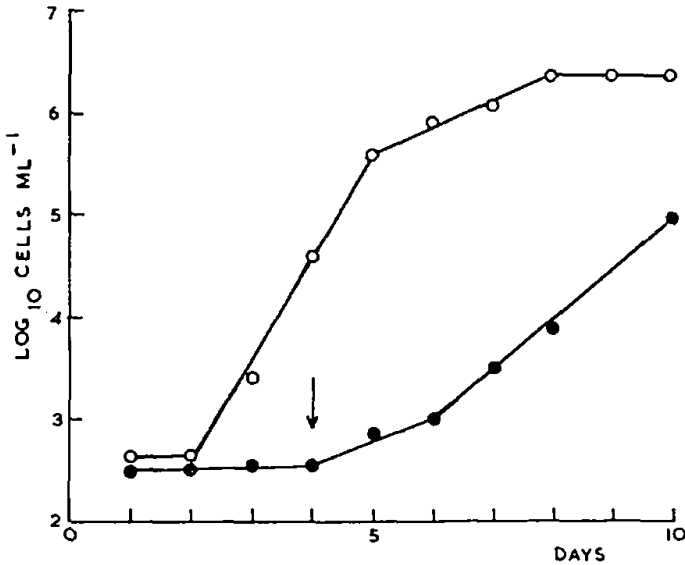


Fig. 2. Growth of *Skeletonema costatum* under continuous illumination of 6000 lux at 20 °C in the presence (●) and absence (○) of 2 g aluminium oxide 50 ml<sup>-1</sup> of medium: arrow indicates the point of transfer of the culture to fresh medium without alumina and with 1 mg l<sup>-1</sup> sodium glycollate.

TABLE I

Relative growth constants,  $k'$ , in log<sub>10</sub> day units and standard deviation for *Skeletonema costatum* during the exponential phase in bacterized culture at three different light intensities and in the presence and absence of 1 mg l<sup>-1</sup> of glycollic acid:  $P$ , probability of difference between control and experimental results being due to chance.

Light intensity (lux)	Without glycollate	With glycollate	$P$
600	0.170 ± 0.006	0.207 ± 0.004	< 0.001
1000	0.236 ± 0.008	0.270 ± 0.012	≈ 0.02
4000	0.277 ± 0.047	0.316 ± 0.011	≈ 0.30

Nalewajko, Chowdhuri & Fogg (1963) found an approximately two-fold increase in uptake by *Chlorella* of <sup>14</sup>C from glycollate at 500 lux as bicarbonate concentration was increased from 0.05 to 1.5 mM. No appreciable effect of bicarbonate concentration on glycollate uptake by *Skeletonema* has been found (Table II) but a correspondence between a marine diatom and a chlorophycean from oligotrophic freshwater in respect of their relations to bicarbonate concentration are scarcely to be expected. Less <sup>14</sup>C was taken up from glycollate at the higher light intensity, as would be expected from the results of the growth experiment.

All three fractionation experiments showed an incorporation of  $^{14}\text{C}$  from glycollate into alcohol-soluble-benzene-insoluble material and alcohol-insoluble material (polysaccharide and protein) but not into benzene-soluble material (lipid). A time-course experiment (Fig. 3) showed relatively higher labelling in the first of these three fractions during the first 30 min of exposure, which would be expected if glycollate is incorporated *via* the glycollate pathway into water-soluble substances such as serine and glyceric acid (Tolbert, 1974). Substantial incorporation of label into insoluble material agrees with the supposition that glycollic acid acts as a substrate for growth in the light.

TABLE II

The effects of bicarbonate concentration and light intensity on the uptake of  $^{14}\text{C}$ -glycollate by *Skeletonema costatum* during a 3-h period: radioactivity of the cells in counts  $\text{min}^{-1}$ .

NaHCO <sub>3</sub> conc. (mM)	600 lux	3000 lux
0.0	21,500 ± 8000	1900 ± 103
4.9	25,500 ± 3500	1900 ± 71
9.8	21,500 ± 500	2000 ± 71
19.6	20,000 ± 3000	2500 ± 1100

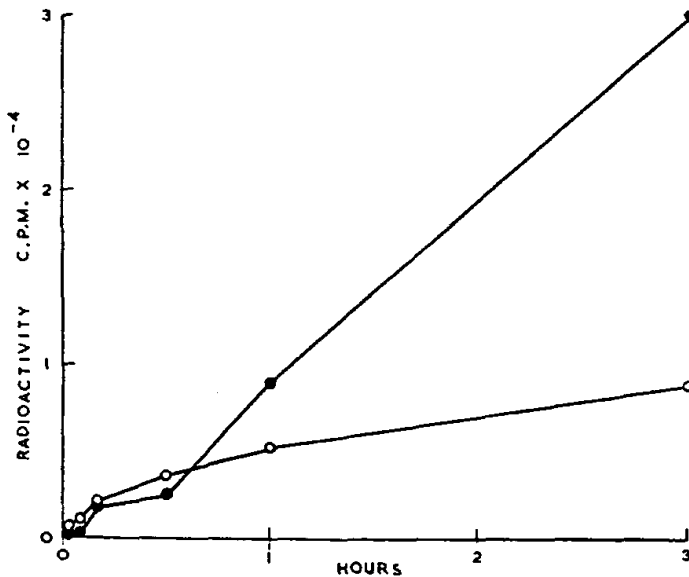


Fig. 3. Time course of incorporation of  $^{14}\text{C}$  from Na-1- $^{14}\text{C}$ -glycollate into alcohol soluble-benzene insoluble (O) and alcohol insoluble (●) fractions of *Skeletonema costatum* at 3000 lux and 20 °C.

These results are all consistent with the hypothesis that glycollate is assimilated by the *Skeletonema* itself and not by bacteria associated with it. The effects of light intensity on growth stimulation by, and assimilation of, glycollate are, therefore,

precisely those to be expected if the substrate were being photoassimilated. The absence of any marked effect of bicarbonate concentration on the uptake of glycollate carbon makes it clear that this is not being assimilated as carbon dioxide after bacterial degradation. Attempts by us to assess the relative importance of bacterial and algal uptake by the method of Hobbie & Wright (1965) have yielded confusing results and Bunt (1969) did not find this method satisfactory; it seems to us that the use by Hobbie & Wright (1965) of amounts of substrate assimilated at the end of a fixed period to estimate rates of uptake is liable to give unreliable results unless it is established that the rate of uptake is constant over the experimental period.

TABLE III

Distribution of  $^{14}\text{C}$  supplied as glycollate in three cell fractions of *Skeletonema costatum* incubated at 3000 lux for 3 h: cell concentration used  $\approx 4 \times 10^4$  cells  $\text{ml}^{-1}$  in all three experiments: all values are corrected for adsorption as described in the text (p. 229).

Fraction	% total uptake		
	Expt 1.	Expt 2.	Expt 3.
Alcohol-soluble- benzene-insoluble	19.4	8.77	11.6
Alcohol-soluble- benzene-soluble	0.0	0.0	0.0
Alcohol-insoluble	80.57	91.23	88.42

Hellebust (1965), using axenic cultures of *S. costatum*, was unable to find any uptake of glycollate and our own unpublished observations show that such uptake by this diatom is distinctly less in the absence than in the presence of bacteria. The explanation of this contradiction is not obvious. It may lie in the physiological state of the cells. Work on synchronized cultures of *Scenedesmus* (Nelson & Hess, 1969; Bruin, Nelson & Tolbert, 1970) has shown the uptake of glycollate to be largely confined to the dividing phase and if also true of *Skeletonema* it may be that the uptake observed by us is related to our use of actively growing cultures and to the better growth which this alga usually shows when in bacterized culture. A further factor which may be important is that glycollate dehydrogenase activity, necessary for glycollate assimilation, is derepressed in algae grown with forced aeration (Nelson & Tolbert, 1969; Codd, Lord & Merrett, 1969). It may also be that associated bacteria supply some factor necessary for assimilation of glycollate by *Skeletonema*.

It is possible that because some of our cultures were bubbled with air that glycollate dehydrogenase activity was enhanced as compared with that in natural populations and that we have over-estimated the ability of the alga to assimilate glycollate. Nevertheless, the results obtained suggest that the re-assimilation of excreted glycollate may occur under natural conditions so that to some extent this substance acts as an extracellular storage product.

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