J. mar. biol. Ass. India, 1975, **17** (1): 206 - 212

PRODUCTION OF DISSOLVED CARBOHYDRATE (DCHO) IN THREE UNIALGAL CULTURES

SUMITRA VIJAYARAGHAVAN, K. J. JOSEPH, V. K. BALACHANDRAN AND V. CHANDRIKA

Central Marine Fisheries Research Institute, Cochin 682 018

ABSTRACT

Production of dissolved carbohydrate (DCHO) in the unialgal cultures of *Tetraselmis gracilis*, *Chlorella salina* and *Synechocystis salina* was studied in the laboratory for a period of 31 days. DCHO values ranged from 0.5 to 25.0 mg/l in *T. gracilis* culture, the range of variation in *C. salina* and *S. salina* was 0.5 to 8.5 mg/l. Both in *T. gracilis* and in *S. salina* cultures, the DCHO values showed an increasing trend towards the end of stationary phase, whereas in *C. salina*, the production of DCHO could not be attributed to any particular phase of growth of the culture. The fluctuations of DCHO values for a given number of cells showed that it is not the number but the physiological state of the organisms that influences the production of DCHO. No definite relationship or trend was evident between DCHO and the bacterial counts.

INTRODUCTION

EARLIER studies of Lewin (1956), Guillard and Wankersky (1958) and Collier *et al.* (1953) have shown that pure algal cultures produce extracellular carbohydrates. Barring in view some information available on the liberation of extracellular products by phytoplankton (Samuel *et al.*, 1971) and the occurrence of dissolved carbohydrate in Cochin Backwater (Sumitra *et al.*, 1972), no detailed work has been undertaken on unialgal cultures so far. In view of the importance of utilisation of extracellular DCHO by the phytoplankton and the role they play in the transfer of energy in an ecosystem, the present work was considered necessary. This study aims at finding out how much DCHO is produced at different phases of unialgal cultures.

We wish to extend our gratitude to Dr. S. Z. Qasim, former Director and Dr. E. G. Silas, Director, Central Marine Fisheries Research Institute, Cochin for their keen interest, encouragement and help in the preparation of this manuscript. It is a pleasure to acknowledge Dr. P. V. R. Nair for the supply of the cultures.

MATERIAL AND METHODS

Three unialgal cultures, *Tetraselmis gracilis* and *Chlorella salina* of Chlorophyceae and *Synechocystis salina* of Cyanophyceae were used for the present study. These cultures were maintained in the laboratory for a period of 31 days. Simultaneously chlorophyll-*a*, and bacterial counts were also made. These cultures were inoculated into 1.5 litres of Miquels medium (modified by Ketchum and Redfield, 1938). The cultures were exposed to subdued daylight and maintained in the laboratory at room temperature. Aliquots of algal cultures were drawn at two days interval for 31 days to study the changes in the cell numbers, chlorophyll-*a* content, quantity of DCHO produced and bacterial counts.

A few days after inoculation, 25 ml of the aliquots of the culture medium containing the algae in suspension were used for the above mentioned determinations;

but as the culture grew dense, the volume was reduced to 10 ml to avoid clogging during filtration. For DCHO determination, the culture medium was filtered through Whatman (GF/C 4.25 cms) glass fibre filteres and the method of Umbreit

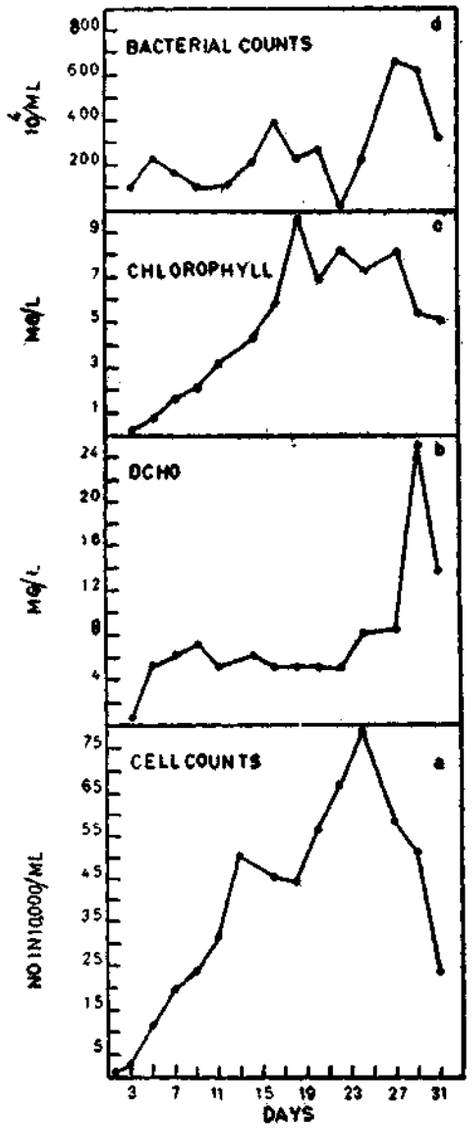

Fig. 1. Changes in the cell numbers, chlorophyll content, production of DCHO and bacterial counts for a period of 31 days in *Tetraselmis gracilis* culture.

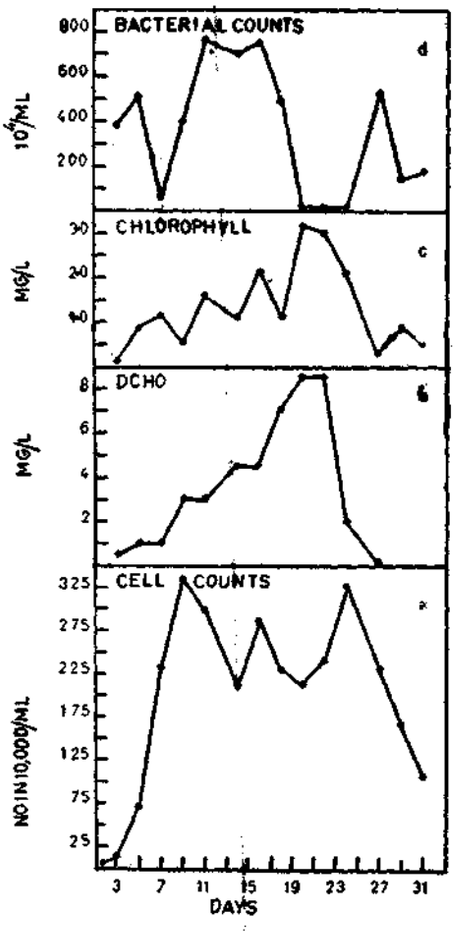

Fig. 2. Changes in the cell numbers, chlorophyll content, production of DCHO and bacterial counts for a period of 31 days in *Chlorella salina* culture.

et al. (1959) was used. Chlorophyll-*a* was determined according to Strickland (1963). Enumeration of total viable bacterial count was made 72 hours after incubation at room temperature.

RESULTS

Figure 1, gives the changes in the cell numbers, chlorophyll-*a* content, amount of DCHO produced and the bacterial count with reference to *T. gracilis*. From an initial concentration of 6467 cells/ ml, the cell concentration reached 25,000/ ml on the 3rd day. From 5th day till 14th day, there was an increasing trend in the cell numbers. Between 16th and 18th day, a slight drop in the cell count was

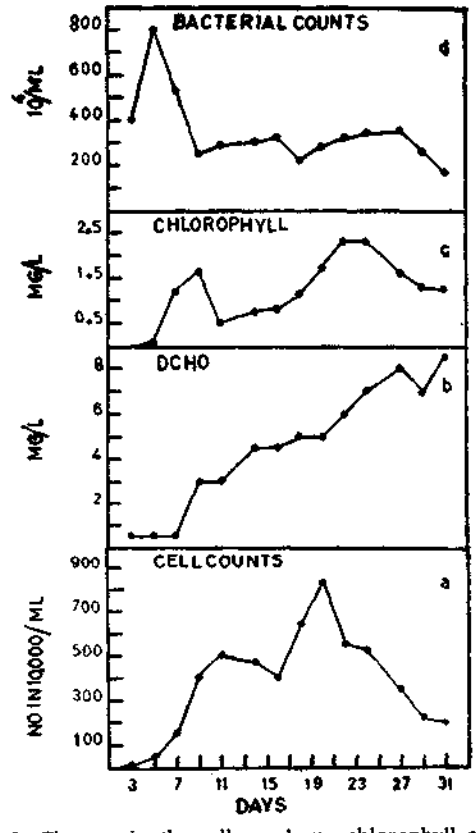

Fig. 3. Changes in the cell numbers, chlorophyll content production of DCHO and bacterial counts for a period of 31 days in *Synechocystis salina* culture.

noticed. This was followed by increase upto 24th day and a decline thereafter (Fig. 1a). The chlorophyll content on the other hand showed a steady increase from 0.231 mg/l on the 3rd day to 9.6 mg/l on the 18th day (Fig. 1c). Between 20th and 29th day, the chlorophyll values were found to fluctuate within a narrow range (7.0 to 8.0 mg/l). From 26th day onwards, as in the case of cell numbers, a declining trend was observed.

The DCHO concentration varied from 0.5 to 25.0 mg/l. From an initial level of 0.5 mg/1 on the 3rd day of inoculation, there was a tenfold increase on the 5th pay (5.0 mg/1). Between 5th and 22nd day, the level of DCHO became more or less steady (6.0 to 8.0 mg/1). When the cell numbers showed a declining trend between 24th and 31days of inoculation, the DCHO showed an increasing trend, reaching a maximum of 25.0 mg/1 on the 29th day (Fig. 1b). The bacterial count (Fig. 1d) from 3rd day till 22nd day fluctuated from 12 to 376 with an average count of 178×10^4/ml. When the cell numbers and chlorophyll began to decline, the level of bacterial count (average) between 24th and 31st day became high (452×10^4/ml).

The cell numbers of *Chlorella salina* (Fig. 2a) showed an increasing trend from an initial count of 24800 cells/ml on the 1st day to 3350000 cells/ml on the 9th day after inoculation. Between 11th day and 24th day, it was more or less in a steady state at an average counts of 2380000 cells/ml. From 24th day a declining trend in the cell numbers was observed. The values for chloropyll-a fluctuated from 0.08 to 1.068 mg/1 between 3rd day and 18th day (Fig 2c). On the 20th day, there was an increase which reached a maximum of 3.204 mg/1, and then declined.

The level of DCHO revealed a steady increase (Fig. 2 b) from 0.5 mg/1 on the 3rd day to 8.5 mg/1 on 22nd day. This was followed by a sudden decline in the DCHO level (2.0 mg/1). Thereafter it was found to be absent till the end of the experiment. No definite trend was found with reference to bacterial counts except that the average counts were rather high (503×10^4/ml) between 3rd and 18th day. It touched minimum counts from 20th to 24th day. On the 27th day, the counts shot up to 528×10^4/ml. This was again followed by reduction (Fig. 2 d).

From an initial concentration of 21533 cells/ml on the 1st day of inoculation, the cell counts of *Synechocystis salina* reached 5070000 cells/ml on the 11th day. Between 14th and 18th day, there was not much variation in the cell counts. The maximum cell concentration of 8380000 cells/ml was reached on 20th day, following which there was a decline in the cell numbers (Fig. 3 a). The chlorophyll-*a* showed an increase from 0.067 mg/1 on the 3rd day to 1.602 mg/1 on the 9th day after inoculation (Fig. 3 c). Thereafter there was a drop (0.534 mg/l.) Between 11th and 20th day there was a gradual rise in chlorophyll values. From 24th day onwards it again declined.

To begin with the level of DCHO (Fig. 3b) was more or less constant (0.5mg/1) till 7th day of inoculation. From 9th day to 31st day, there was a gradual increase in the DCHO level of the medium. The bacterial count was found to be high between 3rd and 7th day. This was followed by more or less uniform counts untill the 27th day, thereafter it decreased (Fig. 3d).

DISCUSSION

Although the values of DCHO, cell numbers and chlorophyll content have been given in results as they appeared, it was felt that production of DCHO in relation to unit number of cells and the relationship of DCHO per unit quantity of chlorophyll content would be more illustrative. From such an analysis it became evident that on the third day, the DCHO content of the three species of algae *T. gracilis*, *C. salina* and *S. salina* were 200 µg, 38.5 µg and 100 µg/10,000 cells respectively. Their corresponding chlorophyll values were 924 µg, 6.8 µg and 13.4 µg/10,000 cells. Thus in *T. gracilis*, the ratio of DCHO to chlorophyll was 2.2: 1

in *C. salina* it was 5.6: 1 and in *S. salina* it amounted to 7.5: 1 This relationship was not maintained at the various phases of growth of the three cultures.

The maximum concentration of DCHO (553 µg/10,000 cells) for *T. gracilis* was obtained on the 30th day towards the end of the experiment. Generally high values of DCHO was recorded both during logarithmic and stationary phases of growth. In the case of *C. salina* maximum DCHO (39.5 µg/10,000 cells) was recorded on 2nd and 20th days after inoculation. *S. salina* gave the highest concentration of DCHO (100 µg/10,000 cells) on the 2nd day and the next high value (40 µg/10,000 cells) on the 30th day. Thus in the present study, the pattern of production of DCHO varied with different species. This has been found to be true by earlier workers also. Guillard and Wankersky (1958) in some marine flagellates reported that accumulation of DCHO occurred at the end of the logarithmic phase of growth and increased during the stationary or senescence phase of the culture. Allen (1956) and Lewin (1956) found that *Chlamydomonas* sp. produced extracellular products throughout the course of its growth. Fogg (1952) also recorded a similar pattern of liberation of nitrogenous compound by *Anabaena cylindrica*. Collier (1959) showed that the concentration of extracellular carbohydrate increased with the growth of algae in culture even during its logarithmic growth phase. Nalewajko *et al.* (1963) showed that the relative production of dissolved organic matter by *Chlorella pyrenoidosa* increased when the cell suspension was diluted. Walsh (1966), however, noted that the concentration of DCHO in natural environment need not be directly related to the standing crop of phytoplankton.

The fluctuations of DCHO values for given number of cells show that probably it is not the number of organisms but the physiological state of the algae that influences the production of DCHO. For *T. gracilis*, the ratio of DCHO to chlorophyll was more than 1 at both their logarithmic and late phases of growth. In the stationary phase of growth, it was nearer to 1. For *C. salina*, the ratio of DCHO to chlorophyll varied from 0.93 to 6.5:1. On the 18th day, the ratio of chlorophyll to DCHO was 6.5:1. This was found to be the maximum value of DCHO in relation to chlorophyll. From then onwards the DCHO showed a gradual decline (0.94:1) and on the 24th day till the end of the experiment, the DCHO was totally absent. From these observations it appears that, unlike *T. gracilis*, the variations in DCHO content per unit quantity of chlorophyll can not be attributed to any particular phase of growth of the culture. It is also clear that in *C. salina* DCHO was not released from the late stationary phase onwards; whereas, in *T. gracilis* the DCHO showed an increase.

In *S. salina*, the maximum DCHO liberated per µg of chlorophyll was 7.5. This was on the 2nd day. The variations in their ratio was not much except on 4th and 6th day when it amounted to 0.43:1 and 0.31:1 respectively. Though there was no large difference in the DCHO–chlorophyll ratios, a slight increase was observed towards the end of stationary phase.

Fogg (1962) reports that isolated strains of bacteria use glycollate as a source of carbon. Thus many bacteria probably derive much of their carbon requirements from the dissolved organic substances, of which algal extracellular elaboration may form an appreciable proportion. Guillard and Wankersky (1958) reported that when the aliquots of culture were deliberately contaminated with bacteria, the production of carbohydrate increased. This probably suggests that bacteria

synthesise carbohydrate from other organic matter produced by the algae. However, in the present study, no clear trend or relationship was evident between DCHO and bacterial counts.

From the present studies, it is clear that the algae produce DCHO. Such a substance released into the environment will have a variety of ecological effects. These have been summed up by Fogg (1962). Some of the ecological implications of the extracellular products are: extracellular products are probably important as nutrients in the symbiotic associations in which the algae participate or various organisms derive a part of their nitrogen from the extracellular products of nitrogen fixing blue green alage; it may also play an important role in the succession of algae by producing growth promoting substances or antibiotics and these may form a complex of extracellular products which may favour algal growth by maintaining nutrients in a soluble state and which would otherwise get precipitated. The variations in the total dissolved organic matter both in lakes (Domogalla *et al.*, 1925) and in the sea (Duursma, 1960) show that much of it is derived from the decay of phytoplankton.

It is therefore necessary to obtain more data on the production of DCHO by the different algal cultures and the knowledge thus gained can be tested in the field to develop a better understanding of the kinetics of energy flow.

REFERENCES

ALLEN, M. B. 1956. Excretion of organic compounds by *Chlamydomonas*. *Arkiv. Microbiol.*, **24** : 163–168.

COLLIER, A. 1959. Some observations on the respiration of the American oyster *Crassostrea virginica* (Gmelin). *Publ. Inst. Marine Sci. Taxas*, **6** : 92–108.

————, S. M. RAY, A. W. MAGNITZKY AND J. O. BELL 1953. Effect of dissolved organic substances on Oysters. *U. S. Fish wild life serv. Fishery Bull.*, **54** : 167-185.

DOMOGALLA, B. P., C. JUDAY AND W. H. PETERSON 1925. The forms of nitrogen found in certain lake waters. *J. Biol. Chem.*, **63** : 269–285.

DUURSMA, E. K. 1960. Dissolved organic carbon, nitrogen and phosphorus in the sea. *Neth. J. Marine Research*, **1** : 1–148.

FOGG, G. E. 1952. The production of extracellular nitrogenous substances by a blue green alga. *Proc. Roy. Soc.*, **139** : 372–397.

————,1962. Extracellular products. *In*: R. A. Lewin (Ed.) *Physiology and biochemistry of algae*. Academic press. pp 475–489.

GUILLARD, R. R. L. AND P. J. WANKERSKY 1958. The production of extracellular carbohydrates by some marine flagellates. *Limnol. Oceanogr.*, **3** : 449–454.

KETCHUM, B. H. AND A. C. REDFIELD 1938. A method for maintaining a continuous supply of marine diatoms by culture. *Biol. Bull. Woodshole*, **75** : 165–169.

LEWIN, R. A. 1956. Extracellular polysaccharides of green algae. *Can. J. microbiol.*, **2** : 665–672.

NALEWAJKO, C. N., N. CHOWDHURI AND G. E. FOGG 1963. Excertion of glycollic acid and the growth of a planktonic *Chlorella*. *In : Studies on microalgae and photosynthetic bacteria*. Japan. Soc. Plant physiologists, Tokyo Univ. press, Tokyo. pp 171–183.

SAMUEL, S., N. M. SHAH AND G. E. FOGG 1971. Liberation of extracellular products of photosynthesis by tropical phytoplankton. *J. mar. biol. Ass. U. K.*, **51** : 793–798.

STRICKLAND, J. D. H. 1963. Marine plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. *J. Mar. Res.*, **21** : 155.

SUMITRA VIJAYARAGHAVAN, K. J. JOSEPH, D. C. V. EASTERSON AND V. K. BALACHANDRAN 1972. Studies on dissolved carbohydrate content (DCHO) in a tropical estuary. *J. mar. biol. Ass. India*, **14** (2): 864–866.

UMBREIT W. W., R. H. BURRIS AND J. E. STAUFFER 1959. *Manometric techniques*. Burgess publishing company; 305 pp.

WALSH, G. E. 1966. Studies on dissolved carbohydrate in Cape Cod waters. III. Seasonal variation in oyster pond and Wequaquet Lake, Massachusetts. *Limnol. Oceanogr.*, **11** : 249-256.