Marine Biology 4, 208—214 (1969)

Carbohydrate metabolism in the marine diatom *Skeletonema costatum*

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Abstract

This is a study on the metabolism of the algal biochemical constituents of a marine diatom, *Skeletonema costatum*. The carbohydrates, protein, and lipid, of the diatom grown under light and dark conditions were analyzed. The composition of the organic compounds did not vary in the alga grown under continuous light conditions throughout the experiment, although a little accumulation of carbohydrate and lipid was observed in the stationary phase of the algal growth. When the diatom, at maximum growth stage, was transferred to darkness, 44.4% of the carbohydrate, 57.8% of the protein, and 27.0% of the lipid were used by respiration within a few days. Detailed analysis of the algal carbohydrate demonstrated that low molecular weight carbohydrates, consisting of glucose, and β-1,3-glucan, were readily metabolized by algal respiration, leaving cell-wall carbohydrates consisting of mannan and pentosan which are immune to algal respiration. The respiratory utilization of carbohydrates is discussed in relation to the rapid decay of glucan from marine particulate matter during the course of its sinking to further depths in the ocean, as observed previously by the present author.

Introduction

Studies on the organic constituents of marine particulate matter from Sagami Nada, off the eastern coast of Honshu, Japan, have been conducted and reported by the present author (HANDA, 1967; HANDA and TOMINAGA, 1969). It has been shown that particulate matter consists of laminarin type 1,3-glucan and hemicellulose as the main components of the particulate carbohydrate. A detailed analysis of the particulate matter from various depths indicated that the glucan decayed more rapidly than the other carbohydrates during the course of sinking to the depths, leaving the hemicellulose as the main carbohydrate constituent of deep-water particulate matter.

The behaviour of algal biochemical constituents under both light and dark conditions has been studied by several authors (RICKETTS, 1966; WERNER, 1966; EPPLEY et al., 1967). They reported that carbohydrate content in algae was decreased by algal respiration, and also conversion into other biochemical constituents such as protein and lipid, when the algae were placed in the dark. Thus, algal respiration is assumed to play an important role in the decay process of the 1,3-glucan in marine particulate matter, although bacterial decomposition processes and zooplankton grazing may be involved in this process to a certain extent.

The present paper is a preliminary study evaluating the significance of algal respiration which utilizes biochemical constituents. This study is especially focussed on the metabolic behaviour of the storage carbohydrates during the various stages of algal growth under light and dark conditions.

Materials and methods

A marine diatom, *Skeletonema costatum*, was cultured in a 10 l vessel fitted with glass tubing through an unadsorbed cotton stopper. 10 mg of KNO_3, 10 mg of K_2HPO_4, 10 mg of Na_2SiO_3 and 1 drop of 1% solution of $FeCl_3$ were added to each liter of sea water. All components and culture media were sterilized by autoclaving but, in spite of this precaution, the test for bacteria was positive. The temperature of the culture medium was set at 18 °C by submerging the vessel in running water. Illumination of 3,800 lux, measured at the surface of the culture medium, was provided from above by fluorescent lighting.

At certain intervals, an aliquot of the culture medium was withdrawn. This was used for the determination of the photosynthetic rate and for the collection of algal cells by filtration through 984-H ultra filters (H. Reeve Angel and Co.).

Photosynthetic rate was measured by the ^{14}C-method (STEEMANN NIELSEN, 1952) after exposure under 3,800 lux for 3 h at 18 °C. Twenty ml of the culture medium were used, after being diluted with 80 ml of sea water which was sterilized by boiling. The organic carbon and nitrogen of the alga were measured through the wet combustion method developed by KOYAMA (1954), and the Kjerdahl method, respectively.

Carbohydrate content was determined by the phenol sulfuric acid method, and protein content by the ninhydrin method, which determines the amino acid derived from protein after acid hydrolysis (6N, HCl) at 100 °C for 24 h. Lipid content was then determined by weighing the chloroform-methanol (2:1 v/v) extracts of the alga. Chlorophyll a of the alga was estimated by the RICHARDS and THOMPSON method (1952).

For separation of the carbohydrate components of the alga, the cells collected from 2 l of the culture medium on the ultra filter were treated with 100 ml of water in a boiling water bath for 1 h, to secure the water-extractable carbohydrate. This procedure was repeated until the extract showed negative in the phenol sulfuric acid test. To the combined extracts were added ethanol (1 vol.) and acetone (3 vol.) consecutively. The carbohydrate was separated into soluble and insoluble fractions. The residual material after water extraction was a cell-wall carbohydrate of the alga, and no further isolation treatments were conducted.

The monosaccharide composition of the carbohydrate fractions of the alga, as well as its whole cells,

was determined by the gas chromatographic technique described in a previous paper (HANDA and YANAGI, 1969). The gas chromatograph used in this work was Toyo Roshi Model GC-S-7 equipped with a thermal conductivity detector. Chromatographic peak area measurements were made with a Polar compensation planimeter. A 200×0.5 cm column packed with 3% ECNSS-M on Gas Chrom Q (Applied Science Laboratories) was employed at a helium flow rate of 35 ml/min. The column was operated isothermally at 194 °C with an injection port temperature at 245 °C. The monosaccharides employed as standards for analysis were obtained from Wako Pure Chemical Co. Ltd.

Results

Skeletonema costatum was grown under continuous light conditions for 8 days. Half of the culture medium was transferred to darkness and the rest allowed to stand, successively, in the light for an additional 10 days. Both culture media were withdrawn at intervals; algal organic carbon, organic nitrogen, chlorophyll a and the major biochemical constituents such as carbohydrate, protein and lipid, were determined. The concentrations of the dissolved carbohydrates were measured by use of the culture medium, after collection of algal cells by filtration through an ultra filter.

The variations of the concentrations of algal organic carbon and nitrogen, and of dissolved carbohydrates under continuous light conditions, according to time, is shown in Fig. 1. After inoculation, the algal biomass increased to its maximum in 8 days. A little decrease in the concentration of the organic elements, however, was observed in the stationary phase. The concentration of the dissolved carbohydrates tended to increase, with time, from 0.14 to 0.40 mgC/l. Such an increase in the dissolved carbohydrate may be due to the excretion of carbohydrate by the alga; these values are equivalent to 0.01 to 10.6% of the total fixed carbohydrate. These values were almost identical with those obtained by NALEWAJKO (1966), who made a similar calculation of organic carbon in several classes of algae. EPPLEY et al. (1967) also reported that only 2 to 3% of the radiocarbon assimilated by *Ditylum brightwellii* cells grown in the light-dark cycle, were released to the medium during the dark period.

When the *Skeletonema* cells, at maximum growth, were transferred to darkness, features of organic carbon, organic nitrogen and dissolved carbohydrate differed during the course of growth (Fig 2). The concentration of algal organic carbon decreased from 11.6 to 6.80 mgC/l during the dark period. Rapid decrease in the biochemical element was observed, especially within the first few days, after which the value did not vary so much. Organic nitrogen decreased to 1.5 mgN/l, a drop of 0.6 mgN/l, several days after darkening. No further change of values was observed.

No significant change in the concentration of the dissolved carbohydrate was observed during the light and dark periods in this experiment, although rather low values were measured in the logarithmic phase of the algal growth. Thus, in no case was the production of extracellular carbohydrate sufficient to account for the decline of algal carbohydrate in the dark. This finding can be extended to the cases of the other biochemical constituents in the algal cell.

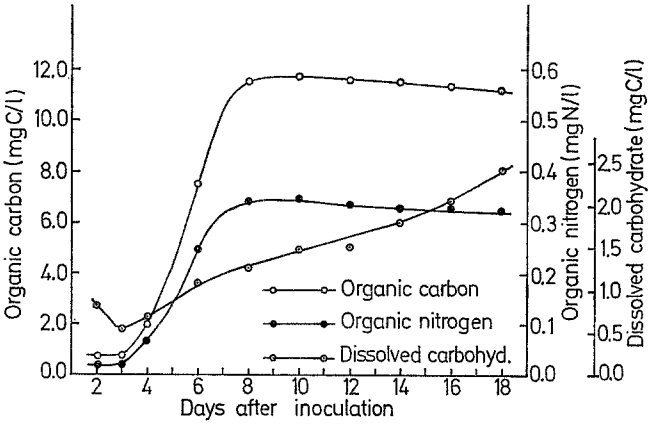

Fig. 1. Cellular content of organic carbon and nitrogen in *Skeletonema costatum* grown in the light and the concentration of dissolved carbohydrate in culture medium

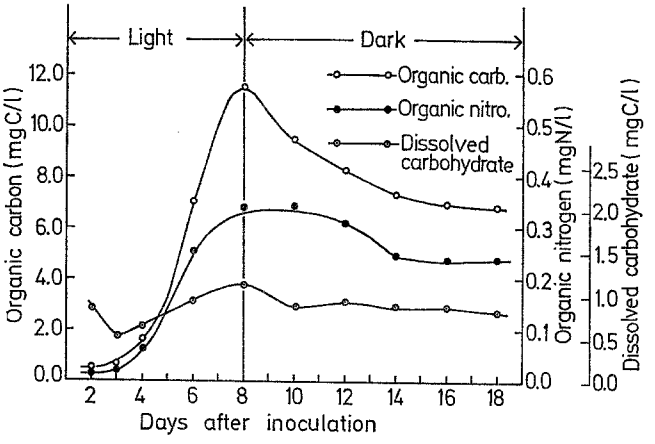

Fig. 2. Cellular content of organic carbon and nitrogen in *Skeletonema costatum* grown in the light and later placed in the dark, and the concentration of dissolved carbohydrate in culture medium

To test the biochemical activity of the alga in various stages of growth during the dark period, relative photosynthetic activity was estimated (Fig. 3). The photosynthetic rate of the culture medium after the inoculation of alga increased with time, until it reached a maximum value in the logarithmic phase of the algal growth, while the concentration of algal organic carbon was still increasing; rather lower values were observed at maximum growth stage. When the alga was transferred to the dark, its photosynthetic activity continued for several days at the level of

values observed at maximum growth stage; thereafter, the alga tended to lose its photosynthetic activity. Even in such physiological condition, the alga contained an adequate amount of chlorophyll a to perform photosynthesis. This indicates that a certain factor, other than chlorophyll a, is affected by the loss of photosynthetic activity in the alga during the dark period. The concentration of the organic carbon tended

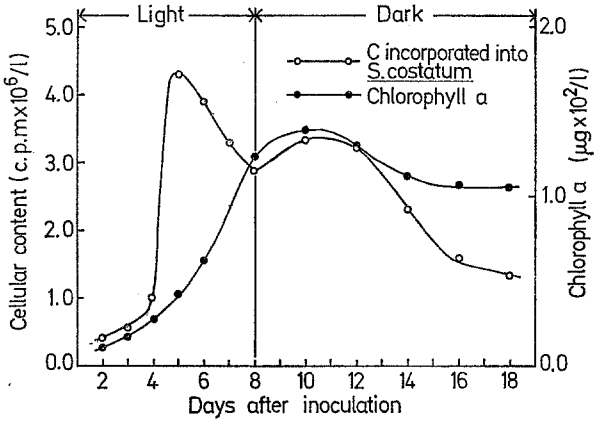

Fig. 3. Photosynthetic activity and chlorophyll a content in *Skeletonema costatum* grown in the light and later placed in the dark

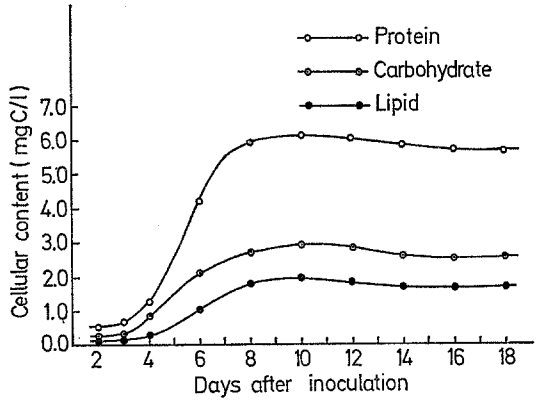

Fig. 4. Cellular content of protein, carbohydrate and lipid in *Skeletonema costatum* grown in the light

to decrease with time in the dark, as well as the photosynthetic activity. Thus it is assumed that loss of organic carbon results in loss of photosynthetic activity in the alga.

Studies on the biochemical composition of *Skeletonema costatum* in various growth stages under light and dark conditions were conducted. The major organic constituents of cells of the alga were carbohydrate, protein and lipid. 85.9 to 97.5% of the algal organic carbon could be accounted for by these biochemical constituents. Protein content of the alga was almost constant during growth, while accumulation of carbohydrate and lipid was observed only in the stationary phase of growth under light conditions (Fig. 4).

When the alga was placed in the dark, the features of its biochemical components were decidedly different from those observed in the light (Fig. 5). During the dark period, losses of 1.64, 1.62, and 0.70 mgC/l of carbohydrate, protein, and lipid, respectively, were ascertained (Fig. 6). The sum of carbohydrate, protein, and lipid utilized during the dark period can account for the total loss of algal organic materials, ranging from 73.0 to 90.0% during this period.

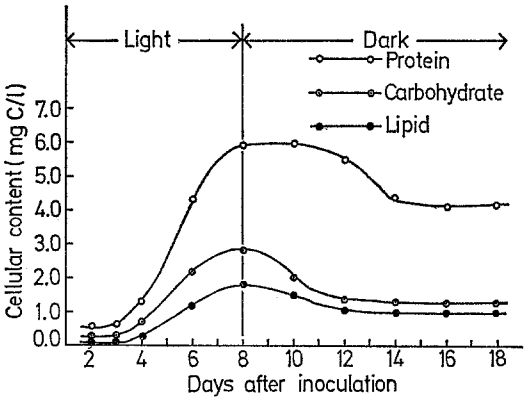

Fig. 5. Cellular content of protein, carbohydrate and lipid in *Skeletonema costatum* grown in the light and later placed in the dark

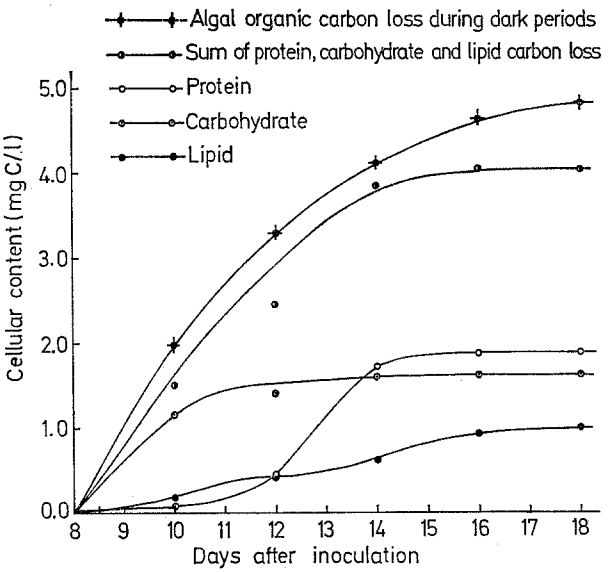

Fig. 6. Decreases in protein, carbohydrate and lipid contents of *Skeletonema costatum* when the culture was placed in the dark

Utilization of these compounds did not seem to occur at the same time, but they were metabolized consecutively. Carbohydrate was the first compound to be utilized in the early stage of darkening, while protein utilization started only when no further change of carbohydrate concentration was observed. Lipid seems to be utilized gradually, and rather independently from the metabolism of carbohydrate and protein.

To obtain more information on carbohydrate metabolism in algal respiration, algal carbohydrate was fractionated with water and organic solvents. The carbohydrate of the algal cells grown during the stationary phase was fractionated into water-extractable carbohydrate (WEC), and residual carbohydrate (RC). The former fraction was divided into carbohydrates which were soluble and insoluble in organic solvents. The soluble fraction was designated OSC and the insoluble fraction OIC (Table 1). The monosaccharide composition of the fractions was calculated from the area of each of the monosaccharides in the gas chromatograms of the acid hydrolysates of these carbohydrate fractions (Fig. 7). The molar ratio of monosaccharides in the whole cell, WEC and RC, were calculated on the basis of the mole of fucose which remained in the RC when the algal cell was treated with hot water (Table 2). Paper chromatographic analysis indicated that the OSC consisted of glucose and its oligosaccharides. The carbohydrate in OIC, which was the main fraction of WSC, was β-1,3-glucan judging from chemical characterization data obtained by means of periodate oxidation and methylation techniques which will be discussed elsewhere. No further investigation on arabinose, beyond the gas chromatographic identification, was made.

Table 1. *Yields of carbohydrates in the fractionation of Skeletonema costatum carbohydrate with water and organic solvents*

Carbohydrate fractions	Yield (%)
Water-extractable carbohydrates	45.6
Soluble in ethanol-acetone	(10.8)
Insoluble in ethanol-acetone	(34.8)
Residue	54.4

The water-insoluble carbohydrate consisted of mannose as the main component, with rhamnose, fucose, and xylose, as minor components. A peak, having identical retention time with glucose, appeared in the gas chromatogram of this fraction. This peak is assumed to be due to both glucose and glucuronic acid for the following reasons, (1) this fraction gave glucose and glucuronic acid by paper chromatographic separation after hydrolysis, (2) glucuronic acid has identical retention time with glucose when 3% ECNSS is used as the liquid phase for the gas chromatographic separation.

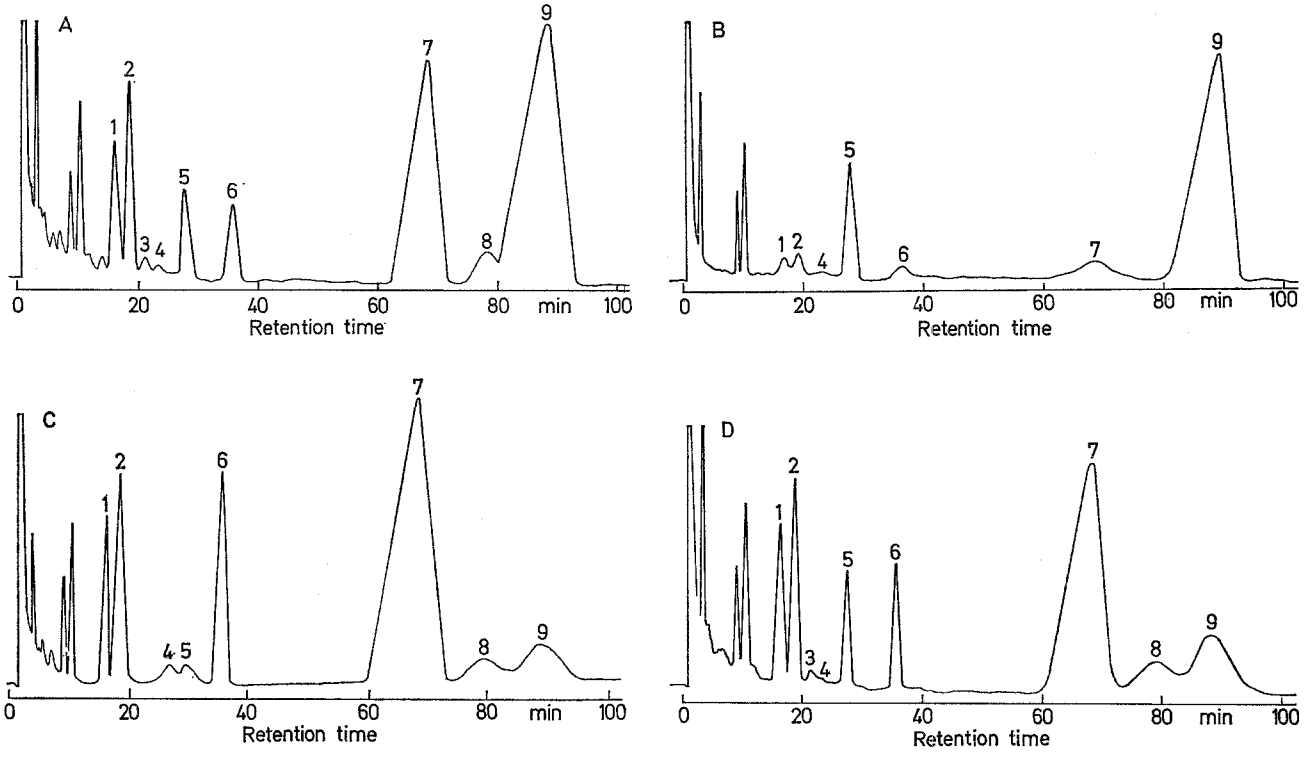

Fig. 7. Gas chromatograms of the acid-hydrolysates of various carbohydrate fractions from *Skeletonema costatum*. (A) Whole cells, (B) water-extractable carbohydrate, (C) residue, (D) whole cells of 18 days, after inoculation shown in Fig. 5. Numbers of the peaks correspond to: 1 Rhamnose, 2 fucose, 3 unknown, 4 ribose, 5 arabinose, 6 xylose, 7 mannose, 8 galactose, 9 glucose

When the culture medium was placed in the light, the amounts of those OSC, OIC and RC fractions tended to increase with time (Fig. 8). Throughout the stationary phase of the algal growth, the RC kept at a constant value and a little decrease was observed

Table 2. *Monosaccharide composition of the carbohydrates of whole cell, water extract, and residue, of Skeletonema costatum*

	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
				Whole cell 35.0 mgC of carbohydrate				
%	5.6	4.9	0.8	3.2	6.7	31.0	1.8	48.4
$\times 10^{-2}$ mMol	1.2	1.2	0.2	0.7	1.5	6.0	0.3	9.4
Mol. ratio	0.9	1.0	0.2	0.6	1.3	4.9	0.3	7.7
				Water extract 15.9 mgC of carbohydrate				
%	0.8	0.4	0.8	4.8	0.2	1.1	0.0	91.4
$\times 10^{-2}$ mMol	0.1	0.0_4	0.1	0.5	0.0_2	0.1	0.0	8.1
Mol. ratio	1.5	1.0	1.8	12.0	0.0	2.5	0.0	20.2
				Residue 18.5 mgC of carbohydrate				
%	11.8	10.2	0.6	2.3	11.3	55.0	3.0	5.8
$\times 10^{-2}$ mMol	1.3	1.1	0.1	0.2	1.4	5.8	0.3	0.6
Mol. ratio	1.2	1.0	0.1	0.2	1.1	4.4	0.2	0.5
Recovery (%)[a]	113.5	95.5	80.0	100.0	93.5	96.5	100.0	98.0

[a] Recovery was calculated as follows: Mol. water extract + residue/Mol. whole cell × 100.

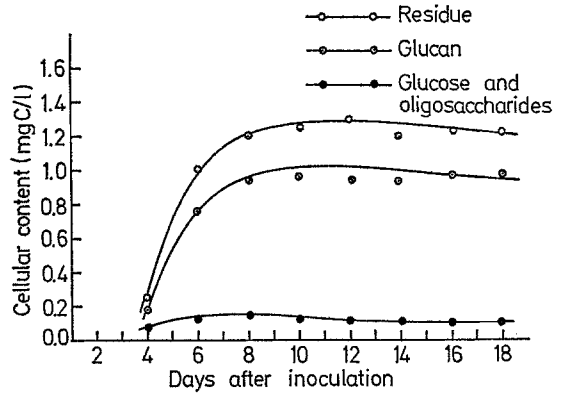

Fig. 8. Cellular content of glucose and its oligosaccharides, glucan, and residue, in *Skeletonema costatum* grown in the light

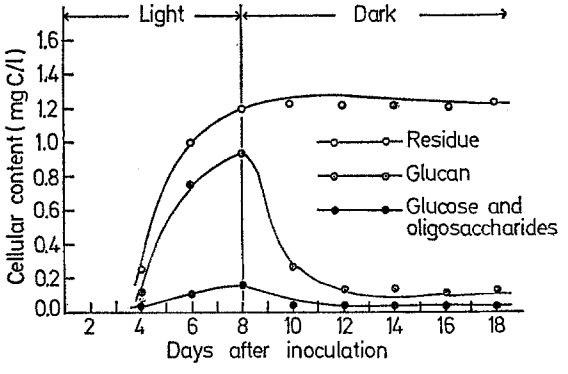

Fig. 9. Cellular content of glucose and its oligosaccharides, glucan, and residue, in *Skeletonema costatum* grown in the light and later placed in the dark

in the OSC, whereas the OIC tended to increase a little with time.

When transferred to darkness, the algal cells at maximum growth stage gave decidedly different features of carbohydrate composition (Fig. 9). The concentration of RC did not vary with time in the dark as much as in the light, whereas the values of OSC and OIC tended to markedly decrease, and only about 10% of the carbohydrate was retained in the alga at the end of the experiment. The algal whole cell kept in the dark for 8 days gave rhamnose, fucose, ribose, arabinose, xylose, mannose and galactose, with a low value of glucose upon acid hydrolysis as compared with the glucose content of the algal whole cell at maximum growth stage (Table 3). These results clearly demonstrated that the water-extractable carbohydrate, comprising glucose, was used by algal respiration, whereas the RC was immune to algal respiration and was left without receiving any biochemical modification of the molecule.

Discussion

In *Skeletonema costatum* grown under continuous light conditions, the protein content ranged from 50.9 to 59.2%, the carbohydrate content from 16.5 to 25.8%, and the lipid content from 9.35 to 16.3% of the algal organic carbon, during the course of growth. These values agree with those obtained by PARSONS et al. (1961) for several classes of marine unicellular algae, by HASE et al. (1957) for *Chlorella ellipsoidea*, and by RICKETTS (1966) for green algae. This suggests that unicellular algae have a relatively similar gross

Table 3. *Monosaccharide composition of the carbohydrates of the whole cell of Skeletonema costatum placed in the dark for 8 days*

	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
%	10.8	10.3	0.7	3.0	10.2	52.0	3.2	10.0
Mol. ratio	1.0	1.0	0.1	0.3	1.0	4.6	0.3	0.8

chemical composition throughout the species. The variation of biochemical composition in the algae seems to change, according to growth stage. Algae harvested during the logarithmic phase of growth gave lower contents of lipid (1.5 to 18% of cell dry weight) and carbohydrate (4.1 to 37.0%) (PARSONS et al., 1961), whereas rather high contents of lipid (14 to 48%) and carbohydrate (5 to 57%) were reported by RICKETTS (1966) when harvested at the stationary phase. This must be due to an accumulation of carbohydrates and lipid, which takes place commonly in various classes of algae as well as in the *Skeletonema costatum* used in this experiment.

When green alga grown under continuous illumination were transferred to the dark, RICKETTS (1966) observed a decrease in the carbohydrate content of 37%, while there were slight changes in protein and lipid. Similarly, it was observed that *Skeletonema* cells showed a decrease in carbohydrate contents of 57% when transferred to the dark. This process of carbohydrate utilization was followed by protein and lipid utilization, with decreases in their contents of 27.8, and 44.4%, respectively. No further change of the contents of these biochemical compounds in the alga was observed throughout the experiments. It was observed that *Skeletonema* cells still had the ability to perform photosynthetic carbon assimilation to a certain extent, when exposed to the light. This indicates that *Skeletonema* cells were still alive, regardless of their having been in the dark for a prolonged period. Thus, the utilization of the biochemical constituents was assumed to be due mainly to algal respiration, although the bacterial attack on these constituents may be ignored when using the incomplete axenic culture medium of the alga.

BIDWELL and GHOSH (1962) suggested that, as the exogenously supplied mannitol was slowly incorporated into the tissue, it became available for respiration. Later, PERCIVAL (1967) reviewed that mannitol is the main respiratory substrate in *Fucus vesiculosus* because of the steady loss of radioactive mannitol from this frond after its initial photosynthesis from $^{14}CO_2$.

Glucose and its oligosaccharides and polysaccharide can be considered to be one of the main respiratory substrates in the *Skeletonema* cells, as mannitol is in the *Fucus vesiculosus* cells, because of the rapid decrease in the percentages of water-extractable carbohydrates in the algal cells when the cells were transferred to the dark. No change in the protein and lipid contents of the cells during this steady loss of carbohydrate content indicates that no carbohydrate-protein and -lipid interconversions occur during the process, as observed by EPPLEY et al. (1967) and WERNER (1966), respectively.

After the exhaustive extraction of *Skeletonema* cells with hot water, the residual material gave mannose as the main component, with some pentoses. Mannose is regarded as a constituent of diatom-glucurono-mannan which has been isolated from *Phaeodactylum tricornutum* and characterized, in part, by FORD and PERCIVAL (1965). Pentoses may occur in the cell as pentosan(s) with mannan. Judging from the consistent contents of these water-insoluble polysaccharides in the *Skeletonema* cells even when transferred to the dark, it is clear that these polysaccharides are fairly immune to utilization due to algal respiration. It can, therefore, be concluded that water-extractable carbohydrates consisting of glucose are exhaustively metabolized by algal respiration within several days after darkening, leaving only water-insoluble carbohydrate in the cells. In addition, the variation of the chlorophyll a content, according to time, in the alga in the dark, suggests that only such exhaustive utilization of water-extractable carbohydrate finally causes a decrease in the photosynthetic activity of the alga.

The metabolic pattern of carbohydrates, as observed in this work, can be applied to explain the decrease in water-extractable carbohydrate content of particulate matter collected from water layers below the euphotic zone, as observed in Sagami Nada (HANDA and TOMINAGA, 1969). When phytoplankton sinks below the compensation depth, it is placed under unfavorable light conditions for the performance of photosynthesis. Thus, water-extractable carbohydrate is preferentially respired by phytoplankton, resulting in particulate matter consisting of water-insoluble carbohydrate with only a small amount of water-extractable carbohydrate, if any.

Zooplankton and bacteria may also be important factors effecting decreases in the water-extractable carbohydrate content of marine particulate matter. LONGHURST (1967) and LONGHURST et al. (1967) reported that over 80% of the net organic matter produced in phytoplankton photosynthesis was eaten by zooplankton under natural conditions; bacteria can also attack organic compounds of marine particulate matter. Thus, it is important in understanding the contribution of these organisms to carbohydrate metabolism in a marine environment, to estimate to what extent phytoplankton, zooplankton or bacteria utilize phytoplanktonic carbohydrate, and at what stage of the degradation processes the organisms participate.

Summary

1. *Skeletonema costatum* was cultured under continuous light conditions for 8 days. Half of the culture medium was then transferred to darkness while the remainder was successively cultured under light conditions.

2. In both light and darkness, organic carbon, organic nitrogen, carbohydrate, protein and lipid of the alga were determined.

3. Carbohydrate, protein and lipid did not vary so much in the alga grown under continuous light conditions. Decided decrease in the contents of these biochemical cellular components was observed when the alga was transferred into darkness.

4. The algal carbohydrates were fractionated into 3 fractions such as glucose and its oligosaccharides, 1,3-glucan, and water-insoluble carbohydrate. Studies on the metabolism of 3 carbohydrates in the alga placed in the dark indicate that the former 2 carbohydrate fractions are readily available for algal respiration, whereas the water-insoluble carbohydrate is fairly immune to biological utilization and becomes the only main carbohydrate component of the alga after a prolonged period in the dark.

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Date of final manuscript acceptance: August 26, 1969. Communicated by M. ANRAKU, Nagasaki