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Development of particulate and dissolved carbohydrates in parallel enclosure experiments with monocultures of *Thalassiosira rotula*

K. Eberlein & U. H. Brockmann

Institut für Biochemie und Lebensmittelchemie der Universität Hamburg, Martin-Luther-King-Platz 6, D-2000 Hamburg 13, Federal Republic of Germany

ABSTRACT: Three large outdoor tanks (3 to 4 m^3, 4 to 5 m deep) were filled with filtered North Sea water (0.2 µm plate filters) and inoculated with monocultures of *Thalassiosira rotula*. Under nearly natural conditions the diatoms showed partial good synchronization of cell division during exponential growth. Concentrations of particulate and dissolved carbohydrates increased slowly at this time. A rapid increase was measured at the beginning of the stationary phase when silicate and nitrogen-containing nutrients in the enclosures were exhausted and proteins and amino acids were synthesized at a lower rate. Particulate and dissolved carbohydrates reached maximum values of 25 µmol Glc Eq (glucose equivalents) dm^{-3} and 18 µmol Glc Eq dm^{-3} respectively, indicating that a relatively large portion of synthesized carbohydrates was released by the diatoms. For synthesis and release a day-night rhythm was found with maximum increase rates during daylight. Carbohydrates were exuded intermittently and in the main by healthy cells. Maximum rates of 2.6 µmol Glc Eq dm^{-3} h^{-1} were measured, corresponding to 1.3 pmol Glc Eq $cell^{-1}$ h^{-1}. The carbohydrates were taken up as fast as they were released, probably by heterotrophic bacteria.

INTRODUCTION

Living marine phytoplankton is known to exude organic substances such as carbohydrates and amino acids, sometimes at a very high rate (Guillard & Hellebust 1971, Thomas 1971, Williams & Yentsch 1976, Fogg 1977, Brockmann et al. 1979, Larsson & Hagström 1979, Hammer & Brockmann 1983, Eberlein et al. 1985). These substances are of great importance for interrelations in marine ecosystems (Wheeler et al. 1974, Williams 1975, Sepers 1977, Smith et al. 1977, Larsson & Hagström 1979). Little is known so far about the release of carbohydrates under natural conditions and about possible changes in release activities during different stages of phytoplankton life cycles. Appropriate means for the investigation of such problems are experiments using large foil tanks, filled with filtered natural sea water, inoculated with monocultures of algae and exposed *in situ* at an anchored sea station (Brockmann et al. 1974). Contrary to laboratory experiments, nearly natural conditions can be kept in such enclosures. Open sea measurements are less appropriate because of the difficulties in taking samples from the same water body over a long period. The enclosure experiments offer the additional advantage that the investigated ecosystem may be reduced to a few or even one phytoplankton species; accompanied, however, by a complex system of microorganisms. Lastly, they permit investigation of phytoplankton in a synchronous physiological state (Hammer & Brockmann 1983).

Such enclosure experiments were carried out near Helgoland (German Bight) in 1973 with monocultures of *Thalassiosira rotula* (Brockmann et al. 1977). The release of amino acids by the diatoms has been described elsewhere (Hammer & Eberlein 1981, Hammer et al. 1981, Hammer & Brockmann 1983). In this paper the release of total dissolved carbohydrates will be reported and discussed.

METHODS

Experimental procedures, nutrient measurements and phytoplankton counts have been described previously (Brockmann et al. 1974, 1977, Eberlein et al. 1983). Samples for carbohydrate measurements were taken at 6 h intervals; during the time of highest

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dynamics at 2 h intervals. Samples were filtered through 0.6 µm polyamid filters (Sartorius SM 11905), fixed with 3 ml of mercury-II-chloride solution (3.5 %) per l and stored at +4 °C. Filters were frozen and stored at −20 °C.

Total particulate carbohydrates were determined by the anthrone method (Brockmann et al. 1977), while total dissolved carbohydrates were analysed by using an automated L-tryptophane/sulfuric acid method (Eberlein & Hammer 1980). Concentrations were measured as glucose equivalents (Glc Eq). The carbohydrate measurements included mono-, oligo- and polysaccharides.

From the carbohydrate data rates of decrease and increase were computed. Percentage deviations from the trend of development were calculated using the running average method (5th order) for smoothing of original values (Hammer & Eberlein 1981). Deviations were also calculated from the regression lines found between concentrations of dissolved carbohydrates and experimental time.

RESULTS

In 3 enclosed monocultures (Tanks F, G, H) *Thalassiosira rotula* showed partly synchronized cell division during exponential growth (Brockmann et al. 1977, Hammer & Brockmann 1983). The stationary growth phase began on the 5th experimental day (July 1).

Concentrations of total particulate carbohydrates showed a nearly parallel increase in the 3 enclosures (Fig. 1) during the exponential growth phase of *Thalassiosira rotula*. Particulate carbohydrates reached maximum concentrations of 18 to 25 µmol Glc Eq dm^{-3}

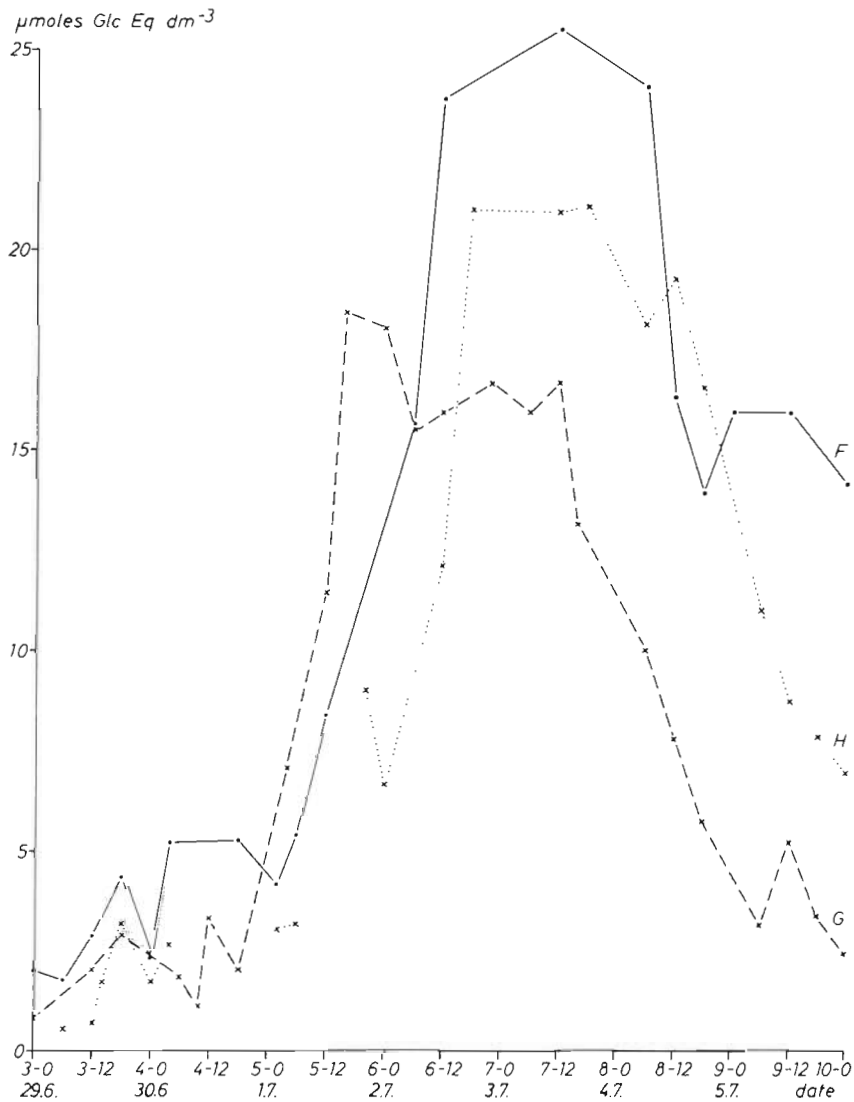

Fig. 1. Concentrations of total particulate carbohydrates in Cultures F (●—●), G (x--x) and H (x···x). Time is given as numbers of experimental days and, after the hyphen, as hours of the day

at the beginning of the stationary phase. For about 2 d concentrations remained nearly constant and then decreased very suddenly (Fig. 1). In Tank G a more rapid development was observed, as also for diatom growth.

Concentrations of total dissolved carbohydrates showed an even more parallel development (Fig. 2). Maximum concentrations of 13 to 18 µmol Glc Eq dm^{-3} were measured, these values being only a little lower than maximum concentrations of particulate carbohydrates. Dissolved carbohydrates showed rapid large fluctuations (Fig. 2), whereas high increases of particulate carbohydrates were followed by short stagnation periods (Fig. 1).

A diurnal rhythm could be demonstrated for changes in concentrations of dissolved and particulate carbohydrates (Fig. 3 & 4). Highest increase rates occurred during daylight; at midnight rates were about zero or negative. On the 5th and 6th days, when silicate uptake decreased due to exhaustion, and diatom growth reached the stationary phase, especially high increase rates were found during daylight. Particulate carbohydrate rates showed no negative values during the stationary phase of diatom growth, whereas dissolved carbohydrates then decreased strongly during the night. At this time particulate carbohydrates started to increase a little earlier in the morning than dissolved carbohydrates.

The representation of short-term variations given in Fig. 5, for dissolved carbohydrates on the 4th and 5th days, reveals that during daylight maxima and minima appeared as several short-termed events. For the different enclosures similar variability patterns were found. Deviations from the regression lines, represented in Fig. 5, showed highly significant linear correlations between the different enclosures.

In one enclosure (F) the fluctuations in cell numbers and in dissolved carbohydrate concentrations showed a very similar pattern (Fig. 6). However, the maxima and minima of the 2 different parameters did not appear exactly simultaneously. Therefore, a significant correlation between them could not be computed.

For dissolved carbohydrates the calculation of rates in the early stationary phase gave a maximum increase of 2.6 µmol Glc Eq dm^{-3} h^{-1} and a maximum decrease of 2.3 µmol Glc Eq dm^{-3} h^{-1} (Tank G). The computation of rates per diatom cell showed a pattern very similar to the fluctuations in Fig. 5. The rates per cell

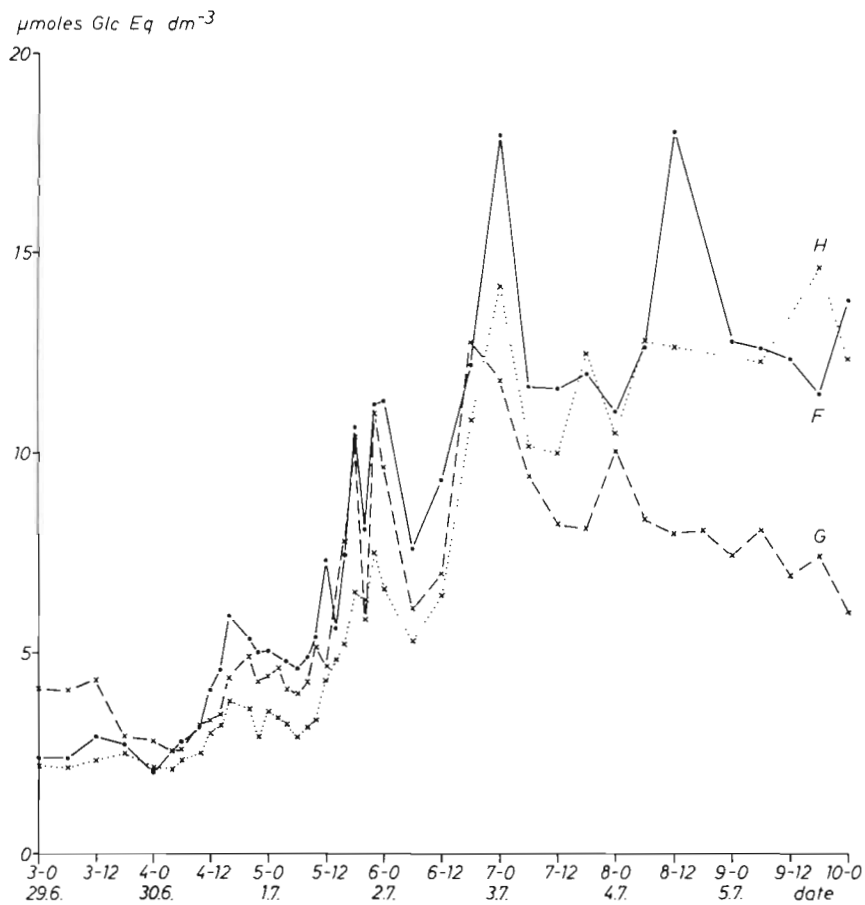

Fig. 2. Concentrations of total dissolved carbohydrates in Cultures F (•——•), G (x--x) and H (x·····x). Time as in Fig. 1

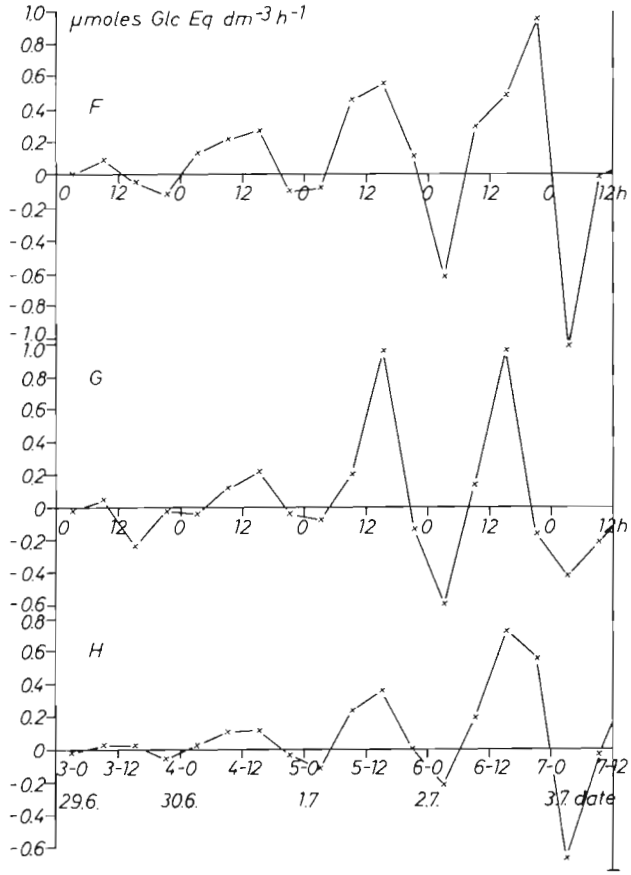

Fig. 3. Increase and decrease rates of dissolved carbohydrates in Cultures F, G and H during the exponential and stationary growth phase of *Thalassiosira rotula*. Time as in Fig. 1

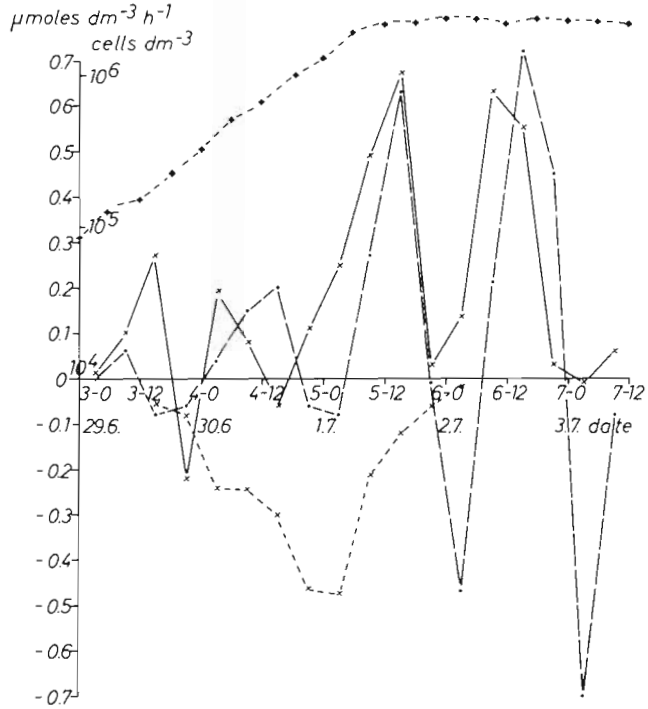

Fig. 4. *Thalassiosira rotula*. Development of cell numbers dm^{-3} (+––+), increase and decrease rates of silicate (x––x), total particulate carbohydrates (x——x) and total dissolved carbohydrates (•——•). (Mean values of the 3 enclosures F, G and H.) Time as in Fig. 1

were also highest during the stationary phase. A maximum increase of 1.3 pmol Glc Eq $cell^{-1}$ h^{-1} was calculated (Tank G).

DISCUSSION

As was found for the development of diatoms, bacteria, chlorophyll, particulate nitrogen, particulate carbon, inorganic nutrients, and dissolved free amino acids (Brockmann et al. 1977, Hammer & Eberlein 1981), concentrations of dissolved and particulate carbohydrates showed a nearly parallel development during diatom growth in the 3 enclosures F, G and H (Fig. 1 to 3). Even the short-term fluctuations of dissolved carbohydrates in the different enclosures were correlated (Fig. 5). All this indicates a very good reproducibility of our enclosure experiments.

Highest rates of increase in particulate carbohydrates were found during the stationary phase of diatom growth (Fig. 4). Since nitrogen-containing nutrients were entirely consumed during this time (Brockmann et al. 1977, Hammer et al. 1983), cells could no longer produce amino acids and proteins at a high rate. As shown by Hammer & Eberlein (1981) concentrations of total dissolved free amino acids in the water decreased strongly at the beginning of the stationary phase indicating a reduced release by the diatoms. Instead of nitrogen-containing substances, carbohydrates were then synthesized at a higher rate, as found by Myklestad & Haug (1972) in *Chaetoceros affinis* cultures.

Since particulate carbohydrates, in contrast to dissolved carbohydrates, showed no negative rates at night during the stationary phase (Fig. 4), it can be concluded that diatom cells did not decay on a large scale until the 7th day and that grazing by zooplankton did not occur in the enclosures at a high rate. However, towards the end of the experiment (7th/8th day) the beginning of cell decomposition was indicated by the sudden decrease of particulate carbohydrate concentrations (Fig. 1).

Attention should be drawn to the day-night rhythm found for rates of increase of particulate as well as of dissolved carbohydrates during the exponential and early stationary phases (Fig. 3 & 4). Several findings lead to the speculation that carbohydrates were released in the main by healthy diatoms. Concentra-

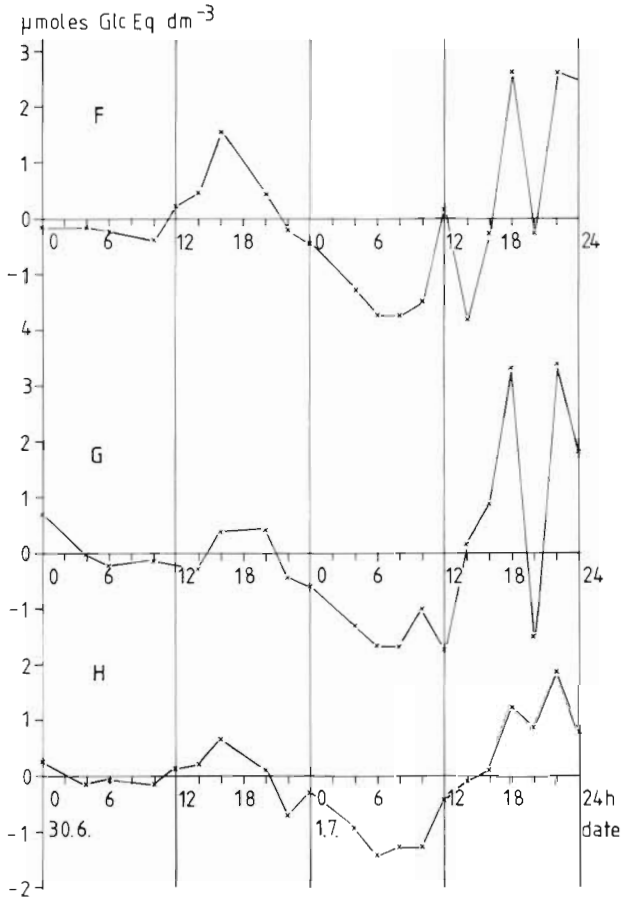

Fig. 5. Deviations from the regression lines computed for concentrations of total dissolved carbohydrates in Cultures F, G and H against experimental time during the late exponential phase and the early stationary phase of *Thalassiosira rotula*

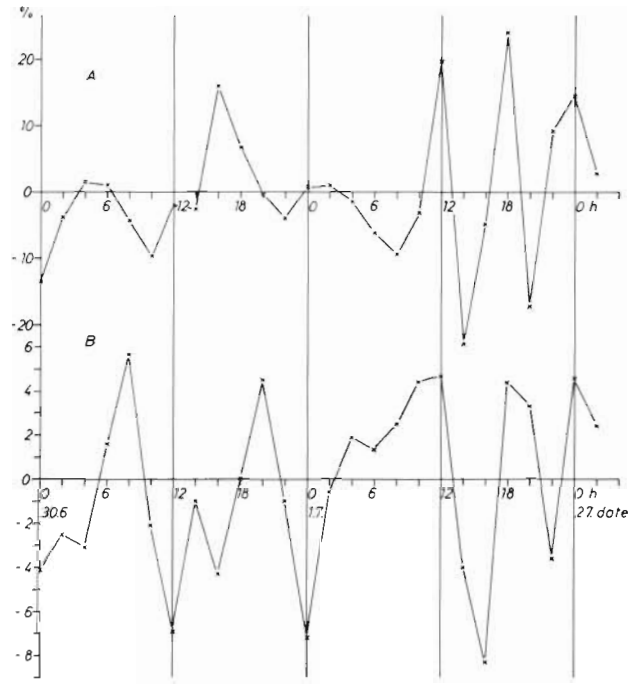

Fig. 6. Percentage deviations from the trend of the development of dissolved carbohydrate concentrations (A) and diatom numbers (B) in Enclosure F during the late exponential phase and early stationary phase of *Thalassiosira rotula*

tions of particulate carbohydrates showed no decrease but only a stagnation during darkness. Dissolved carbohydrates started to increase a few hours later in the morning than particulate carbohydrates. As for carbohydrates, amino acids also were mainly liberated during daylight (Hammer & Eberlein 1981). For both substances an intermittent release was demonstrated pointing to regulation by metabolic condition of the diatoms (Eberlein et al. 1983, Hammer & Brockmann 1983).

Particulate carbohydrates were mainly synthesized during daylight (Fig. 4). Due to the standstill of the light reaction of photosynthesis during darkness the concentrations of intracellular carbohydrates stagnated at night. It is obvious that cells started synthesizing carbohydrates with the beginning of photosynthesis in the morning and that these substances at first were mainly used as internal storage products or wall substances. Presumably, when the possible incorporation of carbohydrates into the cells was nearly finished, biosynthesis was continuing and carbohydrates were then mainly released. Therefore, the daily release started later than the increase of intracellular carbohydrates. The release into the environment may have been merely a liberation of waste material. On the other hand, it may have been of use for the diatom population by promotion of symbiosis with bacteria, by detoxification or disposal of metals or by external storage of reserve products.

It is remarkable that highest increases of dissolved carbohydrates were immediately followed by the highest decreases (Fig. 5). Heterotrophic bacteria may have been responsible for the rapid disappearance of released carbohydrates since they began to increase strongly at the beginning of the stationary phase (Brockmann et al. 1977), when the high decrease rates of dissolved carbohydrates were first found (Fig. 4). When bacteria activities in sea areas are as high as described here, data taken for horizontal mapping at different times of the day are difficult to interpret. However, carbohydrates exuded by phytoplankton are not always taken up so rapidly (Eberlein et al. 1985).

Further work will be done to gain some insight into the metabolism of *Thalassiosira rotula* and to investigate the status and fate of dissolved carbohydrates released during phytoplankton blooms.

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