

# Bacterial Incorporation of Organic Substances Released by Natural Phytoplankton Populations\*

Karin Wolter

Institut für Meereskunde an der Universität Kiel, Düsternbrooker Weg 20, D-2300 Kiel, Federal Republic of Germany

**ABSTRACT:** A modified differential filtration was used to measure extracellular release of primary products by natural phytoplankton populations and subsequent incorporation of these products by bacteria. Water samples were incubated with  $^{14}\text{C}$  labelled bicarbonate for 6 h and 24 h, respectively. A time course experiment showed that 6 h are sufficient for attaining an approximate isotopic equilibrium between the fractions of inorganic carbon and released organic carbon. Results reveal a strong relationship between primary production and release of organic substances. The highest concentrations of exudates ( $239 \mu\text{g C l}^{-1}$ ) released by phytoplankton amounts to 27.6 % of the primary production. Percentage of released substances depends on the composition of the algal populations. *Skeletonema costatum* released between 5.1 % and 12.5 % of the primary products; nanoflagellates, up to 40 %; *Prorocentrum micans*, slightly over 20 %; *Chaetoceros sp.*, about 25 %. Up to 90 % of available exudates were utilized by bacteria. Bacterial incorporation of exudates fluctuated during the year. The highest percentage of exudates was incorporated in the presence of dinoflagellates and nanoflagellates. Bacterial utilization of exudates is compared with that of glucose.

## INTRODUCTION

Release of organic substances by phytoplankton and the physiological and ecological relevance of this mechanism have attracted special interest in recent years. Investigators used phytoplankton cultures (e. g. Hellebust, 1965; Nalewajko, 1966), as well as phytoplankton populations in fresh-water lakes (e. g. Nalewajko et al., 1976, Storch and Saunders, 1978), and in marine environments (e. g. Williams and Yentsch, 1976, Smith et al., 1977).

For better understanding of carbon fluxes in pelagic ecosystems it is necessary to investigate the release of organic substances by natural phytoplankton populations and the subsequent bacterial assimilation of such substrate. For this kind of experiment it has to be assumed that phytoplankton actually releases organic substances and that findings about released material are not due to sample handling. Values of exudate release by phytoplankton obtained from the literature range from 0 to over 50 % of primary production. In his

review Sharp (1977) doubts that phytoplankton releases a significant fraction of assimilated carbon as DOC. He argues that observed exudation is probably an artefact.

Preliminary experiments using a chamber system and radioautograms have shown that release of exudates actually occurs and that bacteria incorporate these substances (Wolter, 1980). To minimize methodological interference during investigations, a careful filtration procedure was used. Correction factors were introduced to overcome difficulties of separating the autotrophic and heterotrophic population using differential filtration. Measuring bacterial utilization of naturally released exudates by adding labelled bicarbonate to the water sample, has the advantage that there is no need for addition of a special substrate, like glucose, to the water sample which might require adaptation by bacteria or cause the selection of special bacteria strains.

Bacteria are able to utilize organic substances released by phytoplankton. Larsson and Hagström (1979) demonstrated direct transfer of exudates from phytoplankton to bacteria. Bacterial incorporation of exudates was up to 25 % of the primary production in a coastal ecosystem. Derenbach and Williams (1974)

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found that bacteria utilize phytoplankton exudates rapidly. Storch and Saunders (1978) and Larsson and Hagström (1979) report extracellular release following an annual pattern. According to Lee and Nalewajko (1978) maximum exudation occurred when mainly phytoflagellates were present in the water sample. In this paper, release of exudates and their bacterial utili-

zation is described and related to the yearly cycle and pattern of phytoplankton species distribution. Bacterial utilization was measured only as incorporation of exudates (without respiration). An experiment was conducted to establish approximate isotopic equilibrium conditions. No differentiation was made between dissolved and colloidal substances in the exudate pool.

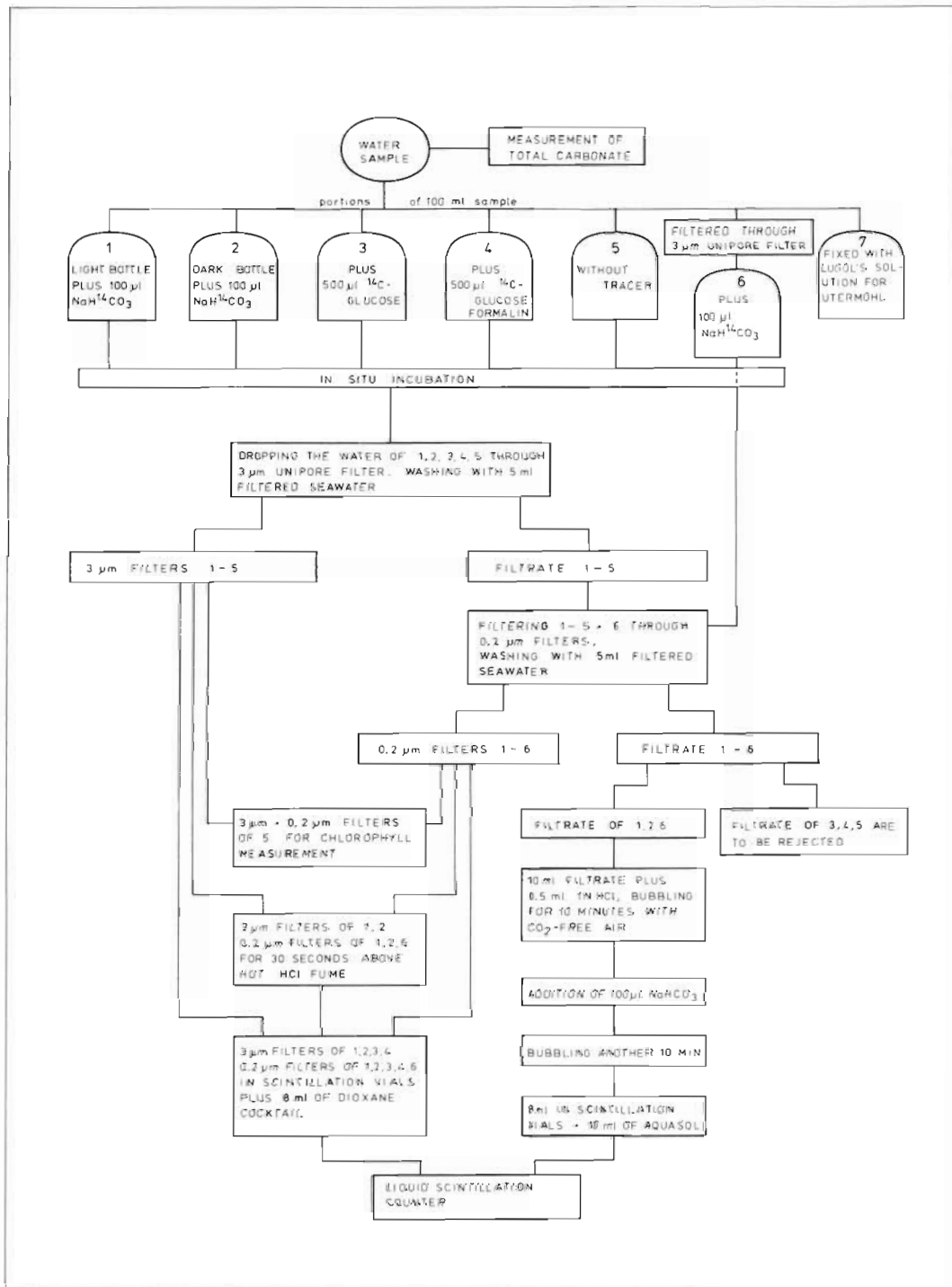


Fig. 1 Filtration procedure

## MATERIAL AND METHODS

Investigations were carried out at a station in the inner Kiel Fjord (Federal Republic of Germany). Every second week, 2-l samples were collected from 1 m depth. Water temperature and salinity were recorded with a TS-recorder (Electronic Switchgear Ltd, Type MC). Two 100 ml subsamples were filled into glass-stoppered flasks and 10  $\mu\text{Ci}$   $\text{NaH}^{14}\text{CO}_3$  added (specific activity 40–60  $\text{mCi mmol}^{-1}$ ). The samples were incubated *in situ* for 6 (9 a.m. to 3 p.m.) and 24 h (9 a.m. to 9 a.m.), respectively (Vial 1, Fig. 1). Following incubation, 1 ml of water was removed and placed in a scintillation vial containing a drop of 1 N NaOH-solution. After adding 10 ml Aquasol, total radioactivity was counted. Aliquots (50 ml) were filtered immediately through 3  $\mu\text{m}$  polycarbonate filters (Biorad Laboratories, Unipore) without applying vacuum. The filters were rinsed with 5 ml of seawater. Filtrates were collected and refiltered through 0.2  $\mu\text{m}$  polycarbonate filters under a vacuum not exceeding 100 mm Hg (0.2  $\text{kpcm}^{-2}$ ). The filters were placed in the fume of hot concentrated HCL for 30 s in order to remove inorganic  $^{14}\text{C}$ . Thereafter the filters were placed in scintillation vials and 6 to 8 ml of dioxane cocktail (120 g naphthalene, 5.5 g permablend and 1000 ml aqua dest.) were added. Radioactivity was measured in a Berthold Betaszint BF 500 liquid scintillation counter. The filtrate (10 ml) was acidified with 0.5 ml 1N HCL, and air was bubbled through the sample for 10 min to remove the inorganic  $^{14}\text{C}$ . Subsequently, 100  $\mu\text{l}$  of a saturated  $\text{NaHCO}_3$ -solution was added and bubbling continued for a further 10 min; 8 ml were then placed in a scintillation vial, 10 ml of Aquasol added, and the radioactivity determined. Quench correction factors were calculated using  $^{14}\text{C}$ -toluene as an internal standard for every type of sample, while the external channel ratio was run routinely to check if the samples were comparable. To correct for adsorption of  $^{14}\text{CO}_3$  on the filter, and dark uptake of  $^{14}\text{CO}_3$  by algae and bacteria, a subsample (Vial 2, Fig. 1) was incubated in the dark.

As it was impossible to separate completely the algae from the bacteria by differential filtration, 4 further subsamples were treated as follows (Fig. 1): A subsample (Vial 3) was incubated with 0.25  $\mu\text{Ci}$   $^{14}\text{C}$ -glucose (specific activity 200–350  $\text{mCi mmol}^{-1}$ ; Derenbach and Williams, 1974). Since bacteria take up the glucose, any radioactivity remaining on the 2 filters after filtration represents bacterial distribution.

I have assumed that bacterial uptake of exudates is equivalent to bacterial glucose uptake. Hence the amount of radioactivity on the 3  $\mu\text{m}$  filter of Vial 1 due to bacteria, can be calculated. This value was then subtracted from the counts of the 3  $\mu\text{m}$  filter of Vial 1 and added to those obtained on the 0.2  $\mu\text{m}$  filter. A

blank (Vial 4) was run to determine  $^{14}\text{C}$ -glucose absorption.

Another correction was made for small algae which may pass through the 3  $\mu\text{m}$  filter. Their radioactivity was measured together with the bacterial radioactivity on the 0.2  $\mu\text{m}$  filter. Therefore, a prefiltered subsample (Vial 6) was incubated with  $\text{NaH}^{14}\text{CO}_3$ . After incubation, aliquots were filtered only through a 0.2  $\mu\text{m}$  filter. The radioactivity on this filter was assumed to be due to small algae. Only this value was used to correct the radioactivity on the 0.2  $\mu\text{m}$  filter of Vial 1. The 4th subsample was incubated without any tracer. Aliquots were also filtered through 3  $\mu\text{m}$  and 0.2  $\mu\text{m}$  filters, which were later used for measurements of chlorophyll a.

The calculations are summarized in the following equations:

$$(1) \text{ Algal incorporation} = \frac{(a_1 - b_1 + c - d) \times 1.05 \times \text{mg C l}^{-1}}{e}$$

$$a_1 = \text{dpm on 3 } \mu\text{m Vial 1}; b_1 = \text{dpm on 3 } \mu\text{m Vial 2};$$

$$c = \text{dpm on 0.2 } \mu\text{m Vial 6} - \text{dpm on 0.2 } \mu\text{m Vial 2};$$

$$d = \frac{a'}{b'} \cdot c'$$

$$a' = \text{dpm on 3 } \mu\text{m Vial 3} - \text{dpm on 3 } \mu\text{m Vial 4};$$

$$b' = \text{dpm on 0.2 } \mu\text{m Vial 3} - \text{dpm on 0.2 } \mu\text{m Vial 4};$$

$$c' = \text{dpm on 0.2 } \mu\text{m Vial 1} - \text{dpm on 0.2 } \mu\text{m Vial 6};$$

$$e = \text{dpm added to the same volume which was filtered. The factor 1.05 is the discrimination factor after Strickland and Parsons (1972).}$$

$$(2) \text{ Bacterial incorporation of exudates}$$

$$= \frac{(a_2 - b_2 - c + d) \times 1.05 \times \text{mg C l}^{-1}}{e}$$

$$a_2 \hat{=} a_1 \text{ for 0.2 } \mu\text{m filter}; b_2 \hat{=} b_1 \text{ for 0.2 } \mu\text{m filter}.$$

$$(3) \text{ Dissolved exudates} = \frac{(a_3 - b_3) \times f \times 1.05 \times \text{mg C l}^{-1}}{e}$$

$$a_3 \hat{=} a_1 \text{ for filtrate}; b_3 \hat{=} b_1 \text{ for filtrate}; f = \text{dilution factor (washing water).}$$

The above equations are combined as follows to yield the required results:

$$1 + 2 + 3 = \text{primary production during the given incubation time (mg C l}^{-1}\text{)}$$

$$2 + 3 = \text{released organic matter (mg C l}^{-1}\text{)}$$

$$\frac{2}{1 + 2 + 3} = \% \text{ bacterial uptake of primary products}$$

$$\frac{2}{2 + 3} = \% \text{ bacterial uptake of total exudates.}$$

Total carbonate, nitrate, nitrite, and ammonium concentrations were determined after Grasshoff (1976), and phosphate after Murphy and Riley (1962). Chlorophyll a measurements were done fluorometrically (Lorenzen, 1966).

Direct counts of bacteria were performed on black stained (sudan black) 0.2  $\mu\text{m}$  polycarbonate filters using the fluorescent dye acridine orange (Zimmermann and Meyer-Reil, 1974, Hobbie et al., 1977). To calculate bacterial biomass, the lengths of bacteria were measured and mean volumes used after Zimmermann (1977). Bacterial carbon was calculated assuming that 10 % of the wet weight is due to organic carbon. Phytoplankton was counted in fixed samples (Lugol's solution; Vial 7, Fig. 1) using the technique of Utermöhl (1958). Cells were measured and carbon content calculated after Smetacek (1975).

### RESULTS

Approximate isotopic equilibrium conditions between labelled  $^{14}\text{C}$ -organic substances and  $^{14}\text{CO}_3$  were obtained within 6 h (Fig. 2). The slope of the curve of dissolved exudate release decreases during incubation. Dissolved exudates as percentage of pri-

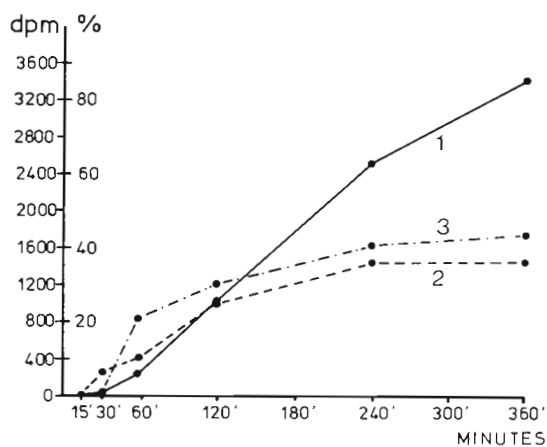


Fig. 2. Time-course experiments with natural water samples. 1 dissolved exudates; 2 activity on 0.2  $\mu\text{m}$  filters; 3 dissolved exudates as percentage of total primary production (dpm: disintegrations  $\text{min}^{-1}$ )

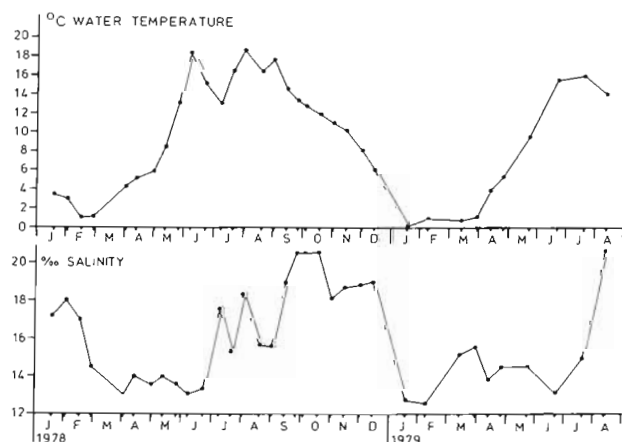


Fig. 3. Water temperature and salinity

mary production, as well as bacterial incorporation of exudates, approach saturation.

Fig. 3 shows the yearly pattern of water temperature and salinity at the station during the period of investigation. Changes in salinity are influenced by water exchange (Lenz, 1977). The yearly pattern of inorganic nutrients (Figs. 4 and 5) is mainly caused by phytoplankton development. The trend is for nutrients to decrease in spring when phytoplankton starts to grow. The concentrations remain low during summer; regeneration occurs during winter (Rheinheimer, 1977, Szwercinski, 1981). Abnormally high peaks (March 1978, February and April 1979) for nitrate may have been caused by a small eutrophied fresh-water river which flows into the Kiel Fjord.

Fig. 6 illustrates the primary production of phytoplankton after 6 and 24 h. The values after 6 h incuba-

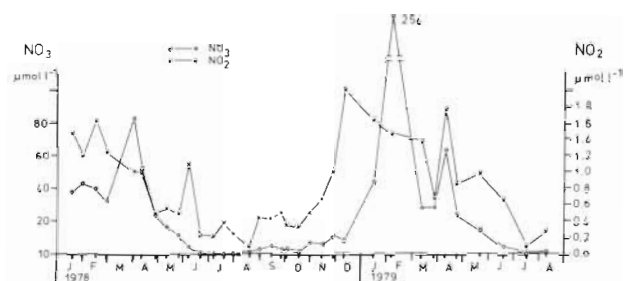


Fig. 4. Concentration of nitrate and nitrite

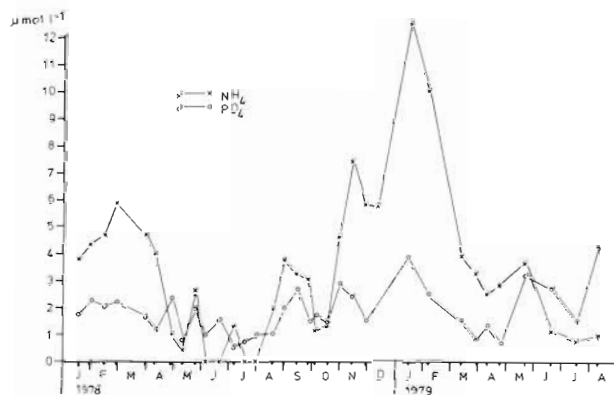


Fig. 5. Concentration of ammonium and phosphate

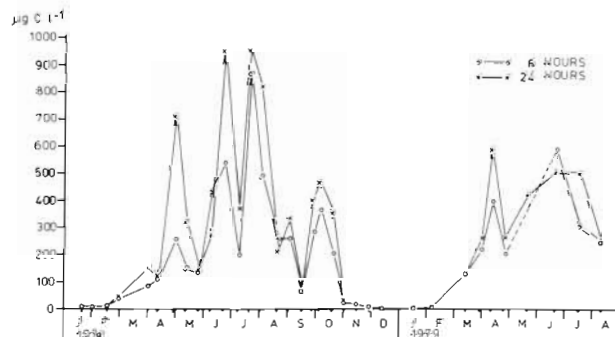


Fig. 6. Total primary production after 6 and 24 h incubation

tion probably represent gross production, as it can be assumed that photorespiration and respiration are negligible during such short incubation; 24 h values represent the production on a daily basis. The 24 h values may also serve as indicators of the physiological condition of the algae: if production after 24 h is far below the production after 6 h it is possible that the algae have a quick catabolism and do not use the primary products for increasing their biomass.

The data in Fig. 6 comprise the particulate production on both 3 µm and 0.2 µm filters as well as the dissolved fraction. During the period of investigation several algal blooms were recorded, some of which had a high production. Fig. 7 shows the carbon content of different species responsible for the primary production. In spring 1978 and 1979 mainly diatoms were found, followed by mixed populations of nanoflagellates, dinoflagellates and diatoms in summer, and again diatoms in autumn.

Fig. 8a shows the total exudates released (dissolved exudates plus particulate bound on 0.2 µm filters); the same data are expressed as percentage of the primary production in Fig. 8b. Release of exudates fluctuates

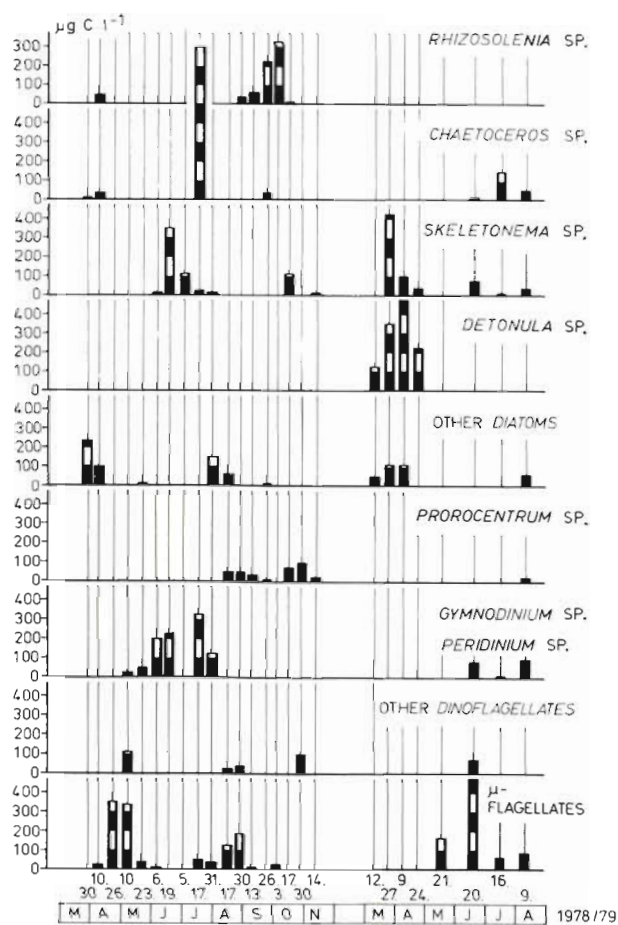


Fig. 7. Composition of algal populations expressed as carbon content for the different species in the water sample

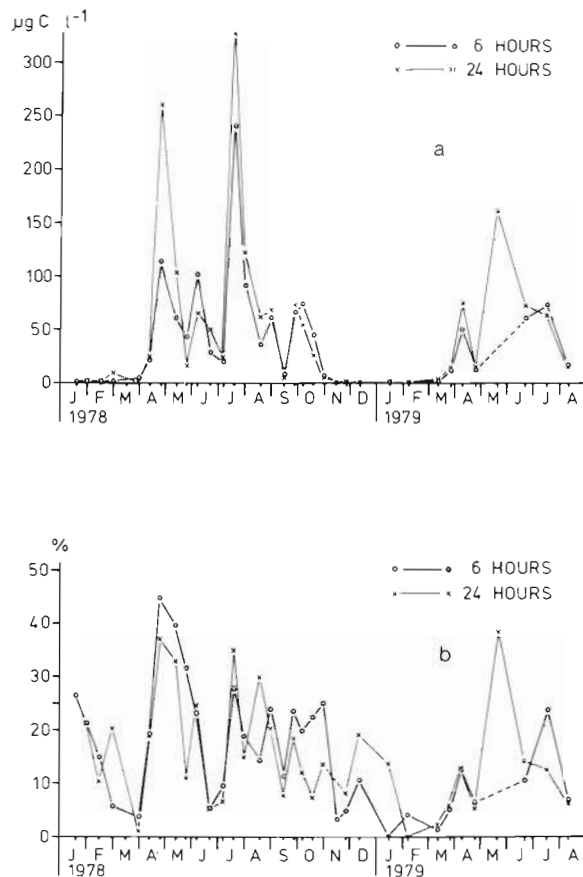


Fig. 8. Total exudates after 6 and 24 h incubation, expressed as µg C l<sup>-1</sup> (a), and as percentage of total primary production (b)

during the year; the higher the primary production the higher the concentration of total exudates. There is little difference in percentage release of exudates between 6 h and 24 h incubation indicating that organic substances are released mainly during the light phase. The highest concentration of total exudates (239 µg C l<sup>-1</sup>) was found in July 1978 after 6 h incubation. This corresponds to 27.6 % of the total primary production. No correlation was found between primary production and percentage release of exudates.

A comparison between percentage release of exudates between the main algal species in the samples shows that some algal species release more organic substances than others. *Skeletonema costatum* was the major algal species on 19. 6. and 5. 7. 78, 27. 3. 79, 9. 4. 79, 24. 4. 79, 20. 6. 79 and 9. 8. 79. The percentage release of the primary products ranged from a minimum of 5.1 on the 19. 6. 78 to a maximum of 12.5 % on the 9. 4. 79. When mainly nanoflagellates were present in the sample (April and May 1979) the release was between 10 and 40 % of primary

production. In late autumn 1978 it appears that the release of slightly over 20 % of primary production after 6 h, is due to *Prorocentrum micans*. *Chaetoceros* sp. which was present on 17. 7. 78 released 27.6 % of its primary production. *Gymnodinium* sp. and *Peridinium* sp. appear to release very small amounts.

Fig. 9a documents bacterial incorporation of exudates during 6 h and 24 h incubation. In spring, there is a time lag of bacterial activity when compared with primary production. The fraction of incorporated exudates is usually higher after 24 h incubation. In those instances where the production after 24 h was more than 4 times higher than the production after 6 h, isotopic equilibrium had probably not been reached after 6 h. In some cases bacterial incorporation of exudates was higher after 6 h than after 24 h. This might be due to different uptake rates by bacteria during the day. It is also possible that the bacteria in these experiments use the organic substances mainly for their energy metabolism.

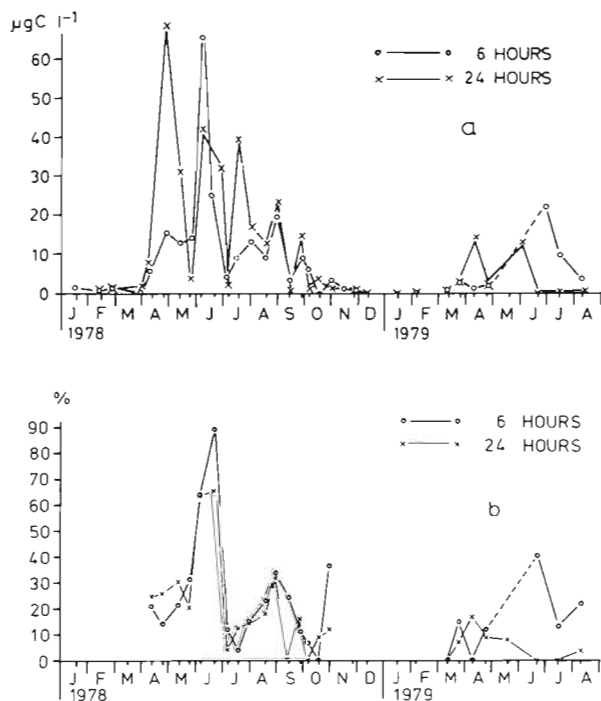


Fig. 9. Bacterial incorporation of exudates after 6 and 24 h incubation, expressed as  $\mu\text{g C l}^{-1}$  (a), and as percentage of total exudates (b)

Maximum bacterial activity occurred in June 78 and in June 79 after 6 h. This was also found by Larsson and Hagström (1979). Although water temperature reaches a maximum in June, this does not fully explain the increase of bacterial activity since water temperature remains high during July and August while the bacterial activity shows strong variations.

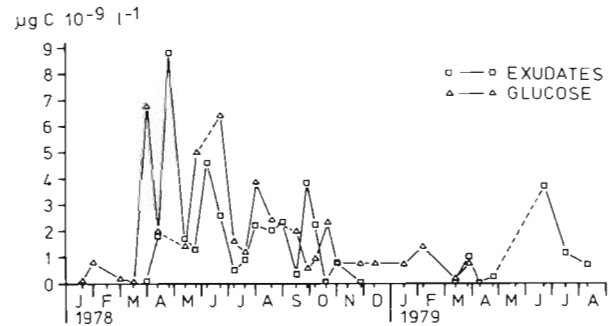


Fig. 10. Incorporation of exudates and glucose per bacterial cell after 6 h (glucose curve after Bölter, 1981)

To demonstrate a relationship between bacterial incorporation of exudates and abundance of algal species the bacterial uptake of exudates is shown as a percentage of total available exudates (Fig. 9b). *Gymnodinium* sp., *Skeletonema costatum* and nanoflagellates were present when the highest percentage of available exudates were incorporated by bacteria. This occurred in June 1978 when 90 %, and in June 1979 when 40 % was taken up. Only 10 % was incorporated when diatoms were present in the sample (i. e. 26. 9. 78). In one sample (5. 7. 78) in which only *S. costatum* occurred, bacteria incorporated 11.4 % of the available exudates. In July 78 during a strong bloom of *Chaetoceros* sp. only 3.5 % of the exudates was incorporated; a higher percentage was incorporated in autumn when *Prorocentrum micans* was present.

Fig. 10 illustrates the incorporation of exudates and glucose per bacterial cell. During summer there is a correlation between glucose and exudate incorporation, whereas in autumn and winter the reverse was observed.

## DISCUSSION

The results show that the release of organic substances by natural phytoplankton populations and their immediate incorporation by bacteria can be measured by using careful differential filtration. Because of certain correction factors used, it is difficult to compare the results with those obtained elsewhere. It was found that the method applied played an important role in these investigations. Preliminary experiments (Wolter, 1980) have shown that there is up to 20 % variation in exudation of primary products depending on whether filtration was carried out under vacuum or not. It was also found that small algae may pass through the  $3 \mu\text{m}$  filter and that they may contribute up to 35 % of the total production.

Larsson and Hagström (1981) found that up to 70 % of the activity on the  $0.2 \mu\text{m}$  filter was due to autotrophic productivity. For this reason it is obvious that a

correction must be made for these small algae. Although the correction factor for bacteria associated with the algae on the 3  $\mu\text{m}$  filter was used in the calculations of the results, the value was found to be almost insignificant since only an average of 6 % of the bacteria remained on the 3  $\mu\text{m}$  filter.

The time-course experiment revealed that an approximate isotopic equilibrium was reached within an incubation time of 6 h; however, this may vary in different water samples. Saunders (1972) and Lancelot (1979) found that there may be a variation in the time for labelled organic substances to appear in the dissolved fraction. This depends on the metabolic pathways responsible for the production of substances released. Release of labelled organic matter, however, may be very rapid after addition of  $\text{NaH}^{14}\text{CO}_3$  to the sample (Fogg, 1966; Lancelot, 1976; Nalewajko et al., 1976; Nalewajko and Schindler, 1976). Mague et al. (1980) found a rapid equilibrium between the compounds released and the new primary products. The results from the 24 h parallel incubation, done to obtain data on a daily basis and to signify the physiological condition of the algal population, revealed that in most cases 6 h were sufficient for attaining isotopic equilibrium. This was also found by Wiebe and Smith (1977), Lancelot (1979) and Larsson and Hagström (1979).

The 24 h incubation also showed that a higher percentage of exudates is released during the light phase of the incubation. The results of primary production, exudate release and bacterial incorporation of exudates are presented for the whole incubation time. To obtain an estimate of the hourly rate of exudate release, the linear slope of the dissolved exudates in Fig. 2 was used. The rate of exudate release was  $7.4 \mu\text{g C l}^{-1}\text{h}^{-1}$ .

The rates calculated by Lancelot (1979) ranged from 0 to  $16 \mu\text{g C l}^{-1}\text{h}^{-1}$ , and Larsson and Hagström (1979) found a release of  $3 \mu\text{g C l}^{-1}\text{h}^{-1}$ . Using the calculation of Larsson and Hagström (1979) bacterial respiration was estimated from the hourly rate of exudate release (assuming that this rate is constant for the first 6 h) and from the difference between dissolved exudates found after 6 h and the theoretical pool of released substances. The difference between available exudate and the measured incorporation of exudates by bacteria was probably due to respiration during incubation. This difference was calculated to be 29 %. Respiration of glucose by bacteria of the Kiel Fjord varied between 5 and 45 % (Bölter, 1981). Herbst (1976) found that between 67 and 74 % of *Oscillatoria redekei* exudate was respired.

During spring and summer 1978 incorporation plus respiration of glucose in the Kiel Fjord ranged from 0.8 to  $15.3 \mu\text{g C l}^{-1}\text{h}^{-1}$  (Bölter, 1981). It appears therefore, that bacteria utilize the main portion of exudates

released. The similarity between the curves of bacterial uptake of exudates and exudate release is caused by the response of the bacterial population to the substances released. Bacterial activity begins to increase shortly after the first phytoplankton bloom even if the water temperature is low. In autumn bacterial activity decreases with a decrease in algal activity, while the water temperature remains relatively high. Similar observations were made by Sieburth (1967) and Saltzmann (1980). Significant positive rank correlations could be found between bacterial incorporation of exudates and glucose (Bölter, 1981). The natural glucose concentrations (Liebezeit, 1980) only show a positive correlation with bacterial exudate incorporation and primary production during spring, summer and autumn – not in winter. These results indicate that phytoplankton seems to play a major role in nutrient supply for bacteria, and that it releases substances which are taken up similarly to glucose.

Several authors (Guillard and Wangersky, 1958; Guillard and Hellebust, 1971; Stabel, 1976; Bolze and Soeder, 1978, Jüttner and Matuschek, 1978) have reported that glucose was a constituent of algal exudates. Moshiri et al. (1979) also found a positive relationship between dissolved glucose and primary production in surface water of an estuary in Florida.

On a 24 h basis the bacteria incorporated 0–65 % (mean value 20.9 %) of the total exudates for biomass production. This corresponds to 0–15.6 % (mean value 3.6 %) of total primary production. Larsson and Hagström (1979) found that the bacterial production was 27 % of total primary production. Their calculations are based on a 4 h incubation and are related to the total water column.

Although water temperature is fairly constant during summer, considerable variations in bacterial activity were found. This can be explained by the composition of the algal flora. The amount of exudate release varies with the algal composition in the Kiel Fjord. Fogg et al. (1965) and Steinberg (1978) could not find such a relationship, whereas Lee and Nalewajko (1978) reported that phytoflagellates and *Scenedesmus* released more primary products than other species. According to Hellebust (1965), cultures of *Chaetoceros pelagicus* and *C. simplex* released 20 % of their primary products, whereas *Exuviella* sp. released only 3 %. My investigations have shown that *Chaetoceros* sp. released relatively high amounts ( $\approx 27.6$  %), dinoflagellates – except *Prorocentrum micans* – low concentrations and *Skeletonema costatum* between 5.1 % and 12.5 %. No definite relationship could be found with this method between the physiological condition of the algae and the percentage release of exudates. Bacteria incorporated between 0 and  $65.6 \mu\text{g C l}^{-1}\text{d}^{-1}$ . The values of the bacterial standing stock during the

investigation were of the same order of magnitude (Palmgren, 1981). They varied between 10 and 69.2  $\mu\text{g C l}^{-1}$  with 2 exceptions of 92.9 and 112.05  $\mu\text{g C l}^{-1}$ .

The data presented here confirm conclusions of other authors (e. g. Bauld and Brock, 1974; Derenbach and Williams, 1974; Gocke, 1977; Rheinheimer, 1977; Hoppe, 1978; Larsson and Hagström, 1979) that a considerable portion of dissolved organic substances flows directly from phytoplankton to bacteria. The importance of bacteria is therefore not only to be seen in their remineralization of organic substances but also in their transformation of dissolved organic material to a particulate form which is then available for bacterial feeders.

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