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Carbohydrates in the North Sea during spring blooms of *Phaeocystis*: a specific fingerprint

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ABSTRACT: Regional and temporal variation in the composition of water-soluble carbohydrates from *Phaeocystis* colonies sampled in the southern North Sea was small during spring 1994, except for a high variability in the contribution of glucose. Glucose is universally present in storage products of microalgae; the relative constancy of the carbohydrate pattern of the other monosaccharides suggests that these are part of the more refractory colony mucus. In all *Phaeocystis* samples arabinose dominated, followed by xylose (Belgian coast) or galactose and mannose (Dutch coast). Rhamnose, glucuronate and O-methylated sugars were present in lower amounts. The latter, always present in samples containing *Phaeocystis*, may be typical for North Sea strains. The sugar patterns we report here differ from those presented in the literature concerning *Phaeocystis*-derived material, and also from the sugar fingerprint in the preceding diatom bloom. The *Phaeocystis* mucus apparently behaves as particulate matter since it was retained on filters of over 1 µm. This characteristic together with its refractory nature, typical of 'transparent exopolymer particles' (TEPs), must have consequences for the heterotrophic microbial community in terms of adherence and substrate availability.

KEY WORDS: *Phaeocystis* · Mucus · Carbohydrates · Sugars

INTRODUCTION

The marine prymnesiophyte *Phaeocystis* has a world-wide distribution (Baumann et al. 1994). In Arctic and Antarctic regions, but also in the North Sea, it is capable of forming massive blooms that often dominate the plankton population (Gieskes & Kraay 1975, 1977, Lancelot et al. 1994). The taxonomic position and life cycle of *Phaeocystis* continue to be the subject of studies that started many decades ago (Kornmann 1955, Baumann et al. 1994, Medlin et al. 1994, Rousseau et al. 1994, Vaulot et al. 1994). The alga undergoes phase changes between flagellated single cells and non-motile cells organized in gelatinous colonies. The predominant form during the blooms is the colonial ('palmella') phase.

Colony mucus can contribute up to 90% to the total *Phaeocystis* biomass (Rousseau et al. 1990). This cell-surrounding gel is of a polysaccharide nature (Guillard & Hellebust 1971, Painter 1983). Guillard & Hellebust (1971) reported the carbohydrates galactose, glucose, mannose, arabinose, xylose, ribose, rhamnose and a hexuronic acid. The contribution of each sugar to the total amount was different in the Surinam and the Massachusetts (USA) strains that they analyzed. In a seawater sample dominated by *Phaeocystis*, Painter (1983) found a proteoglycan containing hemiester sulphate residues of galacturonic acid, glucosamine, galactose, arabinose, xylose, and rhamnose. The gelling properties of the matrix polysaccharides of *Phaeocystis* are promoted by salt bridges that involve calcium and magnesium ions that interlink the matrix polysaccharides which, by alcian blue staining, have been shown to contain carboxylated and sulphated groups (van Boekel 1992).

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The physical properties of *Phaeocystis* colonies change through successive stages of the bloom, from regularly shaped colonies, essentially free of bacteria, to irregular colonies with sticky mucus (Verity et al. 1988, Rousseau et al. 1994, Thingstad & Billen 1994). Decaying colonies are often abandoned by the cells, leaving empty 'ghosts' that eventually turn into aggregates. It is likely that changes in the physical appearance of the colonies are reflected in their chemical structure and composition. The matrix composition and the possibly related firmness of colonies are of physiological interest since they may affect nutrient uptake and metal speciation (Lubbers et al. 1990), aggregation and sedimentation (Wassmann et al. 1990), and may even affect resistance to grazing by heterotrophs, ranging from protozoa to copepods (Weisse et al. 1994).

Heterotrophic bacteria interacting with the mucus might alter the matrix properties (Thingstad & Billen 1994). As a first step towards revealing possible *Phaeocystis*-bacteria interactions we investigated the carbohydrate composition of *Phaeocystis* colonies in the course of the spring bloom in the North Sea. Another purpose was to find a possible relationship between structural characteristics of the colony mucus matrix and its biochemical composition.

MATERIAL AND METHODS

Culturing conditions. *Phaeocystis* strain BCZ was isolated in 1993 by V Rousseau at Stn 330 (51° 26' N, 2° 49' E; Fig. 1). The strain was cultured in 0.5 l serum bottles containing 0.4 l seawater (S = 33‰) from the Dogger Bank area that was enriched with 'minor salts', trace elements, and nutrients according to Admiraal & Werner (1983) and also 0.4 ml of a vitamin solution (Veldhuis & Admiraal 1987) and additional bicarbonate (0.2 g l^{-1} $NaHCO_3$). Bottles were incubated in a culture cabinet (11°C, 14:10 L:D cycle) on a rolling device (8 rpm) at an irradiance of 40 mmol m^{-2} s^{-1}, measured with a cosine collector.

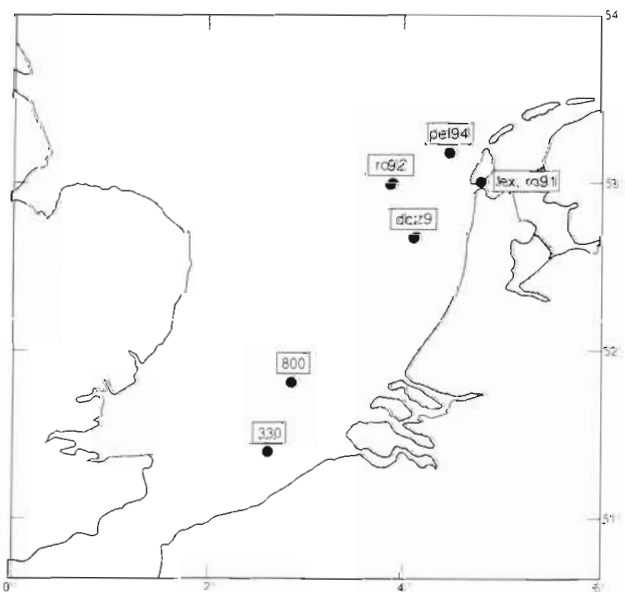

Fig. 1. Sample sites along the North Sea coast

Sampling. Samples were taken at regular intervals off the Belgian coast (Stn 330) and from the Marsdiep close to Texel, The Netherlands (Fig. 1). Samples were gently taken with a bucket in order to avoid colony disruption; they were then transferred to plastic bottles. In the laboratory, the samples were treated within 5 h in order to obtain the following fractions:

(1) Total organic matter, i.e. dissolved and particulate, obtained after homogenization of a frozen (–20°C) and thawed sample.

(2) Dissolved organic matter containing the <1 µm fraction obtained by applying gravity filtration, on preashed (4 h, 450°C) GF/C glass fibre filters (Whatman). Small volumes (<10 ml) were filtered on relatively large filters (17.3 cm^3) to prevent clogging of the filters. With larger volumes the filters were changed regularly (vacuum pressure was never applied).

(3) Material in which colonies of *Phaeocystis* dominated was usually obtained by gentle filtration of 5 to 20 l over a 250 µm mesh net. At some locations (Stns 800, dcz9, ro91, ro92, and pel94) material was obtained by towing a plankton net (100 µm mesh) through a dense *Phaeocystis* bloom (>10^6 cells l^{-1}). Collected material was extracted for 5 min at 100°C after freezing and thawing. Samples were homogenized on a Vortex, centrifuged (29 000 × *g* for 20 min), and dialyzed (Viskin size 1 8/32" Medicell International, pore size 1000 D) prior to sugar analysis.

For determination of the composition of polysaccharides present in the total seawater fraction, similar homogenization, centrifugation, and dialysis steps were performed prior to concentration using a tangential flow membrane filter (Mini-ultraset, Filtron Technology B.V., The Netherlands) with a pore size of 1 kD (4°C).

Phytoplankton abundance. The amount of phytoplankton cells was determined by counting of Lugol-preserved samples with an inverted microscope after gentle ultrasonic treatment of the samples to provide an even distribution of cells on the bottom of the counting vessel. Carbon biomass estimates were based on amount and size of cells and colonies. For *Phaeocystis* the calculation method described by Rousseau et al. (1990) was used, and for diatoms the factor of 0.11 pg C μm^{-3} recommended by Edler (1979) was used.

Sugar analysis. Sugars present in a sample were analyzed by the modified phenol-sulphuric acid method described by Liu et al. (1973) with glucose as a refer-

ence. For the determination of the relative sugar composition, samples were methanolyzed (2.0 M methanolic HCl, 24 h, 85°C) prior to analysis of the trimethylsilated (N-reacylated) methyl glycosides on a Varian 3600 gas chromatograph equipped with a J&W DB-1 capillary column (diameter 0.32 mm, length 30 m) and a flame ionization detector Identification of sugar derivatives was performed by comparison with known standards; it was confirmed by GLC/MS (Kamerling & Vliegenthart 1989). Unknown peaks were identified by GLC/MS as O-methylated hexoses and pentoses. These sugars were not available as reference sugars, so the molar ratio was estimated by assuming a degradation factor similar to those of hexoses and pentoses; this will result in an unknown systematic error in the exact amount of these methylated sugars.

RESULTS

At Stn 330 (Belgian coast) an unexpectedly dense diatom bloom (*Rhizosolenia delicatula* predominant) was present in spring 1994 (Fig. 2A). Also near Texel the *Phaeocystis* bloom was preceded by this massive diatom bloom (Fig. 3). At both monitoring stations the phytoplankton community was dominated by *Phaeocystis* colonies only during a short period of time, from 28 April (Day 118) until late May. The unusually successful development of diatoms over *Phaeocystis* colonies in 1994 has been attributed by V Rousseau (pers. comm.) to the very rainy weather conditions prevailing in the preceding winter, flushing more riverborne silicate into the North Sea than normal (Schaub & Gieskes 1991).

Based on microscopic observations, the *Phaeocystis* species blooming at all sites sampled in this study was identified as *Phaeocystis globosa* (Baumann et al. 1994). The variation in biomass and cell numbers of *Phaeocystis* at Stns 330 and tex are presented in Figs. 2A & 3A (data courtesy of V. Rousseau and G. C. Cadée). Maximum cell numbers at the Stns 330, tex, ro91, ro92, pel94, 800 and dcz9 were 6.5, 18.3, 52, 3.2, 1.0, 14.7 and 13.1 × 10^6 cell^{-1}, respectively.

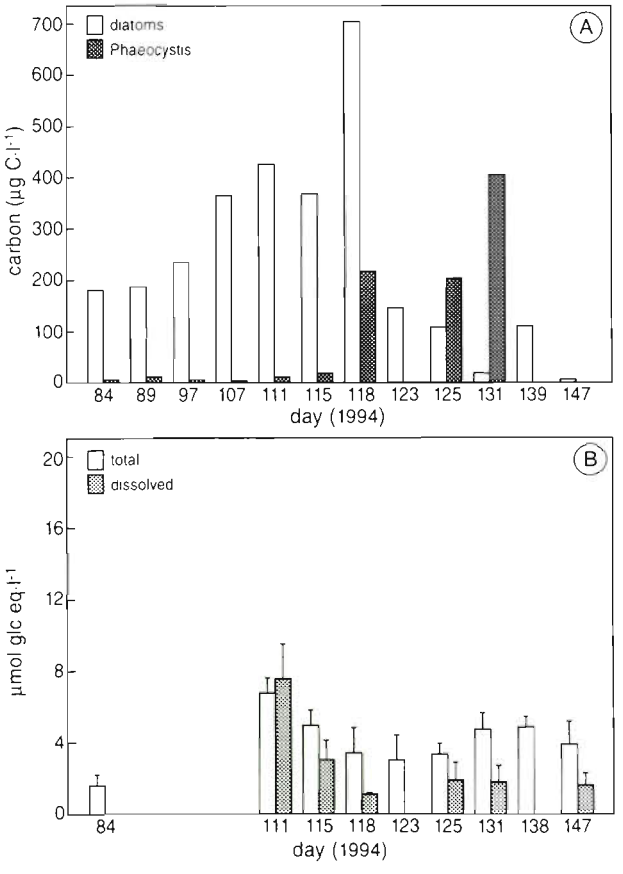

Fig. 2. Stn 330 (Belgian coast), spring 1994. (A) Diatom and *Phaeocystis* colony biomass. Data from V. Rousseau. (B) Total amount of sugars measured in seawater and the <1 µm 'dissolved' fraction, expressed in glucose equivalents

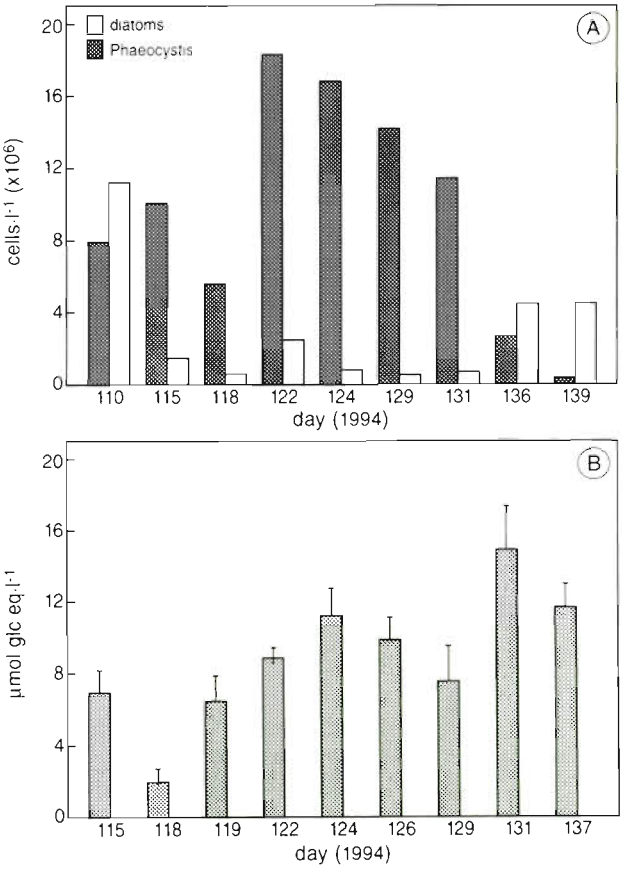

Fig. 3. Stn tex (Texel, Dutch coast), spring 1994. (A) Diatoms and *Phaeocystis* colonial cells. Data from G. C. Cadée. (B) Total amount of sugars in the water column. All sugars were retained on GF/C filters

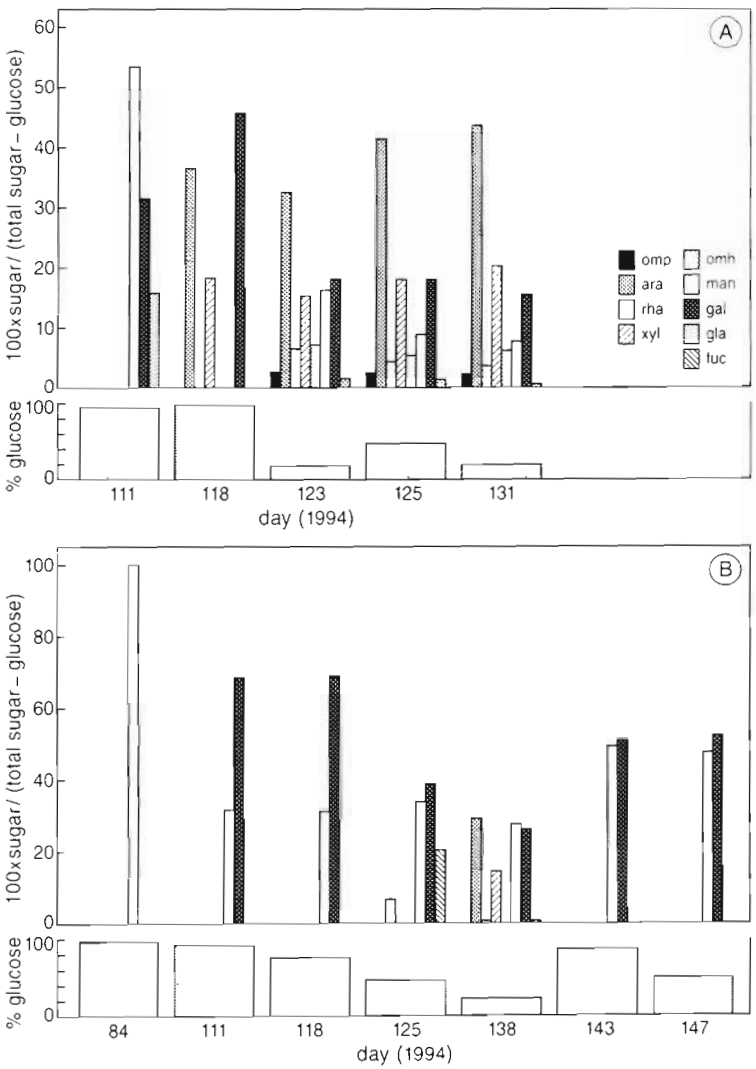

Fig. 4. Stn 330 (Belgian coast). (A) Upper panel: patterns of carbohydrates other than glucose measured in the >250 µm fraction. Lower panel: percentage of glucose to total amount of sugars in the samples. (B) Upper panel: patterns of sugars other than glucose present in the total polysaccharide pool of the water column. Lower panel: percentage of glucose in the samples. Omp: O-methylated pentoses; ara: arabinose; rha: rhamnose; xyl: xylose; omh: O-methylated hexoses; man: mannose; gal: galactose; gla: glucuronic acid; fuc: fucose

High concentrations of total sugar were found during the spring phytoplankton growth phase, i.e. 0.7 to 7.5 mmol m^{-3} glucose equivalents (50 to 540 mg C m^{-3}) at the Belgian reference station (330). One peak coincided with the diatom bloom of *Rhizosolenia delicatula* (Day 111) and one with the *Phaeocystis* bloom (Days 131 and 138; Fig. 2A). At Stns 800 and dcz9, 11.6 and 6.1 mmol m^{-3} were found, of which 100 and 89%, respectively, were in the fraction retained by GF/C filters. There was a notable shift from the sugars present predominantly in the 'dissolved' fraction, when the diatom was blooming, to the 'particulate' fraction (>1 µm), i.e. total minus 'dissolved' when *Phaeocystis* became dominant (Fig. 2). Near Texel, all sugars were present in the >1 µm fraction at concentrations varying between 1.9 and 14.9 mmol m^{-3} glucose equivalents (137 to 1070 mg C m^{-3}; Fig. 3). The changes in the amounts of sugars in the water column corresponded to the changes observed in phytoplankton biomass (Fig. 3).

The *Phaeocystis* colony fractions that were obtained on a 250 µm mesh filter (or 100 µm in case of stations only sampled once) formed a green and viscous layer. The water-soluble carbohydrates that were extracted from this layer contained arabinose, rhamnose, xylose, mannose, galactose, glucose, and glucuronate as well as unidentified O-methylated pentoses and hexoses and traces of ribose (Figs. 4 to 6). N-acetyl-glucosamine was not found in these >250 µm samples, indicating that the colony fractions were essentially free of chitin-containing plankton such as copepods.

The diatom bloom sugar pattern (fraction >250 µm; analyzed on Days 111 and 118 at Stn 330) differed considerably from that of the *Phaeocystis* bloom that followed. Only mannose, galactose and relatively large amounts of glucose (385 and 165 times the total amount of other sugars) were present; O-methylated sugars, always found during the *Phaeocystis* bloom, were lacking during the diatom bloom (Fig. 4A).

Glucose was also the dominant sugar in the total polycarbohydrate pool present in seawater (Fig. 4B), except for the period of the *Phaeocystis* bloom. At that time (Days 125 and 138) a number of sugars also found in the colony fraction (Fig. 4A) became predominant, which suggests that *Phaeocystis* contributed most to the polysaccharide pool.

The *Phaeocystis* bloom at Stn tex showed, apart from variable amounts of glucose, a rather constant pattern for the sugars in the >250 µm colony fraction (Fig. 5). The glucuronic acid content seemed to increase slightly over time while mannose decreased. The anomalous pattern on Day 118 was possibly caused by aggregates of the waning *Rhizosolenia delicatula* bloom that were caught in the >250 µm fraction.

The composition of non-glucose sugars in colony fractions of *Phaeocystis* sampled in 1994 at different locations were compared (Fig. 6). Arabinose was pre-

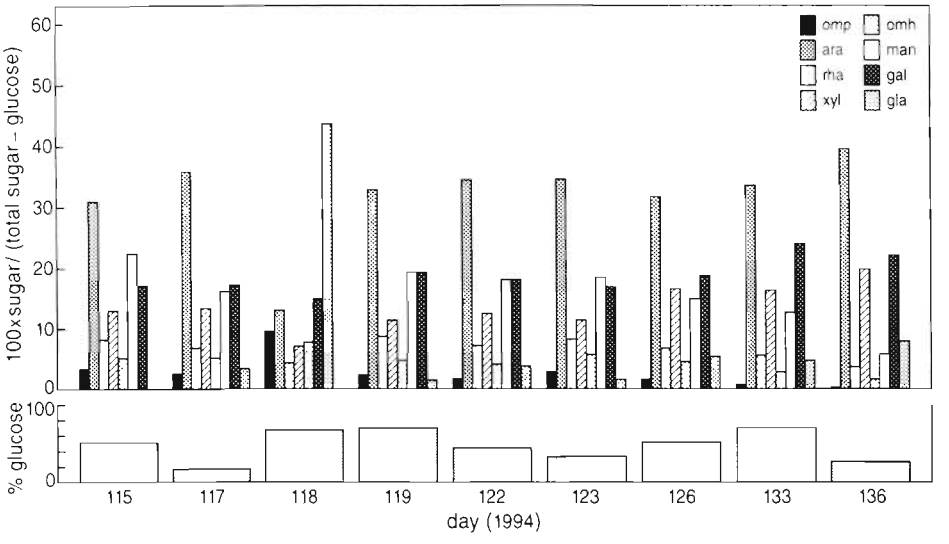

Fig. 5. Stn tex (Texel, Dutch coast). Upper panel: patterns of carbohydrates other than glucose measured in the >250 µm fraction. Lower panel: percentage of glucose in the total amount of sugars in the samples. Abbreviations as in Fig. 4.

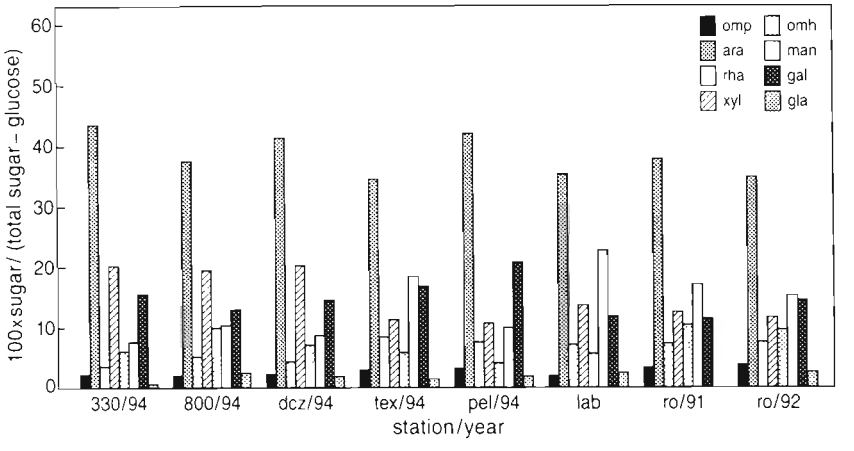

Fig. 6. Comparison of the carbohydrate patterns in different samples collected along the North Sea coast (stations as in Fig. 1). Glucose left out for reasons explained in the text. Abbreviations as in Fig. 3; lab: laboratory

dominant in all these fingerprints; the second most predominant sugar in the Belgian samples was xylose, of which the relative contribution to the total sugar amount decreased northward, while the relative amounts of galactose and mannose increased such that they became the second most predominant sugars in the Dutch samples. The O-methylated sugars were present in all *Phaeocystis*-containing samples, making up approximately 10% of the total amount of sugars (glucose not included). The samples ro91 and ro92, although taken in 1991 and 1992, resembled pel94 and tex94 taken in the same area. The laboratory culture of a strain isolated from Stn 330 (Belgian coast) in 1993 but cultured in water from the Dogger Bank (central North Sea) also resembled the Dutch fingerprints.

DISCUSSION

The sugar composition of the *Phaeocystis* colonies fraction revealed a complex fingerprint. Apart from the highly variable amounts of glucose, the sugar pattern appeared to be similar along the entire continental North Sea coast, but it was completely different from those reported for *Phaeocystis* strains originating from the English Channel (Thingstad & Billen 1994), Surinam and Massachusetts (Guillard & Hellebust 1971), as well as from the diatom bloom preceding the *Phaeocystis* bloom (Fig. 4). Samples taken in the North Sea during the *Phaeocystis* bloom contained arabinose as the most abundant sugar, not mannose as reported by Guillard & Hellebust (1971). Furthermore, only traces of ribose and fucose were found in our samples. Instead, we identified O-methylated pentoses and hexoses in all our *Phaeocystis*-containing samples, including a laboratory culture originally isolated from Belgian coastal waters. These methylated sugars may be indicative of the *Phaeocystis* strains in the North Sea. The sugar pattern of the Belgian *Phaeocystis* samples is similar to that found by Haug et al. (1973) in a phytoplankton sample from the Trondheimsfjord on 30 March 1971, of which 63% of the cells was *Phaeocys-*

tis, except that the O-methylated sugars were not reported.

Despite the overall similarity of the fingerprints along the continental North Sea coast, some regional differences could be identified. In the Belgian samples, arabinose was twice as abundant as xylose and galactose, while in the Dutch area, mannose and galactose were most abundant, next to arabinose. Such local variability may reflect the presence of different strains. Cadée (1991) has suggested that regional blooms are inoculated by just a few colonies wintering in the area. This could be the mechanism by which local strains can develop. Strain differences in genome size and pigment contents have recently been reported for North Sea and English Channel isolates (Vaulot et al. 1994). Carbohydrate fingerprint differences might also be induced by environmental conditions (such as nutrient availability); an indication of this is the fingerprint of the strain isolated from the Belgian coast cultivated in water from the Dogger Bank area (central North Sea) which resembled the Dutch fingerprints.

The relative amounts of glucose were highly variable for the different sugar patterns, ranging from 0.2 to 380 times the amount of the other sugars. There are 3 possible explanations for this. Firstly, glucose polymers (glycogen, starch and laminaran) are universally produced as storage polymers by algae (Painter 1983). In fact, *Phaeocystis* produces a glucan as reserve polymer (Janse et al. 1996). The amount of glucose reserve polymers produced by algae depends highly on the environmental conditions (light and nutrients; Cuhel et al. 1984). Secondly, a contamination of the *Phaeocystis* colony fraction with other algae can cause a relatively high contribution of glucose to the sugar pool. Thirdly, the consumption of storage glucans can be expected to be rapid when they are consumed by bacteria that are adapted to this energy source. The amount of β-glucosidase at Stn 330 followed the total phytoplankton biomass development, not the seasonal pattern of any single species (S. Becquevort pers. comm.). Because processes that influence the glucose pool are not *Phaeocystis*-specific, glucose has been omitted from the comparison of sugar patterns here.

The relative amounts of sugars other than glucose present in *Phaeocystis* polymers were rather constant in the North Sea area. This suggests that these sugars are part of a structural rather than a storage polymer, needed to form the colony matrix. A structural polymer in *Phaeocystis* together with a storage glucan (Janse et al. 1996). resembles the situation in diatoms in which an acid-extractable storage glucan is commonly found in addition to more complex structural polymers (Haug & Myklestad 1976, Brockmann 1982, Hoagland et al. 1993).

The dramatic change of the colony shape during a bloom reported by Verity et al. (1988) and Rousseau et al. (1994) is not reflected in the sugar composition, which remained rather constant. Also, similar sugar fingerprints were found at the Stns 800 and dcz9, where healthy and decaying colonies dominated, respectively (V. Rousseau pers. comm.). Changes in colony firmness may depend on alterations in the amount of negatively charged carboxyl and sulphate groups (van Boekel 1992) or changes in polymer chain length and branching rather than on sugar composition per se.

The concentration of total sugars found during the *Phaeocystis* bloom is well within the range of 5 to 25 mmol m^{-3} glucose equivalents found earlier by Eberlein et al. (1985). Although *Phaeocystis* is known to be capable of high excretion rates of sugars (Guillard & Hellebust 1971, Lancelot & Mathot 1985, Veldhuis & Admiraal 1985) all carbohydrates were retained upon GF/C filters regardless of the state of the bloom that was sampled. In contrast, most of the carbohydrates present during the preceding diatom bloom passed these filters, except at the very beginning of the bloom when they were apparently associated with the cells. The reason for the different behavior of the carbohydrates produced by the diatoms and *Phaeocystis* probably lies in the production of matrix polysaccharides, which is so characteristic for *Phaeocystis*.

The constant sugar composition of this gel-forming colloidal material throughout the bloom may be indicative of its degradation-resistant nature, which results in a relatively long residence time. The result could be the formation of particles large enough to be retained on the filters, thus resembling the transparent exopolymer particles (TEPs) reported by Alldredge et al. (1993). The absence of dissolved sugars during and at the end of the *Phaeocystis* bloom can be explained by scavenging of dissolved organic matter by such particles (Decho 1990). Since bacteria have been shown to adhere to TEPs (Alldredge et al. 1993) and because the substrates available to the microbial loop are concentrated in these microenvironments (Azam & Smith 1991), the observation of a high concentration of TEP-like polysaccharides during *Phaeocystis* blooms in the North Sea suggests an important role of this mucus producing alga in the microbial loop.

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