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Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat

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ABSTRACT: Two field studies were conducted to study the *in situ* net production of extracellular carbohydrates and the distribution of benthic diatoms over a period of 24 h per study. A comparison was made between a situation where a clear surface biofilm of diatoms had developed and a situation where this was not the case. Vertical profiles were made by sampling the top 2 mm of the sediment at depth intervals of 0.2 mm using the 'cryolander' technique. In the presence of a biofilm, diatom distribution showed a consistent pattern when the sediment was emersed. In the light, most of the diatoms were present in the top 0.2 mm while in the dark, diatoms were homogeneously distributed in the upper 2 mm of the sediment. When a biofilm was absent, no clear patterns were observed. Extracellular carbohydrates were extracted from the sediment and separated in 2 operationally defined fractions (colloidal and EDTA-extractable). The 2 carbohydrate fractions showed a different dynamic behaviour. The colloidal carbohydrate fraction was highly variable while the EDTA-extractable fraction behaved more conservatively. Only in the light and in the presence of a diatom biofilm, was production of extracellular carbohydrates observed. The maximum rate of chlorophyll-normalized production of extracellular carbohydrates, expressed in glucose equivalents (g g^{-1}), amounted to 20 h^{-1} in the upper 0.2 mm. The molecular size distribution of both carbohydrate fractions was similar. The monosaccharide composition was also similar, except that the EDTA-extractable fraction contained a higher percentage of uronic acids. Carbohydrates produced during tidal emersion were rich in glucose and were rapidly turned over.

KEY WORDS: Extracellular polymeric substances (EPS) · Polysaccharides: microphytobenthos · Mud flat · Monosaccharide composition · Cryolander

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INTRODUCTION

Benthic epipelic diatoms are the most important group of primary producers in intertidal mudflats (Admiraal 1984, Smith & Underwood 1998). They are known to produce copious amounts of extracellular polymeric substances (EPS) mainly consisting of carbohydrates (Hoagland et al. 1993). By so doing, diatoms form a biofilm that serves to produce their own microenvironment which protects them from the rapidly changing conditions in intertidal mudflats (Decho 1994). The excretion of EPS plays a role in the movement of epipelic benthic diatoms (Edgar & Pickett-Heaps 1983) and allows the organisms to adhere to sediment surfaces (Wang et al. 1997). EPS-production may also be controlled by nutrient availability, for instance when the organisms grow under unbalanced conditions (Ruddy et al. 1998), a situation likely to occur in intertidal environments (Flothmann & Werner 1992). The presence of diatom biofilms increases the stability of the sediment surface (Kornman & de Deckere 1998, Paterson 1989) and can have a profound

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effect on the morphodynamics of mudflats (Dyer 1998, de Brouwer et al. 2000). Through the excretion of EPS, diatoms are responsible for a considerable input of high-quality organic carbon into the sediment that may be utilized as a food source for heterotrophic consumers (King 1986, van Duyl et al. 1999).

The dynamics of extracellular carbohydrates in intertidal mudflats have been studied at different temporal and spatial scales (Underwood & Paterson 1993, Taylor & Paterson 1998, Taylor et al. 1999, Blanchard et al. 2000, de Brouwer et al. 2000, Staats et al. 2000). By using different extraction protocols, different operationally defined carbohydrate fractions are obtained (Underwood et al. 1995). Colloidal carbohydrate, a water-soluble fraction that is commonly used for the determination of extracellular carbohydrates, shows a significant correlation to chlorophyll *a* (chl *a*) in intertidal mudflats that are dominated by epipelic diatom assemblages (Underwood & Smith 1998a, Blanchard et al. 2000). Other fractions have been obtained using EDTA to recover extracellular carbohydrates that are more tightly associated with cell walls or sediment particles (Decho & Lopez 1993, de Winder et al. 1999). Finally, total hydrolyzable carbohydrate contents can be measured by direct hydrolysis of sediment samples (Taylor & Paterson 1998, Taylor et al. 1999). Production of extracellular carbohydrates has been observed to occur when the sediment is exposed to the light (de Winder et al. 1999, Taylor et al. 1999, Staats et al. 2000), and is dependent on a number of factors including time of exposure (Underwood & Paterson 1993), growth stage (Staats et al. 1999, Smith & Underwood 2000) and morphology of the mudflat (Taylor & Paterson 1998, Blanchard et al. 2000). Furthermore, increased production of extracellular carbohydrates has been observed concurrant with changes in diatom numbers at the mudflat surface, typically at the start and end of the emersion period (Smith & Underwood 1998, Underwood & Smith 1998b).

To elucidate the factors that control the excretion of carbohydrate, it is important to sample in a way that provides sufficient resolution within the area were diatoms are active (Paterson et al. 2000). Epipelic diatoms show rhythmic migrations in response to light and tidal cycles (Happey-Wood & Jones 1988, Serôdio et al. 1997), and the amplitude of vertical migration has been reported to vary between 1.6 mm (Paterson 1986) and 12 cm (Kingston 1999). In intertidal mudflats, migration generally occurs within the upper 2 mm of the sediment. The cryolander method (Wiltshire et al. 1997) is a sampling technique that allows the cross-sectioning of undisturbed sediment cores at a depth resolution of up to 100 µm and is therefore the method of choice to investigate carbohydrate and diatom dynamics in surface sediments.

In situ studies have so far measured only daytime emersion periods (Taylor et al. 1999) or lacked sufficient vertical resolution when comparing day- and nighttime emersion periods (van Duyl et al. 1999, Staats et al. 2000). In addition, there is very little information on the biochemical properties of extracellular carbohydrates in mudflat sediments. Information about the monosaccharide composition of carbohydrates excreted by benthic diatoms comes mainly from work on cultures (de Winder et al. 1999, Staats et al. 1999), although Taylor & Paterson (1999) analyzed the monosaccharide composition of colloidal and bulk carbohydrates in the Eden estuary, Scotland.

In addition to compositional data, size-distribution analyses can provide information on the properties of extracellular carbohydrates and may therefore be useful in understanding the dynamics of these sugars in intertidal mudflats. Ethanol precipitation of colloidal carbohydrates is often used to obtain the high molecular weight fraction, which is termed EPS (Underwood et al. 1995) and is composed of the >100 kdaltons polysaccharides (pers. obs.). However, in marine sediments it has been demonstrated that polysaccharides are rapidly decomposed, resulting in smaller polymers with a broad range of molecular weights (Arnosti 1995, 1996) that are well below the size that precipitates in ethanol. Furthermore, EPS-excreting organisms may produce polymers that are variable in chain length. For example, Bender et al. (1994) found that the benthic mat-forming cyanobacterium *Oscillatoria* sp. excreted polysaccharides of 2 different sizes.

In this paper, diurnal dynamics in diatom distribution and extracellular carbohydrate in the upper 2 mm of intertidal mudflat sediments are described. The main questions that we address are: (1) What are the effects of light and tidal inundation on diatom distribution and carbohydrate standing stocks? (2) What are the dynamics of extracellular carbohydrates in terms of biochemical properties? (3) Are the 2 extracellular carbohydrate fractions comparable in terms of dynamics and biochemical properties?

The studies that we conducted represented 2 different situations that occur in intertidal mudflats. In June 1998 the mudflat was devoid of a distinct visual layer of microphytobenthos, while in May 1999 extensive mats were observed that altered the sediment properties of the mudflat surface, changing bedform structure and increasing sediment stability (de Brouwer et al. 2000).

MATERIALS AND METHODS

Site description. The Biezelingse Ham mudflat is situated along the northern shore of the Westerschelde estuary in the south-west of the Netherlands

(51° 56.47′ N, 3° 55.51′ E). The mudflat has an area of ca 1.5 km^2 and the tidal range is ca 4 m. A small saltmarsh and a freshwater inlet are situated on the north side of the mudflat. Sampling was carried out at the middle station of a transect established at the southern end of the mudflat. The emersion time at this site was ca 6 h. Sediment at this part of the mudflat was typically muddy, with a median grain size of <20 µm; the surface was planar, with major bedforms generally absent. A detailed survey, examining the dynamics in biology and sedimentology of this mudflat was carried out by de Brouwer et al. (2000).

Sampling methodology. The sampling site was sampled twice over a period of 24 h each. In both studies, low tide during daytime emersion occurred at 14:00 h, while low tide during nighttime emersion occurred at 02:30 h. The first study took place on 16 June 1998, about 3 wk after the collapse of a benthic diatom bloom (see also de Brouwer et al. 2000). At that time, no diatom biofilms were found at the sediment surface. In contrast, well-developed diatom films were present during the second study, which was carried out on 20 May 1999. Pigment analysis of the sediment samples revealed that in both studies diatoms were by far the dominant phototrophic organisms present. In May 1999, sediment samples were taken every 30 or 60 min, starting 15 min after the sediment became exposed; the last sample was taken 15 min before immersion. In June 1998, 3 sediment samples were taken during daytime emersion (1.25, 4.00, and 5.00 h after emersion), while 7 cores were taken during nighttime emersion (15 min to 5.5 h after emersion at regular time intervals).

Sediment cores were collected using the cryolander coring method (Wiltshire et al. 1997). Briefly, the sediment surface was frozen with liquid N_2 vapour, ensuring minimal disturbance of the structure of the sediment. After the sediment surface had been frozen, liquid N_2 was poured over it in order to freeze deeper sediment layers (down to 1 cm). The frozen sediment samples could be removed easily, and were wrapped in aluminium foil and transported to the laboratory in liquid N_2. Prior to sectioning, the cryolander samples were cut into pieces of approx. 1 cm^2 with a diamond grinder. Subsequently, the top 1 mm of the sediment was sectioned into 0.2 mm slices and the second 1 mm into 2 slices of 0.5 mm each, using a freeze-microtome (Leica, SM 2000 R). Samples were lyophilized prior to analysis of carbohydrate (n = 3) and chl *a* (n = 3).

Results are expressed as contents (µg g^{-1} dry wt of sediment) and would thus be sensitive to changes in water content. In the 2 field studies, water content (calculated from the wet and dry weights of the sediment) decreased with increasing depth (from 55.5 ± 8.4 [SD] and 75.1 ± 6.5% in the 0 to 0.2 mm depth horizon to 47.6 ± 5.0 and 70.0 ± 4.7% in the 1.5 to 2.0 depth horizon in June 1998 and May 1999, respectively). Over the course of the 2 field studies, the water content of the separate depth layers did not change; therefore the time-series data were not influenced by water content.

Chl *a* analysis. Lyophilized sediment samples (10 to 100 mg) were extracted with 2 ml 90:10 (v/v) acetone:water for 1 h at 0°C. Extraction was aided by sonication. The extracts were centrifuged for 10 min at 1000 ×*g*. Chl *a* was measured fluorometrically, applying a correction for pheopigments, using the equations of Lorenzen (1966).

Carbohydrate analysis. The lyophilized sediment (5 to 55 mg) was extracted with 400 µl distilled water for 1 h at 30°C. The extract was centrifuged for 5 min at 6000 ×*g*, and the colloidal carbohydrate fraction of the supernatant was determined. Subsequently, the pellet was incubated with 500 µl 0.1 M Na_2EDTA for 16 h at room temperature. The extract was centrifuged for 5 min at 6000 ×*g*, and the EDTA-extractable carbohydrate fraction of the supernatant was assayed. Carbohydrate was measured spectrophotometrically using the phenol-sulphuric acid assay (Dubois et al. 1956). Glucose was used as the reference. To a 200 µl sample, 200 µl 5% (w/v) phenol and 1 ml concentrated sulphuric acid were added, and the mixture was incubated for 35 min.

Size distribution and monomeric composition of carbohydrate fractions. Sediment samples taken 15 min after the start and 15 min before the end of the daytime emersion period of the May 1999 study were used for molecular size distribution analyses and determination of the monomeric carbohydrate composition. Samples were taken at depth intervals of 0 to 0.2, 0.6 to 0.8 and 1.5 to 2.0 mm. Ten replicate samples were pooled, lyophilised and subsequently extracted with 1 ml distilled water and 1.5 ml 0.1 M Na_2EDTA as described above.

Size distribution was determined by ultrafiltration using Centricon centrifuge tubes (Millipore) with molecular weight cut-off filters of 100, 50, and 10 kdaltons. These filters are commonly calibrated using protein standards. Carbohydrates have different space-filling molecular properties, and therefore their molecular weight cut-off may be slightly different and their size classes must be regarded as operationally defined. Samples were centrifuged for 30 min, 1000 ×*g*; 15 min, 5000 ×*g*; and 2 h, 5000 ×*g*, through 100, 50 and 10 kdalton filters, respectively. The fractions retained by these filters were desalted by adding 2 ml of distilled water and repeating the ultrafiltration. The recovery was determined by weighing the amount of original sample in the ultrafiltration device, the amount of filtrate and retentate after ultrafiltration, and the carbohydrate concentrations in these fractions. Recoveries of the ultrafiltration varied between 85 and 115% and were normalised to 100%.

The monosaccharide composition of the carbohydrates was determined by HPLC. Methanolysis and subsequent hydrolysis of the carbohydrates was carried out according to de Ruiter et al. (1992). Briefly, lyophilised samples were methanolyzed with 0.5 ml 2 M HCl in methanol for 16 h at 80°C. Subsequently, the methylglycosides were hydrolyzed to the monosaccharides with 2 M trifluoro-acetic acid (1 h at 120°C). The sugars where separated on a Carbopack-1 column (Dionex) and detected by a pulse amperometric detector (PAD). Neutral sugars were separated running the following gradient: 0 to 20 min, 15 mM NaOH; 20 to 25 min, 300 mM NaOH, 25 to 35 min, 15 mM NaOH. Uronic acids were analyzed in a separate run using 100 mM NaOH/100 mM sodium acetate as eluent. The peaks of xylose and mannose co-eluted. Quantification was based on the assumption that both monosaccharides were present in equal amounts. The analytical reproducibility was within ±5% (n = 8) for all monosaccharides analyzed. The relative contribution of the different monosaccharides was calculated using external standards, and is expressed in weight%.

Statistical analyses. Statistical analysis was performed on log(n+1)-transformed data. A MANOVA (multiple analysis of variance) design was used to test the effects of duration of emersion and day or night on the content of carbohydrates and chl *a*. Dependent variables were the content of chl *a* and carbohydrate at all depth intervals. The duration of emersion, day/night situation and the interaction were used as independent variables, where time is a numerical and day/night a categorical variable. The interaction between the duration of emersion and the day/night situation examines to what extent the evolution of the measured contents over the daytime emersion period are different from that measured during the nighttime emersion period. *F*-tests for the total depth layer were based on Wilkinson's lambda *F*-statistics. Statistical results are given for the total depth layer, unless stated otherwise. The data were analyzed using Systat 5.02 (Systat Corporation, Evanston, Illinois).

RESULTS

Extracellular carbohydrates

In June 1998, the dry sediment content of colloidal carbohydrate over the whole depth layer analyzed averaged 206 ± 32 (SD) µg g^{-1} sediment, while the EDTA-extractable carbohydrate averaged 670 ± 201 µg g^{-1} (Fig. 1). There was no interaction between duration of emersion and day/night in either carbohydrate fraction (colloidal carbohydrates, $F_{[7, 11]} = 0.49$, $p = 0.83$; EDTA-extractable carbohydrates, $F_{[7, 10]} = 2.02$, $p = 0.15$). The contents of both carbohydrate fractions did not vary over the emersion periods (colloidal carbohydrates, $F_{[7, 11]} = 0.49$, $p = 0.83$; EDTA-extractable carbohydrates, $F_{[7, 10]} = 2.31$, $p = 0.11$). The colloidal content differed between day- and nighttime emersion periods ($F_{[7, 12]} = 6.09$, $p < 0.01$). However, when the separate depth intervals were considered, none of the tests revealed significant differences ($F_{[1, 18]} < 3.48$, $p > 0.08$ for all depth intervals). Vertical profiles of colloidal carbohydrate showed no significant differences with increasing depth. In contrast, the content of EDTA-extractable carbohydrate decreased from 900 µg g^{-1} at the surface to 600 µg g^{-1} below 1 mm depth (Fig. 1).

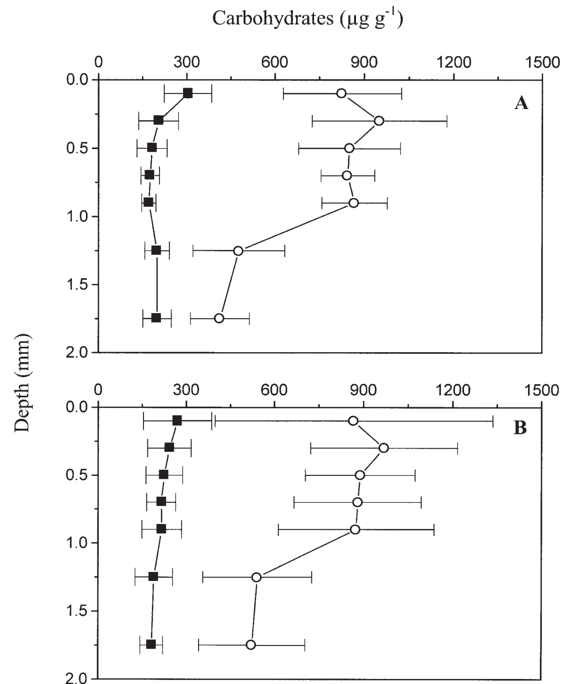

Fig. 1. Vertical profiles of average (± 1 SD) content (µg g^{-1} dry sediment) of colloidal (■) and EDTA-extractable (○) carbohydrates on 16 June 1998 during daytime (A) and nighttime (B) emersion periods

In May 1999, the content of both colloidal and EDTA-extractable carbohydrate was higher than in June 1998. At the start of the emersion, colloidal carbohydrate in the top 2 mm averaged 1644 ± 443 µg g^{-1} sediment, and EDTA-extractable carbohydrate 1893 ± 305 µg g^{-1}. In the light, the content of colloidal carbohydrate increased almost 10-fold in the upper 0.2 mm over the emersion period (Fig. 2). When considering the whole depth layer sampled, the interaction between duration of emersion and day/night was significant ($F_{[7, 36]} = 5.59$, $p < 0.001$) as a result of carbohydrate production during daytime emersion but not during nighttime emersion (cf. Figs 2 & 3). When the

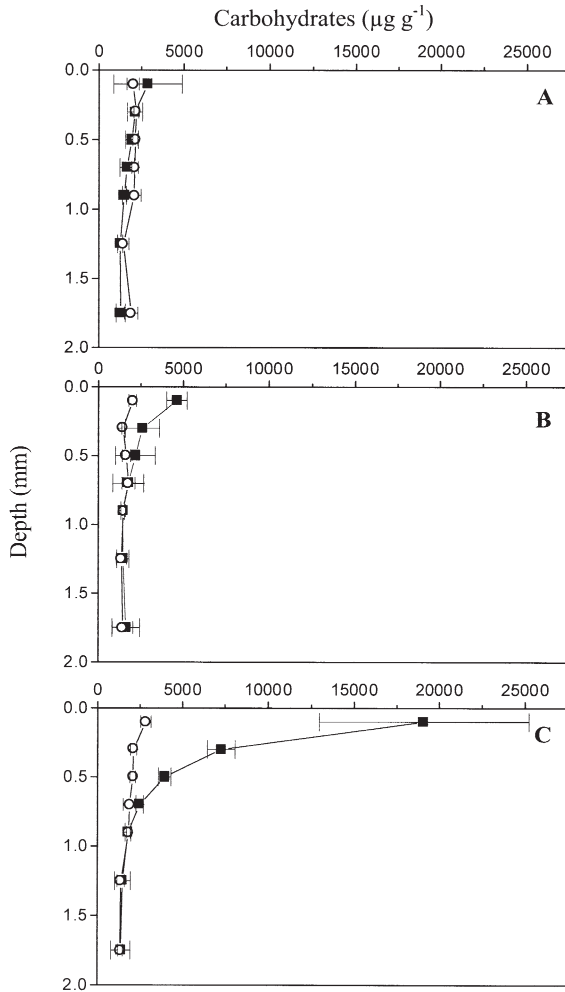

Fig. 2. Vertical profiles (means ± 1 SD) of colloidal (■) and EDTA-extractable (○) carbohydrates over daytime emersion period on 20 May 1999, 15 min (A), 2.05 h (B) and 5.5 h (C) after emersion

different depths were examined individually, a significant interaction effect was evident in the top 1 mm of the sediment ($F_{[1, 42]}$ = 5.6, p = 0.022 at 0.8 to 1.0 mm). The interaction between time of emersion and day/night was not significant for EDTA-extractable carbohydrates ($F_{[7, 37]}$ = 0.67, p = 0.70) and the amount did not vary during emersion ($F_{[7, 37]}$ = 1.75, p = 0.13). A significant increase in EDTA-extractable content during emersion was observed only in the top 0.2 mm ($F_{[1, 43]}$ = 7.35, p = 0.01). The content of EDTA-extractable carbohydrate was higher during daytime than nighttime emersion ($F_{[7, 37]}$ = 5.43, p < 0.001), but again this was mainly due to the higher content in the upper 0.2 mm layer during the daytime emersion ($F_{[1, 43]}$ = 29.96, p < 0.001). At the start of the nighttime emersion period, the carbohydrates produced in the light had disappeared, and the levels had dropped to those at the start of the daytime emersion period (Figs 2 & 3).

During the night, the content of colloidal as well as EDTA-extractable carbohydrate did not vary, with average values of 1648 ± 408 and 1610 ± 277 µg g^{-1} respectively.

Chl *a* content

In June 1998, the chl *a* content in the top 0.2 mm of sediment during emersion varied from 3.3 to 12.3 µg g^{-1} during daytime and 5.0 to 9.4 µg g^{-1} during nighttime. Chl *a* content varied only marginally during the emersion periods ($F_{[7, 5]}$ = 4.86, p = 0.05). No significant changes were observed between chl *a* content during daytime and nighttime emersion ($F_{[7, 5]}$ = 3.11, p = 0.12) (Fig. 4).

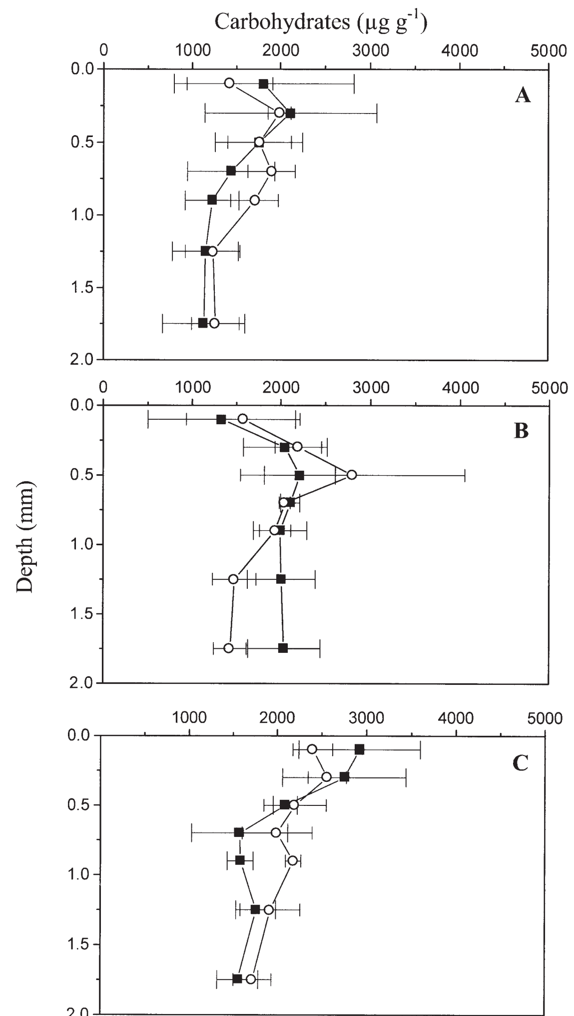

Fig. 3. Vertical profiles (means ± 1 SD) of colloidal (■) and EDTA-extractable (○) carbohydrates over nighttime emersion period on 20 May 1999, 5 min (A), 2.05 h (B) and 5.5 h (C) after emersion

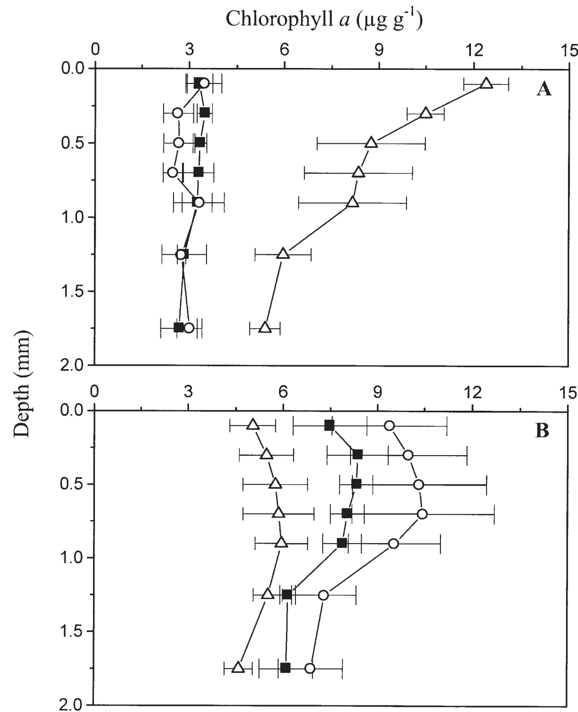

Fig. 4. Vertical profiles average (± 1 SD) of chl *a* contents on 16 June 1998 during daytime (A) and nighttime (B) emersion periods, 1.25 h (■), 4 h (○) and 5 h (△) after emersion

In May 1999, the content of chl *a* was 5 to 10-fold higher than in June 1998. In the top 0.2 mm, the chl *a* content during emersion was typically in the range of 155 µg g^{-1} during the day and 51 µg g^{-1} during the night. Although there were no significant changes between samples within the emersion periods ($F_{[7,\,30]}$ = 0.41, p = 0.89), vertical profiles differed markedly between day and night ($F_{[7,\,30]}$ = 31.23, p < 0.001), (Fig. 5). In the light, the chl *a* content was highest in the top 0.2 mm, decreasing steeply with increasing depth. During the night, the content of chl *a* remained constant over the depth range analyzed. The content of chl *a* in the top 2 mm during the night was about 75% of the content during the day.

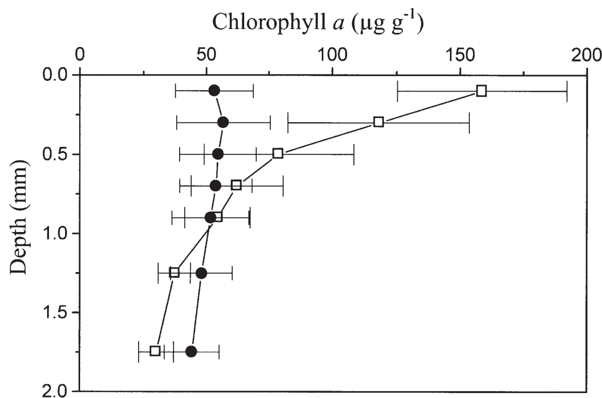

Fig. 5. Vertical profiles of average (± 1 SD) chl *a* contents on 20 May 1999 during daytime (□) and nighttime (●) emersion periods

Carbohydrate production in the light

During daytime emersion in May 1999, the carbohydrate content was normalized to chl *a* in order to obtain production estimates of extracellular carbohydrates at the measured depth intervals (Fig. 6, Table 1). Colloidal carbohydrate production was significant in the top 1 mm, being highest in the top 0.2 mm. In this layer, the chlorophyll-normalized production rate of colloidal carbohydrate (glucose equivalents) was 20 h^{-1}. For EDTA-extractable carbohydrates, a significant increase was only found in the top 0.2 mm sediment layer. However, compared to colloidal carbohydrates, production of EDTA-extractable carbohydrates was low (1.2 h^{-1}). Typically, the intercepts of the linear regressions for both colloidal and EDTA-extractable carbohydrates increased with increasing depth (Table 1).

Size distribution

Table 2 gives the results for the size-distribution analyses. The colloidal and EDTA-extractable fractions

Table 1. Chl *a*-normalized carbohydrate production ($y = ax + b$) during daytime emersion period on 20 May 1999. ns: not significant

Depth (µm)	*a* (h^{-1})	*b*	R^2	p
Colloidal carbohydrates				
0–200	19.9	7.2	0.74	<0.001
200–400	7.6	12.7	0.59	<0.001
400–600	5.6	19.1	0.37	<0.001
600–800	3.4	23.9	0.23	<0.05
800–1000	2.2	24.3	0.17	<0.05
1000–1500	1.2	31.8	0.03	ns
1500–2000	–0.5	40.7	0.03	ns
EDTA-extractable carbohydrates				
0–200	1.2	13.2	0.44	<0.001
200–400	1.6	16.8	0.09	ns
400–600	0.8	22.0	0.12	ns
600–800	0.4	31.1	0.007	ns
800–1000	0.05	33.5	0.0002	ns
1000–1500	–0.1	35.0	0.0008	ns
1500–2000	–3.2	49.0	0.12	ns

Table 2. Size distribution of colloidal and EDTA-extractable carbohydrates (μg g^{-1} dry sediment) in 3 depth intervals at start and end of daytime exposure period on 20 May 1999. Values in parentheses are percentages of whole fraction. nd: not detected

Depth (μm)	Size class (kDa)							
	>100		100–50		50–10		<10	
	Start	end	start	end	start	end	start	end
Colloidal								
0–200	836 (29)	4774 (24)	375 (13)	1719 (9)	173 (6)	3819 (20)	1529 (53)	8975 (47)
600–800	413 (25)	523 (21)	314 (19)	323 (13)	132 (8)	124 (5)	793 (48)	1518 (61)
1500–2000	477 (37)	465 (34)	115 (9)	123 (9)	90 (7)	82 (6)	605 (47)	698 (51)
EDTA-extractable								
0–200	441 (22)	512 (18)	180 (9)	199 (7)	260 (13)	114 (4)	1142 (57)	2019 (71)
600–800	nd	320 (17)	nd	301 (16)	nd	245 (13)	nd	1036 (55)
1500–2000	419 (22)	295 (22)	190 (10)	147 (11)	190 (10)	268 (20)	1104 (58)	643 (48)

showed a comparable size distribution. Both fractions contained high amounts of carbohydrates <10 kdaltons (47 to 71%), while carbohydrates in the size classes 50 to 100 and 10 to 50 kdaltons were less abundant. During the emersion period, the relative contribution of the >100 kdaltons size class decreased slightly compared to the other size classes (Student's t-test, $p < 0.05$). The size distribution of the carbohydrate fractions did not vary as a function of depth.

Monosaccharide composition

The depth-averaged monosaccharide compositions for the different size classes of carbohydrates are shown in Fig. 7. Comparison of the size fractions per sample in terms of monosaccharide composition size classes >100, 50 to 100 and 10 to 50 kdaltons revealed no differences. However, the colloidal carbohydrates of <10 kdaltons were enriched in galactose and depleted in xylose/mannose, rhamnose and arabinose compared to the larger polymers. EDTA-extractable carbohydrates of <10 kdaltons were enriched in glucose, while the contribution of xylose and mannose dropped to zero. Furthermore, the relative contribution of fucose decreased with decreasing size class in both

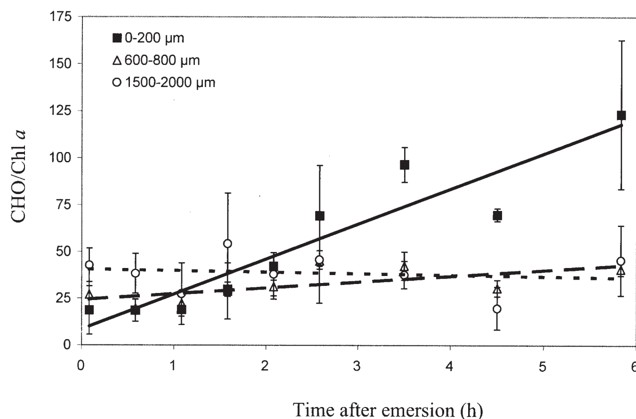

Fig. 6. Average (± 1 SD) chl a-normalized colloidal carbohydrate (CHO) production during daytime emersion period on 20 May 1999

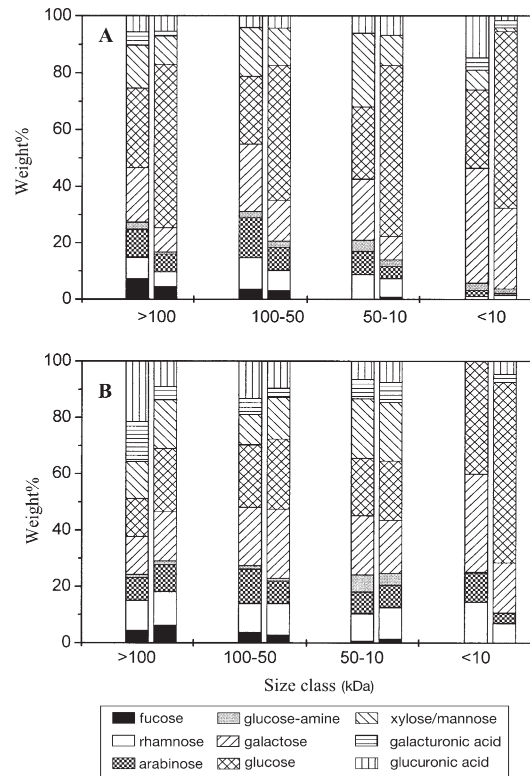

Fig. 7. Relative monosaccharide composition (depth-averaged) of different size fractions. Samples were taken during daytime emersion period on 20 May 1999. (A) Colloidal carbohydrates; (B) EDTA-extractable carbohydrates. For each size class, left-hand bar represents monosaccharide composition at start of the emersion period, right-hand bar represents monosaccharide composition at end of the emersion period

carbohydrate fractions. The EDTA-extractable carbohydrates contained at least twice as much uronic acids as the colloidal fraction, regardless of size, except for the <10 kdaltons size class.

Fig. 7A shows changes in the sugar composition of colloidal carbohydrates during the emersion period. In all size classes, the relative contribution of glucose had increased about 2-fold by the end of the emersion period. Hence, the extracellular carbohydrates produced during emersion were relatively rich in glucose. The EDTA-extractable fraction of >10 kdaltons did not change in composition during emersion, whilst some variation in the <10 kdaltons fraction was apparent.

The composition of the carbohydrates at different depth intervals is shown in Fig. 8. At the end of the emersion period, glucose represented up to 85 % of the sugars in the colloidal fraction in the top 0.2 mm of the sediment. The contribution of glucose decreased rapidly with increasing depth. At 1.5 to 2 mm, the contribution of glucose was only 28 %, and galactose became the most abundant monomer. In the EDTA-extractable fraction, the contribution of glucose increased during emersion, although this was much less pronounced than for the colloidal fraction. Again, most of this increase was associated with the surface 0.2 mm of the sediment.

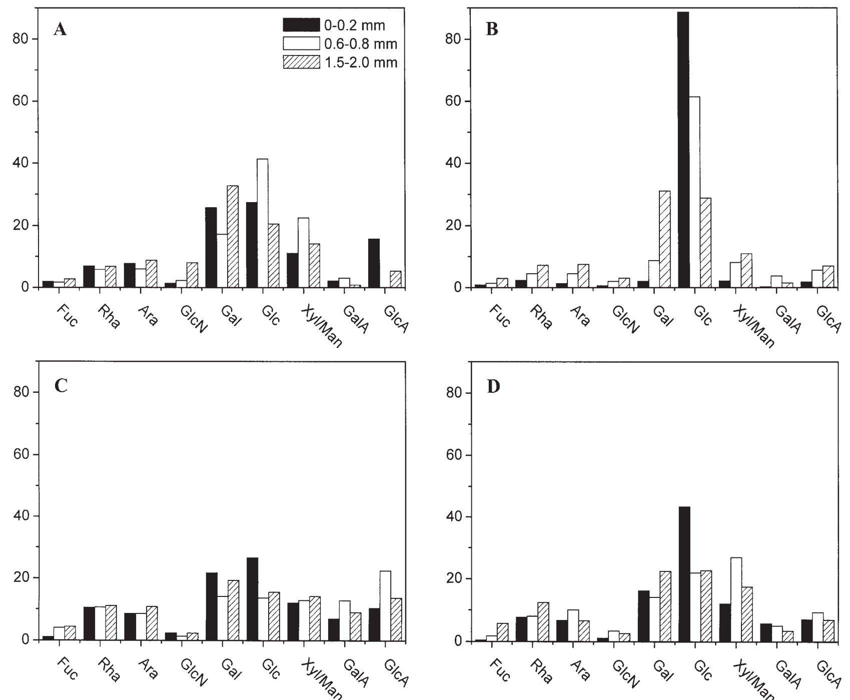

Fig. 8. Relative monosaccharide composition in the 3 sampled depth-intervals during daytime emersion period on 20 May 1999. (A) Colloidal carbohydrates, start of the emersion period; (B) colloidal carbohydrates, end of the emersion period; (C) EDTA-extractable carbohydrates, start of the emersion period; (D) EDTA-extractable carbohydrates, end of the emersion period. Fuc: fucose; Rha: rhamnose; Ara: arabinose; GlcN: glucose-amine; Gal: galactose; Glc: glucose; Xyl/Man: xylose/mannose; GalA: galacturonic acid; GlcA: glucuronic acid

DISCUSSION

Carbohydrate dynamics

In this study, the dynamics of colloidal and EDTA-extractable carbohydrate fractions in an intertidal mudflat were investigated. Measurements of carbohydrate contents in the presence and absence of diatom biofilm structures revealed different dynamics between the 2 carbohydrate fractions. This was clear both on the time scale of a tidal cycle, and also when comparing the samples with and without a diatom biofilm. In June 1998, colloidal carbohydrates, which have been suggested to be indicative of microphytobenthic production (Underwood & Smith 1998a), were present in lower amounts than EDTA-extractable carbohydrates. No production of extracellular carbohydrates was observed over the tidal cycle. In May 1999, the contents of colloidal as well as EDTA-extractable carbohydrate were considerably higher than in June 1998. Colloidal carbohydrate content was higher by a factor of 8, while EDTA-extractable carbohydrate content had increased by a factor of 2 to 3. In contrast to June 1998, net production of EPS (mainly colloidal) was observed, resulting in a 10-fold increase in carbohydrates by the end of the daytime emersion period. This daytime production had disappeared at the start of the nighttime emersion period. These data suggest that colloidal carbohydrates are recently produced and disappear rapidly from the system, being either washed away during immersion (Smith & Underwood 1998, de Winder et al. 1999) or utilized by heterotrophic consumers (van Duyl et al. 1999, Middelburg et al. 2000). EDTA-extractable carbohydrates are less dynamic and represent a more conservative fraction of these sediments.

Although clear differences in the dynamics of the 2 carbohydrates were observed, both the size distribution and the monosaccharide composition of carbohydrate fractions >10 kdaltons were remarkably similar. The monosaccharide composition of the smallest size fraction differed from that of the larger fraction, galac-

tose being most abundant in the colloidal fraction, while glucose was the predominant sugar in the EDTA-extractable fraction. Paterson (1986) showed that lyophilisation may damage diatoms present in the sediment. Also Janse et al. (1996) observed that internal carbohydrates present in the cells of *Phaeocystis globasa* were released when cells were frozen. Therefore we cannot exclude that storage carbohydrates (e.g. chrysolaminaran, molecular weight = 3 to 4 kdaltons) and other low molecular weight carbohydrates were present in the <10 kdaltons fraction.

The similarities in the monosaccharide composition and size distribution of the 2 fractions may hint at a common source of these carbohydrates or a rapid utilization of the degradable part of the carbohydrates. Although both the composition and size of the 2 fractions were broadly similar, the uronic acids in the EDTA-extractable fraction were twice as abundant as in the colloidal fraction. Anionic sugars (sulfonic acids, uronic acids) are considered to be important for the adsorption of EPS to sediment particles (Decho 1990); therefore, it is possible that EDTA-extractable carbohydrate originates from the same source and may be produced by the conversion of colloidal carbohydrate, involving interactions with sediment particles and metal ions (Decho 1994).

The production of carbohydrate was only evident in the light; it was confined to the top 1 mm, and showed its highest rates of production in the top 0.2 mm. This correlated with diatom biomass, which was also concentrated in this top layer of the sediment during daytime emersion. This clearly emphasises the importance of light, which is strongly attenuated in silty sediments (Kühl et al. 1994). De Winder et al. (1999) and Staats et al. (2000) demonstrated that the production of extracellular carbohydrate by benthic diatoms was coupled to photosynthesis, and our results support this conclusion. Smith & Underwood (1998) observed an increase in carbohydrate content associated with the vertical migration of diatoms. In our study, vertical diatom migration occurred at the start and end of the daytime emersion period, as judged from the coloration of the sediment. This was not directly coupled to changes in carbohydrate content. Therefore, the excretion of extracellular carbohydrates as part of the mechanism of diatom motility is probably small compared to that produced through unbalanced photosynthetic growth.

In the light, the chlorophyll-specific rate of production of colloidal carbohydrate carbon (g g^{-1}) amounted to 8 h^{-1} in the top 0.2 mm of the sediment. Assuming chlorophyll-specific carbon-fixation rates for benthic diatoms of 20 h^{-1} (Blanchard & Montagna 1992), we calculate that about 40% of the carbon fixed may be excreted as carbohydrates. This percentage was measured experimentally by Middelburg et al. (2000) using *in situ* enrichments of the stable carbon isotope ^{13}C: however, the assumed rate of carbon fixation was for a subtropical mudflat. Blanchard et al. (1997) calculated a much lower rate of 11 h^{-1} in a temperate mudflat. Using this lower number we calculated that as much as 73% of fixed carbon might be excreted as carbohydrates. Using radioisotope ^{14}C incubations, Goto et al. (1999) reported that 42 to 73% of fixed carbon was excreted. This confirms our estimates. As this extracellular carbohydrate only forms in the colloidal fraction, it follows that the vast majority of this material rapidly disappears. Whether these carbohydrates dissolve in the water column or are degraded in the sediment remains to be determined.

The major component of the EPS produced during the emersion period was glucose, which represented about 90% in the excreted carbohydrates. This was the case for carbohydrates of all size classes, indicating that the polysaccharides produced are highly variable in size or that the polymers are rapidly hydrolysed into smaller molecules. Glucose is a preferred substrate for many heterotrophic consumers such as bacteria (Sawyer & King 1993). The first step in polymer degradation is its hydrolysis to molecules smaller than 0.6 kdaltons (Weiss et al. 1991) by exo-enzymes. King (1986) and van Duyl et al. (1999) measured the activity of β-glucosidases in intertidal mudflats and demonstrated that benthic heterotrophic bacterial production depended on colloidal carbohydrates in a diatom biofilm-dominated mudflat. Aluhiware & Repeta (1999) found that the EPS excreted by 3 phytoplankton species were subject to rapid partial degradation. The residual polysaccharides that remained resembled metabolically stable acyl heteropolysaccharides (Aluhiware et al. 1997) that accumulate in seawater. Our results are very similar. At the start of the emersion period and deeper in the sediment the contribution of glucose was considerably lower than that of the EPS excreted during the emersion period. This indicates that glucose is preferentially removed from the system. Therefore, EPS present at the start of the emersion period and deeper in the sediment seem to represent a polysaccharide fraction that is more resistant to microbial degradation.

Diatom migration

In June 1998, the sediment chl *a* content was low (<13 µg g^{-1}) and no clear pattern in the vertical distribution of benthic diatoms over a tidal emersion period was observed. This was probably the result of the low biomass and high spatial variation. In May 1999, chl *a* content reached up to 150 µg g^{-1} sediment in the surface 0.2 mm during daytime emersion. During this

period, the majority of the diatoms were present in the surface sediment layer, allowing a maximum rate of photosynthesis. During nighttime emersion, the microphytobenthos biomass was equally distributed throughout the upper 2 mm (Fig. 4), the diatoms having migrated deeper into the sediment. Upward migration towards the sediment surface is probably triggered by light, while in the dark the direction of diatom movements may be random, resulting in a homogeneous distribution. Alternatively, diatoms may sense chemical gradients that trigger the direction of migration.

Diatom migration occurs at the start and end of the emersion periods, as shown by Serôdio et al. (1997) and Smith & Underwood (1998). In our study, this migration was also observed. During the daytime emersion period, the coloration of the sediment surface changed 30 min after the site became exposed and again 1 h before immersion. Remarkably, this was not reflected in the vertical profiles of chl *a*, which remained unchanged over the whole emersion period, indicating that the migration of diatoms at the time that the sediment changed colour was only within the top 0.2 mm (see also Wiltshire et al. 1998). Therefore, the differences in chl *a* distribution between the day- and nighttime emersion periods must have emerged during tidal immersion.

Guarini et al. (2000) reported that microphythobenthic biomass is subject to short-term cyclic variations. In their study, biomass production occurred in the light while losses were due to natural mortality, grazing and resuspension. Our results show that microphytobenthic biomass did not increase during the light period, suggesting that fixed carbon was predominantly excreted in the form of extracellular carbohydrates while little was used for balanced growth (see above). During tidal immersion, 23% of the microphytobenthic biomass was lost, probably because of resuspension and migration to below the 2 mm zone.

Summary

In this study, the dynamics of 2 extracellular carbohydrate fractions were examined. Biochemically, both fractions were of a similar composition. However, they behaved differently, and ecologically these fractions have different impacts. The colloidal carbohydrates represent a fraction that were highly dynamic. EPS produced during tidal emersion is rich in glucose (about 90%), disappears rapidly. EPS are turned over in the sediment (particularly the glucose portion) but may also be washed out of the sediment during tidal immersion. The EDTA-extractable fraction is enriched in uronic acids and is associated with sediment particles. This fraction behaved more conservatively. EPS-production was strongly light-dependent and associated with the presence of diatoms in the uppermost surface layer. During the nighttime emersion period no EPS production was observed and the diatom distribution did not vary over the depth investigated. Between 40 and 73% of the carbon fixed during the light period was converted to extracellular carbohydrates that are rapidly removed from the system and therefore are likely to represent an important labile organic carbon pool in intertidal sediments and the associated estuarine and coastal environments.

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