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Sorption of EPS to sediment particles and the effect on the rheology of sediment slurries

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Abstract. Extracellular Polymeric Substances (EPS) are considered to play an important role in the stabilization of intertidal mudflats. In this study the role of EPS as a binding agent in intertidal sediments was investigated. For this purpose two EPS fractions (termed coll-SF and EDTA-SF) were isolated from intertidal sediment and characterized in terms of monosaccharide- and size distribution. In slurry addition experiments the sorption characteristics of these EPS-fractions as well as their effect on sediment properties were examined under varying Ca^{2+}-concentrations. Results showed more EDTA-SF adsorbed to the sediment compared to coll-SF. For both fractions more EPS adsorbed to the sediment when Ca^{2+}-concentration increased. This effect was stronger for EDTA-SF. The differences in sorption between the two fractions could not be explained in terms of monosaccharide- and size distribution, which were largely similar. The addition of EPS in the presence or absence of Ca^{2+} did not alter the rheology of the sediment slurries indicating that there was no effect of EPS on the sediment properties. This contradicts results of experiments with bacterial EPS as well as field observations in which the presence of EPS/biofilms leads to an increase in the erosion resistance of the sediment. Possible causes for this discrepancy in results are discussed.

Introduction

Intertidal mudflats are highly dynamic areas and changes in the morphology are governed by complex interactions between biological and physical processes (Amos et al. 1998; Paterson and Black 1999; De Brouwer et al. 2000). The organisms that make up the biological component in these environments are often able to modify surface sediment properties, thereby changing the sediment stability. This biological mediation may result in destabilization of the sediment, for example due to bioturbation by macrofauna (Paterson and Black 1999). On the other hand, many organisms including macrofauna (Meadows et al. 1990; Mouritsen et al. 1998), bacteria (Dade et al. 1990) and benthic diatoms (Underwood and Paterson 1993; Sutherland et al. 1998) are able to stabilize the sediment. In general, stabilization of intertidal sediments has been related to the excretion of extracellular polymeric substances (EPS) by benthic organisms. The EPS may exert its stabilizing effect via grain to grain adhesion (Yallop et al. 1994; Paterson 1997), drag reduction

(Paterson and Black 1999; De Deckere et al. 2001) or the formation of networks of EPS reinforced tubes (Meadows et al. 1990). Dade et al. (1990) directly showed the effect of bacterial EPS as a binding agent by adding polymers isolated from the bacterium *Alteromonas atlantica*. In cohesive sediments, information on the role of EPS in sediment stabilisation comes solely from correlative studies in which benthic diatoms are considered to be the most important EPS producers (Kornman and de Deckere 1998; Tolhurst et al. 1999; Paterson et al. 2000). A predictive relationship was formulated by Amos et al. (1998) calculating the critical erosion threshold from bulk density and colloidal carbohydrates. Yallop et al. (2000) also proposed a predictive relation that explained sediment stability in terms of colloidal carbohydrate, chlorophyll *a* and water content. Although in these correlative studies EPS was considered to be important as a stabilizing agent, the effect of the EPS as such could not be established. Rather, the effect of the diatom biofilm as a whole is determined using these methods. In this study the role of EPS as a binding agent in intertidal mudflats was addressed.

For this purpose bulk quantities of two commonly extracted EPS-fractions (Underwood et al. 1995; De Winder et al. 1999; De Brouwer et al. 2000) were isolated from an intertidal mudflat and subsequently used in slurry addition experiments. These EPS fractions were used to study the sorption of EPS to sediments under varying Ca^{2+}-concentrations. Divalent cations like Ca^{2+} and Mg^{2+} play a role in the interactions between exopolymers and mineral surfaces and between the exopolymers themselves (Tipping 1981; Decho 1994). In colonies of *Phaeocystis* it was observed that these cations were essential for the gelling of the colony mucus while potassium showed no effect (Van Boekel 1992). The effect of EPS as an adhesive in intertidal sediments was examined using rheological methods. James et al. (1988) stated that knowledge of the rheological properties of cohesive sediments is important in relation to sediment transport processes. In addition, Ruddy et al. (1998) showed that changes in sediment structure (e.g. erosional events) at the sediment-water interface could accurately be followed using rheological methods. The dynamic viscosity, fluidity and rigidity of the sediment slurries were measured, providing data on the visco-elastic properties of the sediment slurries.

Methods

Isolation of EPS fractions

Carbohydrate material for the addition experiments was isolated from sediment collected from Banc de St-Vivien, an intertidal mudflat situated in the Gironde estuary, France. The surface layer of the sediment was sampled to a depth of approximately 2 mm and lyophilized prior to the extraction procedures. At the time of sampling extensive diatom biofilms were not visible and chlorophyll *a* concentrations varied between 10.8 and 39.0 $mg \cdot m^{-2}$. Production of extracellular carbohydrate occurred during emersion and contents increased on average with 27% during exposure of the mudflat. Although extracellular carbohydrates may originate from different sources (including diatoms, bacteria, meio- and macrofauna) it was assumed that the extracellular carbohydrates that were isolated mainly originated from benthic diatoms (see also Underwood and Smith (1998) and Blanchard et al. (2000)). It should however be noted that the fractions that were sampled may to some extend originate from other sources than benthic diatoms (see Yallop et al. (2000)). Two operationally defined carbohydrate fractions were isolated according to procedures described by De Brouwer et al. (2000). In short, the colloidal fraction was obtained by extracting the lyophilized sediment with distilled water (1 h, 30°C). Subsequently, the sediment was extracted with 0.1 M Na_2-EDTA (16 h, 20°C) yielding the EDTA-extractable fraction. Both fractions were filtered through a 0.2 μm filter to remove the remaining particulate matter. Subsequently, the fractions were filtered through a 1000 dalton filter (Amicon) using a tangential-flow filtration set-up (Amicon). During this procedure the sample volumes were reduced to 100 ml after which the samples were diafiltered with 1 l milli-Q water to remove inorganic salts and low molecular weight carbohydrates. Finally, the sample volumes were reduced to 50 ml and the concentrates lyophilized. The carbohydrates that were isolated using this procedure had a molecular size > 1000 dalton, which means that the smallest sugars consisted of 5.6 sugar monomers (glucose equivalents). Because these fractions were devoid of monosaccharides and small oligosaccharides that may pass cell membranes (< 600 dalton, Weiss et al. (1991)) they were designated EPS. The colloidal EPS that was isolated and purified in this way was termed coll-SF (colloidal Sugar Fraction), while EDTA-extractable EPS was termed EDTA-SF (EDTA-extractable Sugar Fraction). For both fractions the lyophilized isolates were pooled and dissolved in 500 ml milli-Q water.

Characterization of the EPS fractions

The EPS fractions were characterized for their size distributions by means of ultrafiltration using Centricon centrifuge tubes (Millipore) with molecular weight cut-off filters of 100, 50 and 10 kdalton (De Brouwer and Stal 2001). In all size fractions the monosaccharide distribution was analysed by HPLC-PAD. Prior to analysis the polymers were hydrolyzed and converted to their monosaccharides by means of acid methanolization followed by trifluoroacetic acid-hydrolysis (De Brouwer and Stal 2001).

Sorption experiments

Sediment collected from Banc de St-Vivien in the Gironde estuary, France was lyophilized and sieved over a 280 μm mesh sieve and the sediment fraction < 280 μm was used as the solid phase in the sorption experiments. No further manipulations were carried out in order not to disturb the sediment. For the sorption experiments, slurries were prepared with a solid to solution ratio of 333 $g{\cdot}l^{-1}$. This is equivalent to a water content of 75% which is within the range of what is commonly found in surface sediments in the presence of diatom biofilms (De Brouwer et al. 2000). The slurries were prepared using 4 different EPS contents (1313.6 and 2627.1 $\mu g \cdot g^{-1}$ coll-SF, 821.3 and 1642.5 $\mu g \cdot g^{-1}$ EDTA-SF) and a control without EPS added. For each EPS addition the Ca^{2+}-contents were varied between 0–300 $\mu mol \cdot g^{-1}$ by adding $CaCl_2$. The ionic strength was kept constant at a value of 0.3 $mol \cdot kg^{-1}$ by the addition of NaCl. For each treatment five replicates were taken. The slurries were shaken for 12 h at 20°C and subsequently frozen and lyophilized. The partitioning of EPS was determined by extracting the sediment as described above. The EPS that was recovered in this way after the incubations are termed colloidal EPS and EDTA-extractable EPS (note the difference in terminology with the EPS that was added at the start of the incubation experiment, which are called coll-SF and EDTA-SF). Carbohydrate contents in the colloidal and EDTA-extractable fractions were analyzed using the phenol-sulfuric acid method (Dubois et al. 1956). From these data the percentage of EPS in the colloidal, the EDTA-extractable fraction could be calculated. The difference between the amount of EPS (coll-SF or EDTA-SF) added at the start of the incubations and the amounts recovered in the colloidal and EDTA-extractable fractions after the incubations was operationally defined as the irreversibly adsorbed EPS fraction. The adsorption data were fit with a Freundlich constant partitioning model:

$$C_s = K_d C_1 \tag{1}$$

where C_s is the adsorbed carbohydrate content, C_1 is the dissolved carbohydrate concentration and K_d is the partition coefficient for the EPS. Two partition coefficients were calculated. One for the partitioning of EPS between the dissolved and the adsorbed phase ($K_{d\text{-coll}}$) in which the adsorbed phase is the sum of the EDTA-extractable and irreversibly bound EPS, and the other for the partitioning of adsorbed EPS between the EDTA-extractable and the irreversibly bound fractions ($K_{d\text{-EDTA}}$). K_d-values were determined using linear regression analysis.

Rheological experiments

The rheological properties of sediment slurries were measured using a Carrimed controlled stress rheometer equipped with a concentric cylinder geometry with a gap width of 2 mm. The properties of the sediment slurries were examined using two modes of operation. First, the apparent viscosity of the sediment slurries was measured as a function of decreasing shear strength. Secondly, the visco-elastic properties of the sediment slurries were measured using variable oscillation frequency with an amplitude of 4 milliradians. This mode of operation yields two independent rheological parameters: the storage modulus G' and the dynamic viscosity n'. G' characterizes the elastic while n' characterizes the viscous contribution to the visco-elastic properties of the sample. Tan Δ is an indicator of the structure of the material and is defined as:

$$\mathrm{Tan}\Delta = n'\omega / G'$$

where ω is the frequency (rad·s^{-1}). For a perfect elastic solid Tan Δ is 0 (Δ=0°) while for a Newtonian fluid tan Δ is infinite (Δ=90°).

The effect of EPS (coll-SF and EDTA-SF) on the structural characteristics of the sediment slurries was investigated using slurries with a sediment concentration of 700 g·l^{-1}. The sediment was diluted with milli-Q water, a solution of EPS and a solution of EPS with 143 μmol·g^{-1} $CaCl_2$. The contents of coll-SF and EDTA-SF were 834 μg·g^{-1} and 522 μg·g^{-1}, respectively. The slurries were incubated for 12 h. After measuring the rheological properties, the slurries were lyophilized, extracted and subsequently analyzed for carbohydrates in the colloidal and the EDTA-extractable fraction (n = 3).

Results

Characterization of the EPS-fractions

The coll-SF and EDTA-SF were analyzed for their size and monosaccharide distribution (Figure 1). Size fractionation experiments (Figure 1A) showed that in both fractions polysaccharides in the size class > 100 kD were most abundant and made up 73 and 62% of the total in the colloidal and EDTA-extractable fraction, respectively. In the size fractions of 1–10, 10–50 and 50–100 kD sugars were present in lower relative amounts and varied between 7 and 15%.

The monosaccharide distribution in the coll-SF was roughly constant when comparing the different size classes (Figure 1B). EDTA-SF was more variable among the different size classes (Figure 1C). In particular the 1–10 kD size class contained more uronic acids while arabinose was absent. The relative distribution of monosaccharides in the two EPS fractions was very similar. For the size classes > 10 kD (93% of the total EPS in both fractions) the difference in relative abundance of the various monosaccharides in the two EPS fractions were within 5% (except for galactose in the 10–50 kD size class, which differed 7%).

Sorption experiment

The sorptive behaviour of the coll-SF and EDTA-SF fractions was investigated under varying Ca^{2+}-concentrations. The sorption was linear over the range of EPS-contents that were investigated (Figure 2). Clear differences were observed between the sorption of coll-SF and EDTA-SF (Figure 2, Table 1). For all Ca^{2+} treatments, the major part of the coll-SF was recovered in the colloidal EPS-fraction (62.7–85.7%), while this was 24.7–48.6% for the EDTA-SF. In the EDTA-extractable fraction contents of coll-SF and EDTA-SF were comparable and varied between 13.5–35.8%. Little of the coll-SF material was irreversibly adsorbed onto the sediment (–6.2–8.0%). In contrast, the relative amount of irreversibly adsorbed EDTA-SF was substantial and varied between 31.8–49.7%. The addition of Ca^{2+} clearly affected the sorption of the EPS fractions. The addition of 300 μmol·g^{-1}

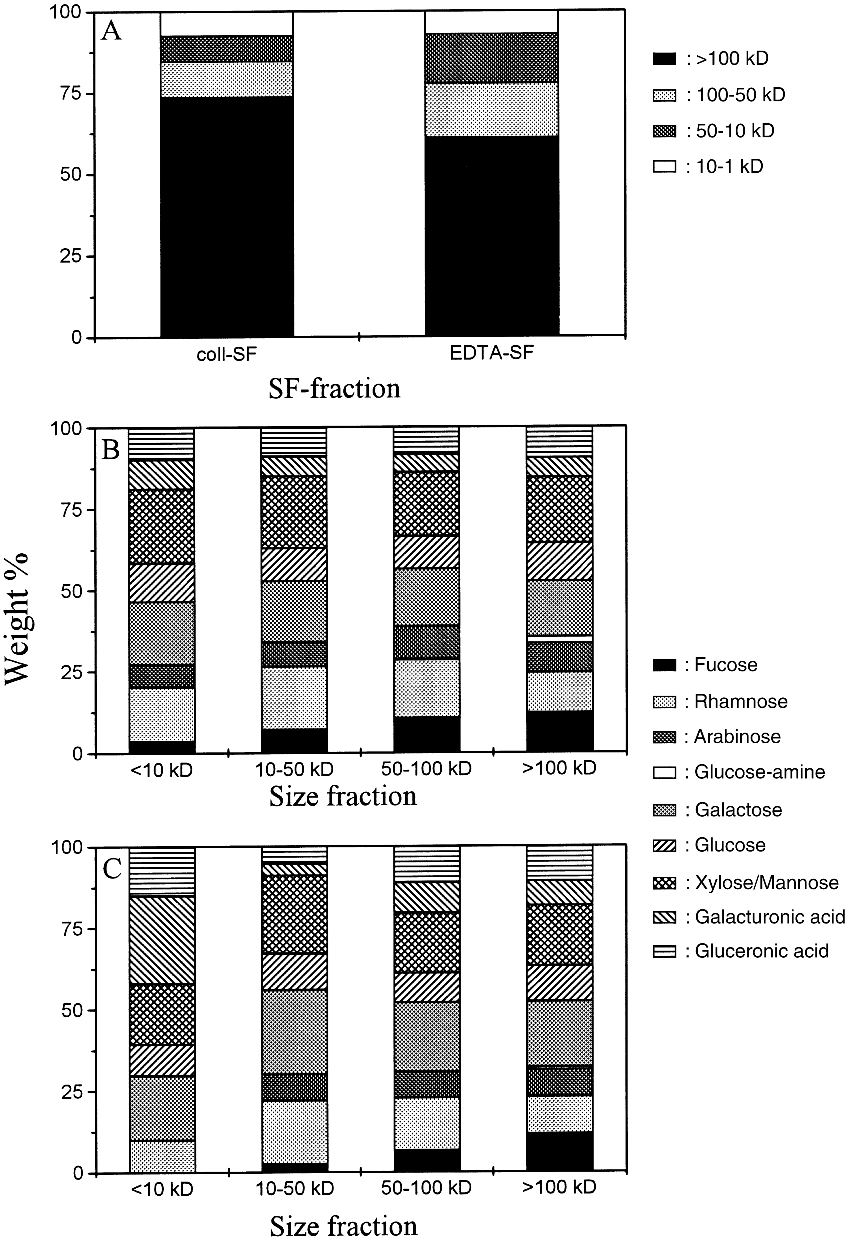

Figure 1. Size distribution of coll-SF and EDTA-SF (A), and monosaccharide distribution of coll-SF (B) and EDTA-SF (C).

Ca^{2+} resulted in increased sorption of the coll-SF and EDTA-SF with 15 and 24%, respectively (which was recovered either in the EDTA-extractable or irreveribly sorbed EPS-fraction). The $K_{d\text{-coll}}$-values varied between 5.8–8.3 $l \cdot kg^{-1}$ and 11.9–27,6 $l \cdot kg^{-1}$ for coll-SF and EDTA-SF, respectively (Table 2). The addition of Ca^{2+} resulted in an increase in $K_{d\text{-coll}}$, which was higher for EDTA-SF (131% increase at 300 $\mu mol \cdot g^{-1}$ Ca^{2+}) than for coll-SF (43% increase at 300 $\mu mol \cdot g^{-1}$ Ca^{2+}). The $K_{d\text{-EDTA}}$-values give an indication of the partitioning of adsorbed matter between the EDTA-extractable and the irreversibly sorbed fraction. The addition of Ca^{2+} resulted in a decrease in $K_{d\text{-EDTA}}$, showing that the carbohydrate fraction that adsorbed under conditions of increasing Ca^{2+}-concentrations was preferentially bound in the EDTA-extractable fraction.

Rheological experiments

Results of the rheological experiments are shown in Figure 3. Flow curves for the different slurries were identical and no effect of the addition of EPS (either coll-SF or EDTA-SF) in the presence or absence of Ca^{2+} was detected. Similar results were obtained for the oscillation experiments. Δ values for all treatments were close to 80 degrees. Therefore, the slurries behaved as viscous, inelastic fluids.

Carbohydrate contents for the additions of coll-SF and EDTA-SF are shown in Figure 4. Clear differences in sorption characteristics were observed between the addition of coll-SF and EDTA-SF. The major part of the coll-SF that was added was recovered in the colloidal fraction (71.4%, Table 3). The addition of Ca^{2+} resulted in extra sorption of this material. Compared to coll-SF, a substantially lower part of the EDTA-SF was recovered in the colloidal fraction (25.8%, Table 3). This colloidal material was bound to the sediment when Ca^{2+} was added (Table 3).

Discussion

In this study, distinct differences were observed in the sorption characteristics of two EPS-fractions that were isolated from an intertidal mudflat. The partition coefficients for colloidal EPS ($K_{d\text{-coll}}$) varied between 5.8–8.3 $l \cdot kg^{-1}$ for the coll-SF, while EDTA-SF adsorbed more strongly to sediment particles with $K_{d\text{-coll}}$-values being 2–3 times higher (11.9–27.6 $l \cdot kg^{-1}$). Arnarson and Keil (2000) studied the adsorption of easily extractable natural organic matter to montmorrillonite. Using slurries with a comparable particle concentration (280 $g \cdot l^{-1}$), these workers showed that the NOM-fractions measured possessed K_d's that varied between 1.6–2.7 $l \cdot kg^{-1}$. In our study, the $K_{d\text{-coll}}$-values for coll-SF were somewhat higher which was possibly because the dissolved phase that determines the K_d is defined differently between the two studies. $K_{d\text{-coll}}$-values for EDTA-SF were substantially higher compared to values found by Arnarson and Keil (2000) indicating a higher affinity of this organic matter fraction for sediment surfaces.

The difference in the sorptive behavior was also reflected in the response of the two EPS-fractions to varying Ca^{2+}-contents. A higher relative amount of the EDTA-SF adsorbed with increasing Ca^{2+}-content. Also, the adsorbed coll-SF was re-

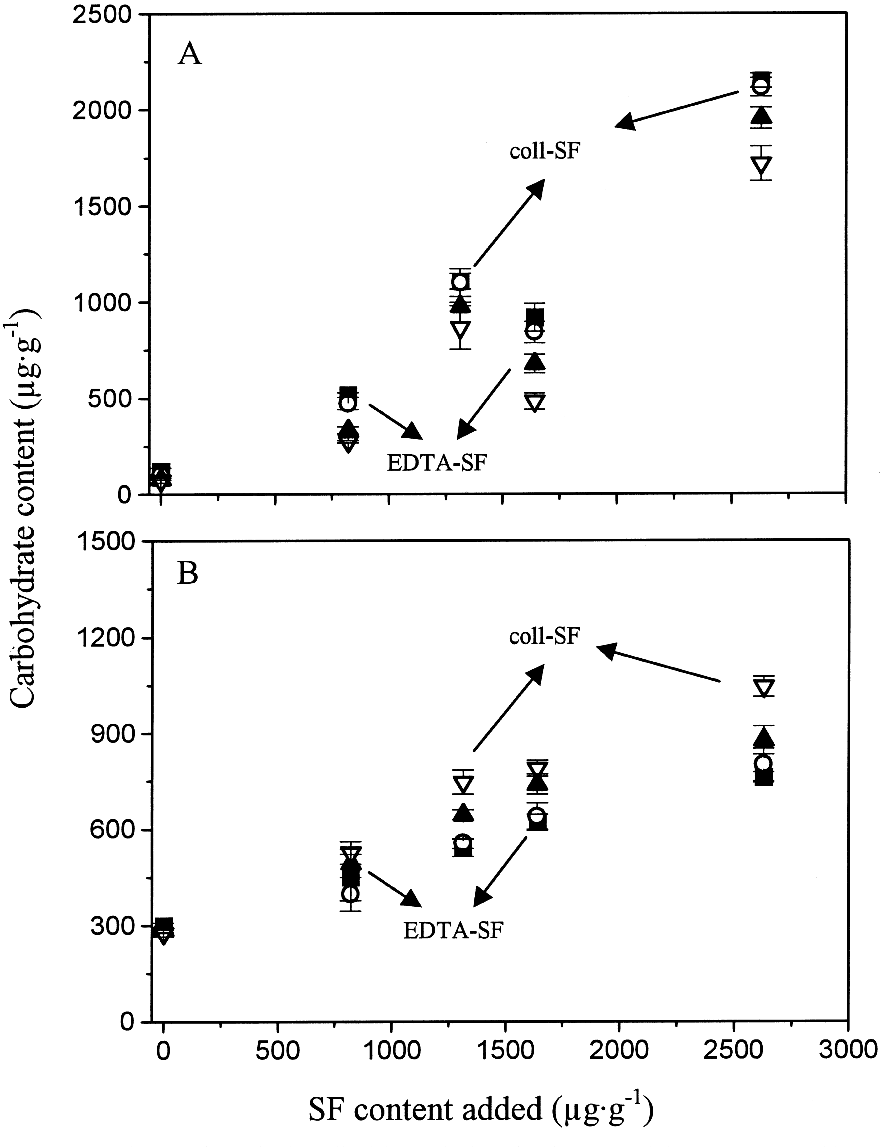

Figure 2. Partitioning of EPS in the colloidal carbohydrate fraction (A) and the EDTA-extractable carbohydrate fraction (B). Ca^{2+}-contents of 0 μmol·g^{-1} (■), 30 μmol·g^{-1} (○), 150 μmol·g^{-1} (▲), 300 μmol·g^{-1} (▽).

covered mainly from the EDTA-extractable carbohydrate fraction while the major part of adsorbed EDTA-SF was irreversibly bound to the sediment. Henrichs (1995) stated that biologically produced polymers might bind to sediments via site specific adsorption. Irreversible adsorption then occurs when the polymer is attached to the sediment at many sites per molecule (Podoll et al. 1987). Our results indicate that

Table 1. Partitioning of coll-SF and EDTA-SF over the colloidal, EDTA-extractable and irreversible fraction for the adsorption experiment. Values are given as the percentage of the total sugars added, corrected for blank adsorption. Values between brackets indicate standard deviation (n = 5).

Ca^{2+} (μmol·g^{-1})	Coll-SF added		EDTA-SF added	
	1313.6 μg·g^{-1}	2627.1 μg·g^{-1}	821.3 μg·g^{-1}	1642.5 μg·g^{-1}
Percentage of sugars in the colloidal fraction				
0	84.5 (4.6)	77.3 (1.5)	48.3 (3.3)	48.6 (4.4)
30	85.7 (5.9)	76.8 (1.8)	45.6 (3.7)	45.4 (3.4)
150	76.7 (1.9)	71.4 (2.1)	30.8 (1.9)	36.5 (2.8)
300	68.1 (10.5)	62.7 (3.1)	24.7 (1.1)	25.1 (3.2)
Percentage of sugars in the EDTA-extractable fraction				
0	18.3 (1.6)	17.5 (0.8)	18.2 (9.1)	19.6 (1.5)
30	20.4 (1.3)	19.5 (1.8)	13.5 (7.1)	21.4 (2.3)
150	27.3 (0.7)	22.5 (1.7)	25.2 (5.0)	27.6 (2.1)
300	35.8 (2.6)	29.3 (1.5)	30.4 (5.0)	31.4 (1.7)
Percentage of sugars irreversibly adsorbed to the sediment				
0	−2.8 (3.7)	5.2 (1.5)	33.5 (10.7)	31.8 (3.9)
30	−6.2 (6.5)	3.7 (1.2)	40.9 (9.8)	33.2 (3.8)
150	−4.0 (1.9)	6.1 (2.5)	44.0 (5.3)	35.9 (1.4)
300	−3.9 (11.5)	8.0 (2.5)	45.0 (5.6)	49.7 (13.3)

Table 2. Partition coefficients for coll-SF and EDTA-SF in the colloidal and EDTA-extractable carbohydrate fractions under different Ca^{2+}-concentrations.

Ca^{2+} (μmol·g^{-1})	$K_{d\text{-coll}}$ (l·kg^{-1})		$k_{d\text{-EDTA}}$ (l·kg^{-1})	
	coll-SF	EDTA-SF	coll-SF	EDTA-SF
0	5.8 (0.2)	11.9 (0.6)	54.3 (0.9)	59.7 (5.5)
30	5.9 (0.2)	13.2 (0.6)	48.1 (1.9)	55.2 (5.5)
150	6.6 (0.2)	17.5 (0.9)	42.7 (1.9)	48.5 (2.4)
300	8.3 (0.4)	27.6 (1.3)	35.2 (1.9)	47.3 (1.8)

Ca^{2+}-ions contributed to the sorption of EPS probably through the formation of cation bridges (Tipping 1981).

The clear differences in sorption between the coll-SF and the EDTA-SF suggest that these EPS-fractions are chemically different. Therefore, coll-SF and EDTA-SF were characterized in terms of monosaccharide composition and size distribution. As was shown in Figure 1, both EPS-fractions were similar. Therefore, the difference in sorptive behavior between the two fractions could not be explained in terms of these compositional parameters. Structures of microbially excreted polysaccharides are generally complex and their physical behavior depends on various properties of the molecule. For example, the arrangement of monosaccharides within a molecule, linkage types and interactions with inorganic molecules have an effect on the properties of the EPS (Decho 1994) and could influence the sorption to sedi-

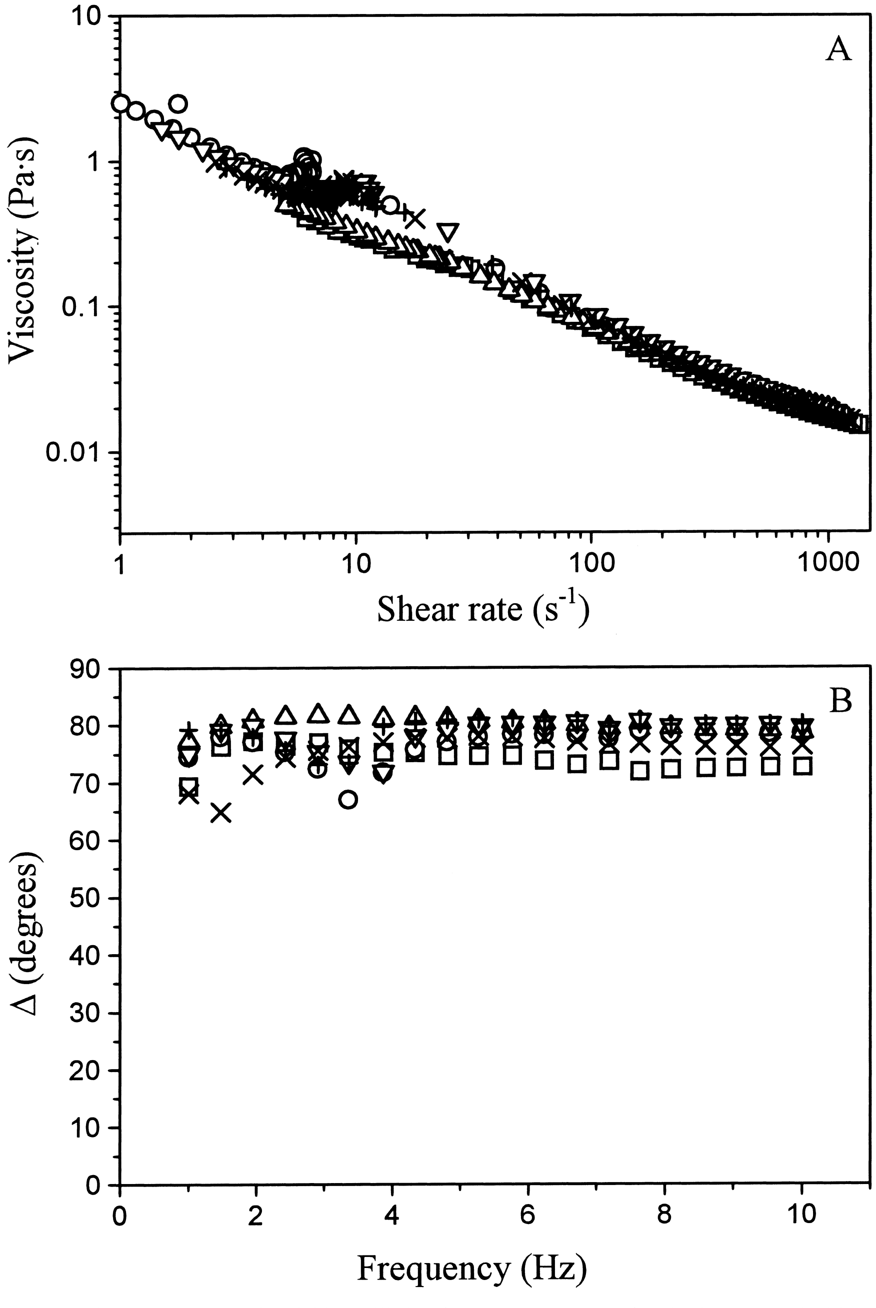

Figure 3. Flow curves (A) and variable oscillation frequency curves (B) for slurries prepared with mQ (□, ▽), coll-SF (○), coll-SF + Ca^{2+} (△), EDTA-SF (×), EDTA-SF + Ca^{2+} (+).

ment surfaces. Dade et al. (1990) reported that erosion resistance of sandy sedi-

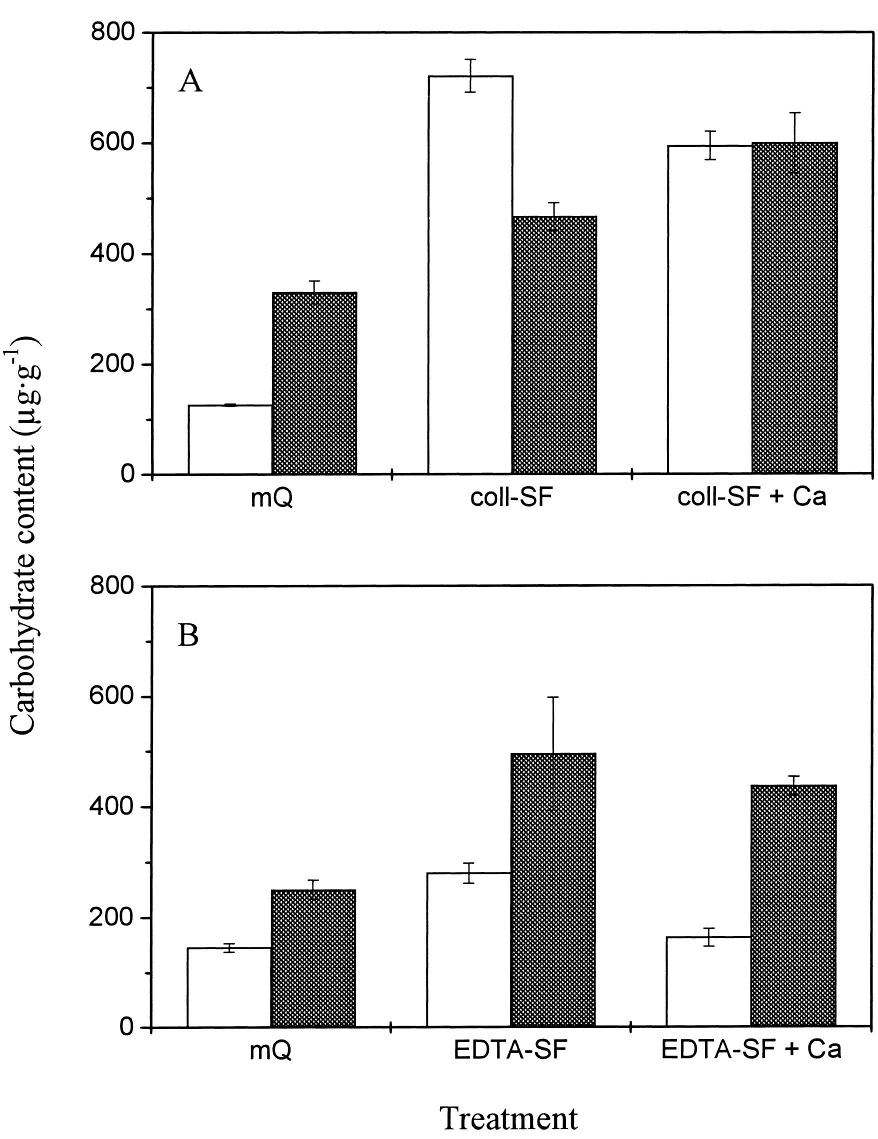

Figure 4. Carbohydrate contents in slurries used for the rheology experiments with coll-SF (A) and EDTA-SF (B). White bars: colloidal carbohydrates, grey bars: EDTA-extractable carbohydrates. Error bars indicate standard deviations (n = 3).

ments increased with increasing uronic acid content of the polymers. In our study, a relation between EPS-sorption and the uronic acid content was not found but other negatively charged moieties like sulfated sugars and ketal-linked pyruvate groups could also be involved in the process of cation bridging (Sutherland 1990). These compounds were not measured in this study. EPS excreted by benthic diatoms are

Table 3. Partitioning of coll-SF and EDTA-SF over the colloidal, EDTA-extractable and irreversibly adsorbed carbohydrate fraction for the rheological experiments. Values are given as the percentage of the total sugars added, corrected for blank adsorption. Values between brackets indicate standard deviation (n = 3).

Treatment	Colloidal fraction	EDTA-extractable fraction	Irreversibly adsorbed fraction
834 $\mu g \cdot g^{-1}$ coll-SF added			
Coll-NOM	71.4 (3.5)	16.4 (3.0)	12.2 (4.6)
Coll-NOM + Ca	56.3 (3.1)	32.3 (6.5)	11.4 (7.1)
521.9 $\mu g \cdot g^{-1}$ EDTA-SF added			
EDTA-NOM	25.8 (3.5)	35.6 (1.3)	40.2 (8.6)
EDTA-NOM + Ca	3.4 (3.1)	35.7 (3.3)	60.9 (2.2)

often considered to act as adhesives that bind sediment particles together in intertidal mudflats. In this way, sediment properties are modified resulting in an increased stability of the surface sediment layer. In our experiments, the addition of the EPS isolated from an intertidal mudflat did not alter the rheological properties of the sediment (Figure 4). Flow curves for slurries incubated with EPS in the presence or absence of Ca^{2+} were similar to the blank slurries, while oscillation experiments showed that little structure was present. This contradicts results from Dade et al. (1990) and Tolhurst et al. (1999) who found that the addition of EPS from the bacterium *Alteromonas atlantica* and of commercially available xanthan gum, respectively, resulted in an increased erosion resistance of the sediment. These contradicting results may reflect differences in properties of bacterial EPS compared to the EPS produced by benthic diatoms. Extensive literature exists on the physicochemical properties of bacterial EPS (Decho 1990; Sutherland 1990; Decho 2000) whereas little is known about the properties of diatom EPS. It has been suggested that mucilage produced by benthic diatoms either for the purpose of movement (Webster et al. 1985) or as a result of overflow metabolism (De Winder et al. 1999) easily dissolves in water. Its effect on sediment stability could be limited because this EPS would dissolve with every tide. On the other hand, Lewin (1956) showed that gel formation of EPS isolated from green algae occurred when divalent cations were added. Also, microscopic observations using low-temperature scanning electron microscopy (Yallop et al. 1994; Paterson 1995; Taylor et al. 1999) revealed a polymeric matrix between sediment grains in which EPS strands bound sediment particles together. In addition, the presence of EPS in amounts comparable to those used in this study, resulted in an increase in erosion threshold of natural intertidal sediments (De Brouwer et al. 2000). Therefore, another possibility that must be considered is that the extraction procedure used to isolate and purify EPS from the sediment, irreversibly changed the tertiary structure preventing it to form a gel-like state. However, Dade et al. (1990) used comparable extraction methods, including ultrafiltration and freeze drying, to isolate purified bacterial EPS. Their results showed a clear effect of the EPS on the properties of the sediment. Also, Moreno et al. (2000) used comparable methods to isolate EPS from the cyanobacterium *Ana-*

baena sp. Rheological measurements indicated that this EPS behaved as a weak gel.

Alternatively, it can be hypothesized that the action of the diatoms is necessary to structure the EPS in such a way that grain to grain adhesion is established. Decho (1994) emphasized that biofilms are heterogeneous, consisting typically of highly ordered and disordered regions. This suggests that the EPS matrix can be structured by the organisms themselves (see also Tolker-Nielsen and Molin (2000)). Wang et al. (1997) reported that the EPS produced by *Achnanthes longipes* allowed these organisms to move and to attach to surfaces. It was observed that this EPS was highly structured. Disruption of the synthesis of these polysaccharides resulted in the inability to attach to substrata. Also, the polymers played a critical role in the development of colony morphology. Edgar and Pickett-Heaps (1984) suggested that diatoms secrete strands of EPS that attach to a surface, thereby facilitating movement through the sediment. In this way diatoms may physically alter sediment properties by binding sediment particles together via a “structured” type of EPS.

In summary, clear differences in sorption were observed between the two types of EPS (coll-SF and EDTA-SF) that were extracted from an intertidal mudflat. For both fractions sorption increased with increasing Ca^{2+}-concentrations indicating the importance of cation bridging. Although the sorption of EPS shows the potential of EDTA-SF and to a lesser extend coll-SF to modify sediment properties in a way that can be observed in field situations, this was not observed by means of rheological measurements that were performed in this study. Therefore it is considered that other aspects than the presence of EPS itself (e.g. active structuring of the biofilm by the organisms present) are equally important when looking at mechanisms of biogenic stabilization in intertidal mudflats. This is currently under investigation.

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