

BIOFILM FORMATION BY BENTHIC DIATOMS AND THEIR INFLUENCE ON THE STABILIZATION OF INTERTIDAL MUDFLATS

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Introduction

Estuaries and other low-energy coastal environments are characterized by extended intertidal mudflats. These mudflats are composed of very small mineral clay and silt particles, which deposit when wave and current energies are low. However, storm events may cause erosion and transport of sediment. Depending on the local conditions, intertidal mudflats can cover large areas. For instance, the Brouage mudflat near Marennes-Oléron in France extends over 100 km² (GALOIS *et al.*, 1996). Mudflats have been regarded as wasteland but this is a misconception. They possess an enormous water-carrying capacity and therefore serve as natural coastal defence systems. Moreover, intertidal mudflats are extremely productive areas, which may provide up to 50% of the primary production of estuaries and thereby sustain large fish and shellfish stocks (UNDERWOOD & KROMKAMP, 1999).

The silt particles have charged surfaces and are coated with organic matter and nutrients (DECHO, 1994). Because of their charged nature, the particles adhere to each other (cohesive sediment). Usually, intertidal flats are mixtures of cohesive and non-cohesive sediments. Hence, depending on the composition, mudflat sediments can be classified as sandy mud or muddy sand.

The cohesive nature of the silt particles provides the mudflat with some stability. Consolidation of the sediment occurs for instance when the particles are packed more densely through drainage of water. However, probably equally important is the stabilization of the sediment through the action of microorganisms, notably the diatoms (PATERSON, 1989). Diatoms are microalgae, which form extensive biofilms or mats on the surface of intertidal mudflats. They exude large quantities of extracellular polymeric substances (EPS), which form the matrix in which the biofilm organisms are embedded. In this paper we will consider the processes that lead to the formation of diatom biofilms and the production of EPS and discuss their role in the stabilization of intertidal mudflats.

Composition of EPS

EPS is composed largely (95%) of polysaccharides, and the balance is made by (lipo)protein. The polysaccharides may be composed of neutral sugars, uronic acids, sulfonated sugars, or ketal-linked pyruvate groups (SUTHERLAND, 1999). EPS can be extracted from cultures or from natural communities of microphytobenthos. This is not trivial, and depending on the procedure used, different EPS is obtained. Moreover, it is important not to contaminate the material with structural cell material or intracellular storage polysaccharide. The procedures of extraction of EPS from cultures or sediments therefore yield what we call an 'operational fraction'.

We distinguish two types of EPS, although the procedures that are used for cultures differ from those used for sediments (DE BROUWER & STAL, 2002; DE BROUWER *et al.*, 2003). In liquid cultures benthic diatoms exude EPS which can be recovered from the medium. Another EPS is associated with the cells and can be obtained by extraction with warm (30°C) distilled water for 1 h. This method is applied to sediments, and analysis of the EPS showed that this material is largely identical for cultures and natural sediment samples. Sediment samples are subsequently extracted with 0.1 M EDTA for 16 h at 20°C. This fraction resembles the EPS that is found in the

medium of cultured diatoms. EDTA-extracted EPS contains a large quantity of uronic acids and other charged sugars. The negatively charged groups of the polymer interact with the sediment particles through bridging with divalent cations. In a liquid culture this EPS is soluble. The bound EPS of a culture of *Nitzschia* sp. was composed of more than 80% of glucose, with small quantities of rhamnose, galactose, xylose and mannose. It contained minor quantities of uronic acids. The soluble EPS contained only 11% glucose and much higher amounts of other neutral sugars and uronic acids (DE BROUWER & STAL, 2002). Atomic force microscopy has visualized and confirmed the presence of two forms of EPS in a marine benthic diatom (HIGGINS *et al.*, 2002).

Another point that deserves attention is the polymeric nature of the extracted material. The extraction procedure would yield any carbohydrate. In order to obtain highly polymeric carbohydrate, an ethanol precipitation step is usually included. DE BROUWER (2002) showed that this yields EPS with a molecular weight of >100 kD, which was less than 20% of the total extracted carbohydrate. However, 50% of the extracted carbohydrate had a molecular weight of >10 kD and is therefore polymeric. Except for the smallest size, the composition of all molecular weight fractions of EPS was identical. Hence, it seems that this EPS is produced in a continuum of molecular weights. These probably represent different points of synthesis and/or degradation of the polymer. Experiments that take only into account the ethanol-precipitated material may therefore underestimate the amount of EPS.

Controls of EPS production

Benthic diatoms are motile, and it is known that motility is associated with the exudation of EPS through the raphe, a longitudinal slit of the diatom siliciferous frustule (LIND *et al.*, 1997). Epipellic diatoms migrate to the sediment surface when it is exposed at low tide during the day. Motility is controlled by both photo- and chemotactic responses. Since epipellic diatoms also migrate in the dark, this EPS is produced independently of photosynthesis, probably at the expense of internal storage carbohydrate. Although dark EPS production has been observed (SMITH & UNDERWOOD, 2000), it seems that the production of the operational fraction of 'cell-associated EPS' as described in the preceding paragraph is under direct control of photosynthesis and CO₂ fixation (STAATS *et al.*, 2000). Moreover, the exudation of EPS is probably the result of imbalanced growth. Imbalanced growth occurs in a phototroph when the availability of light and CO₂ exceeds that of essential nutrients, which are necessary for the synthesis of structural cell material, notably protein. Hence, when nitrogen is limiting, excess fixed CO₂ is diverted to the non-nitrogenous polysaccharide, rather than to protein (STAATS *et al.*, 2000). Presumably, the capacity to store carbohydrate intracellularly is limited and excess is exuded. However, the exuded material is not the same as the intracellular storage carbohydrate chrysolaminaran, and therefore different metabolic processes underlie its synthesis. Also, the production of cell-associated EPS was most pronounced during the exponential growth phase of *Nitzschia* sp., whereas stationary cultures accumulated more intracellular carbohydrate (DE BROUWER & STAL, 2002). This hints at different factors that control the synthesis of intra- and extracellular carbohydrate.

Measurements of CO₂ fixation and the fate of this carbon in a natural microphytobenthos community and a culture of a benthic diatom revealed that respectively 64 and 81% of the fixed carbon was diverted into extracellular carbohydrate (WOLFSTEIN *et al.* 2002). In both cases the cell-associated (glucose-rich) material was quantitatively the most important fraction, and this showed also a strong light-response.

Following the production of this cell-associated (water-extractable) EPS during a tidal emersion period, it was observed that a huge amount of carbohydrate was produced (DE WINDER *et al.*, 1999). The 7 h emersion period was during daytime and lasted from 14:00 to 21:00 (this was in late spring, and daylight was present during this whole period). EPS was extracted from 1 mm slices of the top 10 mm of the sediment. The depth-integrated amount of EPS increased dramatically during this period. Most of this increase was confined to the illuminated part of the sediment. This increase clearly resulted from synthesis and could not be attributed to diatoms migrating to the surface. The strong increase at the surface was the combination of migrating diatoms and light.

Since this production occurred during emersion when EPS increased from low to high content, subsequent immersion led to its loss. This is obvious considering the fact that this EPS is water extractable. It was indeed encountered in seawater that flooded an intertidal mudflat. This represents one way of transport of organic carbon from microphytobenthos to consumers (e. g. filter-feeding shellfish).

In another experiment it was shown that only the water-extractable fraction of EPS was produced during daylight emersion (DE BROUWER & STAL, 2001). EDTA-extractable EPS increased only marginally. In this respect it is noteworthy that water-extractable EPS can also be degraded during the dark by the diatoms themselves. Besides the dissolution during immersion, this could be a second reason why the EPS disappears.

The apparent mobilization of EPS by epipellic diatoms in the dark was demonstrated in pure cultures. Hence, it was not caused by heterotrophic bacteria. Pure cultures that are grown under an alternating light-dark cycle showed a cyclic pattern of production and degradation of 'bound' EPS. It was also demonstrated that only the glucose of this glucose-rich EPS was used (DE BROUWER & STAL, 2002). Hence, at the end of the dark period, this 'cell-associated' EPS contained a considerably lower amount of glucose and was enriched in the other sugar monomers. In fact, we conceive that the degradation of the cell-associated EPS leads to its conversion into the other type of EPS.

EPS in mudflats

On the basis of measurements of chlorophyll *a* and water-extractable EPS from several intertidal mudflats, UNDERWOOD & SMITH (1998) proposed a functional relationship between these two parameters. However, there are arguments against it since this EPS shows a highly dynamic pattern over diurnal and tidal cycles. BLANCHARD *et al.* (2000) performed an extensive study of the relationship between chlorophyll *a* and water-extractable EPS on the Skeffling mudflat in the Humber estuary in the UK. These authors sampled a transect, which covered a number of geomorphological features. On the high shore the sediment consisted of fluid mud (low energy), whereas at the low shore the sediment contained more sand (high energy). The middle part of the intertidal flat was characterized by a ridge and runnel system, which was formed by tidal water currents. When all data were pooled, a significant relationship was found between chlorophyll and EPS. However, differences were observed for the different datasets. For instance, no relationship was observed for samples taken at 5-10 mm depth, whereas the top 5 mm of the sediment gave a significant relationship. In fact, detailed analysis of the depth distribution of EPS in muddy sediments emphasized that the highest content is at the surface, quickly decreasing with depth (TAYLOR & PATERSON, 1998; DE BROUWER & STAL 2001). These analyses have used the cryolander technique, a method that allows instantaneous *in situ* freezing of the sediment, without disturbing its structure (WILTSHIRE *et al.*, 1997). Subsequently, frozen sediment cores can be cross-sectioned using a freezing microtome, with

which easily sections of 100 µm can be obtained and analysed.

Also, no relationship between chlorophyll and EPS was observed in the sandy sediment. In the ridge and runnel system of the intertidal mudflat a significant relationship was only found on the ridges that are exposed during low tide. Water is always standing and flowing in the runnels, and this does not allow EPS to accumulate.

BLANCHARD *et al.* (2000) did not measure EDTA-extractable EPS from this mudflat. This type of EPS does not show the highly dynamic pattern of water-extractable carbohydrate. Firstly, it binds tightly to the sediment by their negatively charged groups and is therefore not washed out during inundation. Secondly, this EPS is not or only slightly subjected to degradation by the diatoms in the dark. Hence, whereas water-extractable EPS may show a strong correlation with diatom biomass (chlorophyll *a*), EDTA-extractable EPS does not. This EPS tends to accumulate in the sediment and its ratio to chlorophyll *a* increases with depth.

The firm correlation of EPS and chlorophyll on the ridges may offer an explanation for these geomorphological structures. Since EPS can bind depositing silt particles, ridges will tend to maintain themselves and may even increase in size, whereas the opposite occurs in the runnels, which are exposed to erosion stress. STAATS *et al.* (2001) observed that diatom biofilms developed only on a relatively narrow band along the tidal channel of the Ems-Dollard estuary. They found that the sediment grain size in that area was smaller than in the rest of the mudflat. These are not isolated observations valid for specific cases. DE BROUWER *et al.* (2003) showed a clear inverse correlation between chlorophyll, EPS and grain size of three European mudflats.

Merging all data from the mudflats of Marennes-Oléron (France), the Humber (UK), Eden (Scotland) and Ems-Dollard (Netherlands), a correlation is seen between the amount of water- and EDTA-extractable carbohydrate (DE BROUWER *et al.*, 2003). However, differences are clearly seen when these mudflats are considered individually. For instance, the Skeffling mudflat in the Humber estuary contained much higher amounts of EDTA- relative to water-extractable EPS than the other sites. It was noted that these sediments were also much stickier and had higher erosion thresholds.

The correlation between sediment grain size and the development of diatom biofilms may be explained in either way. It is likely that diatoms can only develop a biofilm in an area that is characterized by low energy. On the other hand, once a diatom film develops, the exudation of EPS will bind newly depositing silt and increase the erosion threshold. VAN DE KOPPEL *et al.* (2001) presented an elegant theoretical basis for the occurrence of stable states in diatom-sediment interactions. Their model showed that there are two stable states: (1) sediments with a high content of silt and a diatom biofilm and (2) sediments with low silt content and no biofilm. The model assumes that the diatoms grow better on sediments with high silt content and moderate shear stress, and this was also experimentally proven. The better growth on muddy sediments is probably due to the higher nutrient content. Subsequently, this will lead to a cascade of events. The exudation of EPS increases the mud content, its cohesiveness, erosion threshold and, consequently, stimulates the growth of diatoms. Eventually this leads to a stable state with a diatom biofilm. When an erosion event causes a decrease in silt content, it will trigger a cascade of processes eventually leading to sediment with low cohesiveness and low silt content and with no diatom biofilm. This situation will therefore also be stable.

Biostabilization of mudflats

Several publications have emphasized the importance of diatom biofilms on the stability of intertidal mudflats (GRANT *et*

al., 1986; PATERSON, 1989; PATERSON & BLACK, 1999; SUTHERLAND *et al.*, 1998; YALLOP *et al.*, 1994, 2000). It is generally assumed that not the diatoms themselves stabilize the sediment but that the copious amounts of EPS increase the erosion threshold. However, if this is true, this would be effective only for a very short period of the year. In most European mudflats diatom biofilms are particularly important during spring. Grazing by the amphipod *Corophium volutator* can remove the biofilm rapidly.

Although seasonal measurements of the erosion threshold seem to follow the other biological parameters, critical correlation analyses of measurements of chlorophyll *a*, water- and EDTA-extractable EPS, median grain size, bed level height and erosion threshold showed that none of these parameters were significantly correlated with the sediment stability of a mudflat in the Westerscheldt estuary (DE BROUWER *et al.*, 2000). EDTA-extractable EPS correlated strongly with median grain size, giving support to the hypothesis that this material binds strongly to silt and clay particles. If any EPS contributes to sediment stability, EDTA-extractable carbohydrate is probably the best candidate. DE BROUWER *et al.* (2002) showed that this EPS when added to sediment bound much stronger than water-extractable EPS. They also noted that part of the EPS became irreversibly bound to the sediment, i. e. could not be re-extracted by 0.1 M EDTA. The amount that became irreversibly bound to the sediment increased to 50% of the added EPS in the presence of Ca^{2+} . This hints to the involvement of cation bridges in the binding of EPS to the sediment. The strength of the binding depends on the number of binding sites on the EPS molecule and the availability of Ca^{2+} and other divalent ions (Mg^{2+}) to form cation bridges. In spite of this binding, the rheological properties of the sediment did not change as a result of these experiments. A higher viscosity and elasticity of the sediment would be expected when the sediment would have gained more stability. It is of course possible that EPS loses its structure upon extraction and with that part of its properties. Other experiments have shown that EDTA-extractable EPS can increase viscosity and elasticity, but that it depends strongly on the salt concentration (unpublished observations).

In conclusion, although diatom biofilms may locally contribute to the stability of intertidal mudflats, it is uncertain whether they influence the geomorphology of these environments over larger spatial and temporal scales. The majority of the investigations have focussed on water-extractable EPS (colloidal carbohydrate) but this fraction has probably only an indirect effect as the precursor of EDTA-extractable EPS. The latter may accumulate in the sediment and is probably recalcitrant to decomposition. There are some indications that this material may increase the erosion threshold of muddy sediments, even when diatom biofilms are absent.

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