Exopolysaccharide production by marine benthic diatoms
Staats, N.

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CHAPTER 7

General discussion

In this chapter, the results presented in the preceding chapters are discussed in an attempt to answer the two main questions of this thesis as formulated in chapter 1. These questions were:

1. Does the formation of an exopolysaccharide matrix by epipelic diatoms on the sediment surface of intertidal mudflats influence estuarine sediment transport?
2. What are the steering factors for exopolysaccharide production by epipelic diatoms?

The first question may be answered using the data from the field surveys presented in chapters 2 and 3. The monoculture and field experiments from chapters 4-6 are used to answer the second question. Furthermore, the validity of extrapolating results from laboratory culture studies to the field is discussed.

Biostabilization

At high diatom densities, the mud content of the sediment was higher than when diatoms were absent (i.e.: at the mudflat margin in early summer, chapters 2 and 3). From this it was concluded that the presence of the diatom-exopolysaccharide biofilm resulted in the fixation of sediment which would otherwise have been eroded. Removal of a dense diatom biofilm enhances erosion (Underwood & Paterson, 1993b). The stabilizing effect is related not only to the amount of exopolysaccharide but also to the density of charged substituents on the polysaccharide, such as carboxyl and sulphate groups (Sutherland et al., 1998). These substituents were shown to be present on exopolysaccharides produced by two diatom species isolated from mudflats (chapter 4).

Epipelic diatoms generally grow on sediment with a high mud content (Colijn & Dijkema, 1981; Sundbäck, 1984; De Jong & De Jonge, 1995), and these sediments are found only in areas where erosion rates are low (Postma, 1957). Hence, epipelic diatoms are mainly present in depositional areas, where biostabilization will not alter sedimentation/erosion rates to a very large extent. Moreover, the fact that epipelic diatoms were present in high densities only in early summer and on a relatively small part of the intertidal flat obviously restricts the impact of biostabilization on overall sediment transport. Substantial erosion events take place
in autumn, when diatoms are absent (Coles, 1977; Frostick & McCave, 1979; Anderson, 1983; Brown, 1998). Therefore, the conclusion is that the influence of biostabilization by epipelic diatoms on sediment sorting and on sediment transport between tidal flat and channel on a yearly basis is probably small, compared to hydrodynamic factors.

**Steering factors**

In the context of the BOA research theme on tidal areas, knowledge was required on the conditions under which secretion of polysaccharides by benthic diatoms takes place. This could not be achieved without addressing the function of polysaccharide secretion. Therefore, a discussion of the possible steering factors for secretion will also touch upon possible functions of exopolysaccharide production.

**Motility**

As stated in chapter 1, it is clear from literature that the role of exopolysaccharides in motility is to attach the cell to a solid substratum (Edgar & Pickett-Heaps, 1984; Wetherbee et al., 1998). However, it is unlikely that the large quantities of exopolysaccharide that were found attached to the cell (chapter 4) were secreted for motility only. Amounts of exopolysaccharide are generally much higher than the amount that would, theoretically, be required for motility (Edgar & Pickett-Heaps, 1984). Some species produce copious amounts of polysaccharide, but can move with only one strand of exopolysaccharide actually attaching the cell to a substratum (Cohn & Weitzell, 1991). Furthermore, exopolysaccharides are not only secreted through the raphe but also through other openings (e.g. pores) in the silicate frustule (Rosowski et al., 1983). These observations suggest that not all polysaccharides are secreted in order to facilitate locomotion, and that other factors may control exopolysaccharide production.

Smith & Underwood (1998) concluded from monoculture and field experiments that exopolysaccharide production is closely coupled to migratory rhythms. However, their results do not agree with reports showing that epipelic diatoms continue their rhythmic migration for several days when incubated in the dark (Hopkins, 1966; Serodio et al., 1997). It seems unlikely that diatoms are able to produce such large quantities of exopolysaccharide in the dark for several days. Moreover, it has been reported that the inhibitor of photosystem II, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) did not affect motility in the diatom *Amphora coffeaeformis* (Cooksey & Cooksey, 1980) whereas our data showed that this herbicide inhibited polysaccharide secretion (chapter 6). These different
responses of motility and polysaccharide secretion to inhibition of photosynthesis (either by darkness or DCMU) strongly suggest that most of the material secreted is not associated with motility.

Other steering factors: overflow metabolism
Several observations indicated that secretion was dependent on light and nutrient conditions. Accumulation of exopolysaccharides was dependent on growth phase: the highest accumulation rates were found during early stationary phase, which was induced by nitrogen depletion (chapters 4 and 5). Furthermore, secretion of exopolysaccharides occurred only in the light, without inhibition of photosynthesis. These results strongly suggested that exopolysaccharides were produced as a result of overflow metabolism. This is a well known phenomenon in planktonic diatoms (Myklestad et al., 1989; Wake et al., 1995). An excess amount of carbon dioxide is fixed relative to growth requirements, and the resulting carbohydrates are accumulated in the cell or secreted. Overflow metabolism resulting in secretion of polysaccharides was observed as a response to an increase in light intensity (chapter 6) or caused by a decrease in growth rate (chapter 4). In these experiments, the amount of time needed to attain balanced growth was different. When cells were transferred from dark to light, there was initially a rapid secretion of polysaccharides, which decreased however within a few hours (chapter 6). A decrease of growth rate from linear to stationary growth resulted in the accumulation of exopolysaccharides that lasted for a number of days (chapter 4).

Secretion versus intracellular storage: possible functions of secretion
The different experiments described in chapters 3-5 did not reveal any general pattern with respect to the amount of secretion versus intracellular storage of carbohydrate. For instance, in the field experiments described in chapter 6 intracellular carbohydrates as well as exopolysaccharides accumulated during emersion. In cells grown with ammonium as nitrogen source excess carbohydrates were also stored intracellularly and secreted in equal amounts (chapter 5). However, in other culture experiments in which excess carbohydrates were produced these were exclusively secreted and not stored in the cell (chapter 6). The fact that in some cases all excess carbohydrates were secreted suggested that there are additional benefits to secretion of polysaccharides apart from serving as a sink for excess carbon. For instance, secretion of polysaccharides may influence nutrient conditions. Several mechanisms are conceivable through which the exopolysaccharide matrix could bring about a change in nutrient conditions: 1) As
pointed out earlier, a diatom-exopolysaccharide biofilm could substantially increase the mud content of the sediment surface. Because the organic carbon content of the mud is high, this will also increase the concentration of inorganic nutrients (Ruddy et al., 1998b), 2) the exopolysaccharide matrix may bind inorganic nutrients (Admiraal & Werner, 1983; Decho, 1990), 3) at high diatom densities, high oxygen production rates may result in the formation of oxygen bubbles, which create lift forces that induce large parts of the diatom/exopolysaccharide biofilm to erode and be suspended in the water column (Blanchard et al., 1997; Sutherland et al., 1998). This may be a strategy for dispersion or relocation to environments with more favourable nutrient conditions.

Another important function of the exopolysaccharide matrix could be influencing the state of hydration of the sediment surface. Sediment surfaces densely colonized by diatoms generally appear very hydrated, whereas sediments not colonized by diatoms clearly dehydrate during emersion. Epilithic diatoms produce more exopolysaccharides when subjected to desiccation (Peterson, 1987). Protection against dehydration is particularly important for photosynthesis: cells in desiccated or flooded sediment exhibit lower photosynthesis rates than cells in moist (but unflooded) sediment (Holmes & Mahall, 1982).

A comparison of monoculture and field results

The experiments performed with monoalgal cultures indicated that overflow metabolism may be a steering factor for secretion. The question remained to what extent this was true also for secretion by natural populations.

Overflow metabolism in intertidal sediments

In culture, water extractable polysaccharides were produced as a result of overflow metabolism. The question forces itself whether in situ this fraction also accumulated as a result of overflow metabolism. For instance, is nutrient limited growth likely to occur in intertidal sediments? In muddy sediment, concentrations of inorganic nutrients are generally quite high (Admiraal, 1984), suggesting that nutrient limited growth is unlikely. However, pore water concentrations of inorganic nutrients do not necessarily reflect the amount of nutrients available to the diatoms. When biomass is very high, it seems possible that nutrient depletion occurs every now and then. Ruddy et al. (1998a) calculated that especially nitrogen may regularly limit growth of diatoms on intertidal mudflats. In addition to nitrogen, also phosphorous limitation has been shown to occur in intertidal sediments (Pinckney et al., 1995). Moreover, exopolysaccharide accumulation could also be stimulated by
low ambient concentrations of nitrogen or phosphorous that did not limit growth (chapter 5). Hence, low concentrations of nitrogen or phosphorous in intertidal sediments may have stimulated exopolysaccharide production. Obviously, photosynthetic carbon fixation must take place in order for overflow metabolism to occur. Carbon dioxide concentrations probably are not limiting photosynthesis in intertidal sediments (Kromkamp et al., 1998). Also, incident irradiance at the sediment surface must be sufficiently high. During the field experiments reported in chapter 6, incident irradiance at the sediment surface ranged between 1500-2000 μmol photons m$^{-2}$ s$^{-1}$ during the daytime emersion period. Values for $I_{\text{max}}$ (optimal irradiance for photosynthesis) for microphytobenthos were reported by Blanchard & Montagna (1992) and ranged from 380 to 900 μmol photons m$^{-2}$ s$^{-1}$. Therefore, in this particular field experiment (chapter 6) irradiance was probably saturating for photosynthesis, hence overflow metabolism may well have been a steering factor for the observed accumulation of exopolysaccharides.

**Turnover of exopolysaccharides**

In chapter 2, it was shown that the ratio carbohydrate/chlorophyll $a$ was more or less constant throughout the year except for April, when the amount of carbohydrate produced per chlorophyll $a$ increased dramatically. In agreement with the previous conclusions, this could be explained by overflow metabolism occurring during this period. This accumulation of carbohydrates over a longer period of time implied that the water extractable carbohydrate fraction, at least to a very large extent, did not wash out during immersion. At the end of the clear water phase, carbohydrate content rapidly decreased. Probably, this material was rapidly degraded due to heterotrophic activity, which confirmed conclusions drawn in chapter 6, that activity of heterotrophs contributed more to degradation of polysaccharides than wash-out by the tide. This is in agreement with observations by Grant et al. (1986), who found that upon tidal immersion the exopolysaccharide matrix remains more or less intact. Furthermore, Van Duyl et al. (subm.) observed considerable bacterial degradation of polysaccharides in intertidal sediments. In addition to bacterial degradation, it is possible that also diatoms hydrolyze exopolysaccharides and use it as energy and/or carbon source in the dark.

**Chemoheterotrophic uptake of exopolysaccharides**

Benthic diatoms are known to be able to grow heterotrophically, and use a wide variety of sugars as carbon source (Hellebust & Lewin, 1977; Tuchman, 1996). Considering the light climate that benthic diatoms are subjected to (i.e. frequent though irregular exposure to darkness), the ability to grow chemoheterotrophically
may be essential for survival. In chapter 2 it was concluded that photoperiod length was not a factor restricting diatom growth: dense populations were observed in areas where, due to long periods of tidal immersion, photoperiods were short. The ability to grow chemoheterotrophically possibly enables benthic diatoms to grow up to high densities in these conditions. Furthermore, chemoheterotrophic uptake of organic carbon may be related to motility. Cooksey & Cooksey (1980) observed that *Amphora coffeaeformis* cannot migrate in the dark without addition of glucose. This would explain the fact that diatoms are able to continue their regular vertical migration for days even when incubated in the dark (Serodio *et al.*, 1997). Organic carbon may serve as the source for the exopolysaccharides that would be required for this behaviour.

As yet, there is no clear evidence that benthic diatoms are also capable of using their own secretion product as carbon and energy source. However, results presented in chapter 6 supported this hypothesis. The polysaccharides secreted by two isolated species were quite heterogeneous with respect to monosaccharide composition (chapter 4). Do benthic diatoms secrete hydrolytic enzymes that degrade this material? An interesting line of research would be to look for these enzymes, and establish their activity *in situ*.