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# **Structure and dynamics of exopolymers in an intertidal diatom biofilm**

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Running head : Exopolymer in a diatom biofilm

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## **Abstract**

Diatom biofilms growing at the surface of the intertidal mudflat of Marennes Oléron, France, were incubated for 48 h in the laboratory under simulated conditions of high- and low tide (immersed and emersed in seawater) and day and night (illuminated or dark conditions). The biofilms were subsequently sampled using the cryolander technique, without disturbing the structure. The samples were kept in liquid nitrogen until they were transferred to the cooled stage of a field-emission cryo-scanning electron microscope, which was used to study the structural relationships between the sediment particles, the diatoms and the different types of extracellular polymeric substances (EPS) produced by these organisms. The diatoms were most abundant at the sediment surface when incubated in the light under emersed conditions. In the dark or when immersed, the diatoms migrated into the sediment. In the light, the diatoms were coated with EPS, while this was not the case when incubated in the dark. When immersed, the sediment surface appeared smooth as the result of the deposition of mud. Under emersed conditions, the coarser silt grains were prominently present. These grains were wrapped with organic matter and bound together through threads of EPS. This was the case both in light and in dark incubated sediment. It is proposed that this latter type of EPS contributes to the increased erosion threshold of intertidal mudflats colonized by biofilms of diatoms.

**Keywords:** epipellic diatoms, EPS, extracellular polymeric substances, field emission cryo-scanning electron microscopy, intertidal mudflats, microphytobenthos

## Introduction

Intertidal mudflats often harbor extensive communities of benthic diatoms, which are responsible for a major part of the primary production in such environments (Underwood and Kromkamp 1999). Moreover, they have been considered to be important for the morphodynamics of mudflats because they may increase the erosion threshold (Paterson 1989). Benthic diatoms are known to produce copious amounts of extracellular polymeric substances (EPS) that are composed largely of polysaccharides (Bellinger et al. 2005; Hoagland et al. 1993; Underwood and Paterson 2003). Measurements on cultures and in natural communities have shown that 40-80% of the CO<sub>2</sub> fixed during photosynthesis is secreted into the environment (Goto et al. 1999; Middelburg et al. 2000; Wolfstein et al. 2002), where it may become available as food source for other organisms. EPS can be complex and composed of carbohydrates containing various negatively charged groups such as uronic acids, sulfated sugars and ketal-linked pyruvate groups (Sutherland 1999). It has been proposed that this acidic EPS may bind to the charged silt particles and glue them together, producing more stable sediment (Decho 1994; Sutherland 2001). In situ experiments by Vos et al. (1988) demonstrated that stabilization of intertidal sandy sediments persisted after the diatoms were killed, apparently through the sole effect of preserved organic coating of the grains. Although it has been demonstrated that the addition of EPS of the marine bacterium *Alteromonas atlantica* or commercially available xanthan gum resulted in an increased stability of mud sediment (Dade et al. 1990), tests with isolated EPS from benthic diatoms and from intertidal mudflats did not result in a significantly change of the rheological properties of the mud (de Brouwer et al. 2002a). It is therefore believed that the combined action of the diatoms and the EPS, i.e. the biofilm itself, would result in the observed increase of the critical erosion threshold of intertidal mudflats.

Diatoms growing on intertidal mudflats are of the epipellic ('mud dwelling') type. These are relatively large, motile species. Epipellic diatoms migrate in the sediment according to tidal and diurnal cycles (Serôdio et al. 1997). At low tide during the day the diatoms migrate to the sediment surface where they form a thin film of one to a few individuals. This is the period when they photosynthesize. When the tide is coming in, the diatoms migrate into the sediment or they may also become partly suspended in the water column. Although migration occurs mostly in the top few millimeters of the sediment, light penetration in the mud is often less than 0.5 mm and therefore even during the day, photosynthesis is not possible (Garcia-Pichel and Bebout, 1996). Motility of epipellic diatoms is possible through the exudation of polysaccharide through the raphe. Upon secretion it becomes hydrated and through swelling of the EPS the diatom moves with respect to the substratum. This process also takes place in the absence of light, which is important to allow the organism to move from the aphotic zone to the sediment surface. It is conceived that the production of EPS for migration is supported by the mobilization of intracellular carbohydrate storage (Lind et al. 1997). However, epipellic diatoms also produce EPS exclusively in the light, which is dependent on photosynthetic CO<sub>2</sub> fixation. It has been proposed that this EPS is produced because of unbalanced growth, for instance at high irradiances or when nutrients (particularly nitrogen) are limiting (Staats et al. 2000). Interestingly, this EPS has been shown to disappear during the dark, and is probably metabolized in order to cover the energy demand of the diatoms (Staats et al. 2000; de Brouwer and Stal 2002).

The correct way of extraction and measurement of EPS from cultivated diatoms and from natural communities of microphytobenthos has been debated (Bellinger et al. 2005). In cultures, EPS is secreted into the medium and can be collected from the supernatant after centrifugation. However, another fraction of EPS can be obtained by extracting the pellet of

cells of diatoms with water at 30°C. It has been shown that these two fractions of EPS are different in composition (Staats et al. 1999; de Brouwer and Stal 2002). The EPS recovered from the supernatant contains a high amount of uronic acids and reveals a complex composition of monosaccharides. The fraction that is extracted with water from the pellet is less heterogeneous. It is composed largely of glucose, and contains low amounts of uronic acids. This EPS fraction is produced in the light and decomposed in the dark and reveals the typical behavior of a carbohydrate storage compound. From natural communities of microphytobenthos, EPS can be extracted by water (30°C). It appears that this gives the same fraction as by the extraction with water of cultivated cells. When the sediment subsequently is extracted with a solution of 100 mM EDTA, another fraction is obtained. There is some evidence that this fraction is the same as found in the supernatant of the cultivated diatoms (de Brouwer et al. 2002b). Possibly, this complex EPS, which is rich in uronic acids and other charged groups, binds to the charged silt and clay particles through bridging with divalent cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) and can only be recovered by using a strong chelating agent. It is likely that the water-extractable EPS is partly lost from the sediment during tidal inundation, although this will be less efficient at temperatures lower than 30°C. Moreover, this EPS is also decomposed in the dark. The aim of this investigation was therefore to try to visualize these processes by using cryo-scanning electron microscopy applied on samples from the Marennes-Oléron mudflat of the Atlantic Coast of France, that were kept in conditions of light and dark and e- or immersion, simulating day and night and low- and high tide.

## Material and methods

### *Sampling area*

Samples were taken from the Brouage mudflat of Marennes-Oléron in France on April 28, 1998. Samples were taken from the area characterized by ridges and runnels (Galois et al. 2000). Sampling was done during low tide by taking sediment cores with a PVC tube ( $\varnothing = 106$  mm, height 150 mm) from the ridges that were visibly covered with a biofilm of diatoms. After sampling, the cores were kept at 10°C and continuous dim light ( $\sim 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in an incubator. The sediment surface was kept moist with filtered seawater (GF/F glass fiber filters) from the same location until they were used for the experiments 2 days later.

### *Incubation*

The sediment cores were incubated in glass aquaria, filled with filtered seawater (GF/F glass fiber filters) from the same location. Each aquarium contained three sediment cores and four aquaria were used, each for one of the following experimental conditions: light and submersed with seawater; light and exposed to air; dark and submersed with seawater; dark and exposed to air. Hence, all incubations were done in triplicate. Each of these conditions was supposed to mimic low- or high tide and the day or night situation. Incubations were done at ambient temperature and the temperature of the water in the aquaria was 16-17 °C for the duration of the experiment. Light was provided by a 50 W halogen lamp. One lamp was used for each of the two light incubated aquaria. The distance of the lamp to the sediment surface was about 0.75 m, guaranteeing a near homogeneous light field of  $\sim 25 \mu\text{mol m}^{-2} \text{s}^{-1}$ , without heating up the sediment or water in the aquaria. In addition, a fan helped preventing accumulation of heat from the halogen lamp. The temperature of the water was regularly

checked by a thermometer. The dark incubated aquaria were covered by black paper. Each of the aquaria was equipped with an aquarium air pump in order to aerate and continuously mix the seawater. The submersed cores were covered by ~50 mm of water, while the water level of the exposed cores was ~50 mm below the sediment surface. These cores had still exchange with the water through capillary forces, so that the sediment surface remained moist throughout the experiment.

### *Sampling*

After 48 hours of incubation, the sediment surface was sampled using the cryo-lander technique (Wiltshire et al. 1997) in order to study undisturbed samples by scanning electron microscopy. Briefly, a brass tube ( $\varnothing$  54 mm, height 80 mm) with a tripod was carefully positioned on the sediment surface, resembling a moon lander. The bottom of the brass tube was just above the sediment surface and the whole was supported by the three legs of the tripod. Inside the brass tube, a mesh was present on which glass wool was placed. Liquid nitrogen was poured carefully on the glass wool, where it evaporated. The cold and heavy N<sub>2</sub> gas subsequently froze the sediment surface, without disturbing it. Liquid nitrogen was added until the top 5 mm of the sediment was frozen. This frozen sediment disk could easily be taken out of the sediment, wrapped in aluminum foil and kept in a container with liquid nitrogen until further analysis. Samples for analysis of extracellular carbohydrates and chlorophyll *a* were taken after 24 and 48 h of incubation using a corer made of a 10-ml plastic syringe. The top 5 mm of the sediment was sampled and the samples were wrapped in aluminum foil, frozen, and stored in liquid nitrogen until analysis.



### *Field-emission cryo-scanning electron microscopy*

The samples obtained by the cryo-lander were sawed in smaller pieces, using a cooled razor blade, on a metal disk that had been submersed in liquid nitrogen, which prevented thawing of the sample. Very small pieces (1-2 mm<sup>3</sup>) were prepared out of the top layer of the cryo-lander sample, while this was kept frozen on a liquid nitrogen-cooled metal disk and immediately transferred to the cooled (-150°C) stage of the cryo-scanning electron microscope (Hitachi 4200 field emission SEM equipped with a Polaron LT 7400 cryo-system). The surface ice was progressively sublimed away from the sample by temporarily raising the temperature inside the microscope chamber up to -60°C. This operation, known as “heat etching”, was visually controlled in order to avoid any distortion of the natural fabric of the sediment. The sample was then observed at -150°C and 1 kV, without any coating. The field emission device of the Hitachi 4200 allows such examinations at low voltage, which minimizes any possible damage caused by electron beam and decreases beam penetration depth, thus providing pictures representative of the very surface of the sample (Défarge et al. 1999). X-ray microanalyses of relevant elements were performed thanks to an Oxford Isis EDS.

### *Extraction and analysis of extracellular carbohydrates*

The samples taken for the analysis of extracellular carbohydrate and chlorophyll *a* were pooled and lyophilized. The largest uncertainty of these analyses was the heterogeneity of the samples, rather than the error in the analytical procedures. By pooling samples randomly taken from the three replicate cores, the effect of heterogeneity of the biofilm was decreased, while the measurements of carbohydrate and chlorophyll *a* could be related. The uncertainty of the analyses was less than 10% (de Brouwer et al. 2003). From the lyophilized sediment, 200 mg was weighed and put into a glass vial and extracted with 1 ml distilled water for 1 h at 30°C. This yielded water-extractable extracellular carbohydrate (sometimes also called

colloidal carbohydrate). The extract was then centrifuged and the sediment was extracted with 1.5 ml of 100 mM Na<sub>2</sub>-EDTA for 16 h at 20°C. This yielded EDTA-extractable carbohydrate, which is different in composition from the water-extractable fraction and which is tightly bound to the sediment. Both fractions are composed for more than 95% of polymers with a molecular weight of >100 kDa (de Brouwer and Stal 2001). The carbohydrate content was determined using the phenol-sulfuric acid method (Dubois et al. 1956).

#### *Analysis of chlorophyll a*

Chlorophyll *a* was determined from another sub-sample of 200 mg of the lyophilized sediment. The sediment was extracted with dimethylformamide (DMFA) according to the method of (de Winder et al. 1999). The sample was acidified and chlorophyll was corrected for pheophytin. Calculations were done according to Lorenzen and Newton Downs (1986). The values taken for *K* and *R* for DMFA were 2.256 and 1.8, respectively (de Winder et al. 1999).

## **Results and discussion**

#### *Chlorophyll and EPS contents*

The sediment chlorophyll *a* content did not reveal very large differences (Table 1). The overall average was  $16.0 \pm 3.7 \mu\text{g g}^{-1}$ , which was a little higher than  $9.6 \mu\text{g g}^{-1}$  measured by de Brouwer et al. (2003) a year earlier at the same station. There was a tendency of somewhat lower chlorophyll *a* content after 48 h, but this was not significantly different from the overall mean (from  $17.2 \pm 2.0$  to  $14.7 \pm 4.9$ ). It was clear that no measurable increase of phototrophic biomass had occurred. The chlorophyll *a* was extracted from the upper 5 mm of the sediment.

It is known that at low tide during the day, most of the chlorophyll *a* is present in the top 0.2 mm of the sediment and also that the largest changes occur in that layer and during daytime emersion (Taylor and Paterson 1998; Taylor et al. 1999; de Brouwer and Stal 2001). Hence, any changes in biomass at the sediment surface may have been obscured. However, the maximum rate of C fixation at light saturation is low ( $\sim 3 \text{ mg C mg Chl } a^{-1} \text{ h}^{-1}$ ), and a large part (40 – 80%) of the CO<sub>2</sub> that is fixed by microphytobenthos is secreted in the environment and is not used for synthesis of structural cell material (Wolfstein et al. 2002). Hence, although photosynthesis and primary productivity can be high, this does not necessarily lead to substantial increases of microphytobenthos biomass during the relatively short incubation times as were used here.

The content of water-extractable carbohydrate was largest in the light, emersed incubation. This was the case when the carbohydrate was related to chlorophyll *a* (Fig. 1a) as well as to sediment dry weight (Fig. 1c). After 48 h incubation this amount had increased with almost 20% compared to  $t=24 \text{ h}$ , when expressed on the basis of biomass, but the sediment content of water-extractable carbohydrate decreased  $\sim 20\%$ . This was due to the decrease of biomass of almost 40% during the same period. This result is in agreement with the fact that the production of this carbohydrate fraction is light dependent and is driven by oxygenic photosynthesis (Staats et al. 2000). In the light, the diatoms will move upwards and form a film on the sediment surface. However, when the sediment was immersed and illuminated, the amount of water-extractable carbohydrate decreased by more than 50% when compared to the emersed condition. The reason for this may be either that the diatoms had migrated into the sediment or that the carbohydrate became suspended in the water. Migration of microphytobenthos according to the tidal rhythm is known (Serôdio et al. 1997) and photosynthesis would have been impossible and consequently the production of this

carbohydrate fraction. On the other hand, since this fraction of carbohydrate is water-extractable, it may have been lost from the sediment during immersion. Although extraction of this carbohydrate is much less efficient in seawater and at the lower temperature of 16-17 °C, it has been observed that considerable amounts of carbohydrate can be found in seawater that inundated mudflats with diatom biofilms (de Brouwer and Stal, unpublished results).

In the dark, when the sediment was emersed, the biomass-normalized content of water-extractable carbohydrate was more than 60% lower than in the light, further decreasing to 75% after 48 h of incubation. This is in agreement with the fact that the production of this fraction of carbohydrate depends on photosynthesis, while in the dark it is decomposed by the diatoms, who probably utilize it as a storage compound (Staats et al. 2000; de Brouwer and Stal 2002). It has been suggested that water-extractable carbohydrate is intimately associated with the diatom cells (de Brouwer et al. 2002b). This explains why a good correlation between this fraction of carbohydrate and microphytobenthos is usually found in most mudflats that have been investigated (Underwood and Smith 1998; Bellinger et al. 2005).

A correlation with chlorophyll *a* is usually much less evident when considering carbohydrate that is extracted with 100 mM EDTA. The composition of this material is complex. It contains more uronic acids and other acid groups. Fig. 1d shows that the different incubations hardly affected the sediment content of EDTA-extractable carbohydrate. Only in the illuminated incubations, the biomass-normalized amount of this carbohydrate increased by 25 and 19% during 24 h in the emersed and immersed conditions, respectively (Fig. 1b). These results are in agreement with the different structure and function of this material. The EDTA-extractable EPS, because it possesses acid groups, interacts with the charged sediment particles and becomes tightly bound. It is possible that diatoms produce this carbohydrate because of their

migration, which should be independent on light and photosynthesis. The results depicted in Fig. 1 are in full agreement with those obtained by (de Brouwer et al. 2003) in a similar experiment carried out in the same mudflat a year before. However, these authors did not investigate the structural aspects by scanning electron microscopy.

### *Cryo-SEM*

#### Light incubation emersed

Fig. 2 shows the cryo-scanning electron micrographs of the sediment surface from the core that had been incubated in the light, while being exposed to the air. The surface has a smooth appearance, which is due to the matrix of EPS (Fig. 2a). The same sample is depicted at slightly higher magnification in Fig. 2b. Large numbers of diatoms are clearly visible at the sediment surface. The EPS surface layer is shown in its hydrated state in Fig. 2c, which reveals how individual particles are wrapped in this organic matter. This coating with EPS of silt grains is clearly seen in Fig. 2d. When the sediment was emersed and incubated in the light, diatoms were partly covered by EPS (Fig. 2e, f). This EPS probably represents the ‘water-extractable EPS’ that is associated closely with the cells. The other type of EPS (‘EDTA-extractable EPS’) is intimately associated with the sediment and it may be involved in the formation of organomineral aggregates (Fig. 2g). This is for instance illustrated by the halite crystal in Fig. 2h, which is embedded in mucilage.

#### Light incubation immersed

The results of the scanning electron microscopic observations of the immersed sediments incubated in the light are summarized in Fig. 3. When compared to the emersed incubation (Fig. 2a) the sediment surface is enriched by finer-grained mud, mixed with organic coatings (Fig. 3a). Fig. 3b shows the same sample at slightly higher magnification. There are diatoms

visible at the surface but seemingly less than in the emersed incubation. It is also obvious from the micrographs that many diatoms are hardly visible since they are covered by finer-grained mud and organic coating. This is probably because part of the diatoms had migrated down into the sediment or they may also have been sloughed off, due to the turbulence of the overlying water. There is evidence of some coating by EPS (Fig. 3c) but it is less smooth than in the case of the emersed sediment (compare with Fig. 2d). This difference of smoothness is likely due to the presence of the superficial finer-grained mud. The coarser silt particles can be distinguished more easily. Diatoms present in this sample generally did not exhibit EPS coating of the exterior of the organism (Fig. 3d). In the case of Fig. 3e it can be seen that some diatoms did have an EPS coating; however it is usually thinner than in emersed sample (compare with Fig. 2f; see also Fig. 3f). Large amounts of fine-grained minerals adhered to the outer surface of the diatoms in this sample, which is probably the result of the binding by mucilage present in the sediment (Fig. 3g and h). Among the adhering fine-grained minerals, this sample revealed also many halite crystals as was confirmed by EDS analysis.

#### Dark incubated emersed

Fig. 4 shows the images of the sediment that was incubated in the dark and emersed. The individual coarser silt grains can be seen but they seem to be attached to each other by threads of EPS (Fig. 4a and b). The structure of these threads and how they connect the individual silt grains is best illustrated in Fig. 4e and f. There were few areas where diatoms were present at the surface (Fig. 4c). Apparently, they had migrated into the sediment. At higher magnification, the remaining diatoms seemed to be coated by EPS (Fig. 4d). The wrapping of the sediment particles by organic coatings is particularly obvious in samples of this incubation, because the surface does not contain so much finer-grained mud as is the case in the immersed samples. This wrapping by EDTA-extractable EPS is particularly well

illustrated at higher magnification. Fig. 4e and f depict this wrapping and the EPS threads that connect the grains. Part of the EPS thread in Fig. 4e is shown at higher magnification in Fig. 4g. This figure also shows how this thread is connected to the EPS coating of the mud particle. Fig. 4h shows the individual silt grains wrapped in EPS at higher magnification. The very fine-grained mud, as it is found in the immersed incubations, is rare (compare with Fig. 3b or, better, Fig. 5f).

#### Dark incubated immersed

Also when the sediment was incubated immersed in the dark, hardly any diatoms were found at the sediment surface (Fig. 5a). Although at low magnification individual silt grains were hard to distinguish, at high magnification they could be seen (Fig. 5b). It should be noted here that, similarly as in the light immersed samples, the dark immersed samples also contained much more finer-grained sediment on the surface. This is also the reason why the coarser grains are difficult to see. It appeared that the sediment grains were somehow connected in a network of EPS threads (Fig. 5b), which is also illustrated in Fig. 5e. The few diatom cells that were found at the surface were not coated by EPS (Fig. 5c, d). Fig. 5f shows the very fine-grained mud deposited on the sediment surface as was the case in the light immersed incubation. This fine-grained mud also adhered to the outer surface of the diatoms in this sample as illustrated in Fig. 5g and h.

#### *EDS analysis*

Analyzing chemical elements in the cryo-SEM images by using X-ray diffraction showed that the sediment surface of the light emersed samples contained a much higher amount of carbon compared to silicon than in the immersed samples. In the latter the ratio C:Si amounted  $\sim 0.1$ , the same as was found for a non-coated diatom. In the emersed, light incubated sample this

ratio was  $\sim 0.6$ . The higher amount of C relative to Si in the light emerged incubation, agreed with the biochemical analysis of the EPS of the same sample. It was further found that the light immersed sample contained a  $\sim 4$ -times higher amount of Na and Cl, compared to the emerged sample. The crystals found attached to the diatoms in the immersed incubation were therefore probably halite crystals.

## Conclusions

The field-emission cryo-SEM study of the Marennes-Oléron mudflat confirms the migration of diatoms to the sediment surface during low tide at daytime. This was concluded from the fact that the overall biomass (measured as chlorophyll *a*) in the upper 5 mm of the sediment did not reveal any changes (table 1), while clearly high numbers of diatoms were present at the surface in the light under emerged conditions (Fig. 2a, b), while this was not the case in the other incubations. The fact that the overall amount of biomass did not change much, was taken as evidence that sloughing off and re-suspension of diatoms in the water column was not important.

It did not come as a surprise that the production of the light-dependent, photosynthesis-driven, water-extractable EPS fraction is by far largest under the condition of emersion in the light (Fig. 1a, c). It is known that water-extractable EPS from diatoms is intimately associated with the cell. The SEM images show that under emerged, light conditions, diatoms coated with EPS are abundant (Fig. 2f). The EPS coatings of the diatoms seem thinner under immersed conditions in the light (compare Fig. 3e and 2f) or the cells are not coated at all (Fig. 3d). The lower amount of this EPS measured under immersed light conditions (Fig. 1a, c) may either be the result of a lower production because of a downward migration of the diatoms or dissolution of the EPS in the overlying water, or both. Dissolution of EPS is certainly



important as can be concluded from the comparison of the dark immersed and emersed incubations. In the latter situation, diatoms still exhibited EPS coatings (Fig. 4d), while this was not the case in the dark immersed incubation (Fig. 5c).

While water-extractable EPS reveals a dynamic behavior, EDTA-extractable EPS is more conservative. Although there is no doubt that it is also a product of diatom activity, it is not precisely known under which metabolic control it is produced. It may be deposited outside the cell as part of the mechanism that allows motility of the diatom or be produced under another, hitherto unknown, metabolic control. This fraction of the EPS forms organomineral muddy sediment in which carbohydrate compounds and silt particles are intimately mixed and bound.

In both cases of immersion of the sediment, in the light as well as in the dark, the sediment surface was covered by very fine-grained material, mixed with the organic coatings, when compared to the emersed incubations. This can be seen from the Fig. 2a and 4c, depicting the emersed incubations (in the light and the dark, respectively) and comparing them with Fig. 3a and 5a, depicting the immersed incubations. This difference becomes even clearer at higher magnification in the Fig. 2c and 4h and Fig. 3b and 5f for the emersed and immersed incubations, respectively. It is not likely that this is an artifact caused by submersion of the cores in the aquarium. The cores were placed in an empty aquarium, which subsequently was carefully filled with GF/F filtered seawater. The deposition of fine-grained sediment may be the result of the dissolution of EPS in the water and its re-deposition as aggregates, or otherwise small mineral particles may have formed. Another confirmation of the higher abundance of fine mud in the immersed samples relative to the emersed conditions can be found in the Fig. 3b, and 5g and h, representing diatoms in respectively the light and dark immersed incubations, to which very fine-grained minerals adhere. This material is most

likely the same as what was deposited on the sediment surface and it adheres to the diatoms and to the larger sediment particles through the binding by the acidic, EDTA-extractable EPS, which is present under all conditions.

This study shows the aggregation of mineral particles by organic remains and wrapping of coarser silt grains by organic coatings that attach the grains together (Fig. 2g, 3h, 4a, b, e and f, and 5e). These observations provide visual evidence for the processes by which diatom biofilms increase the critical erosion threshold of intertidal mudflats where they develop.

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Table 1. Chlorophyll *a* content of the top 5 mm of the sediment after 24 and 48 hours of incubation.

Extractions were done from 5 individual samples, randomly taken from the each core and subsequently pooled.

Incubation		Chlorophyll <i>a</i> ( $\mu\text{g g}^{-1}$ ) <sup>1</sup>	
		24 hour	48 hour
Light	Emersed	15.5	10.3
	Immersed	17.8	14.5
Dark	Emersed	19.9	21.6
	Immersed	15.7	12.4

<sup>1</sup>Chlorophyll *a*-content expressed on a sediment dry-weight basis

# Figure legends.

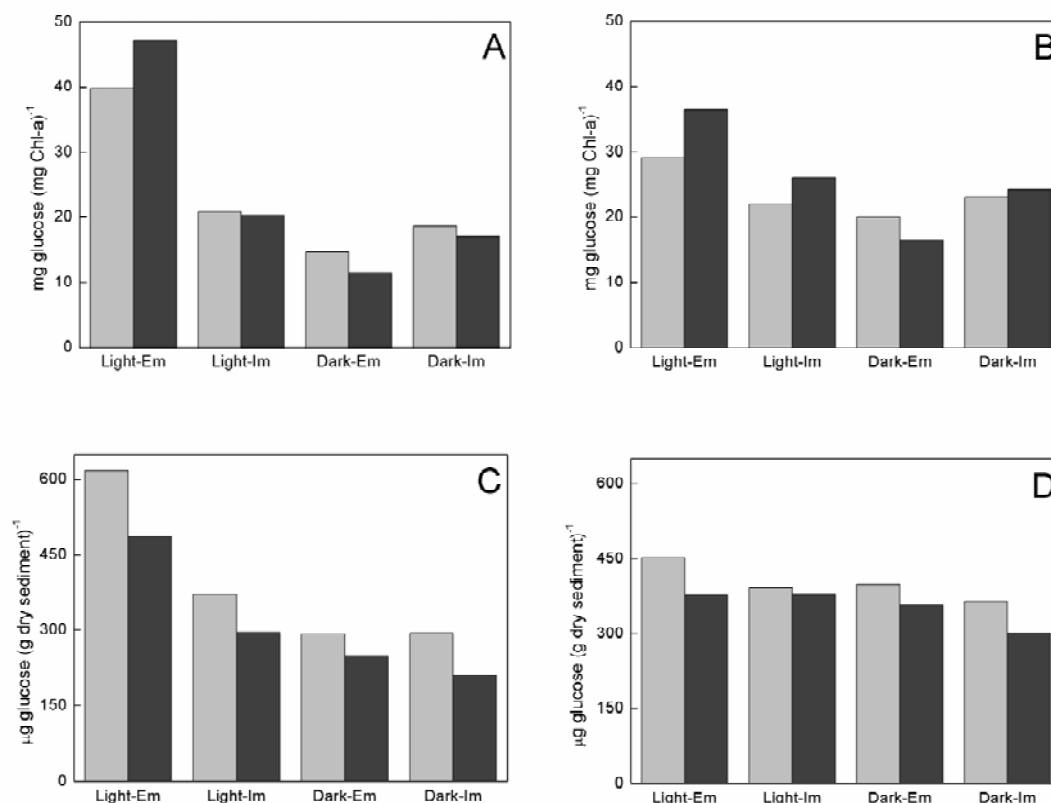
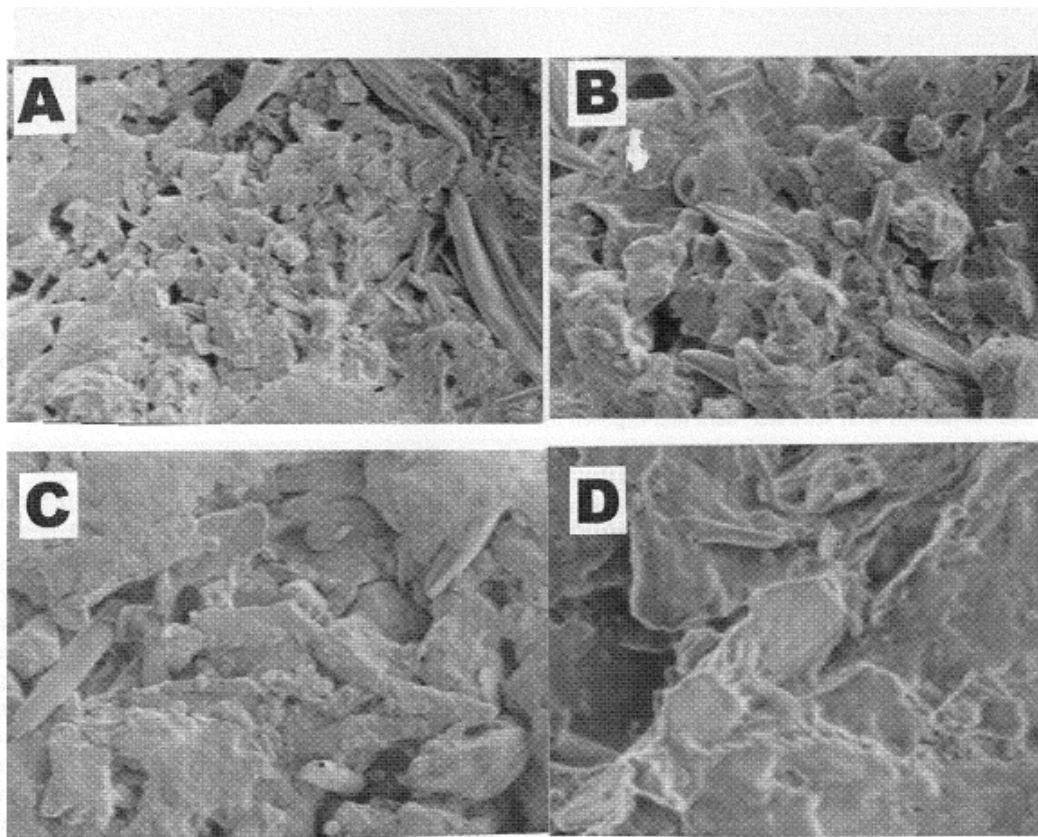


Fig. 1.

The contents of extracellular polysaccharides (expressed as glucose equivalents) in the top 5 mm of the sediment cores incubated emerged in the light (Light-Em) or in the dark (Dark-Em), or immersed in the light (Light-Im) or dark (Dark-Im). The light and dark grey bars represent the carbohydrate contents after 24 and 48 h of incubation, respectively. A and C show the amount of the water-extractable carbohydrate, while the panels B and D depict the EDTA-extractable carbohydrate. The upper panels (A and B) show the biomass-related (chlorophyll-*a*) carbohydrate content, while the bottom panels give the sediment (dry weight)



content of carbohydrate. Extractions were done from 5 individual samples, randomly taken from each core and subsequently pooled.



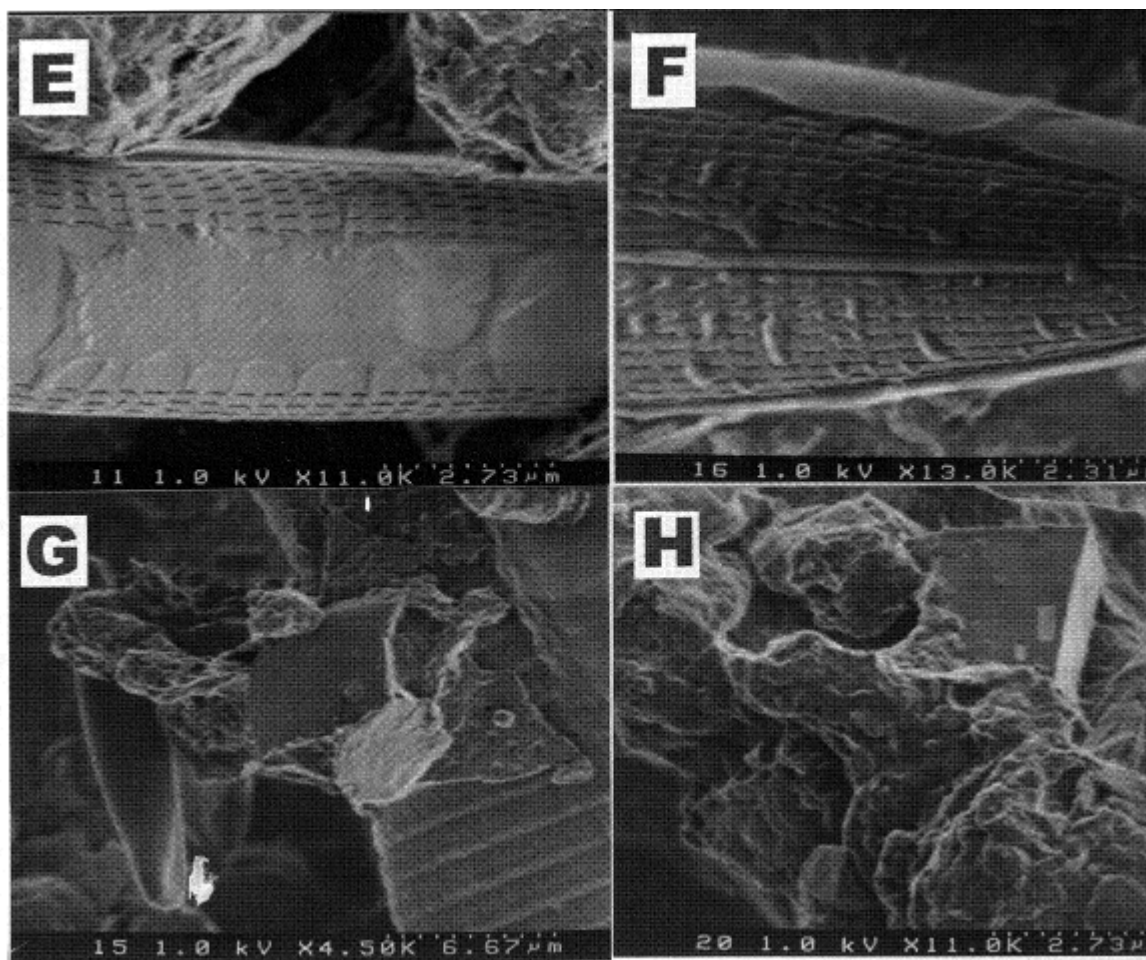


Fig. 2.

Cryo-FEG-SEM images of the sediment surface of the core incubated in the light and exposed to the air. Bar markers: (a) 50  $\mu\text{m}$ ; (b) 25  $\mu\text{m}$ ; (c) 25  $\mu\text{m}$ ; (d) 1  $\mu\text{m}$ ; (e) 2  $\mu\text{m}$ ; (f) 2  $\mu\text{m}$ ; (g) 5  $\mu\text{m}$ ; (h) 2  $\mu\text{m}$ . See text for explanations.

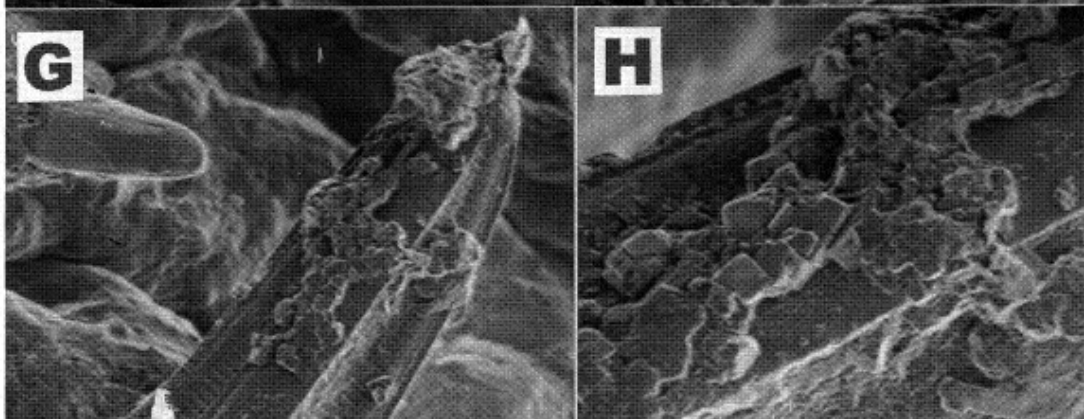
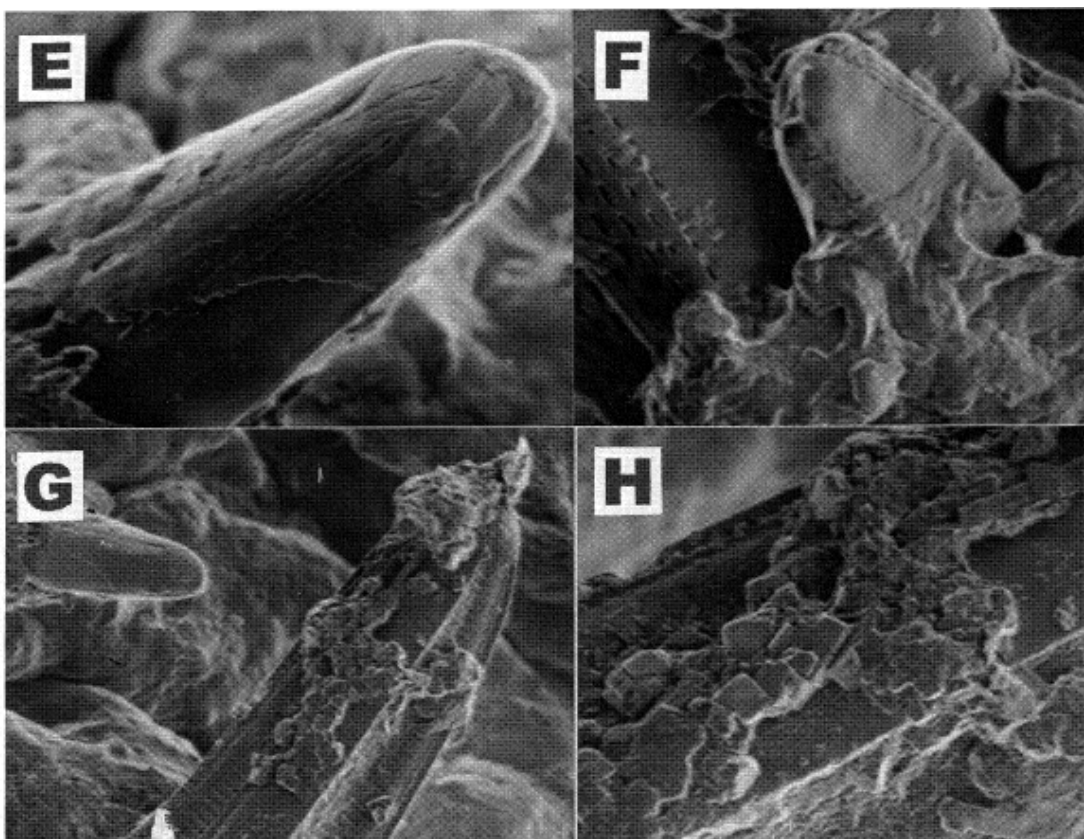
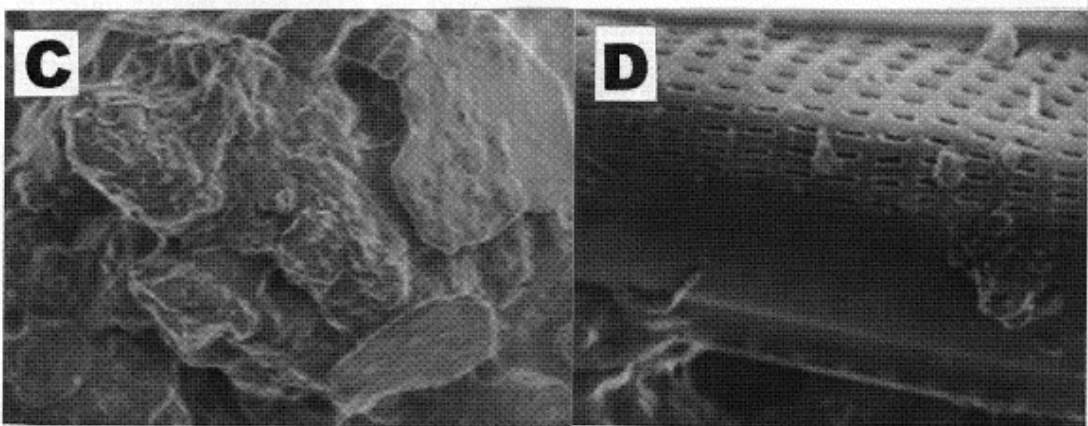
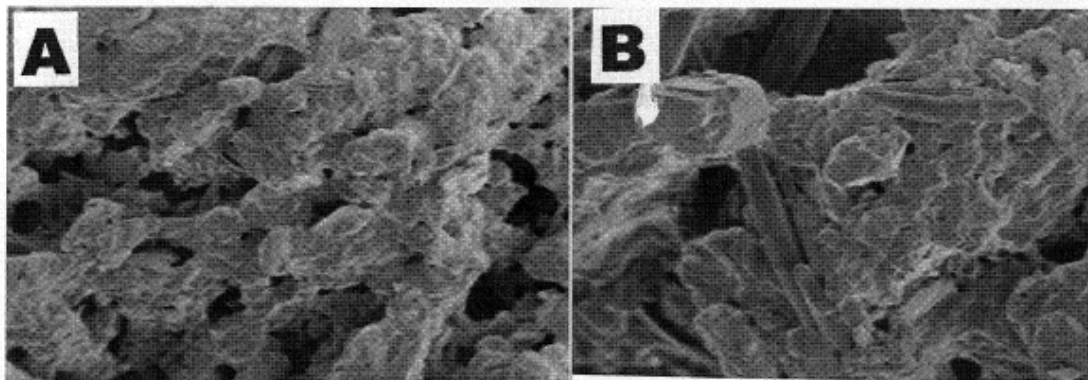
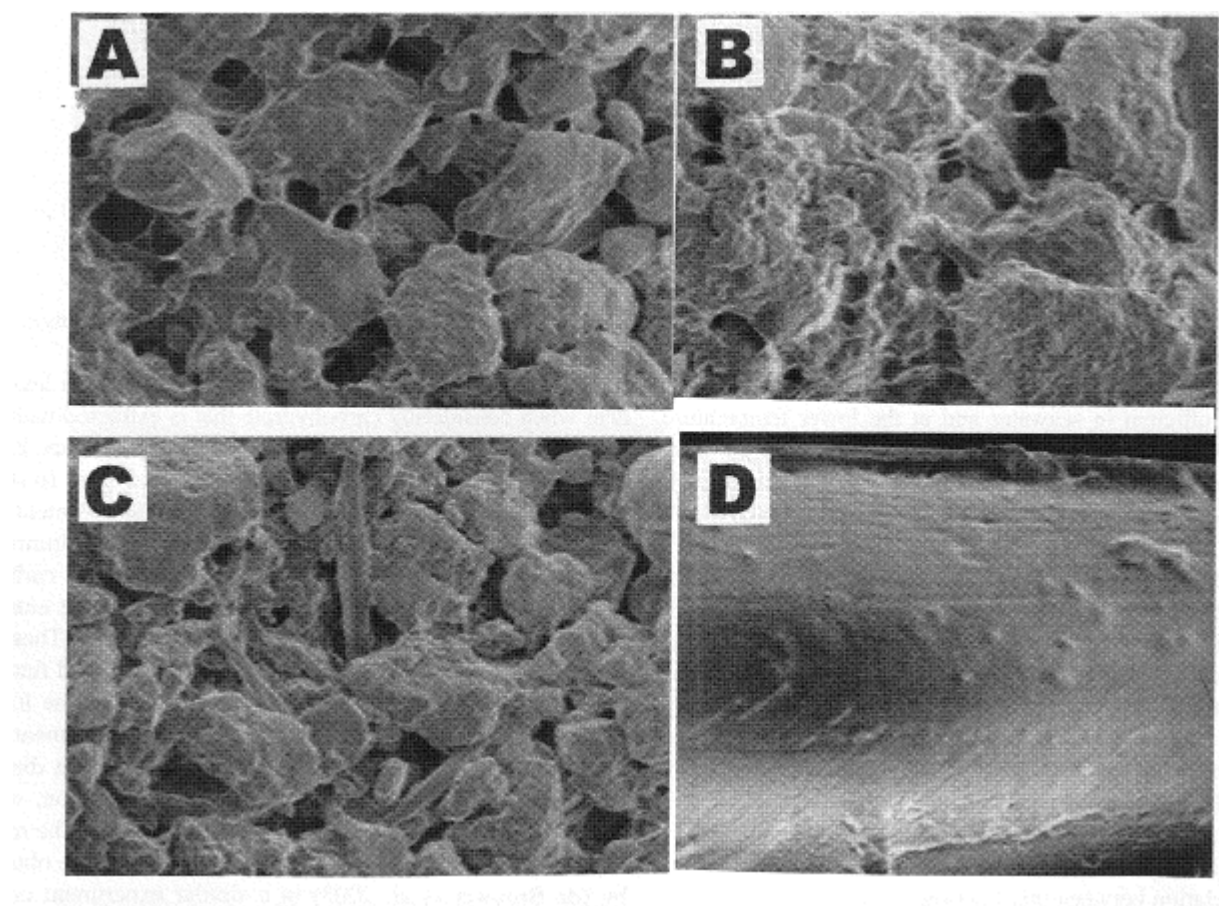


Fig. 3.

Cryo-FEG-SEM images of the sediment surface of the core incubated in the light and immersed. Bar markers: (a) 50  $\mu\text{m}$ ; (b) 25  $\mu\text{m}$ ; (c) 2  $\mu\text{m}$ ; (d) 1  $\mu\text{m}$ ; (e) 2  $\mu\text{m}$ ; (f) 2  $\mu\text{m}$ ; (g) 5  $\mu\text{m}$ ; (h) 2  $\mu\text{m}$ . See text for explanations.



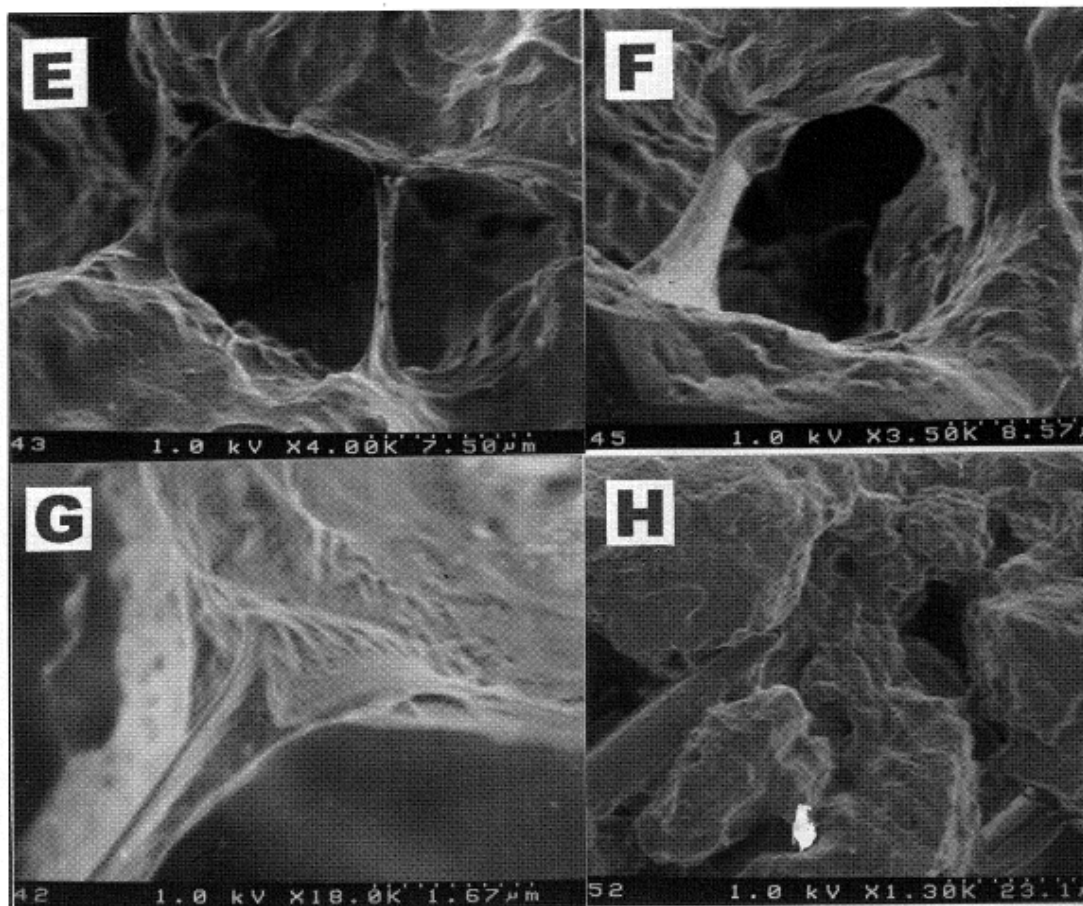


Fig. 4.

Cryo-FEG-SEM images of the sediment surface of the core incubated in the dark and exposed to the air. Bar markers: (a) 50  $\mu\text{m}$ ; (b) 25  $\mu\text{m}$ ; (c) 50  $\mu\text{m}$ ; (d) 2  $\mu\text{m}$ ; (e) 5  $\mu\text{m}$ ; (f) 5  $\mu\text{m}$ ; (g) 1  $\mu\text{m}$ ; (h) 25  $\mu\text{m}$ . See text for explanations.



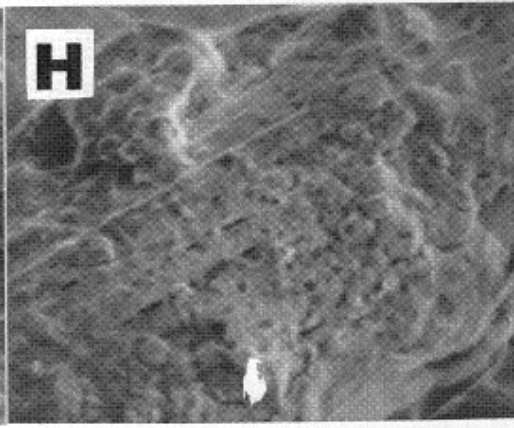
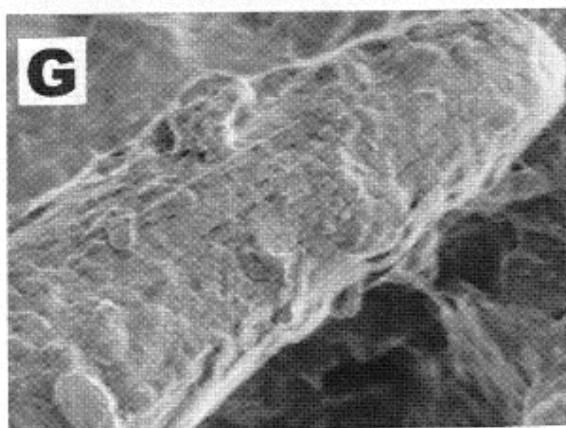
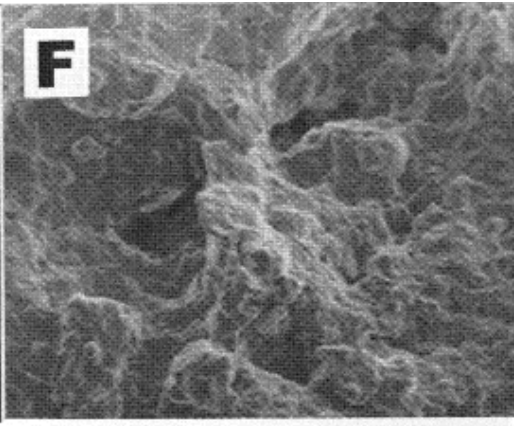
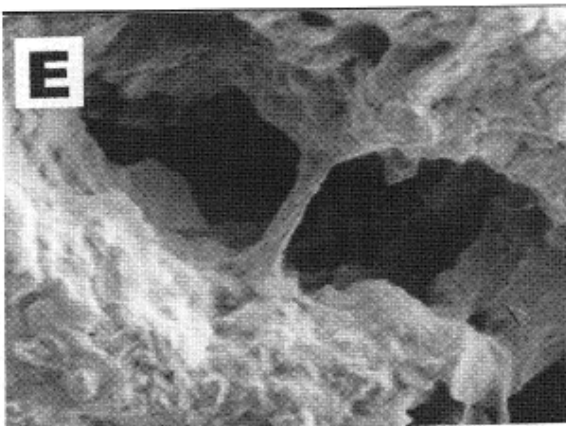
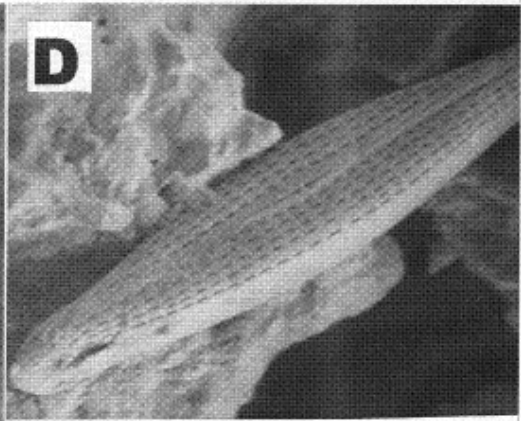
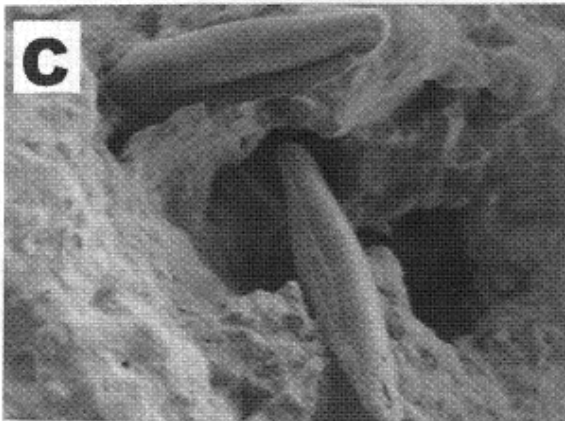
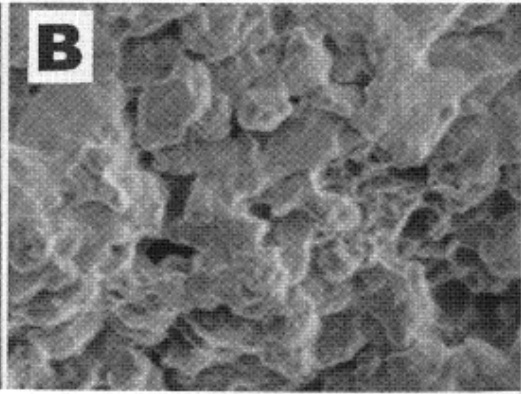
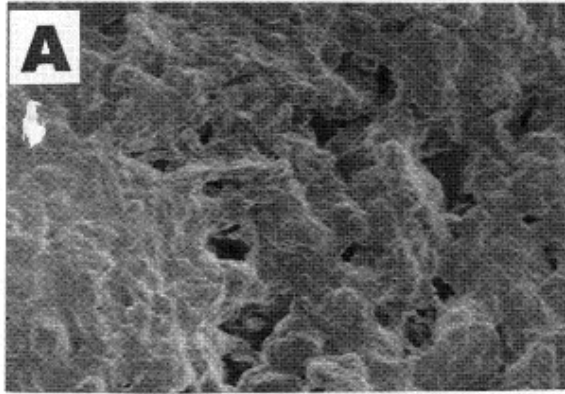


Fig. 5.

Cryo-FEG-SEM images of the sediment surface of the core incubated in the dark and immersed. Bar markers: (a) 50  $\mu\text{m}$ ; (b) 2  $\mu\text{m}$ ; (c) 10  $\mu\text{m}$ ; (d) 5  $\mu\text{m}$ ; (e) 10  $\mu\text{m}$ ; (f) 25  $\mu\text{m}$ ; (g) 2  $\mu\text{m}$ ; (h) 1  $\mu\text{m}$ . See text for explanations.