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Key Points:
- The depth profile over biosedimentary beds of different ages is considered
- Patterns of the bacterial extracellular polymeric substances show natural variation with depth
- Bed stability within the biosedimentary layer varies due to biological effects

Supporting Information:
- Supporting Information S1
- Data Set S1

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Stabilizing Effects of Bacterial Biofilms: EPS Penetration and Redistribution of Bed Stability Down the Sediment Profile

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Abstract Biofilms, consisting of microorganisms and their secreted extracellular polymeric substances (EPSs), serve as "ecosystem engineers" stabilizing sedimentary environments. Natural sediment bed provides an excellent substratum for biofilm growth. The porous structure and rich nutrients allow the EPS matrix to spread deeper into the bed. A series of laboratory-controlled experiments were conducted to investigate sediment colonization of *Bacillus subtilis* and the penetration of EPS into the sediment bed with incubation time. In addition to EPS accumulation on the bed surface, EPS also penetrated downward. However, EPS distribution developed strong vertical heterogeneity with a much higher content in the surface layer than in the bottom layer. Scanning electron microscope images of vertical layers also displayed different micromorphological properties of sediment-EPS matrix. In addition, colloidal and bound EPSs exhibited distinctive distribution patterns. After the full incubation, the biosedimentary beds were eroded to test the variation of bed stability induced by biological effects. This research provides an important reference for the prediction of sediment transport and hence deepens the understanding of the biologically mediated sediment system and broadens the scope of the burgeoning research field of "biomorphodynamics."

Plain Language Summary In many studies, biofilms have been developed on synthetic material such as glass slides. However, natural sediment beds are very different from impermeable surfaces and provide a more extensive substratum for biofilm growth: the porous structure and rich nutrients allow the biofilm and related extracellular polymeric substance (EPS) matrix to extend deeper into the matrix. A sample of field observation on tidal flats proves this: the biosedimentary layer can be found several centimeters below the surface. Therefore, the biological effects on the sediment bed should not be restricted to simply "surficial protection" such as increasing the erosion threshold. Instead, the EPS expansion into the depth profile alters the properties of a layer of sediment, and thus will certainly have an even greater impact on sediment transport. In the light of this, coastal engineers and morphological prediction modelers are concerned with the following: (1) how do these bioeffects change with depth and time? And (2) what will be the changes to bed stability in response? Therefore, the penetration of bacterial EPS down sediment profile and the depth potential of biostabilization are further investigated in this study.

1. Introduction

Biofilms form a heterogeneous matrix consisting of microbial communities and their secreted extracellular polymeric substances (EPSs) (Wingender et al., 1999) and in sediments incorporate particles within the matrix. Microorganisms that inhabit sediments and form biofilms are highly adapted for this habitat and secrete adhesive polymers (EPS). The EPS is primarily composed of organic molecules such as polysaccharides, proteins, lipids, and nucleic acids (Stoodley et al., 2002), and the matrix formed is usually highly hydrated. Biofilms drive a number of important "ecosystem services": they contribute to primary production and play a role in the natural water treatment by particle adsorption, toxin biodegradation, and nutrient cycling (Nicolella et al., 2005; Shannon et al., 2008; Sheng & Liu, 2011; Wei et al., 2012).

Researchers have also increasingly acknowledged the importance of microbial assemblages and their associated EPS as ecosystem engineers in natural environments, especially in depositional intertidal zones such as mudflats and salt marshes (Chen et al., 2017; Gerbersdorf et al., 2009; Passarelli et al., 2014).

EPS affects sediment dynamics by (1) directly enhancing the cohesive forces between sediments, binding them together, and (2) coating sediment particles and changing the micromorphology of individual grains (Paterson et al., 2009; Van Colen et al., 2014). Various sediment processes are mediated by this biological cohesion. In the typical erosion-transportation-deposition-consolidation cycle, erosion is mediated as a result of the redistribution of stability in the sediment bed via the EPS effects. One of the most important effects of biofilms is their ability to stabilize sediments, which then become more resistant to erosion (Fagherazzi et al., 2013). In this respect, a phenomenon called "biostabilization" occurs, defined as "a decrease in sediment erodibility caused by biological actions" (Paterson & Daborn, 1991). Sedimentology and geomorphology have been traditionally considered as research fields in which physical and chemical processes dominate. Recently, biological processes have also been recognized and while the focus has been put almost entirely on vegetation (Kirwan & Megonigal, 2013; Roner et al., 2016). Gradually, it has been realized that microbial communities, including microphytobenthos, should never be regarded as bystanders (Chen et al., 2017; Dodd et al., 2017; Gerbersdorf & Wieprecht, 2015; Le Hir et al., 2007). After being eroded, biological factors can still mediate sediment transport. Fine particles with associated biofilm may promote aggregation, and this alters floc characteristics so that sediment transportation and deposition may be influenced. Larger aggregates will settle more quickly than individual particles, and floc formation may change the dynamics of sediment transport (Tan et al., 2012). During consolidation, the "biosedimentary" matrix undergoes further complex changes that enhance the binding forces (Droppo, 2001, 2004). Therefore, understanding the biofilm growth patterns under controlled conditions is crucial to predict its ecological and morphological roles on coastal and estuarine areas (De Brouwer et al., 2005; Paterson et al., 2008; Underwood & Paterson, 2003).

Pioneering work on the entrainment of a clay-water suspension (Dade et al., 1992) and on the stability of sediment with added EPS (Tolhurst et al., 2002) has shown clear effect of extracellular carbohydrates on the substratum. The EPS forms a cohesive matrix that helps to restructure the physicochemical properties of sediment bed ranging from a loose matrix to a compact, cohesive gel (Decho, 2000). Depending on biotic and abiotic conditions, natural biofilms can be complex, varying with factors such as the availability of nutrients (Wang et al., 2013), intensity and wavelength of light (Paterson et al., 2008), hydrodynamic conditions (Celmer et al., 2008; Chao et al., 2014; Kim et al., 2013; Stoodley et al., 1999), and grazing pressure (Sommer, 1999; Wey et al., 2008). Moreover, while natural sediments provide an excellent substratum for biofilm growth (large surface to volume ratio, rich in nutrients, and porous structure) (Fagherazzi et al., 2013), colonization is very different from impermeable surfaces (such as biofilms growing on glass slides) since EPS may be distributed throughout the porous medium (Davis et al., 2010; Gerbersdorf et al., 2005; Jaiswal et al., 2014; Van Colen et al., 2014; Volk et al., 2016).

In this paper, we investigate the vertical distribution of EPS in intertidal sediments, which has received little attention (Taylor & Paterson, 1998). Current studies on biostabilization have mainly focused on the consequence of the erosion threshold, and little is known about influence of changes in the EPS matrix over depths, which may even have a significant protection role, even for deeper layers (Chen et al., 2017; Gerbersdorf et al., 2005). In the light of this, coastal engineers and morphological prediction modelers are particularly concerned with the following: (1) how do these bioeffects change with depth and time? And (2) what will be the changes of bed stability in response? In addition, this important ecosystem function has commonly been attributed to the microalgae and their associated EPS, while the ubiquitous heterotrophic bacteria have largely been ignored. However, the bacteria are never by-standers as they have suffused all the sedimentary environments from 4.3 billion years ago (Dodd et al., 2017). Recent work has also demonstrated that natural benthic bacterial assemblages can significantly stabilize a test substratum, far exceeding expectations, as based on the limited literature (Lubarsky et al., 2010). Therefore, interdisciplinary study is needed to further understand the interaction between the sediments and bacterial assemblages. In this study, we aim to investigate sediment colonization by bacterial assemblages and follow the accumulation of secreted EPS down the sediment bed profile and unravel how their varying interactions with sediment grains contribute to the overall biostabilization potential.

2. Materials and Methods

2.1. Description of Field Site

Sediments were collected from the Jiangsu tidal flats. The Jiangsu coastal area (119°17′E–122°20′E, 31°33′N–35°07′N) in the south yellow sea comprises rich tidal flats sheltered by radial sand ridges (Figure 1; the topography data were extracted from Xu et al. (2016)). Our previous field observations in the Jiangsu intertidal zones suggested that EPS content displayed natural variation (unpublished data) with depth, indicating that biological effects are not necessarily limited to the surficial cover but influence the bed characteristics for several centimeters in depth.

2.2. Experimental Setup and Operation

The sediments (D_{50}: 98.3 μm) were collected from the lower intertidal zone of Jiangsu tidal flats (Figure 1, Site SM89). Before the incubation in laboratory, the sediment was sieved to remove the cohesive fraction (D_{50}: 108 μm after sieving, Figure S1 in the supporting information provides grain size distribution). The sediment was then washed with hydrogen peroxide to remove organisms and oxidize organic material. The experiment was performed in six identical mixing chambers A–F (29 cm diameter × 25 cm high) with rotating paddles (as used in Chen et al. (2017)). Biofilms developed on the sediment beds (2 cm thick) within the outer annular regions of the chambers, where shear stress was evenly distributed and uniform along the radial direction of flow (measured data of the bed shear stress under different rotating speed and the stress distribution from a simplified 3-D Fluent model are shown in Chen et al. (2017)). During the incubation period, the rotating speed of paddles was modified to generate a constant bottom shear stress of 0.058 Pa. All the chambers were maintained at a temperature of 20 ± 2°C. Chamber A was used as a control experiment where treated sediment was deposited in artificial seawater (ASW, salinity of 23), while sediment beds with bacteria in ASW plus nutrients (biosedimentary beds) were incubated in chambers B–E for different growth periods (5, 10, 16, and 30 days, respectively) (see Chen et al., 2017 for more details). *Bacillus subtilis*, as one of the dominant species in Jiangsu coastal area, was selected as the single bacterial culture in the experiment (bacteria powder supplied by Guangzhou Weiyuan Bio-technology Co, Ltd.). Bacterial powder ($>10^7$ CFU/g) was added with a bulk medium phosphate buffer (pH 7.5). Water was replaced with a fresh medium every 3 days to avoid nutrient limitation and to limit planktonic growth. Chamber E was used for the extraction of sediment samples (every 2–5 days). The sediment beds were sectioned into five layers (0–0.2 cm, 0.2–0.5 cm, 0.5–0.8 cm, 0.8–1.3 cm, and 1.3–1.8 cm) using sediment cores (5 cm in diameter) for extraction and analysis (see Chen et al., 2017, Figure 2). The extracted sediment samples were analyzed for biomass abundance, EPS contents and composition (see section 2.2 for the detailed parameters), and grain morphology (scanning electron microscopy, SEM, HITACHI S-3000 N, 25 kV, sample prepared by freeze drying method). The undisturbed biosedimentary beds in chambers B–D of different incubation periods were used for the erosion tests.

For the erosion tests, the biosedimentary bed was eroded in situ with stepwise increments of bed shear stress (via paddle rotation speed). An optical backscatter sensor (OBS-3+) located 7 cm above the bed surface was used to measure the real-time suspended sediment concentration (SSC). The monitoring range depends on sediment size, particle shape, and reflectivity (Figure S2). For the sediment used in this study, the maximum SSC value was about 65 kg/m^3. The calibration of bottom shear stress for each level of rotating speed was given before erosion by turbulent kinetic energy (TKE) method (Stapleton & Huntley, 1995), using Vectrino Profiler (Nortek AS) to measure instantaneous velocity within an *XYZ* coordinate system (the detail of the velocity measurements and the TKE method used to obtain the bed shear stress are illustrated in Text S1 in the supporting information). TKE is the product of the absolute intensity of velocity fluctuations from the mean velocity and is defined as

$$\boldsymbol{E} = \frac{1}{2}\boldsymbol{\rho}\left(\overline{(\boldsymbol{u}')^2} + \overline{(\boldsymbol{v}')^2} + \overline{(\boldsymbol{w}')^2}\right) \tag{1}$$

Using the constant of proportionality observed in a wide range of flows (Soulsby, 1983), the bed shear stress can be calculated:

$$\boldsymbol{\tau} = 0.19\boldsymbol{E} \tag{2}$$

The data set obtained by the Vectrino Profiler allows calculation of not only the mean velocity over a period of time but also the instantaneous positive and negative fluctuations from the mean velocity (u', v', w') that are

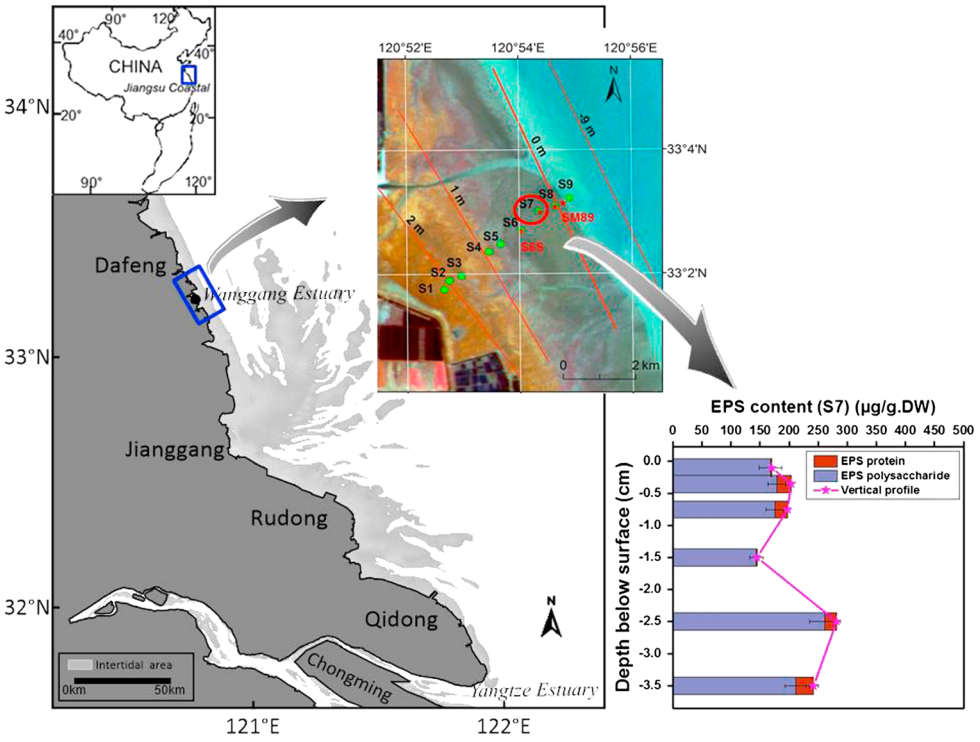

Figure 1. Jiangsu coastal area and the location of the study sites, where Site S6-SM89 marks the transition between the upper and lower intertidal zone. An example of the vertical profiles of extracellular polymeric substances (EPS) was taken at Site S7 in the autumn (19 August 2016).

used to calculate turbulent kinetic energy (E, equation (1)), and the bed shear stress (τ) is then calculated using equation (2).

2.3. Analytical Methods

Dry biomass and volatile suspended solids (VSSs) were measured according to the Standard Methods (APHA, 1998). The EPS extraction method was modified to improve extraction efficiency (Li et al., 2008; Liang et al., 2010). The colloidal EPS, loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS) were extracted step by step. The colloidal EPS was extracted first. A fresh sediment sample of (3 mL) was placed in a 50 mL centrifugation tube, and sterile deionized water was added to a total volume of 30 mL. The tubes were then centrifuged (4,000 g, 10 min, 4°C) after which the supernatant was recentrifuged (13,200 g, 20 min, 4°C) to ensure complete removal of the suspended solids. The colloidal EPS was obtained in the supernatant. The bottom sediments were resuspended to 30 mL using sterile deionized water. Then, 0.06 mL formamide (37%) was added into the suspension (Sunil & Lee, 2008). After incubated in an orbital shaking incubator (150 rpm, 60 min), the suspension was centrifuged (5,000 g, 15 min, 4°C) and filtered through 0.45 μm filters to collect LB-EPS. Bottom sediments were resuspended with an extraction buffer (2 m*M* Na_2PO_4, 4 m*M* NaH_2PO_4, 9 m*M* NaCl, and 1 m*M* KCl, pH 7) to the original volume for the further extraction of TB-EPS by adding 70 g/g VSS of gel cation exchange resin then oscillating (150 rpm, 60 min). With high speed centrifugation (10,000 g) for 15 min, TB-EPS was obtained in the supernatant (after filtered through 0.45 μm filters).

The EPS yields were mainly polysaccharides and proteins, as the main components of EPS (Gao et al., 2008). A modification of the anthrone method was applied for measurement of polysaccharide content in EPS with glucose as the standard (Raunkjaer et al., 1994). The protein content in EPS was measured by the Lowry method using bovine serum albumin as the respective standards (Lowry et al., 1951).

2.4. Methodology

Bacterial biofilm formation usually involves different physiological states, including advection of free-swimming cells to the surface, initial attachment, lag phase, and then exponential growth to a quasi-steady state, and possible detachment (Hall-Stoodley & Stoodley, 2002). A logistic growth pattern is used

to model the growth of the bacteria in different bed layers with increasing incubation periods. The biomass at any time can then be derived as (Cunningham & Cunningham, 2002; Tsai, 2005)

$$X(t) = \frac{K}{1 + Ce^{-rt}} \tag{3}$$

where $X(t)$ is bacteria number or biomass at time t. Here we use bacterial dry biomass per dry weight of sediment sample (mg g^{-1} DW) to represent. K is the maximum carrying capacity of the environment, which can be obtained by the regression of experimental data to the logistic growth model; C is a parameter derived from the logistic growth model; and r is equivalent to the average specific growth rate (SGR) of the bacteria (d^{-1}).

The growth pattern is coregulated by both internal metabolic and external environment factors, and the end population size reflects the adaption of the microbes to the ambient environment. When resources are abundant (e.g., carbon and nitrogen sources) and environmental conditions allow (i.e., light, temperature, and hydrodynamic conditions), the microorganisms can grow exponentially. However, after that, the growth rate (GR) drops asymptotically to zero as the maximum carrying capacity of the environment is reached.

The growth rate (GR) can be obtained as

$$GR = \frac{dX(t)}{dt} = \frac{rKCe^{-rt}}{\left(1 + Ce^{-rt}\right)^2} \tag{4}$$

Similarly, the SGR at time t can be obtained by

$$SGR = \frac{1}{X(t)}\frac{dX(t)}{dt} = \frac{rCe^{-rt}}{1 + Ce^{-rt}} \tag{5}$$

3. Results and Discussion

3.1. Experimental Results of EPS-Sediment Bed Growth

The experimental bacterial biomass development fitted well with the logistic growth curves (Figure 2). The correlation coefficients (R^2) for this regression method were 0.99, 0.98, 0.97, 0.97, and 0.93 for the biomass data of the layers 0–0.2 cm, 0.2–0.5 cm, 0.5–0.8 cm, 0.8–1.3 cm, and 1.3–1.8 cm (depth below the bed surface), respectively. Generally, time required to achieve the equilibrium state was different from layer to layer and increased with the layer depth. The growth patterns for the top two layers (0–0.2 cm and 0.2–0.5 cm) were similar, where biomass both reached a stable state after about 2 weeks of incubation. In contrast, the period was up to about 25 days for the mature biofilm to be developed at a layer depth of 0.8–1.3 cm. In the bottom layer, the biomass value changed slowly with time, with a tendency to increase even at the end of the incubation. Apart from the differences in reaching the asymptotic condition, the final biomass values also showed a divergence in vertical distribution. The value in the surface layer was much greater than that in the bottom layer, almost twice the value (10.2 versus 5.8 mg g^{-1} DW, for the top and bottom, respectively). This phenomenon might be explained as follows: the bed surface was more exposed to the hydrodynamic exchange, which promotes the transport and diffusion of nutrients into the surface biofilm. In addition, oxygen will become limiting to aerobic organisms and is known to decline rapidly with depth in depositional systems, although this effect is more extreme for very cohesive sediments. This stimulation effect and readily supply of nutrient would become weaker in the deeper layers because of cellular uptake and decrease in advective supply.

The growth characteristics of different layers in the established biosedimentary beds with various incubation time reflected the adaptability of *Bacillus subtilis* to the environmental condition. The mean parameters of K, C, and r were obtained by the regression method of the experimental data from the logistic growth model for the $X(t)$ (Table S1 in the supporting information). It is recognized that the microenvironment provides more favorable conditions for growth of the bacteria if higher GR or SGR is observed (Chao et al., 2014; Tsai, 2005) (Figure 3). Generally, the GR curves exhibited different patterns and peak values for the different layers. The peak *GRs* of the top two layers (0–0.5 cm) were evidently greater than that of the bottom layers (nearly 3 times). In addition, the time for reaching the peak GR gradually increased with increased depth. Maximum GR occurred within about 7 days for the surficial biosediments, but this was delayed to more than 10 days

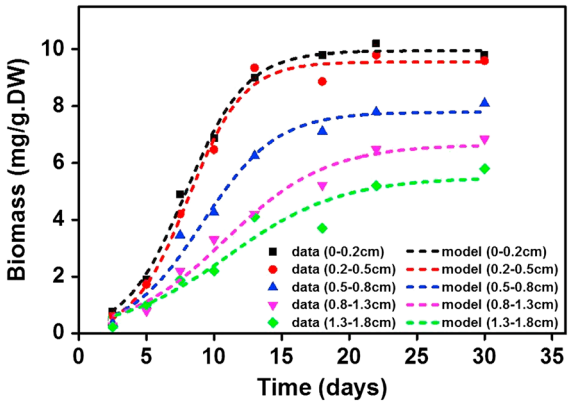

Figure 2. Biomass growth data in sediment bed sectioned in five layers and fitted curves by logistic growth model, respectively. The correlation coefficients (R^2) were 0.99, 0.98, 0.97, 0.97, and 0.93 for the biomass data of the layers 0–0.2 cm, 0.2–0.5 cm, 0.5–0.8 cm, 0.8–1.3 cm, and 1.3–1.8 cm (depth below the bed surface), respectively.

for the 1.3–1.8 cm layer (Figure 3). The SGRs of the surface biomass (at 0–0.5 cm) were much higher than those at the bottom 1 cm before the quasi-steady states were reached (after approximately 10 days). However, the SGR for the top biosedimentary beds decreased rapidly after that, asymptotically to zero. In contrast, the bottom layers showed a tendency to continue a slight increase of the biomass over time (Figure 2), with positive values of GR and SGR even at the end of 20–30 days (Figure 3). The maximum values for K and C from this regression model were obtained at the surficial layers (9.95 and 57.39 mg g^{-1} DW, respectively). This can be explained local condition favoring the growth (Stoodley et al., 1997). Furthermore, the 0.5–0.8 cm layer still retained a relatively high activity (Figures 2 and 3), which reflected biofilm development as more than a "surface phenomenon." Because of the permeability of the sediment, the microbial colonization on this substratum showed a clear vertical penetration (Van Colen et al., 2014).

3.2. Effects of Bed-Age on the Distribution and Composition of EPS

3.2.1. EPS Content on the Bed Surface and in the Bottom Layer

The bacterial biomass down the depth of the biosedimentary beds displayed significant difference in the growth rate ($p < 0.01$), and the surface and bottom layers of the beds were selected to perform further analysis on EPS distribution. The penetration of the biological effects down the sediment beds was then investigated. EPS is composed of polymer materials such as polysaccharide, protein, and humic acid polymers that provide the important binding capacity of the hydrated matrix (Flemming et al., 2000). Basically, EPS can be classified in two main fractions: colloidal EPS that is soluble in water and secreted in the vicinity of cells and bound EPSs that are tightly attached to the cell wall. The total polysaccharide/protein and the colloidal/bound EPS contents varied with incubation time (Figures 4a and 4b), for both the surface and bottom layers in the biosedimentary beds.

Generally, the surface layer of sediment was more abundant in both total polysaccharide and total protein than the bottom layer (Figure 4a). At the steady state (around 22 days of incubation), the total polysaccharide content in the surface layer reached a stable level (about 120 μg g^{-1} DW), outweighing that of the bottom layer (of approximately 80 μg g^{-1} DW). In terms of the growth pattern, both the main components in the surface biofilms showed a rapid increase until around day 10 after which the biofilm became mature, and a stable condition was gradually achieved. In contrast, content in the bottom layer showed limited increases as the growth period advanced. In the surface bed layer, the protein gradually accumulated, but the amounts of total polysaccharide were nearly 5 times higher than those of total protein. Almost no protein developed at the bottom of the sediment bed. The composition of polysaccharide had usually been considered as a main focus in previous studies because the research on biostabilization focused initially on microalgae that secrete large proportions of polysaccharides. In addition, much of this knowledge is derived from investigations on

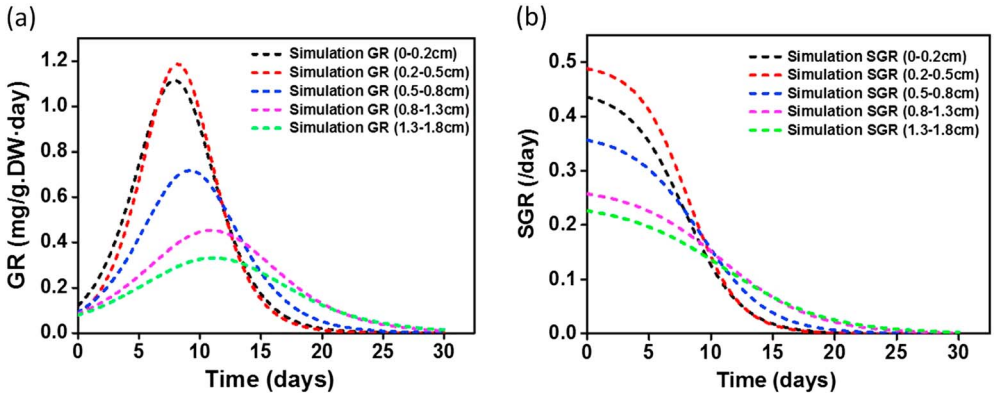

Figure 3. Biomass growth parameters of five sediment bed layers (with depth from the surface). (a) Simulation growth rates (GRs) of biomass in different bed layers. (b) Simulation-specific growth rates (SGRs) of biomass in different bed layers.

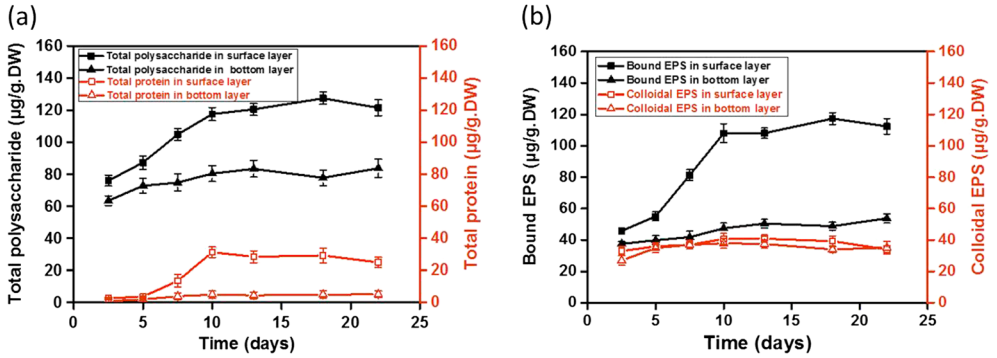

Figure 4. Extracellular polymeric substances (EPSs) in the surface sediment layer and in the bottom layer. The contents varied with bacterial growth, with variation of (a) total polysaccharide and total protein in EPS and (b) soluble and bound EPS fractions.

other forms of microbial aggregations such as planktonic species (Gerbersdorf & Wieprecht, 2015; Underwood & Paterson, 2003).

EPS polysaccharides can form complex networks as shown in the SEM images (in Figure 5). While for the proteins, current knowledge suggests that they promote the growth of microbes and with evidence on the structural role of proteins (Pennisi, 2002). Although some studies show that bacterial EPSs contain a large proportion of proteins (Flemming et al., 2000; Nielsen et al., 1997), the protein/polysaccharide ratio is difficult to predict and might be sensitive to the environment conditions with different microbial species reacting differently. Also, many researchers work on biostabilization using xanthan gum (commercially produced bacterial polymer) as an EPS proxy to provide biocohesion, which contains no protein (Malarkey et al., 2015; Parsons et al., 2016). Even though real biofilms have been cultivated, focus is usually given to the carbohydrate fraction (Orvain et al., 2003; Tolhurst et al., 2008). In our study, attention has been given to both of the polysaccharide and protein. Results showed that the protein eventually comprising nearly 15% of the total EPS in the surface as the biofilm became mature (Goto et al., 2001; Van Duyl et al., 1999). A possible explanation was that the EPS protein was produced in greater quantities as the biofilms ages; thus, protein production lags behind polysaccharide production (De Brouwer & Stal, 2001; Gerbersdorf & Wieprecht, 2015; Nielsen et al., 1997).

After the initial growth period (before 5 days), the contents of the colloidal EPS and bound EPS were similar for both the surface and bottom layers (Figure 4). As the incubation time increased, the bound EPS in the surface layer grew sharply (approximately 110 $\mu g\ g^{-1}$ DW) to as high as 2.5 times of the initial value (less than 50 $\mu g\ g^{-1}$ DW), while the colloidal EPS changed little. This indicated different production and decay patterns in different fractions of EPS: bound EPS was enriched following a logistic growth and became relatively abundant in quasi-steady stage, while colloidal EPS stayed constant over time. A similar phenomenon was observed in a mesocosm experiment of 10 day incubation of benthic diatom on sediments (Orvain et al.,

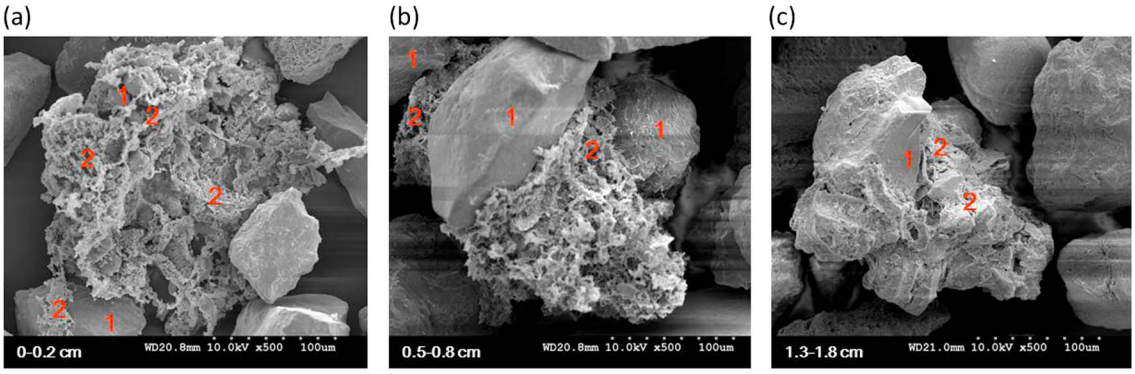

Figure 5. Scanning electron microscope (SEM) images of sediment grain samples taken from three layers (depth below the bed surface: (a) 0–0.2 cm, (b) 0.5–0.8 cm, and (c), 1.3–1.8 cm), in the biosedimentary bed after 22 days of incubation. Some (1) sediment grain surfaces are exposed, but (2) some are submerged in the EPS matrix.

2003). However, in this study, both the colloidal and bound EPS in the bottom layer were generally equivalent but at a much lower level (with a range of only 30–60 μg g^{-1} DW).

It was confirmed that the EPS in newly established biosedimentary bed exhibited different spatial distributions, depending on the bed ages and the bed depth. One possible explanation is that on the top layer, biofilms developed at the bed interface, where nutrients exchange and transport processes were optimal. Therefore, production of EPS was stimulated in response to the better condition, gradually forming the stable biofilm on the bed surface. Consequently, the total EPS in the surface layers were greater than lower in the bed (Figure 4). In addition, in the deeper layers where labile organics were scarce, the naturally produced EPS might be consumed as a substrate for the microbes (Laspidou & Rittmann, 2002), so that the EPS turnover may be much more rapid at the bottom bed.

3.2.2. Evolution of EPS Content on the Depth Profile of Sediment Bed

Microbial secreted polysaccharides are responsible for both adhesion and cohesion, which play a crucial role in maintaining the structural integrity of biofilms (Chen & Stewart, 2002). In biotechnology, many types of EPS polysaccharides have been explored for commercial applications as stabilizers, emulsifiers, or gels to improve cohesive strength. In research work, xanthan gum was commonly used as an EPS proxy to represent biocohesion effect on sediment properties as compared with physical cohesion (Parsons et al., 2016). EPS polysaccharides seem to contribute considerably to the observed binding effects, while proteins may promote the growth of microbes and thus support biofilm formation, with positive effects on sediment stability (Gerbersdorf & Wieprecht, 2015). There is also increasing evidence on the structural role of proteins, but these functions would be highly dependent on the nature of the proteins (Flemming, 2011; Pennisi, 2002). In our study, structural EPS was observed in the upper layers of sediment bed, which extended to a depth of 0.8 cm after an incubation period of 22 days (Figures 5a and 5b), nearly half the bed depth below the bed surface.

Scanning electron microscope (SEM) images of three layers (0–0.2 cm, 0.5–0.8 cm, and 1.3–1.8 cm, of the depth below the sediment surface) for the biosedimentary bed after 22 days illustrated the EPS and sediment grain morphology (Figure 5). Biological effects for the top 2 mm layers were significant (Figure 5a). Almost all grain surfaces were embedded under the EPS matrix. EPS polysaccharides often show various degrees of branching forming complex networks and EPS structure (Pennisi, 2002; Wotton, 2004). As a result, after the boost in EPS production and followed by a period of gradual biofilm maturation, most sediment grains were wrapped with an EPS matrix. In traditional models for sediment transport, only physical and chemical forces are taken into consideration. However, in our study, the bridging effects of EPS strands enhanced the links between individual sediment particles to provide the noncohesive sediment with the characteristics of cohesive sediment. In addition, fine grains were stabilized by being attached to larger particles via this biological binding. In consideration of the above, biological cohesion was demonstrated to play an important role compared to the abiotic factors. From 0.5 to 0.8 cm in depth (Figure 5b), grain surfaces were more exposed from the EPS web, but the effect of EPS filling the pores was still evident. In the bottom layer (1.3–1.8 cm, Figure 5c), the localized effects were only observed as EPS patches, but bridging appeared to be weak (connected only between grains with smaller particle sizes).

The vertical profiles of EPS (colloidal, loosely bound, and tightly bound EPSs) polysaccharide distributed in the sediment beds changed with the increase of bed age, from the initial incubation to the end of 22 days (Figure 6). Biological effects for fine sediment usually reflect an increasing critical shear stress for erosion through high surficial levels of EPS providing an initial layer of protection (Sutherland et al., 1998; Tolhurst et al., 2006, 2009). In this research, noncohesive and very fine sand (D50: 98.3 μm) was investigated under bacterial colonization. As the growth period increased, variations of EPS concentrations on the bed surface reflected the process of biofilm formation. Besides the surface colonization, this EPS matrix was also capable of penetrating downward between the pores, throughout the sediment bed depth. The depth profile of EPS changed little at the initial stages (Figure 6), when the microbes colonized the bed surface and began to grow. During the period of 5–7.5 days, the growth rates (GRs) in all the five layers increased and most rapidly in the top layer, where EPS developed on the bed surface in the form of a biofilm. Consequently, the slope of the EPS vertical profile became greater after 7.5 days of cultivation, which indicated a divergence of the EPS distribution over bed depth. Before the surface biomass reached a stable state, the GR rose to the maximum value and then decreased, but the EPS kept accumulating in the surficial

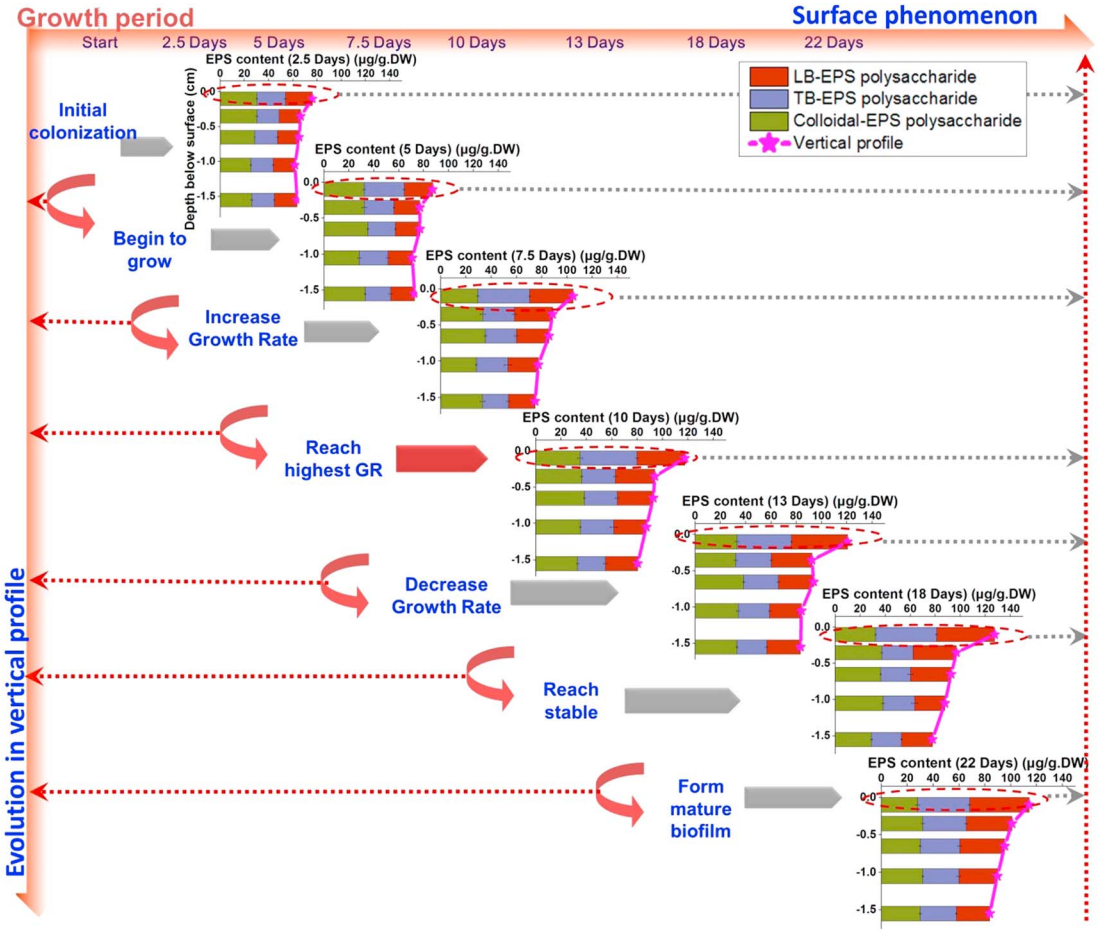

Figure 6. Vertical profiles of EPS in the biosedimentary bed that change with growth stages.

biofilms, resulting in a continuously and progressively increase in the slopes of the vertical profiles. When the mature biofilm formed on the bed surface, the EPS content fluctuated around a constant (i.e., 120 $\mu g\ g^{-1}$ DW in this study), but the EPS in the sublayers continued to increase. In response, the slope of the EPS vertical profile flattened. This implied that the EPS had the potential to extend to greater depths over time, despite the high concentration on the surface. For sand with larger grain diameter (e.g., D50 $>$ 250 μm), such a surface phenomenon becomes weak, but a pervasive distribution of low levels of EPS throughout the sediment plays the key control on bed form dynamics (Malarkey et al., 2015). This means that biostabilization may have different effects and working mechanism mediating sediment movement, depending on grain size, which might be related to the penetrating ability of EPS matrix because of different void size between particles.

By comparing with one of the field samples taken in autumn (Figure 1), it was noticed that the vertical profiles of EPS on tidal flats differed from those obtained in laboratory. The biological processes in the natural sedimentary environment are much more complex than that for the incubation and are clearly related to the variations of coastal environmental conditions and related ecology. One of the inevitable factors is the high biodiversity and variation in dominant species of the mixed assemblages with seasonal changes. Consequently, EPS production fluctuates, with microbial growth and metabolism as they are adapting to the temperature, irradiance, and nutrient supply. Another dynamic condition in the intertidal zones is caused by the daily variation (i.e., the flood and ebb tides) and the cyclic variation (i.e., spring-neap tidal modulation). The alternation between high and low levels of flow velocity (bed shear stress) determines the sediment movement and transport, accompanied by the microbial communities; during which, the EPS spatial distribution would be recasted (Mariotti & Fagherazzi, 2012). Under this hydrodynamic redistribution, the period of

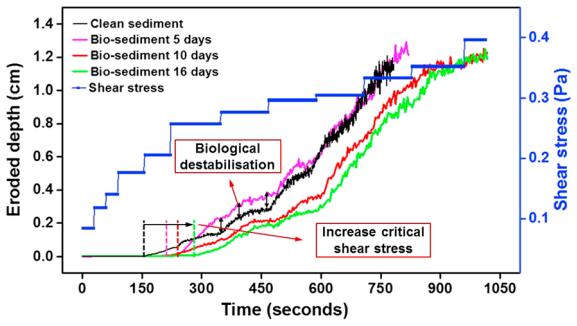

Figure 7. Erosion curves of biosediment (5, 10, and 16 days) and controlled clean sediment represented by eroded depth value increasing with stepwise increment of shear stress (Chen et al., 2017).

time needed for the development of permanent biofilms and microbial mats could be unpredictable. Therefore, the features of EPS profiles in the natural conditions are also difficult to predict.

3.3. Bed Stability Reprofiled by EPS Adhesion

As the vertical profiles of EPS evolved with time inside the biosedimentary, the biofilms and sediments became coupled within the system, increasing the resilience of the surface to hydrodynamic forcing. Erosion processes were significantly mediated by bed age-associated biostabilization (Figure 7) (see Chen et al., 2017 for a full and detailed discussion). The effect of EPS on time-dependent eroded depth was derived from the SSC plot (the original data obtained from OBS3+ can be found in Table S2, and the assumptions used for the calculation can be found in Text S2). The erosion curves provide direct evidences of how the accumulation of bacterial EPS altered the sediment properties along the depth profile (~cm); hence, the main findings from Chen et al. (2017) are summarized herein. Generally, as the erosion time increased, the erosion depth in the sediment beds was restricted for biosedimentary beds as compared to treated cleaned. First, it was noted that the erosion threshold (the point that the eroded depth value became positive) increased due to biological stabilization. The antierosion effect was strengthened with time as biosedimentary beds tended to withstand higher shear forces with the extension of growing period. However, for the very young biosedimentary beds, the early biological activity reduced the bed strength. It was shown that the biosedimentary beds were eroded to deeper layers after 5 days than the control. This indicated that initial biological activities destabilized the subsurface layers of the sediment bed, although, despite this, the erosion threshold for the bed surface was increased. This was attributed to the properties of different types of EPS. Colloidal EPS, which is soluble in water, maintains high pore water in sediment bed, while bound EPS provides more cohesion (de Brouwer et al., 2005; Gerbersdorf et al., 2008; Orvain et al., 2003). During the early period of biofilm development, the colloidal EPS contents along the profile were similar to loosely bound and tightly bound EPSs (represented by EPS polysaccharide). Consequently, at this stage, water content effects played the leading role as opposed to binding effects, which was deemed to be a negative factor for bed consolidation hence decreasing the bed strength and sediment stability.

As discussed in section 3.1, some flow in the biosedimentary system favored biofilm development with more exchange of nutrients. Whereas for the deeper layers, the stimulation effects became weak because of a decrease in the hydrodynamic exposure and change in oxic conditions. On the other hand, the surface biofilm was eroded first and the critical shear stress varied dependent on the structure, biomass, and thickness (Hall-Stoodley & Stoodley, 2002). Therefore, in the erosion experiment, the surface biofilm acted as the first-layer of protection. The erosion on the sublayers only occurred after the failure of the structure and the detachment of the surface biofilm. Furthermore, we observed that biostabilization did not disappear after the erosion of top layers. Measurements of time series demonstrated that erosion was limited with the cultured biosedimentary beds, suggesting that biostabilization was still effective (hindered erosion). As the incubation period increased, a limit was reached for the time (and shear stress) of the initial surface erosion. Smaller differences were then observed between the biosedimentary experiments after 10 days and 16 days, demonstrating that under the experimental conditions, biostabilization reached (or approached) a stable state after 2 weeks of. This quasi-steady state for stability can be linked to the slow changes in EPS profile during this period, as the biofilm progressively reached the final equilibrium in its vertical distribution (Figure 6). It should be noted that in natural cases where applied shear stress, temperature, light, etc. vary temporally, different periods might be needed to reach equilibrium, or it may not be reached at all.

4. Conclusion

This study confirmed that the bacterial (*Bacillus subtilis*) secreted EPS in newly established biological-sedimentary bed exhibited different depth distributions and properties as bed age increased. Apart from the accumulation on the surface, EPS also penetrated the depth profile, but the content in the surface layer was almost double that in the bottom layer. Scanning electron microscope (SEM) images of layers also showed the different micromorphological properties of sediment-EPS matrix. The two distinct fractions of

colloidal and bound EPS accumulated at different rates. The accumulation rate for bound EPS changed considerably during the transition from the logistic growth phase to the stable phase, while almost no change in the colloidal EPS content was observed. As incubation time increased, the effects on the bed stability changed from negative to positive increase in terms of erosion resistance, which might be attributed to the different functions of colloidal and bound EPSs. The bed stability was reprofiled because of the depth distribution of different EPS fractions. The engineering function of the EPS in sediment bed was not only surficial but also reflected in the erosional behaviors of subsurfaces and varying with the bed age. In addition, by comparing with one of the field samples, it was noted that the vertical profiles of EPS on tidal flats differed from the laboratory. The features of the EPS profiles in natural systems are dependent on a variety of environmental factors and much more complex to predict. Nevertheless, this study contributes to the growing body of work exploring the role of the bacterial communities on sediment erosion and provides evidence of the importance of the microbial community in one type of system. Further research is needed to better understand the natural variation of the EPS distributions in terms of different biological effects and to move toward predictive capability for engineering purposes.

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