The effect of cyclic variation of shear stress on non-cohesive sediment stabilisation by microbial biofilms: The role of "biofilm precursors"

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Abstract

Biofilm mediated intertidal sediments exhibit more complex erosional behaviour than abiotic systems. A major feature of intertidal systems is the exposure to repeated cycles of high and low shear created by tidal conditions and also less predictable episodic events, such as storms. There is very little information on how biofilm-forming communities respond to these conditions. In this study, the effects of both single and repeated-cycles of shear on the stability of newly developed bio-sedimentary beds was examined. Cleaned sand, removing any potential biostabilisation, was used as the control. For the single-cycle scenario, biofilms were incubated on a non-cohesive sandy bed under prolonged low shear periods varying between 5 and 22 days after which erosional stress was applied. No significant biostabilisation was observed for the youngest bio-sedimentary bed (after 5 days of low shear incubation). After 22 days, microbial communities were characterized by a firmly attached surface biofilm. To cause erosion, greater hydrodynamic stress (0.28 Pa) was required. The erosional behaviour of the underlying sand was also affected in that bedform ripples noted in the control system were no longer observed. Instead, a sudden "mass erosion" took place (0.33 Pa). The one-cycle scenario indicated that significant biostabilisation of sand only occurred after a relative long calm period. Under repeated cycles of stress (5 days of low

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stress followed by high stress event and re-incubation, repeated for 4 cycles =20 days), frequent cyclic disturbance did not degrade the system stability but enhanced biostabilisation. The properties of the sub-surface sediments were also affected where erosion rates were further inhibited. We hypothesize that organic material eroded from the bed acted as the "biofilm precursor" supporting the development of new biofilm growth. A conceptual framework is put forward to highlight the dynamics of bio-sedimentary beds and the effects of growth history under repeated-cycles.

Keywords

Biostabilisation, biofilm detachment, sediment erosion, biofilm regrowth, bio-sedimentary system

Highlights

- Biostabilisation of bio-sedimentary beds with and without cyclic stress is compared
- The erosional behaviour of the underlying sand was affected and transition of bed structures were observed
- A conceptual erosion framework is proposed for bio-sedimentary systems recognizing the variation of different growth stages and cyclic effects

Introduction

The early attempts to interpret processes that operated in sedimentary environments hinged on empirical observations, and were based almost exclusively on non-cohesive sand. The relationship between hydrodynamic stress and particle size, such as the Shield's curve, is widely used to predict sediment transport [*Miller et al.*, 1977; *Soulsby*, 1998]. Such a relationship has proved to be accurate for abiotic well-sorted quartz sands, but becomes less reliable as the context of natural environments is included such as natural deposits being composed of mixtures of sediment deposits and the further influence of their microbial assemblages [*Flemming*, 2002]. At times these assemblages develop extensive biotic structures, which provide biological cohesion and influence the microstructure and behaviour of the bed [*Paterson*, 1994]. Intertidal biofilms, consisting of microorganisms and their secreted extracellular polymeric substances (EPS) suffuse almost all aquatic sedimentary environments and have been shown to influence coastal surface processes and bedforms [*Gerbersdorf et al.*, 2009; *Malarkey et al.*, 2015; *Paterson*, 1989; *Paterson and Daborn*, 1991; *Van Colen et al.*, 2014]. In this respect, *Paterson and Daborn (1991)* defined "biostabilisation" as "a decrease in sediment erodibility caused by biological action" and this is found to be commonplace. A major mechanism of biogenic stabilisation is the production of extracellular polymeric substances (EPS) by sediment-inhabiting organisms, that can cohere sediment particles, hence increasing sediment stability [*Paterson*, 1989]. The accumulation of EPS can form an organic polymeric network that provides a three-dimensional architecture [*Barzeev et al.*, 2015; *Flemming*, 2011; *Hall-Stoodley and Stoodley*, 2002; *Stoodley et al.*, 2002]. The role of EPS in biofilms is complex but includes cell-to-cell bridging, and transient and irreversible attachment to the surfaces [*Barzeev et al.*, 2015; *Hans-Curt and Jost*, 2010; *Nadell et al.*, 2009].

Natural sediments support varying populations of organisms which significantly alter the response of sediment to physical forcing. As influenced by microbial metabolism, the bio-sedimentary matrix responds to both ecological (light, nutrients, pollutants) and physical (hydrodynamic conditions and meteorological forcing) drivers and exhibits more complex and variable characteristics than abiotic systems [*Celmer et al.*, 2008; *Chen et al.*, 2017b; *Fang et al.*, 2017; *Montague*, 1986; *Paterson et al.*, 2008; *Schmidt et al.*, 2018; *Stone et al.*, 2008]. For instance, one of our field observations, on a tidal flat, showed that natural biofilms were heterogeneously distributed and that biofilm thickness varied across the flat, leading to a highly complex surface structure (**Figure 1**). As a result, areas with biofilm cover were better protected against hydrodynamic forces and required greater energy to be eroded (**SI Video 1**). Sediment grains sheltered underneath the biofilms remained stable until the flow exceeded the upper critical value to slough off the biofilms and then erode the sediments. In contrast, the uncovered surface was easily eroded, with particles either directly suspended or transported as bedload.

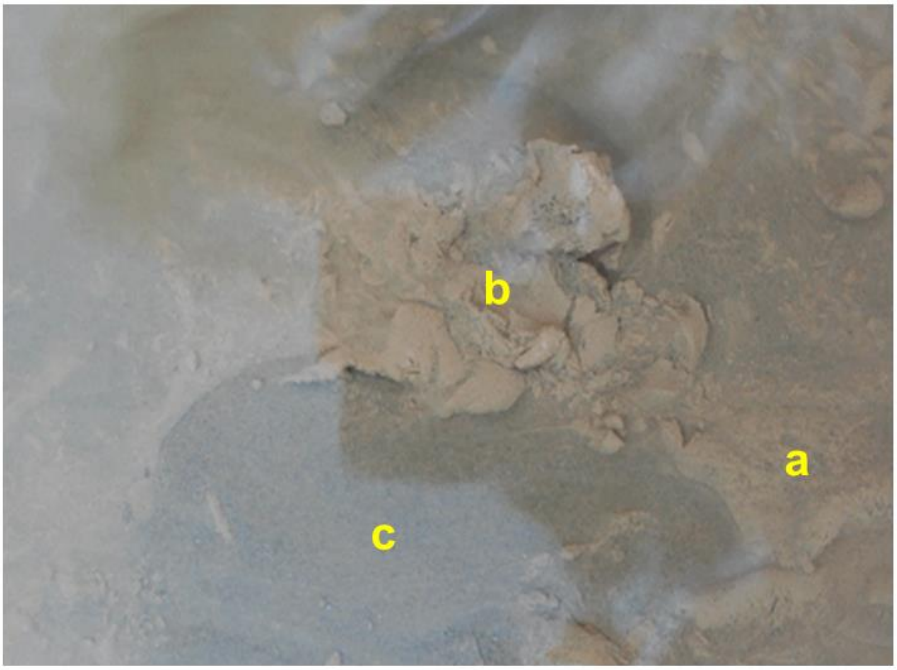

Figure 1. Example of the surface sediment at Site S7 in winter (see site information in *Chen et al.*, 2017b). Tidal flat surface with three different features are shown (a) of natural sediment surface covered with biofilms which form extensive brown mats of bacteria and microphytobenthos, (b) with eroded but not fully detached (flipped-over) biofilm and (c) of the exposed sediment beneath the biofilm.

Despite the many studies that have investigated biofilms *in situ* [*de Winder et al.*, 1999; *Montanie et al.*, 2014; *Orvain et al.*, 2014; *Passarelli et al.*, 2015; *Underwood and Paterson*, 1993], much of our understanding has come from laboratory experiments [*Hagadorn and Mcdowell*, 2012; *Orvain et al.*, 2003; *Tolhurst et al.*, 2008]. Most laboratory experiments tended to examine biofilm growth under steady shear stresses [*Chao et al.*, 2014; *Cochero et al.*, 2015]. This more accurately represents conditions in freshwater riverine system. Examining bio-sedimentary bed development under constant flow is a natural place to start when studying biostabilisation. However, a major feature of intertidal systems is the multiple cycles of high and low turbulent shear with the tidal rise and fall. Biota living in the intertidal zone and coastal embayments are adapted to this highly dynamic environment dominated by the periods of exposure to high stress, particularly the spring-neap tidal cycles and/or storm conditions [*Keyvani and Strom*, 2014; *Valentine et al.*, 2014; *Widdows and Brinsley*, 2002]. Calm periods of low shear forces allow biofilm growth while episodic events (i.e., high shear) can lead to biofilm destruction and subsequent erosion [*Mariotti and Fagherazzi*, 2012]. The disturbance intensity often varies as a result of natural variability, i.e., different lengths of calm periods may allow biofilm growth to different levels of biostabilisation. In addition, the variations of the tidal modulation may lead to cycles of bio-sedimentary bed production, breakdown, and redistribution [*Chen et al.*, 2017; *Malarkey et al.*, 2015; *Underwood and Paterson*, 1993]. Such cyclic hydrodynamic conditions are quite different from the steady-state condition often examined in the laboratory [*Le Hir et al.*, 2007; *Mariotti and Fagherazzi*, 2012]. This study attempts to reveal the effect of cyclic changes in stress on the integrity and biostabilisation capacity of bio-sedimentary systems.

This study was aimed at understanding biostabilisation development on newly formed bio-sedimentary beds with no incubation history, and then the effects of incubation under repeated cycles of potential disturbance. Both single and repeated-cycles of incubation and erosion were reproduced in the laboratory where applied bed shear stress can be precisely controlled and the suspended sediment concentration conditions accurately measured. The specific research questions were: (1) How do erosional behaviours of newly developed bio-sedimentary beds change with age? (2) How does growth history affect the re-establishment of biostabilisation and bed structure? (3) Does biostabilisation still exist under frequent repeated disturbance, or do beds eventually decay to the non-biological condition?

Material and methods

The experimental setup in this study used seven identical 20 L benthic chambers (290 mm diameter × 300 mm high), each with a rotating paddle (**Figure 3**). The device is an improved version of the UMCES Gust Erosion Microcosm System (U-GEMS) [*Thomsen and Gust,* 2000] and Core Mini Flume (CMF) [*Thompson et al.,* 2013]. The chamber was used for both bio-sedimentary bed cultivation and for determining sediment erosion thresholds by adjusting the rotation speed of the paddle. Details of the benthic chamber can be found in *Chen et al.*, 2017b. One chamber was used as a control in which the treated clean sediment was eroded in artificial sea water (ASW, salinity of 23 ‰); five were used for single-cycle scenarios with different cultivation periods (four for incubation days of 5, 10, 16 and 22 respectively and one for sediment sample extraction and analyses); and another one was for the repeated-cycle scenario. For bio-sedimentary bed growth, clean (treated) sands were incubated in microalgae-rich (>10^6 cell·m^{-3}) ASW with added nutrients. Microalgae species were selected from the Jiangsu coastal region (algal powder supplied by Jiangsu Zhenxing Bio-technology CO.，LTD, and was activated before experiment). The chambers were maintained at a temperature of 26 ± 2 ℃ in ASW. The nutrient media consisted of: 0.05 g·L^{-1} tryptone (0.075 g tryptone m^{-2}·day^{-1}), 0.03 g·L^{-1} NH_4Cl (0.045 g NH_4Cl m^{-2}·day^{-1}), 0.006 g·L^{-1} KH_2PO_4 (0.009 g KH_2PO_4 m^{-2}·day^{-1}), 0.015 g·L^{-1} Na_3SiO_3·$9H_2O$ (0.0225 g Na_3SiO_3·$9H_2O$ m^{-2}·day^{-1}) and 0.003 g·L^{-1} $FeC_6H_5O_7$·$5H_2O$ (0.0045 g $FeC_6H_5O_7$·$5H_2O$ m^{-2}·day^{-1}). Planktonic growth was limited by replacing half of the reactor volume with a fresh medium every 2~3 days. The light intensity was permanently set using fluorescent tubes (I = 36 μmol·m^{-2}·s^{-1}, reproducing moderate "natural" conditions), following a 10/14 hour day-light cycle.

We conducted single- and multiple- cycle scenarios. For the single stress scenario, biofilms were incubated on non-cohesive sands under calm low shear periods varying between 5 and 22 days before the erosional stress was applied (**Figure 2a**). Calm low shear referred to a constant bed shear stress (BSS) of 0.06 Pa. A BSS of 0.06 Pa was selected as a reasonable value during neap tide, allowing biofilm growth and was lower than the critical shear stress to avoid the re-suspension of the constituent sand grains [*Shi et al.*, 2016; *Chen et al.*, 2017b]. Scanning electron microscopy (SEM, HITACHI S-3000N, 25 kV, using freeze drying for sample preparation) was employed to visualise the microstructure of the bed grains

at the end of each incubation period. After incubation, the developed bio-sedimentary bed was immediately exposed to a series of stepwise increments of BSS (IS from 0.06 to 0.33 Pa) for approximately 14 min at each level (see full details of the shear stress applied in *Chen et al.*, 2017b). The BSS was dependent on the rotational speed of the chamber skirt and this was not precisely linear with BSS, leading to slight variations between BSS level values. This phase was referred to as "bio-bed erosion". The experimental range of BSS is consistent with field measurements on the Jiangsu mudflat [*Shi et al.,* 2016]. An optical backscatter sensor (OBS-3+) was employed to measure the real-time suspended sediment concentration (SSC) (see Chen *et al.*, 2017b Supporting Information for details of the location and calibration of the OBS). The maximum SSC in this study was 60 kg/m^3 (3.5% by volume).

For the repeated stress cycle scenario, the bio-sedimentary beds were developed under the same calm low shear for 5 days, eroded under high shear and allowed to recover for another 5 days (**Figure 2b**). For cycle 1, the bed was incubated from an initial abiotic state to the first measurement. This cycle was referred to as "no prior growth" (npg) condition. Bed erosion was determined by the same stepwise increment shear stress as for the single-cycle scenario. After erosion occurred, the applied BSS was reduced to zero to allow the settlement of suspended material. The bed was then manually flattened before the next cycle of incubation when the biofilm was allowed to redevelop (low shear value). The flattening procedure (hand scraper) insured a uniform initial bed morphology for each cycle, which allowed better comparison of the erosional behaviour of the bio-sedimentary beds formed during different cycles. This sequence of bio-sedimentary bed incubation (referred to as "bio-bed growth" in **Figure 2b**) at BSS of 0.06 Pa followed by erosion (referred to as "bio-bed erosion" in **Figure 2b**) at BSS increasing to 0.33 Pa was repeated for 4 times.

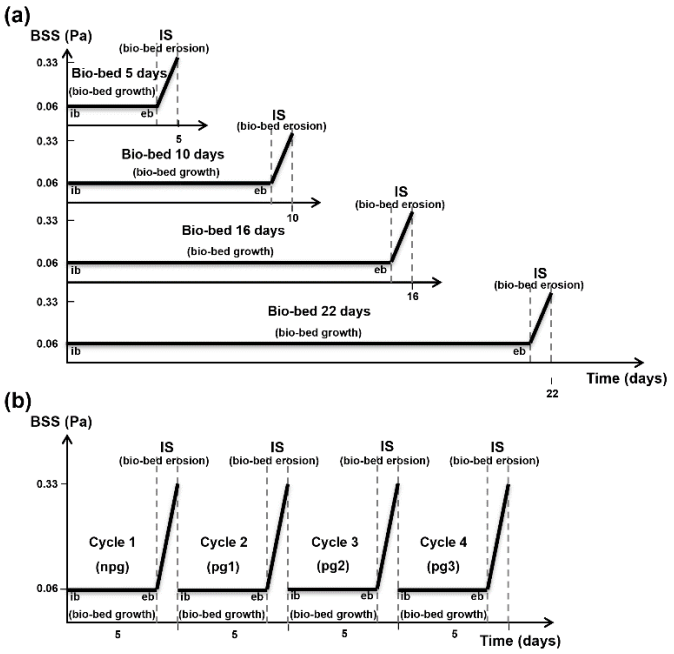

Figure 2. Sequence of experiments with (a) a single-cycle of bio-sedimentary bed incubation for different growth periods (5, 10, 16, 22 days) under low shear stress, after which all the bio-sedimentary beds were eroded by stepwise increment of higher shear stresses; and (b) repeated-cycles of growth (applying low shear stress) and erosion (applying higher shear stress). The abbreviations used in this schematic plot are: "Bio-bed" =

bio-sedimentary bed; “BSS” = bed shear stress; “IS” = stepwise increments of shear stress applied in erosion experiments; “npg” = incubation of clean sediment with non-prior microbial growth; “pg1” = re-established with prior growth 1; etc.; “ib” = initial state of bio-bed at the beginning of each growth phase, and “eb”: ultimate state of bio-bed at the end of each growth phase.

Sediment grains used in this experiment were collected from the lower intertidal zone of tidal flats in the Jiangsu Province (China) and were predominantly non-cohesive fine grains with low threshold for erosion (at Site SM89, see site information in *Chen et al.*, 2017a). The sampled sediment was sieved to remove all the cohesive fraction and washed with hydrogen peroxide to exclude organic material. The median grain diameter (D_{50}) after the treatments was 108 μm (for the grain size distribution see Supporting Information in *Chen et al.*, 2017b).

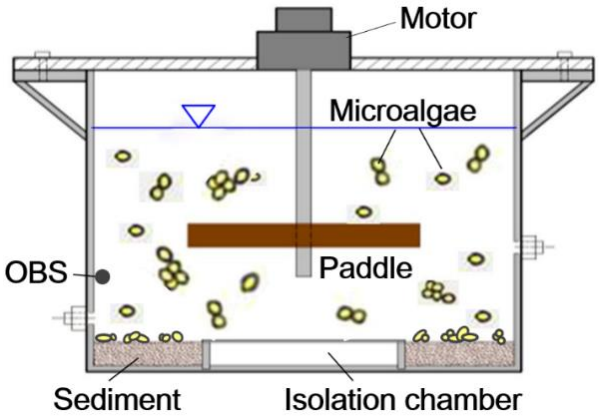

Figure 3. Bio-sediment cultivation and erosion chamber.

Results

For the single-cycle scenario, the erosional behaviour of abiotic quartz sands was progressively altered via microbial colonization (**Figure 4**). A continuous increase in critical erosion threshold was obtained for bio-sedimentary beds aged from 5 days to 22 days, as compared to clean sediment. The threshold for motion of the sand used in this study (D_{50}=108 μm) was 0.15 Pa, obtained from Shields diagram (see *Chen et al.*, 2017b for more parameter information for the calculation). Thresholds of motion obtained from the Shields diagram range from 0.15 to 5.28 Pa (calculated using the function in *van Rijn*, 1993), therefore the sample used exhibited a low resistance to erosion in the absence of biofilm effects. However, the bed strength was greatly enhanced after 22 days of incubation. The bio-sedimentary bed was capable of withstanding an applied BSS up to the last step of 0.33 Pa, an increase of 117 % compared to the clean sand (**Figure 4**). For clean sand and a bio-sedimentary bed of 5 days old, the SSC approached the maximum level (60 $kg \cdot m^{-3}$) after a high stress value of 0.30 Pa. In contrast, only half the amount of bed sand was suspended for bio-sediment after 16 days (with SSC of 30 $kg \cdot m^{-3}$), while the bio-sediment after 22 days only just began to erode. Consequently, the final SSC of the bio-sedimentary systems after 22 days decreased to 40 $kg \cdot m^{-3}$ (33 % of erosion was inhibited compared to the clean sediment characterized by 60 $kg \cdot m^{-3}$).

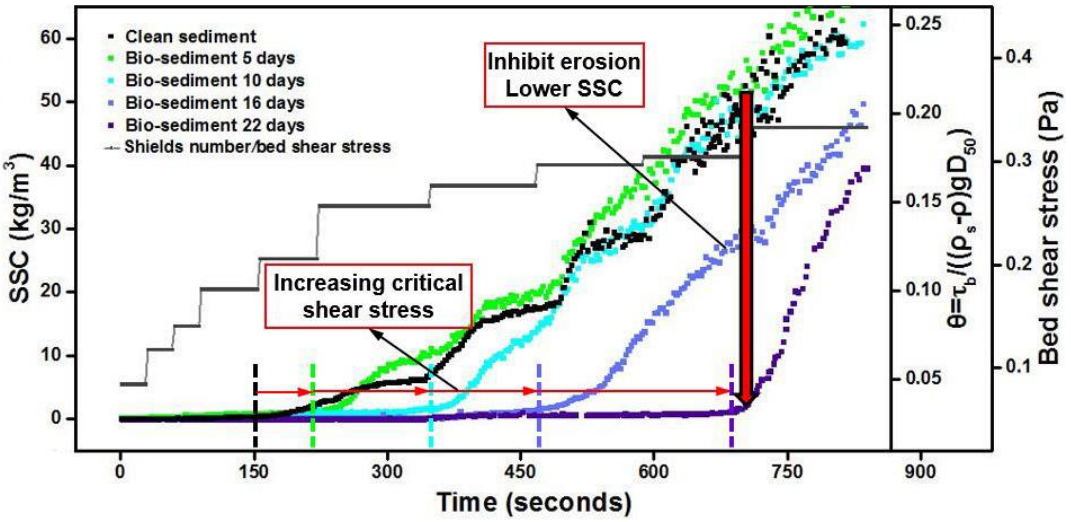

Figure 4. Erosion curves of bio-sedimentary systems developed in single-cycle scenario with different ages (5, 10, 16, 22 days) and clean (control) sediment represented by SSC values and eroded depth increasing with stepwise increments of shear stress during the entire erosion experiment.

After 22 days, the microbial communities were characterised by a firmly attached surface biofilm over the sand (**Figure 5a**). These biofilms, when exposed to greater shear stress at or above 0.28 Pa, were eroded from the sediment surface. However, the detachment of the mature biofilm was not straightforward. It was no longer the case of a "sudden effect" in an "all-or-nothing" fashion, very different from the "fluff layer" which was easily suspended under low shear [*Amos et al.*, 1997; *Mariotti and Fagherazzi*, 2012]. Instead, erosion occurred first through undercutting of the biofilm from a marginal area (**Figure 5b)**. After that, the biofilm was gradually eroded in small fragments from the bed surface (**Figure 5c**). A similar process was illustrated as the formation of "rip-up clasts" in a previous study [*Hagadorn and Mcdowell*, 2012]. The clasts were reported to present quasipolygonal shapes, similar to what was observed here (**Figure 5c and 5d)**. Subsequently, clear erosion edges were formed which were sub-rounded to serrated in shape at their cut ends. This is often referred to as a "Type 1a" erosion (erosion of the surficial organic matters). During this process, surface biostabilisation gradually diminished even though the bio-sedimentary bed still appeared to maintain an overall integrity. No sediment erosion could be determined by naked eye (**Figure 5d)**. However, previous studies have noted that few of the surficial grains entangled and reticulated in the biofilm matrix are likely to be suspended when the biofilm is lifted [*Mendoza-Lera et al.*, 2016].

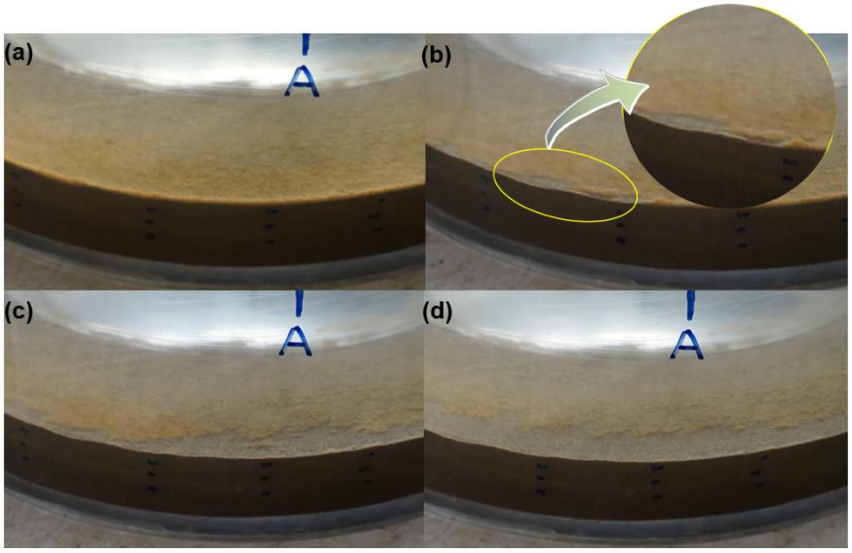

Figure 5. Detachment of biofilms on the surface of the sand bed after 22 days during erosion experiments. Start from (a) low bed shear stress of BSS=0.06 Pa (t=0 s), with stepwise increment of applied forces to (b) BSS=0.28 Pa (t=397 s), (c) BSS=0.30 Pa (t=480 s) and (d) BSS=0.31 Pa (t=600 s).

Low-resolution Scanning Electron Microscope (SEM) images showed an obvious increase of biological infiltration through the sand grains as incubation time increased from 5 days to 22 days (**Figure 6**). After a short incubation period (i.e., 5 days), there were only few visual EPS patches on the exposed clean grain surfaces. This indicated that such a limited period was not enough to establish biological cohesion (in agreement with the erosion experiment showing that the erosion curve for bio-sedimentary systems after 5 days varied little from the experiment using clean sediment, **Figure 4**). As EPS was produced during microbial growth, biological effects appeared in a patchy manner, where grain surfaces were covered and there was localised filling of pores, but many particle faces still remained abiotic (10 days, **Figure 6b**). As the biofilm matured, its binding effect on sediment increased significantly. While initially EPS was confined to small sediment grains, it later spread connecting the small sediment grains into larger aggregates that sometimes included larger sand grains (16 days, **Figure 6c**). During this period, EPS often exhibit various degrees of branching, creating structural integrity and ultimately the formation of complex EPS networks [*Chen et al.*, 2017a; *Gerbersdorf and Wieprecht*, 2015; *Pennisi*, 2002; *Wotton*, 2004]. Bio-cohesion became evident and substantially increased the sediment incipient shear stress (**Figure 4)**. However, large-grain faces that appeared devoid of EPS could still be easily found. The SEM images for bio-sedimentary sediments after 22 days exhibited comprehensive bio-cohesion for the upper visible layer (**Figure 6d**). Although SEM images were used as qualitative support to the presence of EPS and its effect, it should be noted that the preparation of sediments for sampling (i.e., freeze drying) can distort the natural matrix. Therefore, SEM images of polymeric material should be viewed with caution [*Perkins et al.*, 2006].

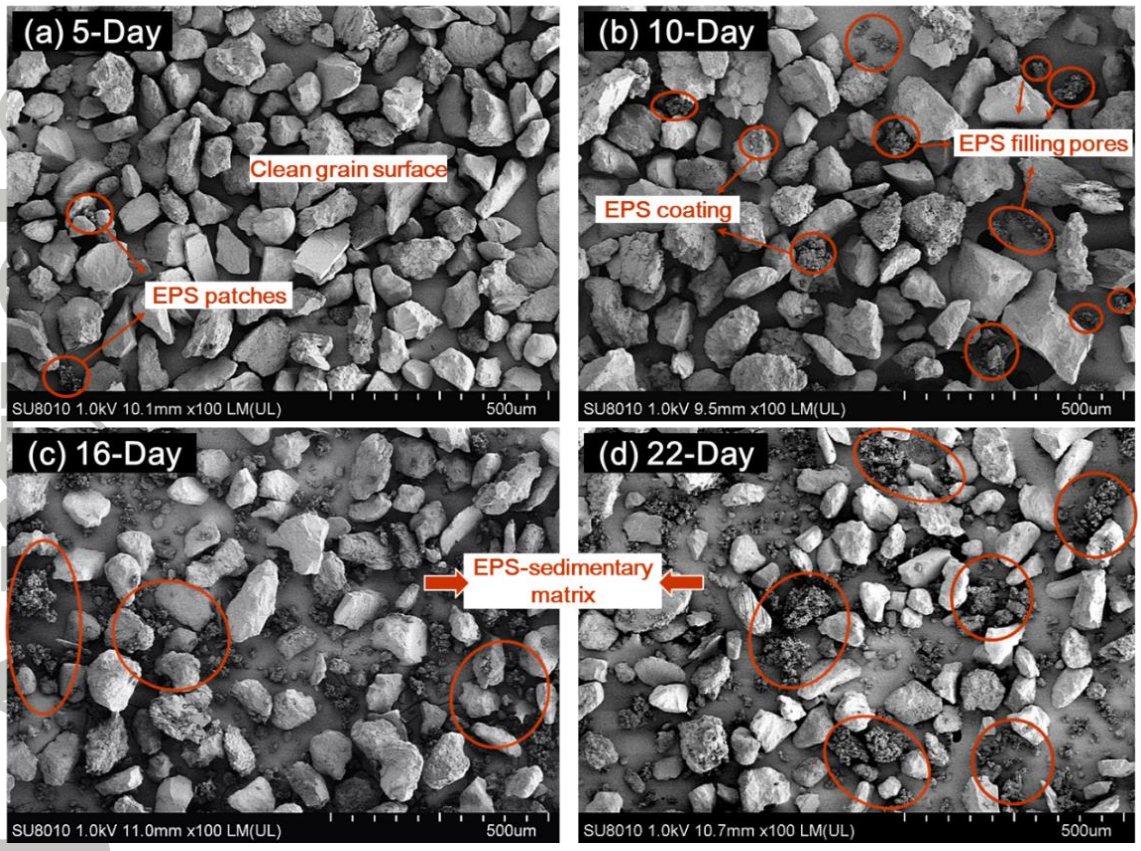

Figure 6. SEM images, illustrating an increase of biological infiltration through the sand grains as incubation advanced from (a) 5 days, (b) 10 days, (c) 16 days to (d) 22 days, samples extracted from the very surficial layer (0-2 mm) at the end of each incubation. Significant accumulation of EPS is shown during bio-sediment development as time advanced. Scale bars are in micrometers (μm).

Results from the erosion test for the repeated-cycle scenario showed a rapid recovery during the calm "growth phase" of the three "pg" cases (**Figure 7**, also referred to as cycle 2-4 in **Figure 2**). Although no obvious biostabilisation effects were observed for the young bio-sedimentary bed (5 days), the critical shear stress began to increase after the second cycle (with one previous growth history "pg1", **Figure 2**). As for the single-cycle scenario, a sharp increase in erosion rates after the surficial failure was observed (**Figure 4**). This supports the early suggestion that the biostabilisation effect was limited as surficial protection [*Le Hir et al.*, 2007]. However, more careful examination of the erosion curves showed that stabilization also developed in the sub-layers under the repeated-cycle scenario, suggesting that the cyclic disturbance may have influenced polymer distribution deeper inside the bed. This was demonstrated by the decline of the gradients of the erosion curves for cycle 2 and 3, compared to the first cycle on a bed with no previous growth history (**Figure 7**). When the BSS of 0.298 Pa was applied to the bed, the gradient of the erosion curve for cycle 1 increased markedly, reaching as high as 0.339 $kg \cdot m^{-3} \cdot s^{-1}$. However, the gradient of the erosion curve for cycle 2 decreased to 0.174 $kg \cdot m^{-3} \cdot s^{-1}$, while the gradient for cycle 3 was only 0.081 $kg \cdot m^{-3} \cdot s^{-1}$. This indicated that the erosion was still obviously inhibited at a greater BSS (0.30 Pa) as the bed eroded deeper down to the sub-layers. However, for cycle 4, the erosion only occurs at a higher BSS (0.31 Pa), where mass erosion was observed suddenly

when the higher stress was applied. As a result, the biological effect disappeared immediately while massive amount of sediment was eroded from the bed and the stability retreated rapidly back to the abiotic condition.

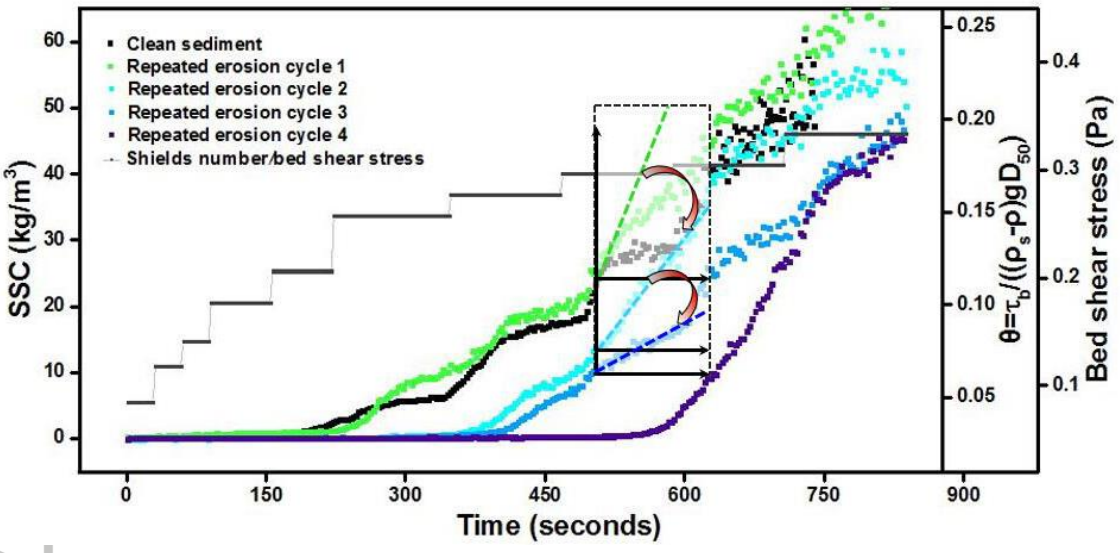

Figure 7. Erosion curves of bio-sedimentary systems developed in repeated-cycle scenario from cycle 1 (no previous growth, "npg") to cycle 4 (with 3 previous cycles, "pg3") and clean sediment as the control represented by SSC values and eroded depth.

Movement of the clean sand started at a very low applied force (BSS=0.14 Pa), and bedforms (ripples) developed (**Figure 8a**). Such sandy surface structures were very dynamic, as ripples migrated and changed shapes constantly over time [*Friend et al.*, 2008; *Noffke and Paterson*, 2008].

In the single-cycle culture of age 22 days, microbial communities were characterized by a tightly-bound surface film (in contrast to the "fluff layer" of young biofilms), which exhibited greater resistance to erosion (**Figure 8b**). At low shear stress that produced ripples in sterile sand, no grain movement occurred in the colonized sand. At or above a shear stress of 0.14 Pa, the microbial film was at first lifted and "flipped over" with the flow followed by detachment of biofilm fragments from the bed surface, indicated by the gradual fade of the visibly golden brown surface color (**Figure 5**). In this case, these thin film-like microbial communities seemed to inhibit the growth of bedforms entirely. However, the biological effects also introduced a horizontal variation of erodibility, caused by the natural heterogeneity in distribution of biofilms. This type of non-uniform biofilm distribution are often observed in the field [*Jesus et al.*, 2005] (**Figure 1)**. At higher shear stress (over 0.31 Pa), flip-overs did not occur, but rather the subsurface part of the mat was torn off, along with amounts of encapsulated sand. Mass erosion was observed at the "weakest point" while the other parts of the bed still remained stable. Sediment erosion began from this weakest point and expanded over the bed (**Figure 8b,** the last image). This indicated the failure of the bio-sedimentary bed structure which subsequently led to immediate erosion of larger amounts of sediment as suggested by a sharp increase of the SSC (bio-sediment 22 days in **Figure 4**).

Production of ripples was either prevented or retarded for the repeated-cycle conditions (**Figure 8c**). For example, in the mats after regrowth over cycle 4, grains went from no movement to mass erosion then passed directly into larger-scale bedform at 0.33 Pa. Flip-over of biofilms was also produced before the erosion point occurred. Bedforms were observed only when BSS was approximately twice as high as that required in sterile sand (0.14 Pa vs. 0.33 Pa). The behavour of the bio-sedimentary system, disturbed by frequent and strong cyclic conditions, did not return to that of the abiotic systems, as shown by the different bedforms formed during erosion compared to the case of clean sediment.

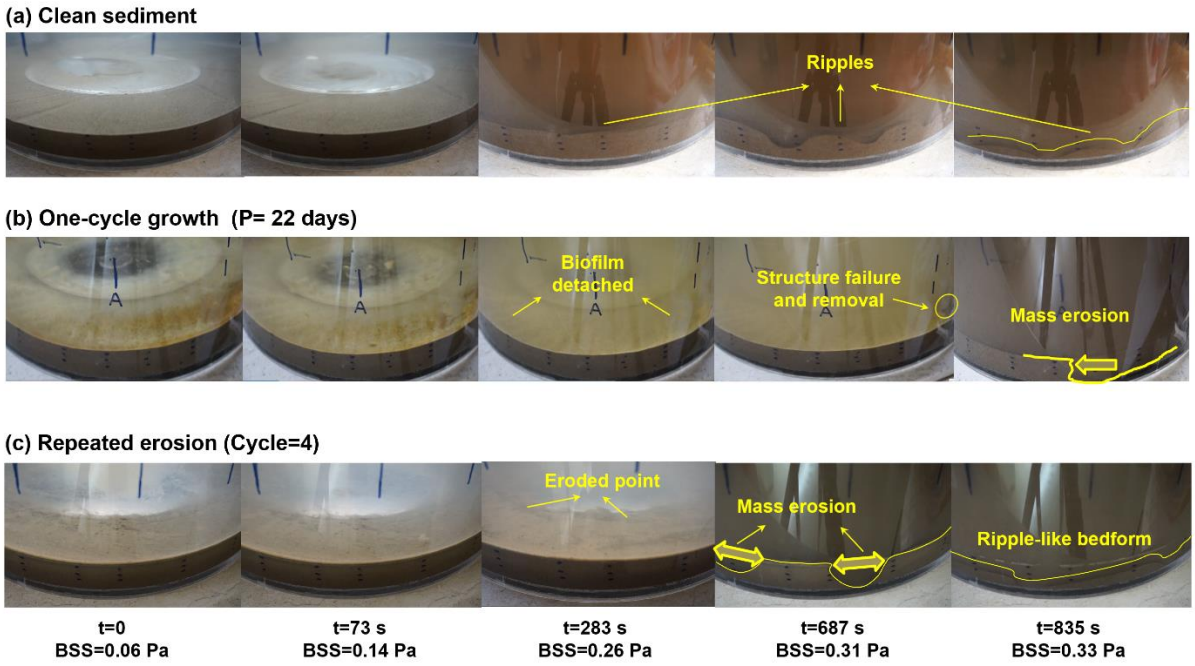

Figure 8. Examples of erosional behaviour of clean sand and microbially-inoculated sand. (a) Erosion of clean sediment. Arrows indicate the formation of ripples. (b) Erosion of bio-sediment with a growth cycle, after 22 days of incubation. The microbial film was visible as a golden brown layer that is thicker in some parts of the bed, which leads to a non-uniform erodibility pattern compared to the clean sand. Arrows indicate the biofilm detachment and the altered erosion pattern to mass erosion. Ripples were not observed in this case. (c) Erosion of bio-sedimentary systems after 4 cycles of repeated growth, erosion and regrowth. The uniform color suggests increasing homogeneity. Arrows indicate the two weak points where erosion was initiated, that consequently changed to mass erosion. Bedform reappeared with a ripple-like structure of greater wavelength as compared to the ripples on a clean sandy bed.

Discussion

Previous *in situ* studies have often shown much stronger anti-erosion properties greatly beyond predicted values based on grain size [*Paterson et al.*, 2000]. This indicates that sediment transport can be significantly reduced in natural microbial cohesive sands, and therefore it is an important piece of information for a modeller to interpret the possible differences between model results and real observations. In addition, some initial comparison can be made with bacterial biofilm biostabilisation. The increase in critical shear stress was

more limited for bacterial systems as compared to algal systems (e.g. the bacterial system had a critical shear stress of some 0.26 Pa after 22 days, which is a 68% increase (see *Chen et al.*, 2017b), compared to the 117% increase for an algal system in this study. Besides, the detachment of the biofilm took a longer time (~minutes) than the bacterial biofilm investigated in our previous work. Bacterial biofilms were generally a thin surface layer which was sheared away within a very short space of time (e.g., <10 s) [*Chen et al.,* 2017b]. This was also shown in the erosion curves (**Figure 4**) where a long period of erosion with low erosion rates was observed in algal systems before any evident increase of SSC.

However, no obvious biostabilisation was observed for "young" bio-sedimentary beds (5 days old) since the erosion curve of the bio-sediment of 5 days old was similar to that of the clean sediment profile (**Figure 4**). Even for the bio-sedimentary beds with longer incubation periods (i.e., ~weeks), the effects on erosion rates are debatable [*Le Hir et al.,* 2007]. It has been argued that once the protective biofilm is eroded, the underlying sediment probably has the same erosion behaviour as bare sediment and this was the case for the single-cycle scenario in our study. Although the surface erosion resistance largely increased for the bio-sedimentary beds after 10, 16 and 22 days, the sub-layers were still unstable, as demonstrated by the sharp slope of the erosion curve (*i.e.*, high erosion rate) occurring shortly after the initiation of surface erosion. After removal of the surface biofilm, the bed stability quickly declined to the abiotic condition, reflecting the reducing effect of biostabilisation. As a result, after 22 days the SSC of the bio-sedimentary bed increased from zero to the final value of 45 $kg \cdot m^{-3}$ within a very short time period (last step of applied BSS). A possible explanation is related to light intensity [*Paterson et al.,* 2008; *Schmidt et al.,* 2018]. Since the vertical penetration of light decreases rapidly with bed depth, microalgae communities are often confined to the sediment surface, in contrast to bacterial communities [*Chen et al.*, 2017a,b] that may be more distributed into the sediment and not dependent on light energy. Consequently, sediment properties were altered by the biofilm but only for the top layer (~1 mm) of sand in this newly-developed bio-sedimentary system (with no previous growth history).

Repeated cycles created a developing resistance in the upper sand layers, as biological colonization increased and the bed became more resistant to erosion. In contrast to single-cycle incubation, the repeated cycles may also have helped impart resilience in terms of redistributing surface material deeper into the bed after erosion. In enclosed systems the eroded material is deposited back to the bed and the surface material redistributed into the depositing layers. Sand particles with surface biofilm may then be deposited deeper into the bed matrix and covered with other particles. This may form deeper innocula that can grow and develop stability before the next erosive cycle. Therefore, miocrobial growth might become more homogenously distributed with depth, at least to the limits of the erosive loss. However, it should be noted that whilst in our experiments all the sediment and microbial growth is retained within the chamber, on an intertidal flat, retention locally will depend on how advection and diffusion processes move material across the flat. Nevertheless, the retained material from previous growth may be the reason that repeated-cycles support resilience and promote a vertical extension of microbial effects. As a result, despite the relatively short calm period (i.e., 5 days), frequent disturbance did not destroy the

biostabilisation effect, but incubation further increased the bed strength helping the biofilm become more robust. The results suggested that growth history played an important role in the development of bed stability.

The conventional interpretation of bedforms, such as ripples, dunes or lower plane-bed lamination, does not account for the microbial binding in microbe-rich intertidal environments [*Flodkvist*; *Greensmith,* 1982; *Hagadorn and Mcdowell,* 2012; *Shields et al.,* 1936; *Southard and Boguchwal,* 1990]. Therefore, a different interpretation may be required of how current-generated sedimentary structures are created in natural environments. Ignoring the delay and reduction in bed form dynamics in response to biological cohesion may lead to flaws in the reconstruction of paleohydraulic variables. Previous bedform data may have been misinterpreted as having been produced under low dynamic conditions if based on predictions of non-cohesive sand without any microbial mediation[*Hagadorn and Mcdowell,* 2012; *Noffke and Paterson,* 2008].

The contribution of EPS to bedform formation have been reported in previous studies. Bedform do not develop because EPS inhibits the grains from moving independently [*Malarkey et al.,* 2015; *Mariotti et al.,* 2014; *Parsons et al.,* 2016; *Schindler et al.,* 2015]. Compared to physical cohesion, even small amounts of EPS exhibits a strong influence on bed form dimension and type [*Friend et al.,* 2008; *Hagadorn and Mcdowell,* 2012; *Schindler et al.,* 2015]. A link was therefore suggested between the delay in the increase in sediment concentration and transport rates, and the delay in ripple formation [*Malarkey et al.,* 2015]. Nevertheless, most work on EPS induced bedform changes was based on the assumption of a homogenous distribution of EPS with depth. Based on this, synthetic EPS (e.g., Xanthan Gum, Carrageenan) was used in physical models and the bio-sediment was prepared creating a homogenous mixture of selected synthetic EPS and quartz sands. However, in intertidal systems, natural spatial and temporal variations of biogenic effects are the status quo [*Taylor and Paterson,* 1998] (**Figure 1**). EPS produced by bacteria and microphytobenthos accumulate and decay in response to physical changes. Under spring-neap tidal cycles bio-sedimentary systems are regularly disrupted, although they can re-establish during calmer building periods. Therefore, shifts occur between deposited sediments of varying levels of organic content. As a result, cells, particles and EPS are mixed and re-distributed supporting a transition from non-cohesive to cohesive states in natural sediment systems. Our study showed that even for a relatively frequent disturbance (i.e., 5 days of low stress interval), biological effects still had a significant role in mediating bed structure formation. The biological mediation responded to the repeated cycles, and continued to further influence sand erosion. Erosion patterns present in our study were quite different from the results obtained using synthetic homogenously mixed EPS-sediment beds [*Malarkey et al.,* 2015; *Parsons et al.,* 2016]. It should be noted that the ripples observed in our microcosms are not perfect analogues of natural ripples. Similar to all other experimental approaches, the analysis of microcosms has limitations, with scaling issues often identified as the most important. In addition, although the artificial flattening performed at the beginning of each recovery period established uniform initial conditions, this could possibly have affaected the beform formation. In natural system, flow-generated sedimentary structures such as ripples and dunes are created. The traditional sequence of ripple→dune→plane-bed lamination indicates that

bedforms are sometimes preserved after erosion but not always [*Hagadorn and Mcdowell*, 2012]. This means that the cyclic effects in a natural intertidal system are much more complex than considered here. Nevertheless, our study suggests that previous studies based on the EPS proxies may lack accuracy in reflecting the natural biological variations in response to intertidal cyclic erosion conditions.

The structure and function of biofilms has been well-documented in their different developmental stages [*Costerton et al.*, 1978; *Donlan and Costerton*, 2002; *Wolcott et al.*, 2010]. When coupling with sedimentary system rather than surfaces, these different stages are also relevant. A conceptual erosion framework is proposed here for bio-sedimentary systems recognizing the variation of different growth stages and cyclic erosion effects (**Figure 9**). The cycle begins with the first cells becoming associated with the bed. Many cells may attach strongly to sediment particles (bacteria, epipsammon) using EPS while others may be loosely associated with particles inhabiting the interstitial pore spaces (epipelon). Biostabilisation effects are not obvious at this stage, as indicated by the SEM images (**Figure 6a**) and erosion curves (**Figure 4**, bio-sediment after 5 days). The first stage is followed by an increase in cell biomass and the formation of patchy micro-colonies, and EPS begins to accumulate forming a biofilm that begins to encorporate smaller particles and form bridges between larger ones (**Figure 6b**, EPS coating grain surfaces and filling pores). At this point, bio-cohesion becomes significant and the bed surface is strengthened and the erosion threshold increases (**Figure 4**, bio-sediment after 10 days). Subsequently, the mature biofilm develops a protective matrix, which can be derived from a wide variety of organisms and forms a three dimensional structure incorporating the surface grains (**Figure 6c and 6d**). Under these conditions, the critical shear stress is greatly increased (**Figure 4**, bio-sediment after 16 and 22 days).

When the mature bio-sedimentary matrix is destroyed during disturbance events, the biological effects may not be entirely removed and the eroded bio-sedimentary elements enter the "erosion-transportation-deposition-consolidation" (ETDC cycle, **Figure 9**). As the biofilms are broken up into small fragments during erosion, some organic matter and microbes are suspended as potential "biofilm precursors" and act as an active agent for biofilm formation over the next cycle [*Barzeev et al.*, 2015]. The physical disturbance by hydrodynamic forces cannot remove all organic material in sedimentary systems, i.e., EPS coating the grain surfaces, or firm links between small particles and microbes still remain. As a result, bio-aggregates are introduced when they enter water column (**Figure 9**). When a calm period returns, the biofilm precursors can deposit and start a new cycle from the remaining bed material as the "new" inoculum. As the remaining material and the nature of the inoculum will vary between events, the initial state will also vary from the previous cycle but will always promote more rapid microbial development than an axenic system. The increased adhesion may partly be due to the plentiful binding sites provided by the remaining organic matter attached to or between sediment particles and therefore surfaces are more available for microbes to adhere. Similar mechanism have been reported in previous study on the role of cell and particle characteristics in the adhesion of *E.coli* bacteria to suspended intertidal sediments [*Wyness James*, 2017]. This work showed that all strains examined adhered more efficiently to organic sediments than other abiotic sediment (i.e., mud or mixed

sands). However, it should be noted that a constant shear stress was considered to represent subcritical flow condition (cf. neap tide and calm period) in this experiment, while in natural system, the shear stress gradually changes from low to high during a neap-spring cycle. This means that a variable shear stress may better represent the natural condition rather than a constant shear stress as used here. During calm periods, previous work has shown that different hydrodynamic conditions result in different biofilm growth. For instance, EPS in biofilms exhibited different distribution and composition under different flow velocities (i.e., varied from 0.04 to 0.28 cm s^{-1}) [*Chao et al.*, 2014]. Besides, there are also short-term changes in sediment extracellular carbohydrates in relation to migratory rhythms due to the daily cycle of flood-ebb tide [*Orvain et al.*, 2003]. Therefore, the accumulation of EPS in the bio-sedimentary bed could possibly be highly affected when the shear condition changes, and lead to different erosion results. Obviously, this makes the issue even more complex. To start at a basic level and due to the operational limitations of the chamber system, we only considered a constant shear stress in this experiment. However, it is highlighted that the dynamic variability of a bio-sedimentary system has yet to be studied under the influence of multiple stressors in coastal environments, which is the next stage of the journey.

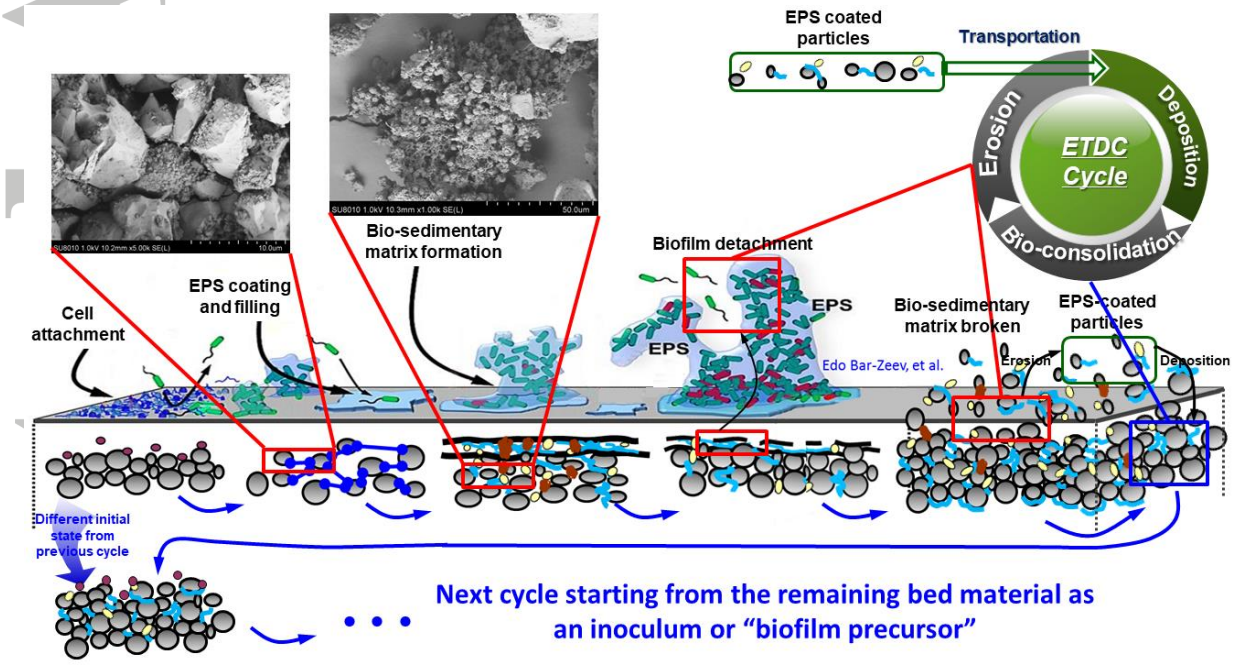

Figure 9. The conceptual model describes how sediment behaviour changes in bio-sedimentary systems from abiotic systems. Different extents of EPS penetration during the growth stages result in increased biostabilisation with a mature biofilm. Biofilms also bridge individual particles so that the stability is enhanced and bed resilience increased. After erosion, EPS-enveloped particles alongside fragments of broken biofilm enter the ETDC cycle and are deposited when the calm period returns. A new bio-sedimentary bed is therefore established and begins the next cycle of bio-cementation [*Barzeev et al.*, 2015].

Conclusions

The erosional behaviour of a newly-developed bio-sedimentary bed with no previous growth history was progressively altered via biofilm development with time. Compared to clean sediment, a continuous increase in critical shear stress was obtained for

bio-sedimentary beds aged from 5 to 22 days. The bed strength was greatly increased (22 days) and was much higher as compared to the protective effect of a bacterial biofilm. Scanning Electron Microscope (SEM) images demonstrated an evident increase in biological penetration with depth and time. Under stronger hydrodynamic stress (0.28 Pa), the biofilm began to slough off, forming clear erosion edges. Although no obvious biostabilisation effects were observed for the young bio-sedimentary bed (5 days), the critical shear stress increased rapidly when the bed surface was eroded, deposited and incubated for another 5 days (repeated cycle). This indicated that the biological activity gradually adapted to the cyclic shear forces so that the biological cohesion re-builds rapidly inside the bed following repeated cycles. As for the single-cycle scenario, erosion rates increased rapidly as soon as the top layer was eroded and approached the abiotic condition. However, greater sub-surface stabilisation effects occurred for the underlying sediment under the repeated-cycle scenario as explained by the biofim precursor effect.

Apart from contributing to increasing resistance to erosion, biological cohesion also restricted small-scale bedform development. Our study reproduced transition of bed structures which may occur in nature. The erosional behaviour of the underlying sand was also affected in that bedform ripples were no longer observed and the bed remained stable with no bed load transport observed. At higher stress, bed integrity was lost suddenly from a "weak point" of the surficial biofilm and rapidly developed towards mass erosion. This was not predicted from the expected behaviour of non-cohesive sandy sediments. However，bedforms re-developed but with greater length under a repeated-cycle scenario as compared to the bedforms observed from the clean sand experiment. Sequences of mixing increased the integrity and the global stability of the bio-sedimentary bed surface and with depth. Ignoring these dynamics in bed form response to biological cohesion may lead to flaws in the reconstruction of paleohydraulic variables. Bedform data could be misinterpreted as being produced under low dynamic stress conditions if based on predictions of non-cohesion sand behaviour. An explanation for the stimulating effects of repeated cycles is that hydrodynamic forces did not entirely remove organic material. As a result, the remains of ESP and microbes contributed to the faster re-development of a new bio-sedimentary beds during the depositional period of the next cycle. Our study indicated that under repeated cycles even with frequent disturbance, EPS accumulated and biostabilisation became more influential as compared to the single-cycle situation. This emphasizes the importance of the complex coupling between sedimentology, benthic biology, and hydrodynamic conditions that govern the ecology and behaviour of coastal sediments.

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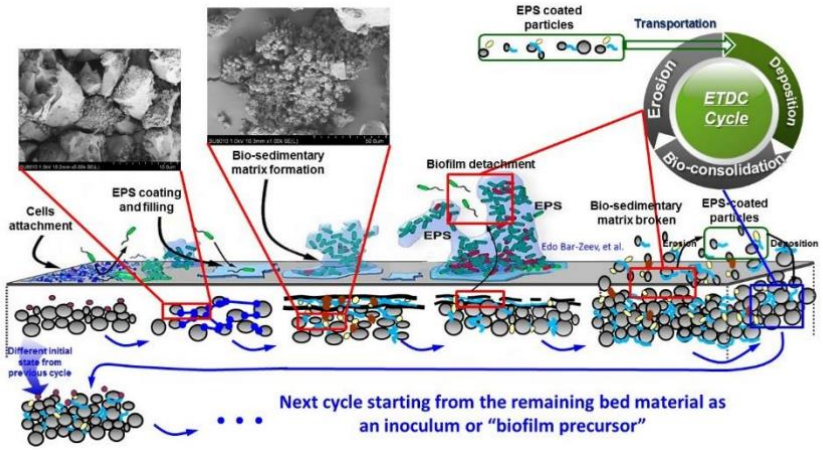
Biostabilisation of bio-sedimentary beds with and without cyclic stress is compared. Results showed that the erosional behaviour of the underlying sand was affected and transition of bed structures were observed. Conceptual erosion framework is proposed for bio-sedimentary systems recognizing the variation of different growth stages and cyclic effects.



The effect of cyclic variation of shear stress on non-cohesive sediment stabilisation by microbial biofilms: The role of "biofilm precursors"

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