Dear Dr. Hodge,

Please find enclosed the revised (MS Reference esurf-2017-59) manuscript entitled *Quantifying biostabilisation effects of biofilm-secreted and extracted extracellular polymeric substances (EPS) on sandy substrate* by W.I. van de Lageweg, S.J. McLelland and D.R. Parsons.

Thank you for your guidance in further improving the manuscript. We included your suggestions to more clearly outline the connections between the two experimental components and also added your suggestions in terms of signposting and clarification to more clearly communicate the results of our work to the reader. Please find a detailed point-by-point response to all your comments (*in italics*) below.

Sincerely,
Wietse van de Lageweg, on behalf of all authors

Page/line

1/17: Change 'provide' to 'provides'
Done

1/18: Change to 'mean biostabilisation effect'
Done

1/20: Clarify that the critical erosion threshold are of colonised sand
Done

2/1: Measured range of what?
Of critical erosion thresholds. We added this information to the main text.

3/11: I think that three author papers should be 'et al' in the text, not written in full.
We used Mendeley as our Reference Manager and selected a citation style in which three paper authors were written in full, and not as 'et al'. We checked the document and corrected all occurrences.

3/13: Consider rephrasing – for example 'sediment stability measured as the threshold for erosion'.
Done. Here and in other places throughout the manuscript.

3/17: 'Yet' is not needed.
We removed 'Yet'.

4/5: Clarify that you are referring to dynamics at the scale of the entire estuary.
Done

4/16: Consider changing to 'sediment settling rates'.
Done

5/4: At some point you need to clearly explain what you mean by extracted EPS – this could be here, or in line 4/11.
We added a definition of extracted EPS and information on its usage in the current study (P4, L18-21).

5/11: Somewhere in this paragraph you need to mention the natural biofilm experiments, e.g. by saying that you will be comparing the extracted EPS results to natural biofilms in complementary experiments. Otherwise the natural biofilms are not mentioned until aim 1, and then it's not clear where they have come from.
Done

6/10: This paragraph also needs some restructuring for clarity. Be explicit that there are two sets of experiments being reported; one using natural biofilms, and the other using extracted EPS. For example: 'In the first set of experiments… In the second set…' Don't use the word 'auxiliary'; this means supplementary, whereas you have identified the extracted EPS experiments as being the main aim of this research. I would also be consistent with how you label the sections headings so that there are clearly two sections of the methods and results.
As suggested, we have made it more explicit that we are reporting on two experiments. Both in the sentences leading up to the specific aims (P6, L3-6) and in listing the aims (P6, L13-17).

7/4: Figures are placed near their first occurrence in the text. Consider moving Figure 1 to here, or removing this reference to it.
We moved Figure 1 to the top of P8.

7/7: You don't refer to the 1 mm and mixed channels anywhere else in the paper, so no need to include them in the methods.
We removed the sentences about the four channels with other substrates not reported on in this study.

7/12: Replace 'auxiliary'
Done

8/6: Consider rephrasing: the flume was inoculated using
Done

8/12: 'From the top 0.01 m' could be interpreted as being from the furthest upstream 0.01 m of the channel. Clarify that you are referring to 0.01 m in the vertical, and explain how the sampling sites were selected. Did you specifically target locations with visible biofilm, or sample from all channel locations?
We clarified now that we are referring to the top 0.01 m in the vertical. Additionally, we clarified that we targeted locations with visible biofilm (P9, L12-15).

8/13 and elsewhere: Where section titles have been inserted into the text there is a missing space afterwards. Remove the title and just leave Section 2.1.1 (in this case).
Done

10/10: It's still not entirely clear how this works. Do you just increase the applied force until the surface is eroded? How do you decide when to stop? Figure 10 has a transmission %, but you don't explain anywhere what this is. How do you go from the datasets in Figure 10 to a single erosion threshold value?
The force of the jet pulse is increased depending on the programmed selected. In our study, we selected the pre-programmed S7 routine, which starts 0 kPa incrementing by 2.068 kPa per step up to 82.74 kPa with a jet being fired for 1 second. 36 other pre-programmed routines consist of other settings for the air increments, duration of the jet, and maximum air pressure applied.

The CSM device works through the program, independent of surface erosion. Therefore, all tests have an identical duration. If no erosion is observed, the maximum air pressure applied can be increased to possibly erode the surface at the next attempt. Initially, some trial-and-error tests are required to obtain the correct CSM routine for the studied material. We found that routine S7 provided a balance between sufficiently detailed measurements for the lower threshold of erosion of some samples and a large measurement range required for some of the engineered substrates (i.e. Xanthan Gum).

A drop in transmission indicates an erosion event. The erosion profile usually has three different parts:

1. An initial horizontal line where the transmission is close to 100%.
2. A slope representing the drop in transmission of light across the measurement chamber as erosion occurs and sediment is being suspended.
3. An asymptotic part where the transmission approaches 0 when the air pressure increases.

These profiles vary depending on the sediment properties. Following Tolhurst et al (1999), the critical erosion threshold was defined as the pressure step at which the transmission drops below 90%.

We added some of the above information to the main text and would like to refer readers to Tolhurst et al (1999) for a full description of the CSM device.

10/14: 'Enabling' is a better word that 'allowing for'.
Done

10/18: Start this paragraph with words or a sentence to tell the reader that you are now moving onto the second set of experiments.
Done

12/15: The start of this line is awkwardly phrase; rephrase.
Done

12/16: Use °C rather than writing out in full.
Done, here and in other places.

12/17: What liquids were used to give these pHs?
We used standard and commercially available buffer solutions to obtain liquids with these pHs. We added this information to the manuscript (P14, L17-18).

13/1: Add a subheading to explain that this section is referring to the natural biofilms experiment.
Done

13/4: Delete 'a' before darker
Done

14/14: How is this theoretical entrainment threshold determined? Delete 'applied'.
We calculated the theoretical entrainment threshold τ_c for our sediment according:

$$\tau_c = \theta_c \cdot (\rho_s - \rho) \cdot g \cdot D_{50}$$

where θ_c is the Shields number (N/m^2), ρ_s is density of sediment (kg/m^3), ρ is the density of water (kg/m^3) and D_{50} is the median grain size (m).

The Shields number θ_c is calculated following Zanke (2003):

$$\theta_c = 0.145 \cdot \mathrm{Re}_p^{-0.33} + 0.045 \cdot 10^{-1100 \cdot \mathrm{Re}_p^{-1.5}}$$

where Re_p is the Reynolds particle number and calculated by:

$$\mathrm{Re}_p = D_{50}^{1.5} \cdot \frac{\sqrt{\Delta \cdot g}}{\nu}$$

where Δ is the relative sediment density (-) and ν is the kinematic viscosity (m^2/s).

We added the above information to the methods in our manuscript and also deleted 'applied' (P12, L6-14).

14/15: How many of these samples came from sections of the flume where no biofilm was present? Without knowing the sampling strategy, this isn't clear. You could use the EPS content values to say something about this.

None. All of the samples were taken from places in the flume with a visible biofilm present. We clarified our sampling strategy in the main text (P9, L14-21) in response to a comment above. Here, we also explain that the sediment entrainment measurements were destructive and sediment samples for determination of the EPS content could therefore not be taken from the same location. As a consequence, we do not feel that the EPS content values can be used reliably to provide answers on why 42% of the sediment entrainment measurements are smaller than the entrainment threshold of the uncolonised sand.

15/3: There is clearly more in these data than you currently present, but I agree that these data are not the key point of the paper. However, as these data are compared to the extracted EPS data, it would be helpful to explore them a little more. Could you add the time data onto Fig 2, for example by colouring the points by week? How did variation over time compare to the spatial variation within and between the flumes at any given time? For all the extracted EPS data you plot shear stress against amount of EPS. Why not also plot the natural biofilm data like this?
There is indeed a wealth of information on natural biofilm behaviour in the data. In another manuscript, we therefore aim to explore the colonisation behaviour of bedforms in more detail because there appears to be an optimum elevation from where the colonisation starts. Also, comparisons with colonisation of other substrates will be made in this other manuscript. As you acknowledge, these detailed colonisation processes are beyond the scope of the current manuscript in which we compare the biostabilisation potential of natural biofilms to that of extracted EPS.

As such, the goal of Figure 2 is to show the probability distribution of the threshold for erosion measurements obtained from the natural biofilm experiment. These data can then be quantitatively compared with the threshold for erosion data obtained from the extracted EPS experiment. The temporal aspect is of smaller importance but, as suggested, we now provided statistics on the weekly measurements. Also, we added some information about the variation in time vs. the variation in space in an effort to explore the spatial and temporal aspects a little more (P17, L4-9).

Regarding your query on plotting shear stress against amount of EPS for the natural biofilm, we now clarified our sampling strategy (see also earlier comments). Since the shear stress and EPS content measurements were not made on the same location, these data cannot be related and plotted in the same manner as was done for the extracted EPS experiments.

16/6: Make it clear that this section is moving onto the extracted EPS experiments. For example, refer to the 'second set' of experiments (or whatever you call them), rather than just small scale experiments (a phrase that I don't think you've used before).
Done

17/1: This paragraph mainly seems to repeat what you have already said in the methods. 17/5 onwards seems to be new information, which should be moved to the methods.
We removed the repetitive statements and moved the new information to the methods.

18/Fig. 4 and other figures: Add space between = and following number.
Done

18/Table 1: How many replicates?
5 repeat measurements. We added this information to the manuscript.

20/Fig 5: A good way of comparing the natural and extracted EPS shear stress values would be to add dashed lines for the mean (and median/standard deviation?) of the shear stresses measured for the natural biofilms. This applies to all figures like this (5/7/8/9).

Also, is mixing different to stirring?
The purpose of figures 5, 7, 8 and 9 is to provide an assessment of the sensitivity of the sediment entrainment threshold of extracted EPS-sand mixtures to the preparation procedure (fig. 5), salinity (fig. 7), pH (fig. 8) and temperature (fig. 9). The highest EPS content (10 g/kg) is used for these sensitivity tests to be able to pick up a difference in entrainment threshold for all these conditions as best as possible. These highest EPS concentrations do, however, not make for a fair comparison with the natural biofilms, since we already know from observations in nature that these EPS contents are on the upper end of the spectrum.

A more meaningful comparison of the shear stresses from natural biofilms and extracted EPS is obtained by contrasting the statistics on shear stress from the natural biofilm experiment with the statistics from the extracted EPS for different contents (as shown in fig 4). This comparison of the shear stresses measured for the natural biofilms and extracted EPS is provided in Table 2.

Stirring and mixing is the same in this context. The procedure is detailed in the methods (P14, 2-10) and therefore removed from the caption of figure 5.

22/1: Rephrase to clarify that you are referring to the extracted EPS experiments.
Done
22/Fig 7: The caption refers to tap water, but distilled water was referred to at the end of 2.3.2.
This should have been distilled water and we now corrected this.

26/5: Explain how a mean biostabilisation index is calculated.
We added an explanation to the methods (P15, L2-10)

26/13: Quote some figures to support this claim that the index is similar.
Done

26/14: Change to 'more suited for replicating'
Done

27/Table 2: The caption still doesn't really explain the biostabilisation index. This table could be clearer. It's a bit confusing that the top row of headings doesn't apply all the way down the columns. What statistic is quoted for the extracted EPS results (mean/median)?
We now added a more detailed explanation of the biostabilisation index to the methods (P15, L2-10). Here, we also define what statistic is used for the extracted EPS results (i.e. mean).

We changed the table so the headings apply all the way down. We considered splitting the data into two separate tables with one for the natural biofilm data and the other for the extracted EPS data, which would have made for simpler tables. However, we believe that it is important to present the data from both experiments in the same table so readers can easily compare the biostabilisation indices obtained in both experiments.

27/4: Give some values.
Done

27/7: I think that you're saying that this technique doesn't measure all of the EPS that is in the sample?
Yes, that is what we meant to say in a rather clumsy way. We rephrased the sentence to clarify that this is indeed the case (P30, L3-7).

29/Fig 10: See earlier comment about explaining what transmission is.
We added this information to the method section, see also earlier comment regarding the transmission and application of the CSM device more generally (P11, L10 – P12, L5).

30/17: Is the change over time primarily from the sediment drying, rather than any sort of degradation of the extracted EPS?
We cannot definitively distinguish between both options based on our experiments. It is well known that the sugars in EPS degrade over time and, as such, it is likely that the change over time is attributable to degradation of EPS. Whether the sediment drying counteracts or amplifies this degradation effect does not become clear from the performed experiments. Rather, the performed experiments raise the question of how important sediment saturation is for the behaviour of extracted EPS and we therefore also call for further research on this topic.

Quantifying biostabilisation effects of biofilm-secreted and extracted extracellular polymeric substances (EPS) on sandy substrate

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Abstract. Microbial assemblages ('biofilms') preferentially develop at water-sediment interfaces and are known to have a considerable influence on sediment stability and erodibility. There is potential for significant impacts on sediment transport and morphodynamics and, hence, on the longer-term evolution of coastal and fluvial environments. However, the biostabilisation effects remain poorly understood and quantified due to the inherent complexity of biofilms and the large spatial and temporal (i.e. seasonality) variations involved. Here, we use controlled laboratory tests to systematically quantify the effects of natural biofilm colonisation as well as extracted extracellular polymeric substances (EPS) on sediment stability. Extracted EPS may be useful to simulate biofilm--mediated biostabilisation, and potentially ~~provide~~provides a method of speeding up time scales of physical modelling experiments investigating biostabilisation effects. We find a mean biostabilisation effect due to natural biofilm colonisation and development of almost four times that of the uncolonised sand. The presented cumulative probability distribution of measured critical threshold for erosion ~~thresholds~~of colonised sand reflects the large spatial and temporal variations generally seen in natural biostabilised

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Met opmaak: Engels (V.S.)

environments. For identical sand, engineered sediment stability from the addition of extracted EPS compares well across the measured range of the critical threshold for erosion and behaves in a linear and predictable fashion. Yet, the effectiveness of extracted EPS to stabilise sediment is sensitive to the preparation procedure, time after application and environmental conditions such as salinity, pH and temperature. These findings are expected to improve bio-physical experimental models in fluvial and coastal environments and provide much-needed quantification of biostabilisation to improve predictions of sediment dynamics in aquatic ecosystems.

Keywords: Biofilm, biostabilisation, EPS, physical modelling, ecology, sediment transport

1 Introduction

Micro-organisms are a fundamental feature of aquatic environments providing a range of ecosystem services (Gerbersdorf et al. 2011; Gerbersdorf and Wieprecht 2015). A large variety of microbial assemblages ('biofilms') such as microphytobenthos, microbial mats and biofouling in pipes (Flemming and Wingender 2010) are representations of microbial communities in aqueous environments. The microbes in biofilms live in a self-formed matrix of glue-like and hydrated extracellular polymeric substances (EPS) such as polysaccharides (often 40-95%), proteins (up to 60%) and minor amounts of acids, lipids and biopolymers (Decho 1990; Flemming 2011; Gerbersdorf et al. 2011). The ecosystem functions of EPS in sediment particle aggregation, increasing sediment stability, altering chemical properties to enable contaminant release or adsorption, and providing a food source for invertebrates are well established for marine environments (Decho 1990; Passow 2002; Bhaskar and Bhosle 2006; Paterson et al. 2008), but remain less well understood for freshwater systems (Gerbersdorf et al. 2011). The ability of biofilms to stabilize sediment and protect sedimentary surfaces against erosion is often

referred to as ‘biostabilisation’ (cf. Paterson 1989). Biostabilisation may result from coverage by microbial mats which protects underlying sediments from fluid forces (Noffke and Paterson 2007) or from micro- to macroscopically thin biofilms that coat, bridge or permeate single grains and pore spaces with their EPS (Gerbersdorf and Wieprecht 2015) which increases sediment cohesion and increases the entrainment threshold. Note that the terms ‘microbial mats’ and ‘biofilms’ are often used interchangeably, the former is not exclusively used to denote a covering of underlying sediments, and the latter is not exclusively used to denote coatings of single grains.

Many studies have attempted to quantify biostabilisation in a variety of environments (Paterson 1989; Dade et al. 1990; Amos et al. 1998; Tolhurst et al. 1999; Tolhurst et al. 2003; Friend et al. ~~2003; Friend, Collins, and Holligan 2003~~2003a; Droppo et al. 2007; Righetti and Lucarelli 2007; Vignaga~~, Haynes, and Sloan~~ et al. 2012; Graba et al. 2013; Thom et al. 2015). These studies generally show a positive correlation between EPS content and sediment stability measured ~~using an erosion~~as the threshold for erosion, although variations in space and time (Friend~~, Collins, and Holligan 2003~~ et al. 2003b; Thom et al. 2015) and between cohesive and non-cohesive sandy environments are large. There are however also examples of buoyant biofilms, which reduce the ~~erosion~~ threshold for erosion of sediments (Sutherland~~, Amos, and Grant~~ et al. 1998; Tolhurst~~, Consalvey, and Paterson~~ et al. 2008). ~~Yet, biostabilisation~~Biostabilisation of coarse sand and gravel may increase the ~~erosion~~ threshold for erosion up to almost three times compared to abiotic sediment (Vignaga~~, Haynes, and Sloan~~ et al. 2012) while a tenfold increase in ~~erosion~~the threshold for erosion compared to abiotic sediment has been reported for fine sands and cohesive sediments (Paterson 1997; Dade et al. 1990). EPS is known to add biostability

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Met opmaak: Tekstkleur: Auto, Patroon: Doorzichtig

in two ways: 1) by physically binding both cohesive and non-cohesive sediment grains together (see Tolhurst~~, Gust, and Paterson (~~ et al. 2002) for low-temperature scanning electron microscopy images of biofilm-secreted EPS strands binding sediment particles together), and 2) by molecular electrochemical interaction with cohesive clay particles (Chenu and Guérif 1991).

Biofilm formation affects sediment erosion, transport, deposition and consolidation (Righetti and Lucarelli 2007; Gerbersdorf and Wieprecht 2015). There is, for example, evidence that diatom blooms alter ~~estuarine~~ sediment dynamics at the scale of entire estuaries (Kornman and De Deckere 1998) illustrating the potential effects micro-organisms can have on system-wide sediment fluxes. At a smaller scale, evidence is growing that biofilms alter their local environment by affecting hydrodynamics (Vignaga et al. 2013), since the biofilm surface changes the bed roughness to either dampen or increase turbulence production (Gerbersdorf and Wieprecht 2015), and sometimes their protruding structures create a buffer layer between the flow and the ~~At a smaller scale, the~~bed that can enhance sediment settling rates (e.g. Augspurger and Küsel 2010).

The introduction of the extracted EPS Xanthan Gum in flume experiments investigating bedform dynamics has been shown to change bedform morphology and behaviour (Malarkey et al. 2015; Parsons et al. 2016). Changes in delta morphology and behaviour were also observed in flume experiments where extracted EPS was added to the sediment mixture (Hoyal and Sheets 2009; Kleinhans et al. 2014). ~~Furthermore~~Extracted EPS are here defined as polysaccharides with a variety of uses (e.g. food additives) that can be extracted from simple sugars using a fermentation process. Extracted EPS are

generally available as a powder and are in this study employed to systematically introduce biological cohesion into physical models.~~, evidence is growing that biofilms alter their local environment by affecting hydrodynamics (Vignaga et al. 2013), since the biofilm surface changes the bed roughness to either dampen or increase turbulence production (Gerbersdorf and Wieprecht 2015), and sometimes their protruding structures create a buffer layer between the flow and the sediment bed that can enhance settling rates (e.g. Augspurger and Küsel 2010).~~

The corollary of the evidence showing the impact of biofilms on sediment stability and flow behaviour is that the inclusion of biological processes and responses is critical to modelling sediment dynamics because micro-organisms are an integral component of the functioning of water and sediment transfer systems~~.~~.. Predicting the potential impacts of climate change on aquatic environments and applying bio-engineering adaptation strategies like '*Building with Nature'* for coastal defence (de Vriend et al. 2015) or flood resilience (Temmerman et al. 2013) requires an understanding of i) the response of micro-organisms to changes in climate-induced hydrodynamic forcing, and ii) the role of micro-organisms in water and sediment transfer systems. ~~Even though it has been demonstrated that the extracted EPS Xanthan Gum is not a perfect analogue for natural biofilms (Perkins et al. 2004), it is~~To date, quantification of biostabilisation effects in space and time remain scarce however. A controlled physical model experiment is therefore employed to systematically investigate and provide further quantification of natural biostabilisation effects. Additionally, the extracted EPS Xantham Gum has proven useful for modelling biological interactions with sediment dynamics (e.g. Hoyal and Sheets 2009; Kleinhans et al. 2014; Malarkey et al. 2015; Parsons et al. 2016)~~. Extracted EPS also has~~, even though it has been

demonstrated that Xanthan Gum is not a perfect analogue for natural biofilms (Perkins et al. 2004). Extracted EPS generally also have the potential advantages over growing natural biofilms that preparation time and experiment duration in physical models can be reduced and biostabilisation effects can be controlled. In assessing the potential of four extracted EPS to mimic natural biostabilisation, the natural biofilm physical experiment is compared to the complementary experiments on extracted EPS.

The objective of this study is therefore to evaluate biostabilisation effects of existing extracted EPS for a range of conditions commonly used in physical modelling experiments. Two sets of experiments are being reported on: the first set of experiments focusses on biostabilisation resulting from colonisation of sandy substrate by natural biofilms ('natural beds'). The second set of experiment focusses on biostabilisation resulting from the systematic addition of extracted EPS to the same sandy substrate ('engineered beds'). In doing so, the study solely focusses on the sediment stabilising aspect of biofilms and does not explicitly intend to replicate and evaluate natural biofilm behaviour ~~and effects~~. The novel outcome of this study is the development of a robust methodology and protocol for the application and resultant impacts of extracted EPS, which can be applied to future experimental studies that require the representation of biological cohesion in a rapid and controlled manner. A sandy substrate was used in this study ~~since~~because this grain size range is most commonly used in physical models of coastal and fluvial systems to date. The specific aims of this study are to:

1. Quantify biostabilisation effects (i.e. ~~erosion~~ threshold for erosion) of natural diatom biofilm-secreted EPS on sandy substrates in a physical model experiment~~.~~ ('natural beds'; first set of experiments).

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2. ~~Using the same sandy substrate, quantify~~Quantify the biostabilisation effects of four extracted EPS~~.~~ using the same sandy substrate ('engineered beds'; second set of experiments).
3. Assess the sensitivity of the biostabilisation effects of the four extracted EPS to:
    a. The preparation procedure
    b. The time after application
    c. Environmental factors that may differ between flume facilities such as salinity, pH and temperature
4. Summarise the key steps and findings into a protocol informing future work on usage and expected biostabilisation effects.

2 Material and methods

This study reports on a flume experiment in which a natural biofilm is allowed to colonise a sandy substrate. The observations made on spatial and temporal dynamics of the sediment stabilising capacity of the natural biofilm provide a reference for auxiliary tests, using the same sandy substrate, on the sediment stabilising capacity of extracted extracellular polymeric substances (EPS). The aim of the auxiliary tests was to quantify the ability of extracted EPS to replicate the sediment stabilising capacity of natural biofilms in a fast and controlled manner. Below we describe the materials and methods used in both experiments.

2.1 Biofilm experiment

2.1.1 Experimental setup and conditions

The biofilm experiment was setup in the Total Environment Simulator flume facility at the University of Hull (Figure 1). Nine parallel channels without an initial gradient were constructed for colonisation. Each channel was 9 m long, 0.48 m wide and contained a 0.1 m thick substrate layer. With a typical flow depth of 0.1 m, the width-to-depth ratio of the channels was about 5. For five channels, the substrate consisted of 110 micron sand. ~~One channel contained a coarser 1 mm sandy substrate and one channel contained a fifty fifty mixture of the 110 micron sand and 1 mm sand. The two remaining channels contained a patterned substrate of alternating patches of the 110 micron sand and 1 mm sand, with different lengths of the patches for the two channels.~~The other four channels contained other substrates and are not included in this study. Here, we will focus on the five channels with the 110 micron sandy substrate that allowed us to investigate the temporal dynamics involved in biofilm colonisation and stabilisation. Importantly, the same 110 micron sand was also used in the ~~auxiliary tests~~second set of experiments with extracted EPS.

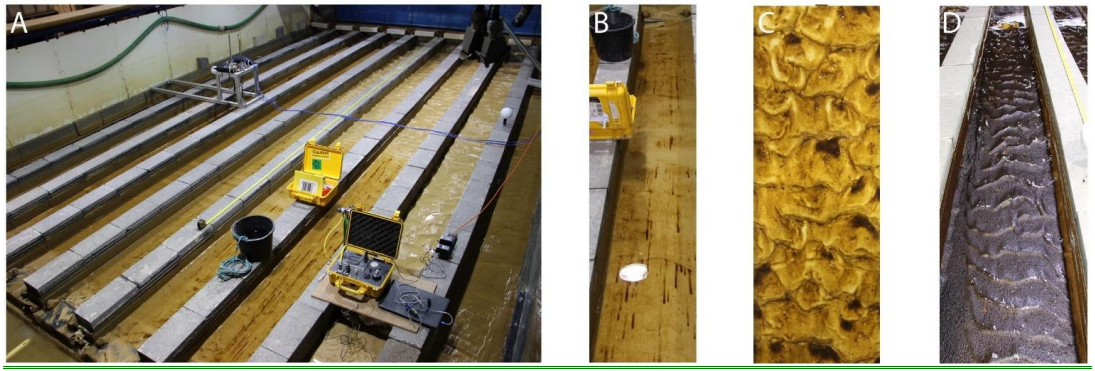


Figure 1: Biofilm experiment in Total Environment Simulator flume facility. A) Overview of experimental setup showing nine (9) parallel channels for biofilm colonisation. Channels are 9 meters long, 0.48 m wide and contain a 0.1 m thick substrate layer consisting of uniform 110 micron sandy sediment. Also visible in the yellow cases is the CSM erosion device. Panels B) – D) show colonisation and development of a diatomaceous biofilm on the sandy substrate from early onset in (B) to a mature and dark biofilm after 6 weeks. Flow in panels A), C) and D) is towards viewer, and away from viewer in panel B).

Brackish water (~30 grams of salt per litre) representative of estuarine, mangrove and deltaic settings was re-circulated at a constant rate. Typical flow velocities were 0.01 – 0.05 m/s with higher flow velocities for the central channels due to the inlet conditions. The Reynold number was generally between 5000 and 10000, indicating turbulent flow conditions. Lighting consisted of ten grow lamps, positioned in two parallel lines of five light sources. Illuminance tests showed that the central channels received the highest light intensity (~3000 lux) with lower intensities towards marginal channels (~1500 lux). Such light intensities correspond to an overcast day. The grow lamps were alternately switched on and off for 12 hours, although the experiment was never completely dark because fluorescent lighting around the flume remained switched on during the night for safety purposes.

The total experimental duration was seven weeks. During the first two weeks, the biofilm community was allowed to establish and no measurements were made. In this two-week period, ~~inoculation of~~ the flume ~~proceeded from~~was inoculated using eutrophic waste water from the local aquarium and by placing rocks with a biofilm sampled from the local Humber estuary in the flume. Then, weekly measurements of EPS content and sediment entrainment were made over a five-week period. The measurements required partial draining of the flume and therefore about 20% of the water volume was replaced weekly with new waste water from the aquarium. This also ensured that high nutrient levels were maintained during the entire experimental duration. ~~Sediment~~When the bed was dry, sediment samples from the top 0.01 m in the vertical of every channel were taken to determine the EPS content from (see section ~~2.1.2 Determination of EPS content~~2.1.2 for details on methodology to determine EPS from sediment samples). Sampling sites were identified based on the visible presence of a biofilm. In total, 80 sediment samples were collected in this way. Similarly, two sediment entrainment measurements for each channel were collected using the Cohesive Strength Meter (CSM) erosion device (see section ~~2.2 Cohesive Strength Meter (CSM) erosion device~~2.2 for details on the CSM erosion device). In total, 61 successful CSM measurements were made. The sediment entrainment measurements were destructive and sediment samples for determination of the EPS content could therefore not be taken from the same location. Sediment sample collection and sediment entrainment measurements took place in the most downstream located meter of the flume channels.

2.1.2 Determination of EPS content

EPS content was calculated using the phenol sulphuric acid method, employing colour differences to determine the amount of carbohydrates (Dubois et al. 1956). The methodology can be subdivided into two main steps. First, 1.5 grams of each sediment sample were weighed and placed into 15 ml centrifuge tubes. Five millilitres of 0.5Mm Ethylene Diamine Tetraacetic acid (EDTA) solution was added to each tube. The sediment-EDTA solution was then centrifuged at 5000 rpm. Following centrifuging, the supernatants were pooled and a placed in a 50 ml centrifuge tube. This was repeated two more times. Then, 35 ml of ethanol was added to the 15 ml of supernatant and left overnight.

The second step started with a 30-minutes centrifuge at 5000 rpm of the ethanol-supernatant solution. Then, the precipitate was dissolved in 1 millilitre of MilliQ water from which the amount of carbohydrates was measured using the phenol sulphuric acid method. This method uses a set of standards to produce a calibration curve. In this study, the standards had glucose concentrations ranging between 0 µg/ml and 40 µg/ml. Standards were produced by mixing 200 µl of the respective glucose solution with 200 µl of phenol solution and 1 millilitre of concentrated sulphuric acid. The samples were prepared according to the same procedure, but by replacing the glucose solution with the aqueous solution. Finally, the absorbance was measured using a spectrophotometer at 490 nm. Using the glucose calibration curve, the measured absorbance was converted to a carbohydrate amount that was assumed equal to the amount of EPS. Dry weight of the sediment sample was used to calculate the EPS content.

2.2 Cohesive Strength Meter (CSM) erosion device

The CSM is an erosion device (https://partrac-csm.com/) that allows for quantification of sediment entrainment thresholds and erosion rates in the laboratory as well as in the field across a variety of environments (Paterson 1989; Tolhurst et al. 1999; Tolhurst~~, Gust, and Paterson~~ et al. 2002). The CSM uses a vertical jet of water that impinges on the sediment surface generating a normal and tangential stress at the interface. These stresses were converted to a critical horizontal shear stress (τ_c) according to the calibrated formulation (Tolhurst~~, Gust, and Paterson~~ et al. 2002):

$$\tau_c = 66.67 \cdot \left(1 - e^{\frac{-C}{310.09}}\right) - 195.28 \cdot \left(1 - e^{\frac{-C}{1622.57}}\right) \quad (1)$$

Where C is the CSM measured vertical threshold stress (kPa).

The CSM allows 39 different test routines making it is possible to vary the jet pulse duration, the pressure increments and the maximum applied pressure. For all data reported in this study, CSM test routine S7 was used as it strikes a balance between fine pressure increments while reaching a high maximum pressure, thus covering a large erosional range within the same setting. Another motivation for selection of CSM test routine S7 is that it was used in Tolhurst~~, Gust, and Paterson~~ et al. (2002) as well, ~~allowing for~~enabling a direct comparison between the data. The CSM test routine S7 starts at 0 kPa, incrementing by 2.068 kPa per step up to 82.74 kPa with a jet being fired for 1 second. A drop in transmission in the measurement chamber is indicative of an erosion event. The erosion profile usually has three different components (Tolhurst et al. 1999):

1. An initial horizontal line where the transmission is close to 100%.

2. A slope representing the drop in transmission of light across the measurement chamber as erosion occurs and sediment is being suspended.

3. An asymptotic part where the transmission approaches 0 when the air pressure increases.

These profiles vary depending on the sediment properties. Following Tolhurst et al. (1999), the critical erosion threshold was defined as the pressure step at which the transmission drops below 90%.

We calculated the theoretical entrainment threshold τ_c for our sediment according:

$$\tau_c = \theta_c \cdot (\rho_s - \rho) \cdot g \cdot D_{50} \quad (2)$$

where θ_c is the Shields number (N/m^2), ρ_s is density of sediment (kg/m^3), ρ is the density of water (kg/m^3) and D_{50} is the median grain size (m). The Shields number θ_c is calculated following Zanke (2003):

$$\theta_c = 0.145 \cdot \mathrm{Re}_p^{-0.33} + 0.045 \cdot 10^{-1100 \cdot \mathrm{Re}_p^{-1.5}} \quad (3)$$

where Re_p is the Reynolds particle number calculated by:

$$\mathrm{Re}_p = D_{50}^{1.5} \cdot \frac{\sqrt{\Delta \cdot g}}{\nu} \quad (4)$$

where Δ is the relative sediment density (-) and ν is the kinematic viscosity (m^2/s).

Gewijzigde veldcode

Gewijzigde veldcode

Gewijzigde veldcode

2.3 Petri dish sediment sample tests with extracted EPS

~~The~~In the second set of experiments, the effect of varying amounts of four different types of extracted EPS on the sediment entrainment threshold and erosion behaviour was tested. The four different EPS Xanthan Gum, Alginic Acid, Carrageenan and Agar were selected for their ease of availability,

differences in chemical properties, and absence of safety issues ensuring the potential for wide usage in future work. Xanthan Gum ($C_{35}H_{49}0_{29}$) is a polysaccharide commonly used as a food additive and has also been included in earlier laboratory tests (Tolhurst~~, Gust, and Paterson~~ et al. 2002; Parsons et al. 2016). Alginic Acid $(C_6H_80_6)_n$, also known as alginate, is a carbohydrate produced by brown algae and also widely used in food. Carrageenan is a sulphate polysaccharide extracted from red seaweeds and also widely used as a food additive. We used the Iota variety that has two sulphate groups per disaccharide ($C_{24}H_{36}0_{25}S_2$). Agar is used as a gelling agent and is obtained from the polysaccharide agarose found in some species of red algae.

A protocol similar to the one used in Tolhurst~~, Gust, and Paterson~~ et al. (2002) was applied to prepare the petri dish sediment samples for CSM testing. A control test with no EPS, and four tests with increasing EPS contents of 1.25 g, 2.5 g, 5 g and 10 g per kg of sediment were performed for the four different EPS. The applied concentrations of the extracted EPS were based on reported values in the literature (Taylor et al. 1999; Tolhurst et al. 2002) and were also compared to the EPS content measured in the natural biofilm experiment. The required EPS amount was added to 330 ml of distilled water and mixed thoroughly by a magnetic stirrer. The EPS solution was then added to 650 g of dry 110 micron sand and mixed with an electric stirrer to distribute the EPS solution throughout the sand. The sand-EPS mixture was then poured into plastic petri dishes (5 cm diameter) to a depth of 1 cm. Irregularities on the sediment surface increase the bed roughness and stress (Tolhurst~~, Gust, and Paterson~~ et al. 2002), therefore care was taken to create a level surface by tapping the side of the petri dishes before testing.

All test conditions were repeated five times and all tests were performed under fully saturated conditions.

2.3.1 Preparation procedure

Protocol development on the application and effects of different extracted EPS required an assessment of the impact of the preparation procedure on the sediment entrainment threshold. To this end, the preparation procedure described above, referred to as 'Wet Mixing', was complemented by a preparation procedure referred to as Dry Mixing. Both procedures used the same sand, EPS and amounts but the order in which they were combined and mixed, was changed. In contrast to the Wet Mixing procedure, in the Dry Mixing procedure the required amount of EPS was first added to the sand and mixed with an electric stirrer. Then, 330 ml of distilled water was added to the dry sand-EPS mixture and a further mixing with the electrical stirrer was performed. Note that the risk of dust formation and associated loss of EPS powder was greater in the Dry Mixing procedure.

2.3.2 Environmental conditions

Protocol development on the application and effects of different EPS also required an assessment of the impact of the different environmental conditions on the sediment entrainment threshold. ~~As temperature~~Temperature, salinity and ~~to a lesser extent~~ pH commonly vary between flume facilities. Therefore, a sensitivity analysis on the ~~effectiveness~~effects of ~~extracted EPS to impact~~these environmental conditions on the sediment entrainment threshold for the four extracted EPS was performed. For temperature, tests were performed at 10° ~~Celsius~~C and 40 ° ~~Celsius~~C in addition to the

control tests at room temperature of 20° ~~Celsius~~C. For pH, tests were performed with a pH of 4 and a pH of 10 in addition to the control tests of a pH of 7. Standard and commercially available buffer solutions were used to obtain liquids with these pHs. For salinity, tests with a salinity of 30 ppm corresponding to brackish conditions were performed in addition to the control tests with distilled fresh water.

2.3 Biostabilisation index

A biostabilisation index (Manzenrieder, 1983; Tolhurst et al., 1999; Friend et al., 2003a; Thom et al., 2010) was calculated to quantify and compare the degree of biostabilisation in the natural biofilm and extracted EPS experiments. The biostabilisation index was calculated from the ratio of the critical erosion shear stress (τ_c) of the relevant experiment, to the τ_c for the uncolonised sand. Since the same sand was used in both experiments, a direct comparison between biostabilisation indices from the natural biofilm experiment and the extracted EPS can be made. For the natural biofilm experiment, the mean, median and maximum critical erosion shear stresses from 61 measurements were used in calculating the biostabilisation index. For the extracted EPS experiment, the mean critical erosion shear stress was used in calculating the biostabilisation index.

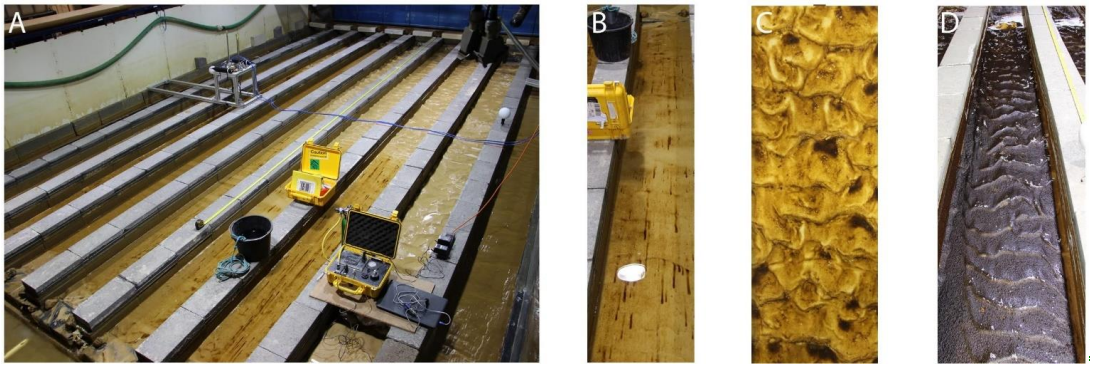
3 Results

3.1 Biofilm colonisation and species ecology of the natural biofilm experiment

The eutrophic water used in the experiment resulted in rapid colonisation and growth of a diatomaceous biofilm on the substrate materials (Figure 1A). After two weeks, biofilm colonisation and growth was

localised and organised into ~~a~~ darker stripes running parallel to the main flow (Figure 1B). Colonisation and development of the biofilm continued over the next five weeks resulting in a more widespread biofilm coverage (Figure 1C). At the end of the experiment after seven weeks, the sandy substrate in the channels was covered by a few millimetres thickness of black biofilm crust (Figure 1D). At this stage, mortality of the biofilm had set in locally, which was illustrated by greyish patches within the black healthy biofilm that were sometimes eroded. This observation ensured that we observed the full life cycle of a diatomaceous biofilm from early colonisation to mortality and subsequent crust erosion.

Microscope investigations of the species ecology confirmed a saline environment that was dominated by halophilous diatoms, which are common in coastal zones (Pan et al. 2004). The diverse flora was dominated by five main species: a) *Nitzschia pellucida*, b) *Nitzschia sigma*, c) *Mastogloia sp*, d) *Navicula perminuta*, and e) *Amphora pediculus*. The *Nitzschia* species are considered early colonisers (Ledger et al. 2008; Ros~~, Marín Murcia, and Aboal~~ et al. 2009), and were indeed found primarily in the samples of the early stages of the experiment. Furthermore, all taxa were benthic rather than planktonic, as expected in lotic conditions (Passy 2001; Schmidt et al. 2016). Some diatoms were attached to the sediment grains while others were motile and unattached to the substrate. Also, ciliates were present and presumably eating the diatoms. Importantly, many of the species observed were obligate and cannot tolerate freshwater, in agreement with the designed experimental conditions.

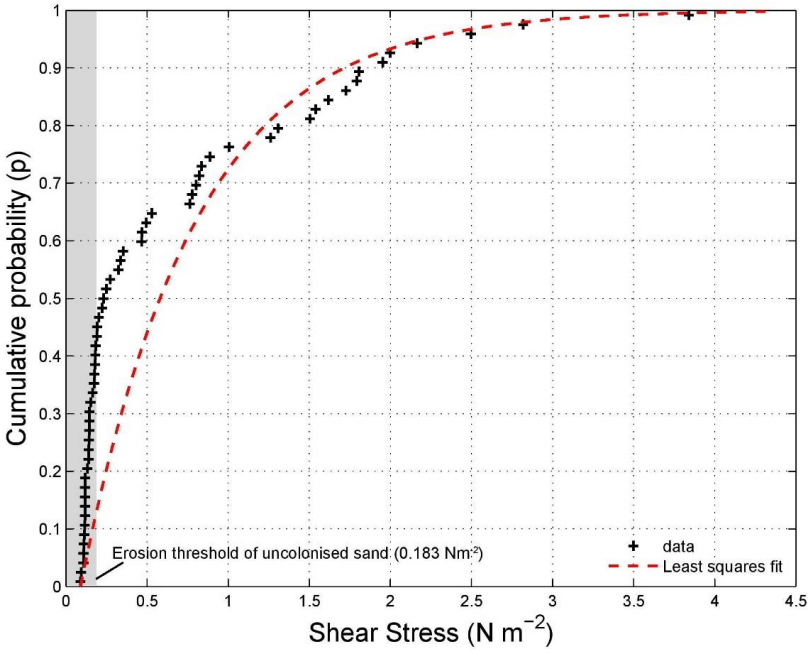


~~Figure 1: Biofilm experiment in Total Environment Simulator flume facility. A) Overview of experimental setup showing nine (9) parallel channels for biofilm colonisation. Channels are 9 meters long, 0.48 m wide and contain a 0.1 m thick substrate layer consisting of uniform 110 micron sandy sediment. Also visible in the yellow cases is the CSM erosion device. Panels B) – D) show colonisation and development of a diatomaceous biofilm on the sandy substrate from early onset in (B) to a mature and dark biofilm after 6 weeks. Flow in panels A), C) and D) is towards viewer, and away from viewer in panel B).~~

3.~~1~~2 Sediment stability from biofilm-secreted EPS

Figure 2 shows a cumulative probability distribution of the CSM sediment stability measurements made during the flume experiment. The average shear stress entrainment threshold was 0.69 $N \cdot m^{-2}$ with a standard deviation of 0.82 $N \cdot m^{-2}$. The distribution is highly skewed towards lower shear stresses, as evidenced by a median shear stress entrainment threshold of 0.23 $N \cdot m^{-2}$. This median value was just above the CSM measured entrainment threshold for the uncolonised sand of 0.18 $N \cdot m^{-2}$, which is in close agreement with the theoretical entrainment threshold for the ~~applied~~ 110 micron sand of 0.15 $N \cdot m^{-2}$ ~~(Zanke 2003).~~(Eq. 2). Notably, 42% of the measurements were smaller than the entrainment threshold ~~of~~for the uncolonised sand, even ~~when~~though a biofilm was clearly visible at the substrate

surface for all measurements. A maximum entrainment threshold of 3.84 $N \cdot m^{-2}$ was measured, which represents a more than 21 times higher erodibility threshold compared to the uncolonised sand. Entrainment thresholds were higher in the first three weeks ~~(~ 1 $N \cdot m^{-2}$ on average) in comparison to the last two weeks (~ 0.3 $N \cdot m^{-2}$ on average).~~(week 1: 0.93 $N \cdot m^{-2}$ (mean value); week 2: 0.84 $N \cdot m^{-2}$; week 3: 1.01 $N \cdot m^{-2}$) in comparison to the last two weeks (week 4: 0.29 $N \cdot m^{-2}$; week 5: 0.34 $N \cdot m^{-2}$). The standard deviations of the erodibility thresholds (week 1: 0.66 $N \cdot m^{-2}$; week 2: 0.94 $N \cdot m^{-2}$; week 3: 1.15 $N \cdot m^{-2}$; week 4: 0.53 $N \cdot m^{-2}$; week 5: 0.40 $N \cdot m^{-2}$) are of similar or larger magnitude as the mean values and indicative of the large spatial variation in and between flumes at any given time.


Cumulative probability (p)
Shear Stress (N m^{-2})
Erosion threshold of uncolonised sand (0.183 Nm^{-2})
data
Least squares fit
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5

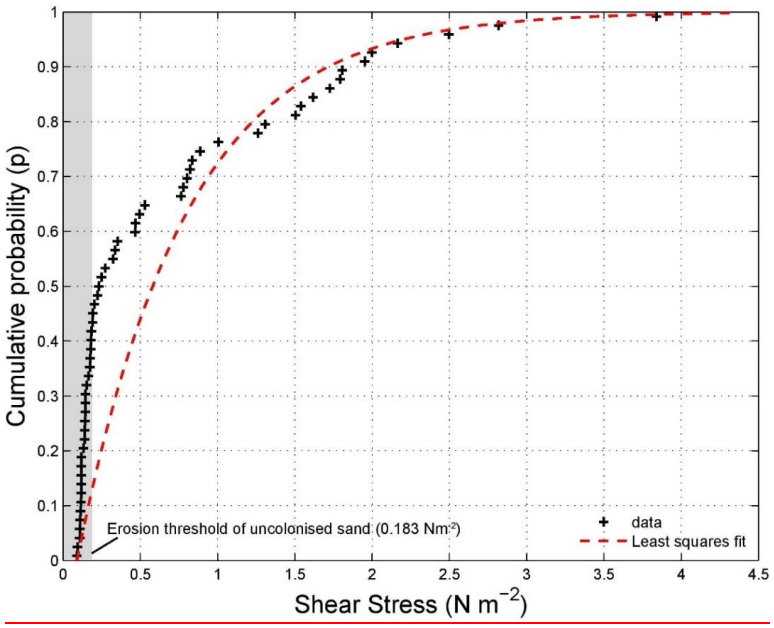


Figure 2. Shear stress measurements made with CSM erosion device during natural biofilm growth experiment. The measurements (n = 61) are best described by a least squares exponential fit with a mean parameter μ of 0.71.

The average carbohydrate content, here equated to EPS content, was 7.8 µg per g of sand with a standard deviation of 7.8 µg per g (Figure 3). The measurements were best described by an exponential fit with a mean parameter μ of 7.88, highlighting the skewed character of the data with many lower content observations and fewer towards higher EPS contents. The maximum measured EPS content was 34.6 µg per g of sand. In contrast to the sediment entrainment threshold (Figure 2), the average EPS content increased on a weekly basis from 5.6 µg per g of sand.in the first week to 11.6 µg per g of sand in the final week.

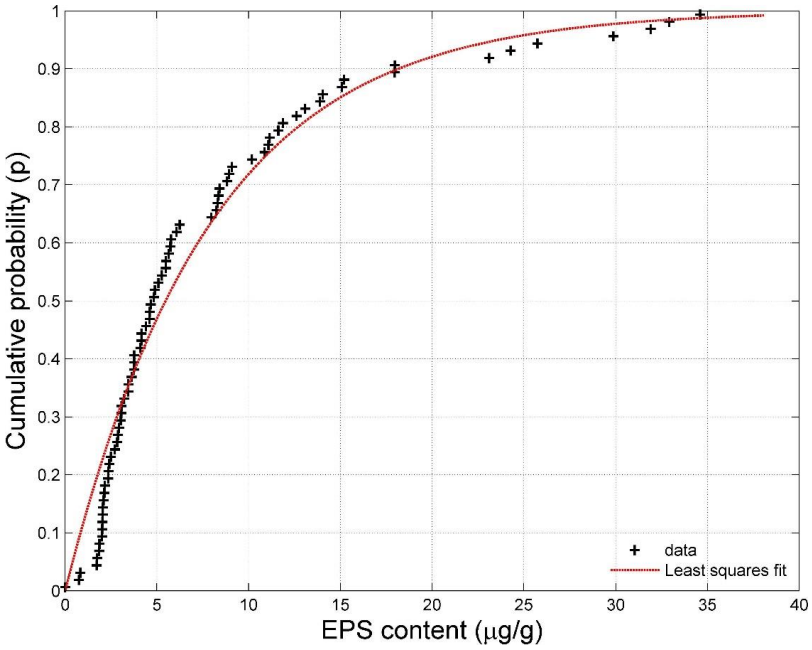


Figure 3. Extracellular polymeric substances (EPS) content measurements made during natural biofilm growth experiment. The measurements (n = 80) are best described by a least squares exponential fit with a mean parameter μ of 7.88.

3.~~2~~3 Sediment stability from extracted EPS

~~The above section 3.1 Sediment stability from biofilm secreted EPSillustrated that experiments involving natural biofilms typically take multiple weeks to capture the complete life cycle. As such flume experiments are costly, extracted EPS has the potential to provide an effective alternative to reproduce the sediment stabilising effects on natural biofilms in a fast and controlled manner. Below, small scale experiments are described quantifying 1) the effect of the different concentrations of four extracted EPS, 2) the effect of the preparation procedure, and 3) the effect of environmental factors such~~

~~as temperature, salinity and pH. All three tests were intended to contribute towards the development of a protocol to guide the use of extracted EPS in experiments as a surrogate to replicate sediment stability from natural biofilms. The applied concentrations of the extracted EPS were based on the measured EPS contents in the natural biofilm experiment (Figure 3) and reported values in the literature (Taylor, Paterson, and Mehlert 1999; Tolhurst, Gust, and Paterson 2002).~~

~~3.2~~The above section 3.2 illustrated that experiments involving natural biofilms typically take multiple weeks to capture the complete life cycle. As these flume experiments are costly, extracted EPS has the potential to provide an effective alternative to reproduce the sediment stabilising effects on natural biofilms in a fast and controlled manner. Below, the second set of experiments focussing on extracted EPS are described.

3.3.1 Effects of extracted EPS content on sediment stability

The four extracted EPS had different effects on sediment stability (Figure 4). Alginic Acid and Agar did not increase the sediment stability above the ~~erosion~~ threshold for erosion of the sand without EPS, for all applied concentrations. For Xanthan Gum and Carrageenan, the ~~erosion~~ threshold for erosion generally increased with increasing EPS content (Table 1). For these EPS, the relation between the critical shear stress for erosion and EPS content was best described using linear models (Figure 4), where the slope of the linear model for Xanthan Gum (0.28) was more than double the slope of the linear model for Carrageenan (0.11).

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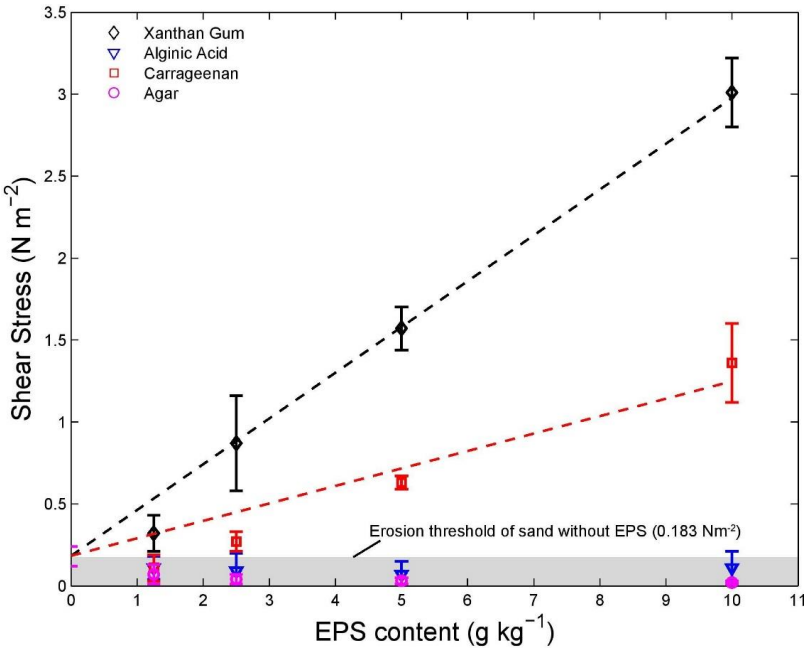

Figure 4. The threshold for erosion ~~thresholds~~ of 110 micron sandy substrate with different contents for four extracted EPS as measured with the CSM erosion device. Best fit curves were fitted using linear models for Xanthan Gum (Shear stress threshold = 0.28 EPS content + 0.18) and Carrageenan (Shear stress threshold = 0.11 EPS content + 0.18). Error bars are standard deviation from n = 5 repeat measurements.

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Table 1. ~~Erosion thresholds~~Threshold for erosion of four extracted EPS measured with the CSM erosion device. Statistics calculated from n = 5 repeat measurements.

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	Average ± St. deviation ~~erosion~~ threshold for erosion (N·m^{-2})			
EPS (g·kg^{-1})	Xanthan Gum	Carrageenan	Agar	Alginic Acid
0	0.18 ± 0.06	0.18 ± 0.06	0.18 ± 0.06	0.18 ± 0.06

1.25	0.32 ± 0.11	0.11 ± 0.08	0.07 ± 0.06	0.11 ± 0.08
2.5	0.87 ± 0.29	0.27 ± 0.06	0.04 ± 0.03	0.09 ± 0.11
5	1.57 ± 0.13	0.63 ± 0.04	0.03 ± 0.02	0.07 ± 0.08
10	3.01 ± 0.21	1.36 ± 0.24	0.02 ± 0.01	0.11 ± 0.10

3.~~2~~3.2 Effects of preparation procedure on sediment stability

The preparation procedure adopted for adding the extracted compounds to the sediment material had an impact on the resultant ~~erosion~~ threshold for erosion (Figure 5). 'Dry mixing' the extracted EPS powder with the sediment prior to adding water resulted in a higher ~~erosion~~ threshold for erosion than 'Wet mixing' the EPS powder with sediment in water for all tested EPS. The difference was greatest for Xanthan Gum with a 67% higher threshold for erosion for the dry mixing procedure compared to the wet mixing procedure.

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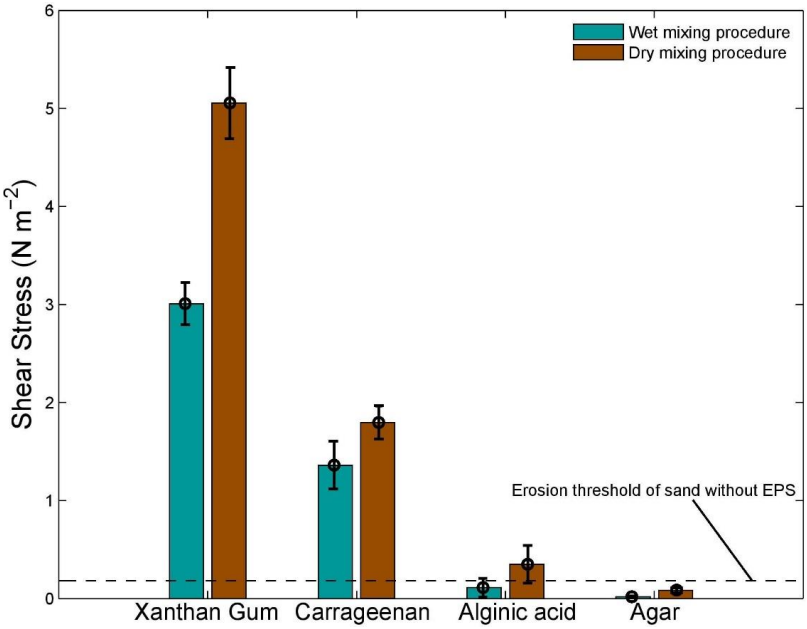

Figure 5. The erosion thresholds as a function of the preparation procedure for four surrogates as measured with the CSM erosion device. ~~Wet mixing involves dissolving the extracted EPS powder in water and stir, then add sediment and mix. Dry mixing involves the addition of extracted EPS powder to sediment and mix, then add water and stir.~~ Error bars are standard deviation from n = 5 repeat measurements.

3.~~2~~3.3 Temporal effects on sediment stability

Time elapsed from initial mixing also affected the sediment stabilising capacity of extracted EPS (Figure 6). Repeat measurements after one day, seven days and fifteen days demonstrated that the ~~erosion~~ thresholds for erosion remained constant throughout the first week. However, the repeat measurements after fifteen days showed a decrease in the ~~erosion~~ threshold for erosion below the

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~~erosion~~ threshold for erosion of sand without EPS. This effectively meant that after about two weeks of initial application of EPS the impact on the ~~erosion~~ threshold for erosion of the sediment ceased to exist.

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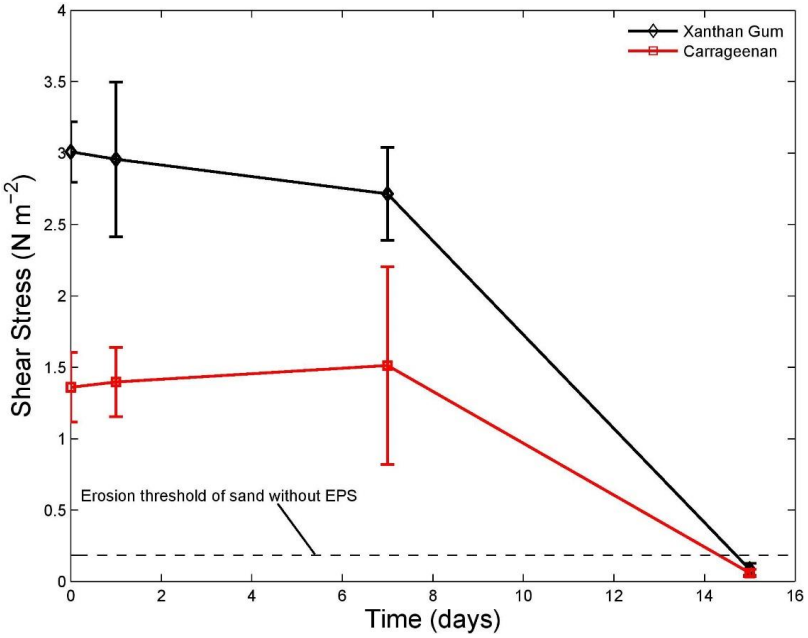

Figure 6. The threshold for erosion ~~thresholds~~ as a function of time for Xanthan Gum and Carrageenan as measured with the CSM erosion device. Error bars are standard deviation from n = 3 repeat measurements.

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3.~~2~~3.4 Effects of salinity on sediment stability

Salinity had a limited effect on the threshold for erosion ~~thresholds~~ (Figure 7). Saline water tended to decrease the threshold for erosion ~~threshold~~ compared to freshwater conditions, though the differences are statistically insignificant for all four EPS. The threshold for erosion ~~thresholds for~~of Alginic Acid and Agar remained below the threshold for erosion ~~threshold~~ of sand without EPS independent of the

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salinity of the water. These findings imply that the results of the extracted EPS experiments, which were mostly obtained for freshwater conditions, can be extrapolated to saline conditions.

~~This implies that the findings of this study that were mostly obtained for freshwater conditions can be extrapolated to saline conditions.~~

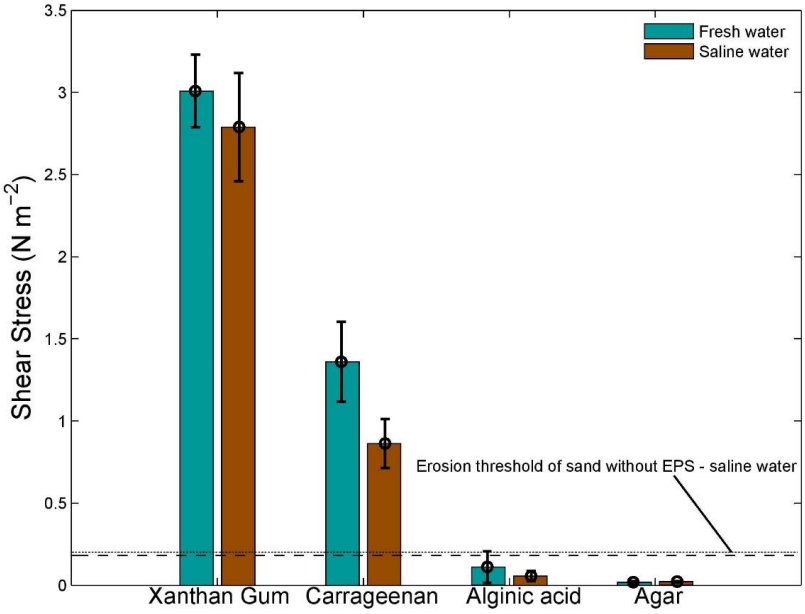

Figure 7. The threshold for erosion ~~thresholds~~ as a function of salinity for four extracted EPS as measured with the CSM erosion device. ~~Tap~~Distilled water was used for the freshwater tests and a salinity of 30 ppt was used for the saline water tests. The horizontal lines correspond to the threshold for erosion ~~thresholds~~ of sand without EPS for freshwater (dashed) and saline water (dotted). Error bars are standard deviation from n = 3 repeat measurements.

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3.~~2~~3.5 Effects of pH on sediment stability

The pH of the applied solution had variable effects on the ~~erosion~~ threshold for erosion (Figure 8). An acid solution with a pH of 4 resulted in a higher ~~erosion~~ threshold for erosion of Xanthan Gum, but in a

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lower threshold for Carrageenan. An alkaline solution with a pH of 10 resulted in a lower threshold for erosion ~~thresholds for~~ of Xanthan Gum as well as Carrageenan. The threshold for erosion ~~thresholds for~~ of Alginic Acid and Agar remained below the threshold for erosion ~~threshold~~ of sand without EPS, independent of the pH of the solution.

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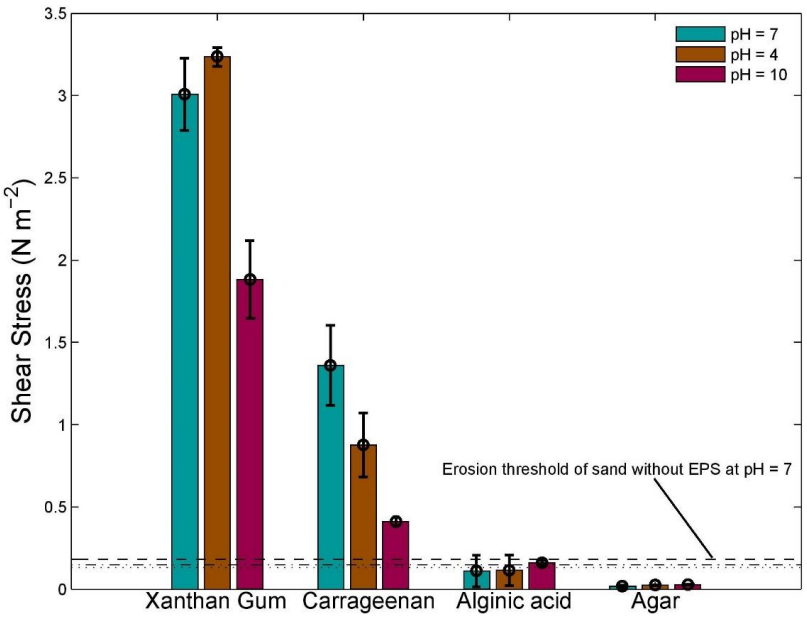

Figure 8. The threshold for erosion ~~thresholds~~ as a function of pH for four extracted EPS as measured with the CSM erosion device. The horizontal lines correspond to the threshold for erosion ~~thresholds~~ of sand without EPS for water with a pH of 7 (dashed), a pH of 4 (dotted), and a pH of 10 (dash-dotted). Error bars are standard deviation from n = 3 repeat measurements.

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3.~~2~~3.6 Effects of temperature on sediment stability

~~Temperature impacted the measured erosion thresholds (Figure 9). Both a~~A lower temperature of 10° ~~Celsius and~~C as well as a higher temperature of 40° ~~Celsius~~C resulted in a lower ~~erosion thresholds.~~ threshold for erosion (Figure 9). For Xanthan Gum as well as Carrageenan, the threshold for erosion ~~thresholds were~~was about ~~half under~~halved during 10° ~~Celsius~~C and 40° ~~Celsius~~C test conditions compared ~~to~~with 20° ~~Celsius~~C test conditions. The threshold for erosion ~~thresholds for~~of Alginic Acid and Agar remained below the ~~erosion~~ threshold for erosion of sand without EPS independent of the temperature.

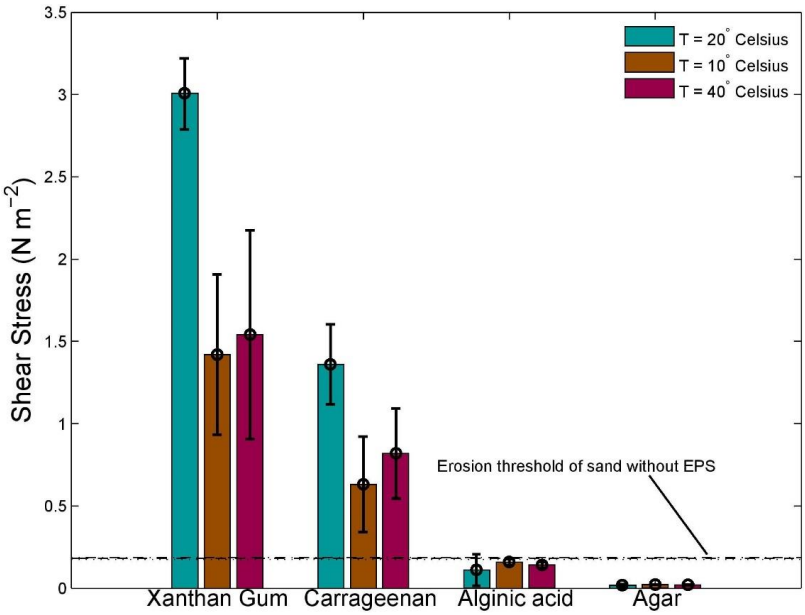

Figure 9. The threshold for erosion ~~thresholds~~ as a function of temperature for four extracted EPS as measured with the CSM erosion device. The horizontal lines correspond to ~~the~~ threshold for erosion ~~thresholds~~ of sand without EPS for a temperature of 20° ~~Celsius~~C (dashed), a temperature of 10° ~~Celsius~~C (dotted), and a temperature of 40° ~~Celsius~~C (dash-dotted). Error bars are standard deviation from n = 3 repeat measurements.

3.~~2~~3.7 Synthesis of the effects of extracted EPS on sediment stability

In summary, extracted EPS Xanthan Gum and Carrageenan increased the ~~erosion~~ threshold for erosion with higher EPS content (Table 1). For these two EPS, the relation between ~~erosion~~ threshold for erosion and EPS content was linearly and predictable (Figure 4). In contrast, the extracted EPS Alginic Acid and Agar did not increase the ~~erosion~~ threshold for erosion (Table 1), independent of the applied concentration (Figure 4), preparation procedure (Figure 5) or environmental condition such as salinity, pH and temperature. Yet, this study demonstrated that the preparation procedure, environmental

conditions and time impacted on the resultant ~~erosion~~ threshold for erosion of the EPS Xanthan Gum and Carrageenan. A dry mixing procedure increased the ~~erosion~~ threshold for erosion while saline water, alkaline solutions and non-room temperature test conditions of 10° ~~Celsius~~C and 40° ~~Celsius~~C decreased the threshold for erosion ~~thresholds~~. The tests also showed that the effects of adding Xanthan Gum and Carrageenan on the threshold for erosion ~~thresholds~~ ceased to exist after about two weeks following initial application (Figure 6). These findings indicate that the effectiveness of extracted EPS to stabilise sediment is sensitive to the applied concentration, the preparation procedure, time and environmental conditions.

4 Discussion

The CSM data show that addition of extracted EPS Xanthan Gum and Carrageenan increases the critical ~~erosion~~ threshold for erosion, even at low EPS concentrations (Figure 4 and Table 1). The observation that the ~~erosion~~ threshold for erosion increased approximately linear with EPS content for Xanthan Gum is in agreement with the findings reported in Tolhurst~~, Gust, and Paterson~~ et al. (2002). We find a similar linear increase in ~~erosion~~ threshold for erosion with EPS content for Carrageenan, though the rate of increase is smaller compared to Xanthan Gum. The approximately linear relation between EPS content and ~~erosion~~ threshold for erosion across the measured range for Xanthan Gum and Carrageenan simplifies the prediction of biostabilisation effects due to extracted EPS. Two other extracted EPS, Alginic Acid and Agar, were also tested and showed negligible biostabilisation for any of the test conditions investigated.

Biostabilisation of the same sandy substrate due to natural biofilm colonisation and due to addition of extracted EPS Xanthan Gum and Carrageenan compares well (Table 2). We find a mean biostabilisation index due to natural biofilm colonisation ~~and development~~ of almost four times that of the uncolonised sand. Such a biostabilisation index is within the reported range for fine sand (Dade et al. 1990; Vignaga et al. 2013). More specifically, 42% of the tested samples did not show biostabilisation compared to uncolonised sand while 10% of the measurements showed a tenfold biostabilisation relative to uncolonised sand (Figure 2). The presented cumulative probability distribution of critical threshold for erosion ~~thresholds~~ reflects the large spatial and temporal variations generally seen in natural biostabilised environments (Paterson 1989; Amos et al. 1998; Tolhurst et al. 1999~~; Tolhurst et al. ,~~ 2003; Friend~~, Collins, and Holligan 2003~~ et al. 2003a~~). The biostabilisation index due to extracted EPS covers approximately the same range of erosion thresholds for the applied EPS contents. Xanthan Gum may be more suited to replicate the higher biostabilisation observations of natural biofilms due to the increased erosion thresholds for the highest applied content of 10 g·kg^{-1}. Carrageenan~~.~~ For the second set of experiments focusing on extracted EPS, we find similar biostabilisation indices as observed in the first set of experiments on natural biofilms (Table 2). For Xanthan Gum, the biostabilisation index of 1.7 for the lowest concentration of 1.25 g·kg^{-1} compares well to the median biostabilisation index of 1.3 in the natural biofilm experiment. The biostabilisation index of 16.4 for the highest concentration of 10 g·kg^{-1} represents the 97th percentile of the biostabilisation index of the natural biofilm experiment, and is close to the maximum biostabilisation index of 21. For Carrageen, the biostabilisation indices are generally lower and the biostabilisation index of 1.5 for the concentration of 2.5 g·kg^{-1} compares well to the median biostabilisation index of 1.3 in the natural biofilm experiment. The biostabilisation index of

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3.5 for the concentration of 5 g·kg^{-1} is close to the mean biostabilisation index of 3.8 in the natural biofilm experiment. Xanthan Gum may be more suited for replicating the higher biostabilisation observations of natural biofilms due to the higher threshold for erosion of the highest applied contentration of 10 g·kg^{-1}. Application of carrageenan may be more appropriate to replicate the lower biostabilisation observations of natural biofilms due to the small effect on the threshold for erosion ~~thresholds for~~ of low concentrations.

Table 2~~. Biostabilisation index resulting from natural biofilm and Xanthan Gum and Carrageenan extracted EPS as measured in this study. The biostabilisation index is defined relative to the erosion threshold of sand without EPS (Manzenrieder 1985).~~

. Biostabilisation index resulting from natural biofilm colonisation and the addition of Xanthan Gum and Carrageenan extracted EPS to sand.

~~Uncolonised~~	*Natural biofilm experiment*~~Median~~	*Extracted EPS experiment*~~Mean~~	~~Maximum~~
~~Biofilm~~	~~1~~	~~1.3~~	~~3.8~~ ~~21.0~~

Bare sand	Mean	Median	Max.	1.25 g·kg^{-1}	2.5 g·kg^{-1}	5 g·kg^{-1}	10 g·kg^{-1}
~~Xanthan Gum~~	~~1.7~~	~~4.8~~		~~8.6~~		~~16.4~~	
~~Carrageenan~~	~~0.6~~	~~1.5~~		~~3.5~~		~~7.4~~	

~~(10 g·kg^{-1})~~				*Wet mix*	*Dry mix*	*Saline*	*pH = 10*	*T = 10° Celsius*~~C~~
Natural biofilm	1	1.3	3.8 21.0	-	-	-	-	-	-	-	-

Xanthan Gum	1	-	-	-	1.7	4.8	8.6	16.4	27.6	15.2	10.3	7.8
Carrageenan	1	-	-	-	0.6	1.5	3.5	7.4	9.8	4.7	2.2	1.6

The concentrations of the EPS derived from the natural biofilm experiment ~~(~~(Figure 3, ~8 µg g^{-1}) are about three orders of magnitude lower than the applied extracted EPS concentrations (2.5 – 10 mg g^{-1}) to achieve the same biostabilisation effect~~.~~ (Table 2). Two reasons may explain these differences. First, the applied phenol-sulphuric acid assay only measures a carbohydrate fraction of the total EPS~~, along with~~ as well as some low-weight sugars that are extracted with the polymeric material (Underwood~~, Paterson, and Parkes~~ et al. 1995)~~. Along with the sensitivity of the EPS extraction methodology~~. As a result, this technique may not measure all of the EPS present in the sample, and is also known to be sensitive to a host of conditions (Perkins et al. 2004)~~, this may be part of the explanation for the lower EPS concentrations in the natural biofilm samples.~~. Second, sediment sampling for EPS concentration analysis typically involved scraping off the top centimetre of the substrate. However, it has been shown that EPS content in nature is highest at the sediment surface (top 200 µm) and decreases with depth (Taylor and Paterson 1998). Our sediment sampling strategy is likely to have diluted the EPS concentration, which may offer another explanation for the lower EPS concentrations in the natural biofilm samples.

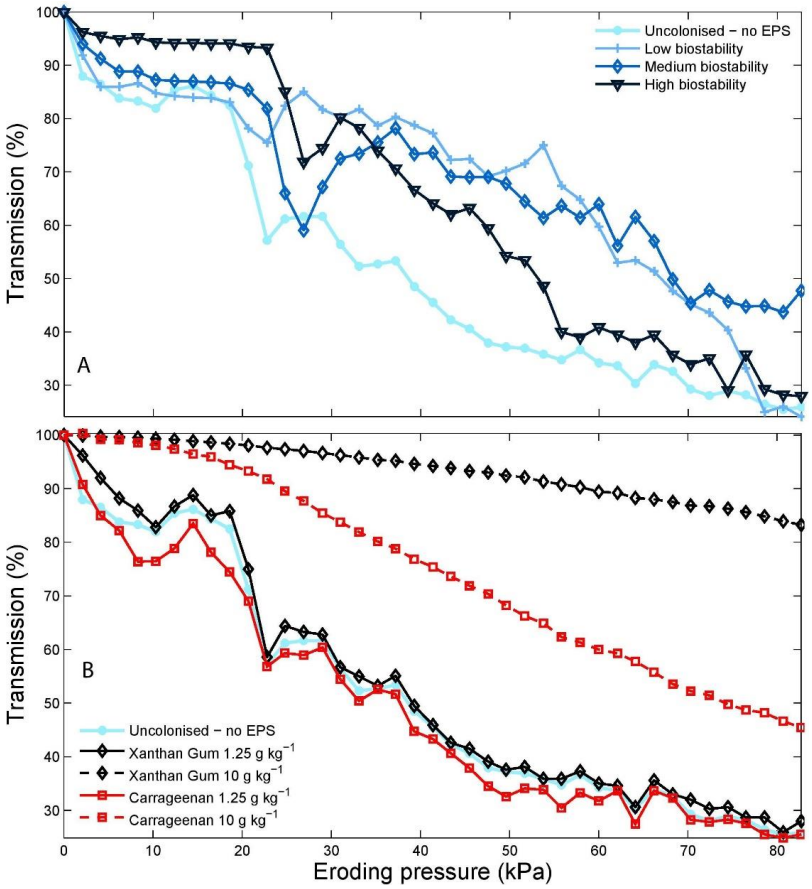

Figure 10. CSM erosion profiles for sediment with different degrees of biostability due to natural biofilm colonisation (A) and due to different Xanthan Gum and Carrageenan extracted EPS contents (B). Following Tolhurst et al. (1999), the eroding pressure corresponding to a 90% transmission is defined as the erosion event.

Erosion profiles for low concentrations of extracted Xanthan Gum and Carrageenan are similar to those measured from the natural biostabilised sediments (Figure 10). For higher concentrations of Carrageenan and particularly Xanthan Gum, the erosion rate is reduced relative to the natural

biostabilised samples. In contrast to the natural samples where EPS concentration decreases with depth (Taylor and Paterson 1998), the extracted EPS were mixed homogenously with depth in this study. As a consequence, the erosion rate for high concentrations of extracted EPS has been reduced more than would be found under natural conditions. To overcome this and to better replicate natural biofilm-mediated erosion behaviour, it may be more appropriate to apply extracted EPS only on the surface in future studies. This will result in the highest EPS concentrations at the sediment surface that decreases with depth depending on the porosity and saturation of the substrate.

The methodologies described herein for preparing engineered sediments and the resultant biostabilisation may serve as protocols to guide the design of future studies that aim to represent biological cohesion. In essence, biostabilisation effects of Xanthan Gum and Carrageenan extracted EPS behave linearly (Figure 4) and are therefore predictable. Different concentrations of these extracted EPS may be used to replicate the temporal and spatial variations generally seen in biostabilisation due to natural biofilm colonisation. Other than biostabilisation, no differences in application or behaviour between Xanthan Gum and Carrageen were observed in this study. Furthermore, the sensitivity analysis performed in this study showed that the effectiveness of Xanthan Gum and Carrageenan for the stabilisation of sediment, not only depends on the applied concentration, but is also is sensitive to the preparation procedure, time after application and environmental conditions. The results for the time elapsed after initial application tests were obtained for samples that dried out between measurements. Temporal behaviour of extracted EPS may be different when the engineered sediments remain wet for the duration of the test, which requires further research. The sensitivity of engineered sediments to

salinity, pH and temperature found in this study indicates that a high level of control of these environmental variables is required for reliable application of extracted EPS in flume facilities.

Physical modelling of the complex flow, sediment transport and ecological interactions within aquatic ecosystems is key to bridge the divide between field observations and numerical models (Thomas et al. 2014; Gerbersdorf and Wieprecht 2015). The implementation of biological processes into sediment transport equations that have traditionally been modelled as abiotic systems is expected to result in better predictions of sediment dynamics (Black et al. 2002; Righetti and Lucarelli 2007; Gerbersdorf et al. 2011; Parsons et al. 2016). Our study confirms that Xanthan Gum and Carrageenan extracted EPS are not perfect analogues of natural biofilms (Perkins et al. 2004), but they are capable of introducing realistic biological cohesion into flume facilities in a fast and controlled manner for a range of commonly used conditions. The reduction in experimental time here is significant since the maximum biostabilisation effects of natural biofilm can easily take 5 weeks or more to achieve, whereas extracted EPS can be introduced at the same time as the sediment minimising time to set-up an experiment. Similarly growth patterns, particularly the effect of increasing biostabilisation can easily be reproduced in a stepwise manner by introducing greater concentrations of the extracted EPS. Although this study has focused on replicating one aspect of natural biofilm behaviour only, future physical modelling studies employing extracted EPS may provide important insights into the role of biological cohesion in sediment dynamics, and how these may be altered in a changing climate.

5 Conclusions

This study aimed to evaluate biostabilisation effects of existing extracted EPS for a range of conditions commonly used in physical modelling experiments. Four extracted EPS were tested and addition of Xanthan Gum and Carrageenan increased the threshold for erosion ~~threshold~~, while the addition of Alginic Acid and Agar did not increase the ~~erosion~~ threshold for erosion for all test conditions. Changes in threshold for erosion ~~thresholds~~ produced by the addition of Xanthan Gum and Carrageenan extracted EPS compared well to measured ~~erosion~~ threshold for erosion resulting from natural biofilm colonisation of the same sandy substrate. The increase of the threshold for erosion ~~threshold~~ with EPS content is linear and predictable for Xanthan Gum and Carrageenan, albeit with a lower rate of increase for Carrageenan. Furthermore, the effectiveness of Xanthan Gum and Carrageenan to stabilise sediment is sensitive to the preparation procedure, time after application and environmental conditions such as salinity, pH and temperature. The methodologies for preparing engineered sediments described in this paper can provide quantifiable biostabilisation effects and may be used as protocols for designing future bio-physical experimental models that seek to represent biological cohesion. This approach will bring the significant advantages of being fast, replicable and controllable, which will improve experimental efficiency and enable experiments that explore a larger parameter space to be undertaken at lower cost.

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