Dear Dr. Hodge,

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

We found the reviews helpful and constructive. Below we describe how we used the reviews (in italics) to improve the manuscript. Detailed textual comments were mostly incorporated and sometimes used as indicator where text clarity had to be improved.

Thank you for your guidance in revising the manuscript. The development of a robust methodology and protocol for the application and impacts of extracted EPS in flume facilities provides the novelty of our work. This novel aspect is explicitly mentioned and has been given emphasis on P5, lines 14-17. We now also more clearly outline the connections between the experiments and the motivation for the petri-dish tests (the importance for preparation time and experimental duration are emphasised on P5 line 7-9). Furthermore, we clarify what type of natural system our flume experiments are intended to reproduce (P7, lines 15-16) and we show in the findings that the biofilm species composition is in agreement with species commonly seen in these natural environments (P13, lines 6-15). Lastly, we provide more details on the flume experiment design conditions (Methods section 2.1.1).

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

Reviewer 1 – Trevor Tolhurst

Specific comments:
Synthetic is used to describe the EPS used in this study, but it is not really synthetic – all of them have been extracted from natural sources, could a more accurate term be used?

We changed 'synthetic' to 'extracted' throughout the manuscript in an effort come up with a more accurate term.

Page 2, line 12: I don't put a hyphen in microphytobenthos. I would not consider flocs and aggregates to be biofilms because to me a biofilm is a thin layer over a surface (although flocs and aggregates could have a biofilm over their surface).

We removed the hyphen in microphytobenthos and deleted flocs and aggregates.

Page 3 lines 2-3: 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. In my own work, I use biofilm to denote a visible (either by eye or microscopically) layer of microphytobenthos on a sediment surface.

Thank you for pointing this out. We added this clarification to the manuscript (P3, lines 5 -7).

Page 3 lines 10-13: there are also examples of buoyant biofilms, which reduce the erosion threshold of sediments (e.g. Sutherland, T. F., C. L. Amos, and J. Grant. "The effect of buoyant biofilms on the erodibility of sublittoral sediments of a temperate microtidal estuary." Limnology and Oceanography 43.2 (1998): 225-235; and Tolhurst, T. J., M. Consalvey, and D. M. Paterson. "Changes in cohesive sediment properties associated with the growth of a diatom biofilm." Hydrobiologia 596.1 (2008): 225-239).

We added this information and the associated references to the manuscript (P3, lines 15 -17).

Page 5 line 14: to clearly differentiate from the synthetic EPS, I would insert 'diatom' before 'biofilm-secreted'.

Done

Page 7 line 20 and throughout: change 'Soil' to 'Sediment'. For me the sand used in this work is not a soil.

Done

Page 9 line 20: being precise, test Sand 7 increments in 2.068 kPa steps, but this probably doesn't matter too much given the error in the actual pressure of the CSM jet.

We corrected this information.

Page 12 line 20: I'm not entirely sure what is meant by 'floated around the substrate' do you mean the diatoms were motile and not attached to the sediment grains?

Yes indeed, that is what we meant to say. We rephrased to clarify the explanation, following the reviewer's suggestion (P13, lines 12-13).

Page 15 lines 9-10: I'm not entirely sure what is meant by 'Added', can the authors clarify?

We removed 'added' here and also in section heading 3.2.

Page 24 line 6: this reads oddly 'non-room temperature test conditions of 20◦' isn't 20◦ room temperature? Should this be conditions of 10 and 40◦?

We rephrased this sentence to correctly represent the temperature conditions (P25, lines 5-7).

Page 25 Table 2: 'Relative biostabilisation' was termed 'biostabilisation index' by Manzenreider, consider using this terminologly instead (Manzenrieder, H. "Retardation of initial erosion under biological effects in sandy tidal flats." 1985 Australasian Conference on Coastal and Ocean Engineering. Institution of Engineers, Australia, 1985).

We changed 'relative biostabilisation' to 'biostabilisation index' in Table 2 in incorporated the citation to the work of Manzenreider (1985).

Page 28 lines 18-19: This is interesting. I looked at the effects of letting diatom biofilms grown on sand drain and 'dry' out for a few hours in my PhD. There were changes in the erosion threshold and some indication that drier samples had a lower erosion threshold, but the effects were largely masked by variability in the biofilms. It is quite possible that the decrease in erosion threshold seen with time in this study is at least partly due to the successive drying. It seems quite likely to me that as EPS dries out it will become less effective at stabilising sediment, but as you say, it needs more research.

Thank you for sharing your experiences on this topic. In our study, the engineered samples with repeat measurements over time showed different behavior after re-wetting the sediment. This may be due to a dilution effect of the EPS, the successive breakdown of the EPS over time, or some other unknown process associated with the successive drying. It would be insightful to further investigate this topic in future work.

Technical corrections:
Page 8 line 9: change 'weighted' to weighed'.
Page 15 line 10: the 'Added' on line 10 should have a lower case A.
Page 21 line 10: insert a comma after 'Gum'.
Page 24 line 14: change 'linear' to 'linearly'.

Page 28 line 13: delete second full stop.
Page 30 line 14: insert a comma after ‘controllable’.

All done.

Reviewer 2

1. The authors provide a very useful review of the literature. However, having done so, I am left wondering what we do not understand, and thus why another study is required? I suggest the authors explain the novelty of their work.

Thank you for reviewing our work and your appreciation of the literature review. The development of a robust methodology and protocol for the application and impacts of extracted EPS in flume facilities provides the novelty of our work (this is explicitly mentioned and has been given emphasis on P5, lines 14-17). Indeed, earlier studies have investigated natural diatom-biofilm behaviour (e.g. Gerbersdorf and Wieprecht, 2015) and also work was done on extracted EPS already (e.g. Tolhurst 2002). Our study builds on this work and explicitly relates the sediment stabilising ability of natural diatom-biofilms to that of extracted EPS. A unique aspect of our study is that we use the same sediment for the natural diatom-biofilms and extracted EPS tests so we can compare the results directly (this is explicitly mentioned on P6, line 1 and P6, lines 11-13). In addition, we expand the existing knowledge on the application and impacts of extracted EPS by testing four different EPS for a range of environmental conditions. Such knowledge is currently lacking and has led to costly and time-consuming trial-and-error approaches in a variety of different modelling facilities. Our findings present a systematic methodology and protocol for a range of commercially available EPS and are therefore expected to inform future studies seeking to introduce biological cohesion in a rapid and controlled manner (the importance for preparation time and experimental duration are emphasised on P5 line 6-9).

2. I was disappointed the Introduction and Methods section did not make it clear what type of freshwater system is investigated. Which system are the scaled flume experiments trying to represent? I think this is especially important because we are told that one of the motivations for this study is that there has been a lot of work on biostabilisation in coastal settings but not in freshwater systems, and yet the studies biofilms are common in coastal zones. Furthermore, how do the studied conditions (e.g. slopes, depth:width, relative roughness, grain size, Reynolds number) pertain to those found in the natural system and match the conditions commonly found where these biofilms grow? Likewise, the authors should comment on how closely the Cohesive Strength Meter systems mimic erosion processes in the natural system? Furthermore little detail is provided on the setup of the small-scale synthetic EPS experiments. For example, I have read over the paper several times and I still cannot establish whether these tests were performed in a flume.

We use brackish water in our experiment. This experimental condition is explicitly mentioned on P7, lines 15-16. The brackish water setting is representative of estuarine, mangrove and deltaic settings within the fluvial-to-marine transition zone. In our literature review, we indeed mention that the role of EPS in freshwater systems is not as well understood as in marine systems (P2, lines 16-20) but our experiment was not aimed at gaining a better understanding of the EPS behaviour in freshwater conditions.

The experimental design conditions are in approximate agreement with natural reference systems. Please note that the experiment was not setup to replicate a specific natural system but rather a collection of shallow brackish environments. The channels had no initial gradient (but the flow may have

created a self-formed gradient in the substrate during the experiment), a width-to-depth ratio of 5, 110 microns sand and a Reynolds number indicating turbulent flow (Re = 5000 – 10000). We added this information to the Methods section 2.1.1. But most importantly, these experimental conditions resulted in a thriving biofilm with a species composition that was consistent with species commonly seen in brackish coastal environments (P13, lines 6-15).

The Cohesive Strength Meter employs a vertical jet to measure the erosion shear stress of sediments. This approach differs from natural erosion processes, which predominantly generate a horizontal shear. Based on a series of systematic tests, Tolhurst et al (1999) provides a calibration of the vertical jet to an equivalent critical erosion shear stress. A full discussion on the strengths and weaknesses of the CSM erosion device as well as the development history and relation to other erosion devices is provided in Tolhurst et al (1999) and we refer the reviewer to this document for full details. In our study, we applied the calibration of the vertical jet to an equivalent critical erosion shear stress, and we would also like to stress that the CSM provides one of the few erosion devices allowing workers to make quantitative and repeat measurements of sediment stability.

The small-scale tests with extracted EPS are performed in petri dishes. This is for example explicitly written on P11, line 11-12 ('The sand-EPS mixture was then poured into plastic petri dishes') and lines 12-13 ('therefore care was taken to create a level surface by tapping the side of the petri dishes before testing'). We also refer to the protocol used in Tolhurst et al (2002) and mention that we follow a similar protocol. To make it more explicit that these small-scale tests were performed in petri dishes, we added this information to section heading 3.1 (Petri dish sediment sample tests with extracted EPS) as well as in referring to the protocol used in Tolhurst et al (2002) on P11, lines 5-6.

3. authors state that synthetic EPS is able "to replicate the sediment stabilising capacity of natural biofilms". However the authors have found that three times more synthetic EPS concentration is needed to replicate the same stabilising effect of natural biofilms, suggesting the capacity is much higher for natural biofilms.

Our findings indeed indicate that extracted EPS can replicate the sediment stabilising capacity of natural biofilms as seen from the biostabilisation index (Table 2). In contrast to the reviewer's suggestion, we do not think that the natural biofilms have a higher stabilizing capacity than observed in our study. The biostabilisation index values are consistent with earlier studies on the sediment stabilising capacity of natural biofilms (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; Droppo et al. 2007; Righetti and Lucarelli 2007; Vignaga, Haynes, and Sloan 2012; Graba et al. 2013; Thom et al. 2015). It is therefore unlikely that the capacity is much higher for natural biofilms, although the observations in our study indicate a substantial internal variation (Figure 2). Rather, the explanation for the different EPS concentration in the extracted EPS tests and natural biofilm experiment must be sought in the determination of the EPS concentration in the natural biofilm experiment. We provide two explanations for the lower EPS concentrations in the biofilm experiment (P27, lines 6 to P28, line 7) and both may explain the difference with the applied extracted EPS concentrations in the small-scale petri dish tests.

4. The calibration curve in equation 1 is important for gaining an accurate estimate of the critical shear stress. To allow readers to have confidence in their estimates, the authors should present a graph showing how this curve has been derived, and the predictive performance of this curve. Small deviations from the curve are likely to produce larger discrepancies in critical shear stress estimates due to the non-linear relationship between critical shear stress and the applied jet force. For example, Figure 4 has error bars to represent the range in estimates from repeats, but hypothetically speaking, how much large would the error bar be if the uncertainty in the estimates themselves was incorporated?

We refer to Tolhurst et al (2002) for details on the how the calibration curve is derived. This article provides a detailed explanation of the performed tests and the quality of the calibration curve. As shown in Tolhurst et al (2002), the uncertainty in the calibration curve is typically in the order of 0.1-0.2 N/m^2, which suggests that the error bars would be 0.4 N/m^2 when, hypothetically, taking this effect into account.

Minor amendments:

P4, lines 14-21: There appears to be mismatch between this paragraph and the approach/results. If the prediction of the potential impacts of climate change on aquatic environments and the application of bioengineering adaptation strategies is important, how does this paper address these needs?

The sentences on P4 (lines 18-21) and P5 (lines 1-9) are included to signal the need for including biological processes in sediment transport predictions. The understanding of these bio-physical relations is currently limited and the relationships may also be different under different climatic conditions. We consider flume experiments a primary tool of researchers to address these bio-physical relationships but the developments are hampered because experiments including real biota are time-consuming and costly, with also some questions raised about the degree of natural behaviour of biota in flume facilities.

Our paper provides the first step to overcome the aforementioned issues by the development of a robust methodology and protocol for the application and resultant impacts of extracted EPS to introduce biological cohesion in a rapid and controlled manner. So although the current paper does not directly address the potential impacts of climate change on aquatic environments, the work described provides an important step in facilitating future work that will do so. We believe that providing this context is helpful to the reader and have checked the manuscript to make sure that the implications of our work are well represented, also with reference to studying the impacts of climate change.

P9, line 16: What is routine S7?

Routine S7 is one of the CSM test routines. To further clarify this, we now explicitly link the use of S7 in the manuscript to the word 'CSM'. See P10, lines 7-12.

Inconsistencies in the use of et al and author names in citations should be corrected.

Done.

Quantifying biostabilisation effects of biofilm-secreted and ~~synthetic~~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 ~~synthetic~~extracted extracellular polymeric substances (EPS) on sediment stability. ~~Synthetic~~Extracted EPS may be useful to simulate biofilm mediated biostabilisation, and potentially provide a method of speeding up time scales of physical modelling experiments investigating biostabilisation effects. We find a mean biostabilisation due to natural biofilm colonisation and development of almost four times that of the uncolonised sand. The presented cumulative probability distribution of measured critical erosion thresholds reflects the large spatial and temporal variations generally seen in natural biostabilised environments. For identical sand, engineered sediment stability from the addition of ~~synthetic~~extracted EPS compares well across the measured range and behaves in a linear and predictable fashion. Yet, the effectiveness of ~~synthetic~~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 micro-phytobenthos, microbial mats, flocs, aggregates 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.~~ (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

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~~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; Droppo et al. 2007; Righetti and Lucarelli 2007; Vignaga, Haynes, and Sloan 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 threshold, although variations in space and time (Friend, Collins, and Holligan 2003; Thom et al. 2015) and between cohesive and non-cohesive sandy environments are large. Biostabilisation of coarse sand and gravel may increase the erosion threshold up to almost three times compared to abiotic sediment (Vignaga, Haynes, and Sloan 2012)~~

while a tenfold increase in erosion threshold 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 in two ways: 1) by physically binding both cohesive and non-cohesive sediment grains together (see Tolhurst, Gust, and Paterson (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 (Kornman and De Deckere 1998) illustrating the potential effects micro-organisms can have on system-wide sediment fluxes. At a smaller scale, the introduction of the synthetic 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 EPS was added to the sediment mixture (Hoyal and Sheets 2009; Kleinhans et al. 2014). Furthermore, 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 synthetic EPS Xanthan Gum is not a perfect analogue for natural biofilms (Perkins et al. 2004), it is 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). Synthetic EPS also has the advantage that enables time scales of physical modelling experiments to be reduced and biostabilisation effects to be controlled.~~

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; Droppo et al. 2007; Righetti and Lucarelli 2007; Vignaga, Haynes, and Sloan 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 threshold, although variations in space and time (Friend, Collins, and Holligan 2003; 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 of sediments (Sutherland, Amos, and Grant 1998; Tolhurst, Consalvey, and Paterson 2008). Yet, biostabilisation of coarse sand and gravel may increase the erosion threshold up to almost three times compared to abiotic sediment (Vignaga, Haynes, and Sloan 2012) while a tenfold increase in erosion threshold 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 in two ways: 1) by

physically binding both cohesive and non-cohesive sediment grains together (see Tolhurst, Gust, and Paterson (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 (Kornman and De Deckere 1998) illustrating the potential effects micro-organisms can have on system-wide sediment fluxes. At a smaller scale, 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 EPS was added to the sediment mixture (Hoyal and Sheets 2009; Kleinhans et al. 2014). Furthermore, 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 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 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.

The objective of this study is therefore to evaluate biostabilisation effects of existing ~~synthetic~~extracted EPS for a range of conditions commonly used in physical modelling experiments. In doing so, the study solely focusses on the sediment stabilising aspect of biofilms and does not explicitly ~~not~~ intend to replicate and evaluate natural biofilm behaviour and effects. ~~The~~The novel outcome of this study is the development of a robust methodology and protocol for the application and resultant impacts of ~~synthetic~~extracted EPS ~~are expected~~, which can be applied to ~~inform~~ future experimental studies ~~seeking to introduce~~that require the representation of biological cohesion in a rapid and controlled manner. A sandy substrate was used in this study since 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) of diatom biofilm-secreted EPS on sandy substrates in a physical model experiment.
2. Using the same sandy substrate, quantify the biostabilisation effects of four ~~synthetic~~extracted EPS.
3. Assess the sensitivity of the biostabilisation effects of the four ~~synthetic~~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 ~~synthetic~~extracted extracellular polymeric substances (EPS). The aim of the auxiliary tests was to quantify the ability of ~~synthetic~~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. 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 with ~~synthetic~~extracted EPS.

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 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. ~~Soil~~Sediment samples from the top 0.01 m of every channel were taken to determine the EPS content from (see section 2.1.2 Determination of EPS content ~~-~~for details on methodology to determine EPS from ~~soil~~sediment samples). In total, 80 ~~soil~~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 ~~-~~for details on the CSM erosion device). In total, 61 successful CSM measurements were made.

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)~~Dubois et al. 1956). The methodology can be subdivided into two main steps. First, 1.5 grams of each ~~soil~~sediment sample were ~~weighted~~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 ~~soil~~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 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_e) according to the calibrated formulation:~~

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

(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 et al. (2002) as well, allowing for a direct comparison between the data. This routine starts at 0kPa, incrementing by 2 kPa per step up to 82~~Tolhurst, Gust, and Paterson (2002) as well, allowing for 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.

2.3 ~~Sediment~~ Petri dish sediment sample tests with ~~synthetic~~extracted EPS

The effect of varying amounts of four different types of ~~synthetic~~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 2002; Parsons et al. 2016)~~(Tolhurst, Gust, and Paterson 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 et al. (2002) was used to prepare the 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 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 et al. 2002), therefore care was taken to create a level surface by tapping the side of the petri dishes before testing.~~Tolhurst, Gust, and Paterson (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 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 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 ~~synthetic~~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, salinity and to a lesser extent pH commonly vary between flume facilities, a sensitivity analysis on the effectiveness of ~~synthetic~~extracted EPS to impact the sediment entrainment threshold was performed. For temperature, tests were performed at 10° Celsius and 40 ° Celsius in addition to the control tests at room temperature of 20° Celsius. 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. 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.

3 Results

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)~~(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 2009)~~(Ledger et al. 2008; Ros, Marín-Murcia, and Aboal 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)~~(Passy 2001; Schmidt et al. 2016). Some diatoms were attached~~, some floated around~~ 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.

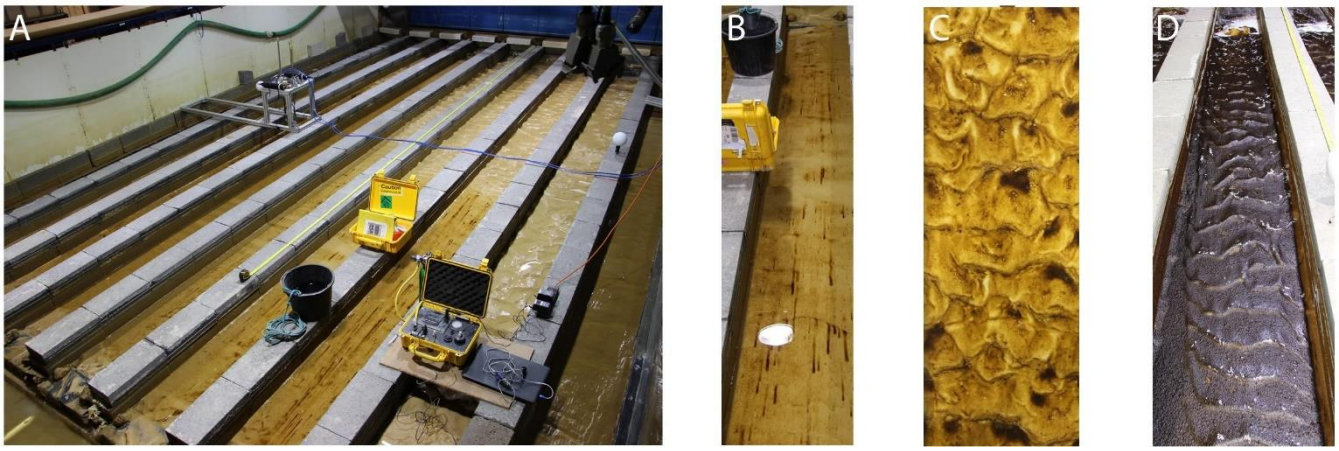

Figure 1: Biofilm experiment in Total Environment Simulator flume facility. A) Overview of experimental setup showing nine (9) parallel channels for biofilm colonisation. Channels are 9 meters long, 0.48 m wide and contain a 0.1 m thick substrate layer consisting of uniform 110 micron sandy sediment. 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 ~~Added sediment~~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).~~(Zanke 2003). Notably, 42% of the measurements were smaller than the entrainment threshold of the uncolonised sand, even when a biofilm was clearly visible at the substrate surface. 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).

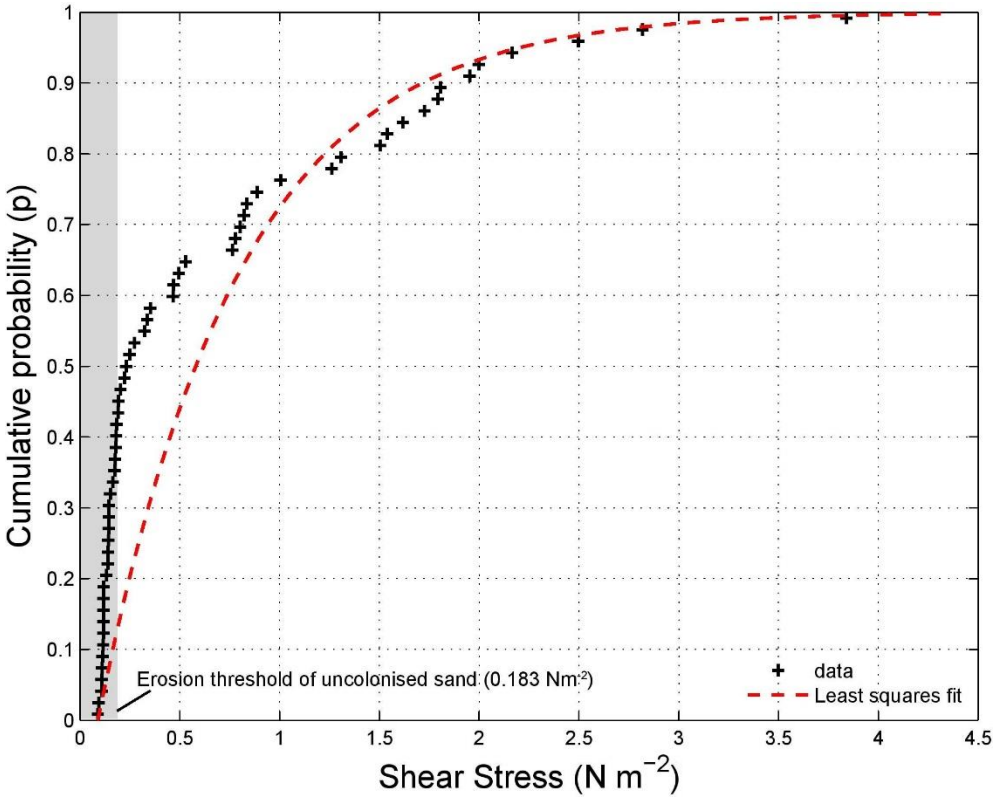

Figure 2. Shear stress measurements made with CSM erosion device during natural biofilm growth experiment. The measurements (n = 61) are best described by a least squares exponential fit with a mean parameter μ of 0.71.

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.

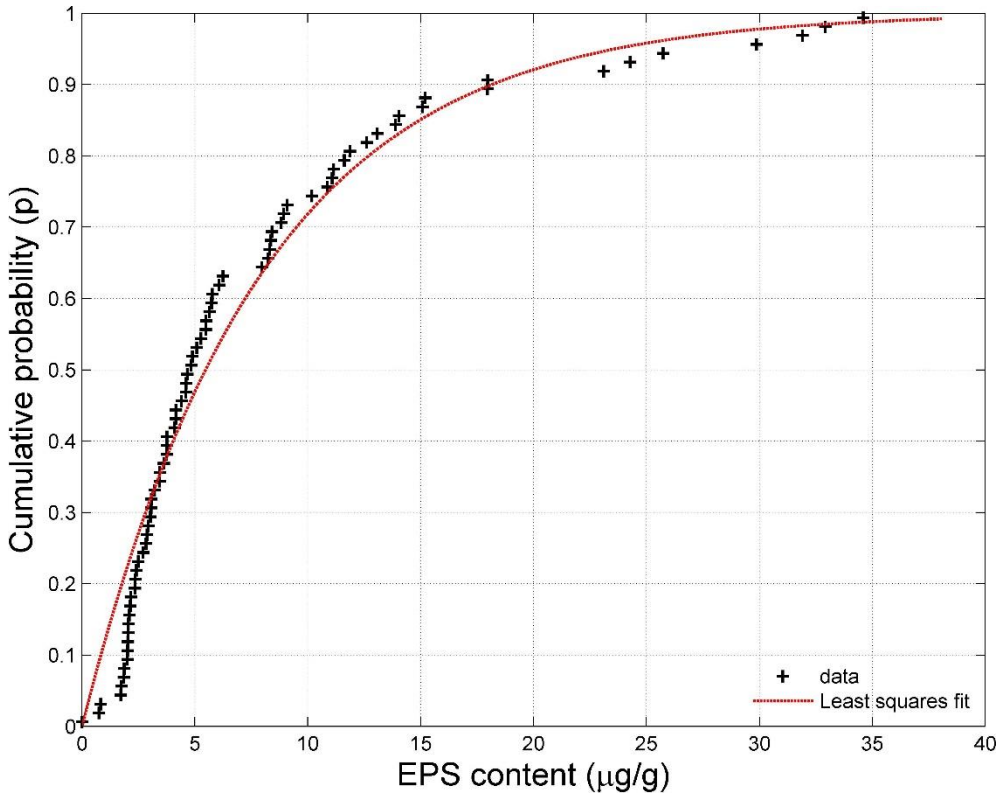

Figure 3. Extracellular polymeric substances (EPS) content measurements made during natural biofilm growth experiment. The measurements (n = 80) are best described by a least squares exponential fit with a mean parameter μ of 7.88.

3.2 ~~Added sediment~~Sediment stability from ~~synthetic~~extracted EPS

The above section 3.1 Sediment stability from biofilm-secreted EPS illustrated that experiments involving natural biofilms typically take multiple weeks to capture the complete life cycle. As such flume experiments are costly, ~~synthetic~~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 ~~synthetic~~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 ~~synthetic~~extracted EPS in experiments as a ~~synthetic~~ surrogate to ~~replace~~replicate sediment stability from natural biofilms. The applied concentrations of the ~~synthetic~~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)~~(Taylor, Paterson, and Mehlert 1999; Tolhurst, Gust, and Paterson 2002).

3.2.1 Effects of ~~synthetic~~extracted EPS content on sediment stability

The four ~~synthetic~~extracted EPS had different effects on sediment stability (Figure 4). Alginic Acid and Agar did not increase the sediment stability above the erosion threshold of the sand without EPS, for all applied concentrations. For Xanthan Gum and Carrageenan, the erosion threshold 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).

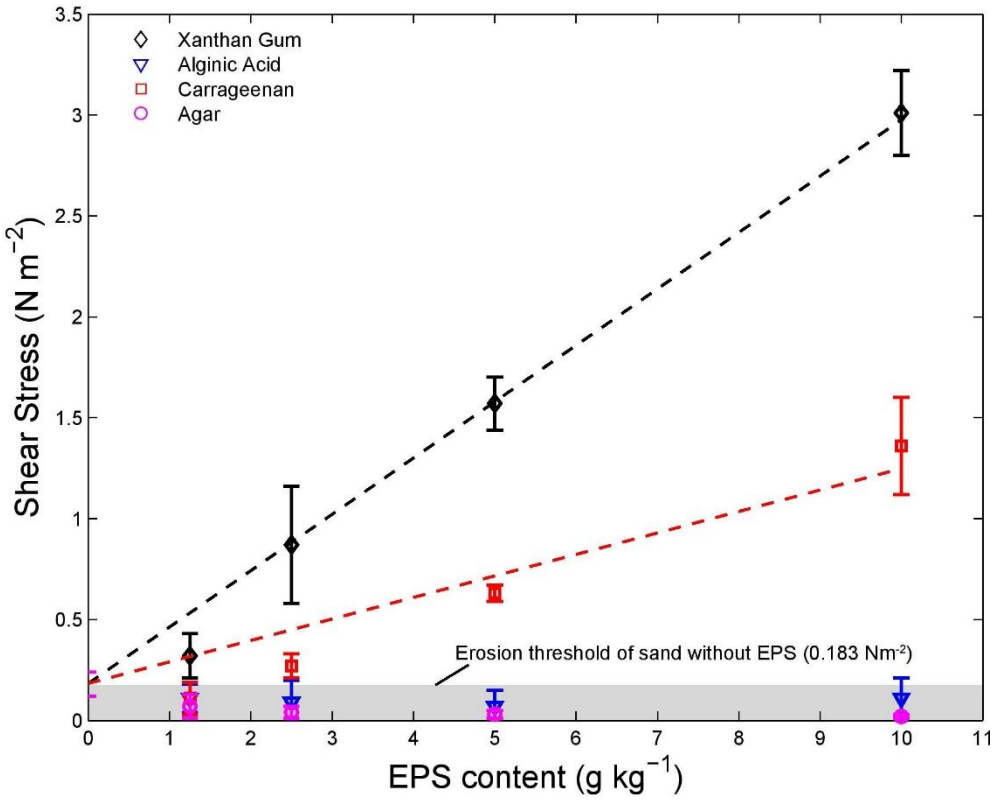

Figure 4. The erosion thresholds of 110 micron sandy substrate with different contents for four ~~synthetic~~extracted EPS as measured with the CSM erosion device. Best fit curves were fitted using linear models for Xanthan Gum (Shear stress threshold = 0.28 EPS content + 0.18) and Carrageenan (Shear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

Table 1. Erosion thresholds for four ~~synthetic~~extracted EPS measured with the CSM erosion device.

	Average ± St. deviation erosion threshold (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

3.2.2 Effects of preparation procedure on sediment stability

The preparation procedure adopted for adding the ~~synthetic~~extracted compounds to the sediment material had an impact on the resultant erosion threshold (Figure 5). 'Dry mixing' the ~~synthetic~~extracted EPS powder with the sediment prior to adding water resulted in a higher erosion threshold 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 the dry mixing procedure compared to the wet mixing procedure.

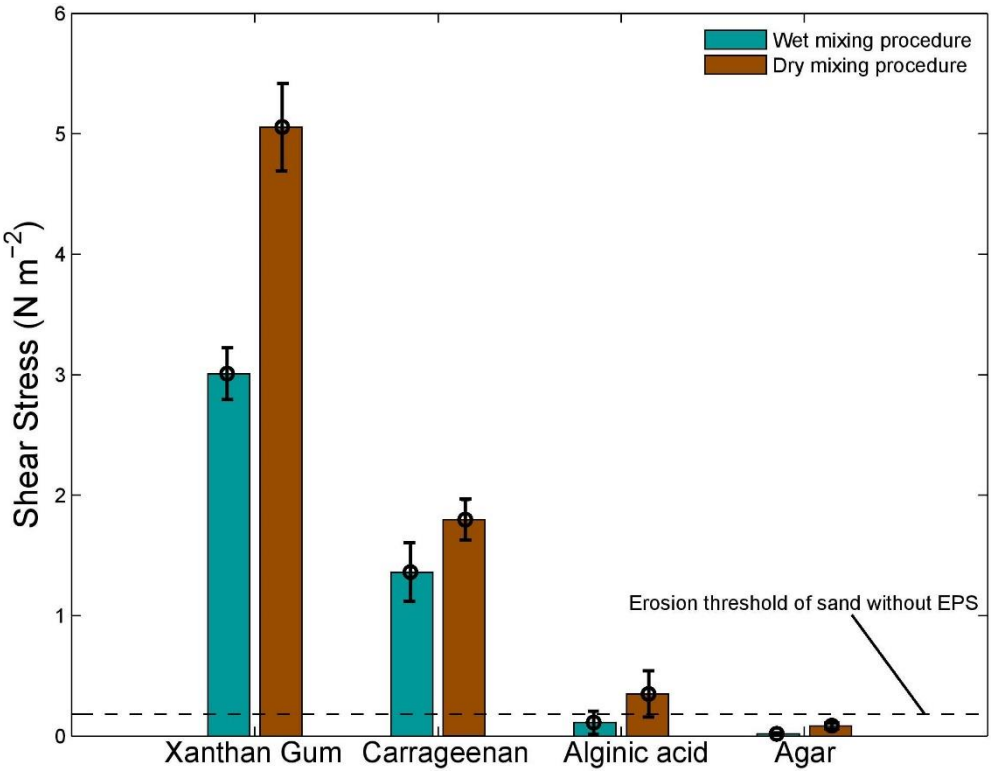

Figure 5. The erosion thresholds as a function of the preparation procedure for four surrogates as measured with the CSM erosion device. Wet mixing involves dissolving the ~~synthetic~~extracted EPS powder in water and stir, then add sediment and mix. Dry mixing involves the addition of ~~synthetic~~extracted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

3.2.3 Temporal effects on sediment stability

Time elapsed from initial mixing also affected the sediment stabilising capacity of ~~synthetic~~extracted EPS (Figure 6). Repeat measurements after one day, seven days and fifteen days demonstrated that the erosion thresholds remained constant throughout the first week. However, the repeat measurements after fifteen days showed a decrease in the erosion threshold below the erosion threshold of sand without EPS. This effectively meant that after about two weeks of initial application of EPS the impact on the erosion threshold of the sediment ceased to exist.

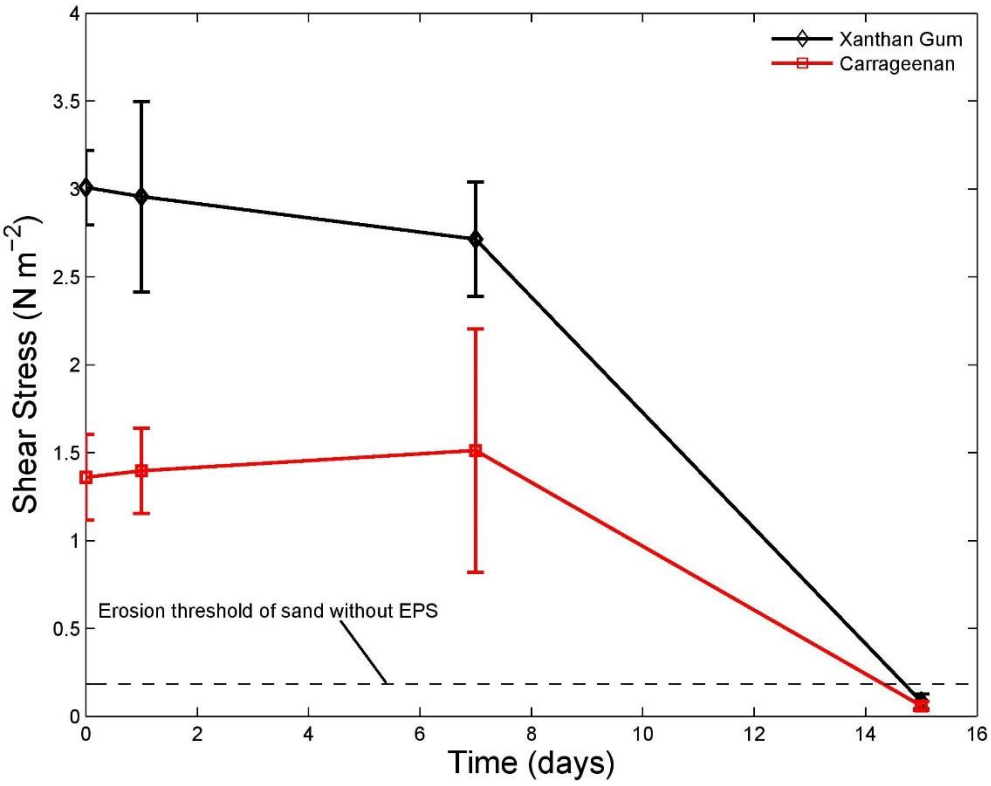

Figure 6. The erosion thresholds as a function of time for Xanthan Gum and Carrageenan as measured with the CSM erosion device. Error bars are standard deviation from n =3 repeat measurements.

3.2.4 Effects of salinity on sediment stability

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

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

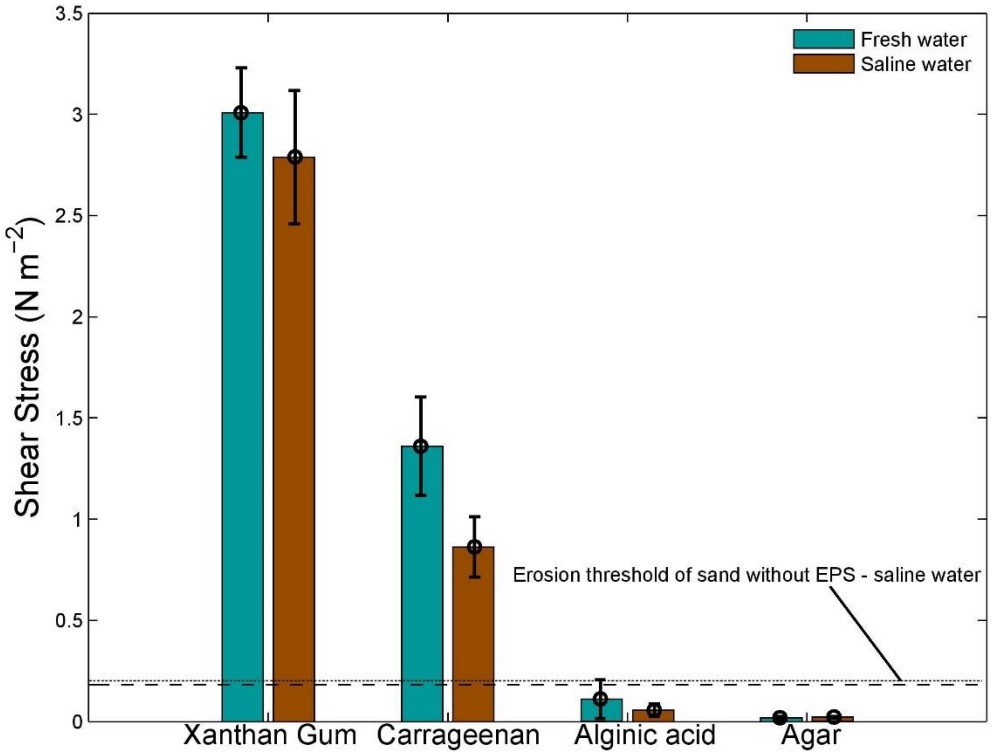

Figure 7. The erosion thresholds as a function of salinity for four ~~synthetic~~extracted EPS as measured with the CSM erosion device. Tap water was used for the freshwater tests and a salinity of 30 ppt was used for the saline water tests. The horizontal lines correspond to the erosion thresholds of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

3.2.5 Effects of pH on sediment stability

The pH of the applied solution had variable effects on the erosion threshold (Figure 8). An acid solution with a pH of 4 resulted in a higher erosion threshold for Xanthan Gum, but in a lower threshold for Carrageenan. An alkaline solution with a pH of 10 resulted in lower erosion thresholds for Xanthan Gum as well as Carrageenan. The erosion thresholds for Alginic Acid and Agar remained below the erosion threshold of sand without EPS, independent of the pH of the solution.

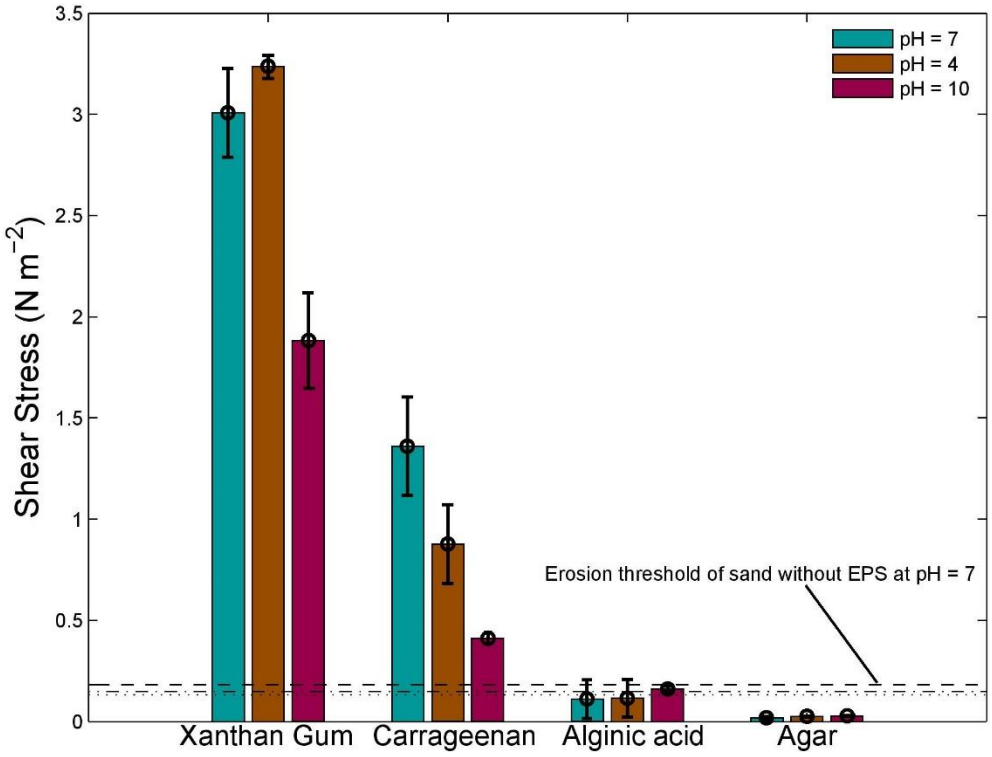

Figure 8. The erosion thresholds as a function of pH for four ~~synthetic~~extracted EPS as measured with the CSM erosion device. The horizontal lines correspond to the erosion thresholds of sand without EPS for water with a pH of 7 (dashed), a pH of 4 (dotted), and a pH of 10 (dash-dotted). Error bars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

3.2.6 Effects of temperature on sediment stability

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

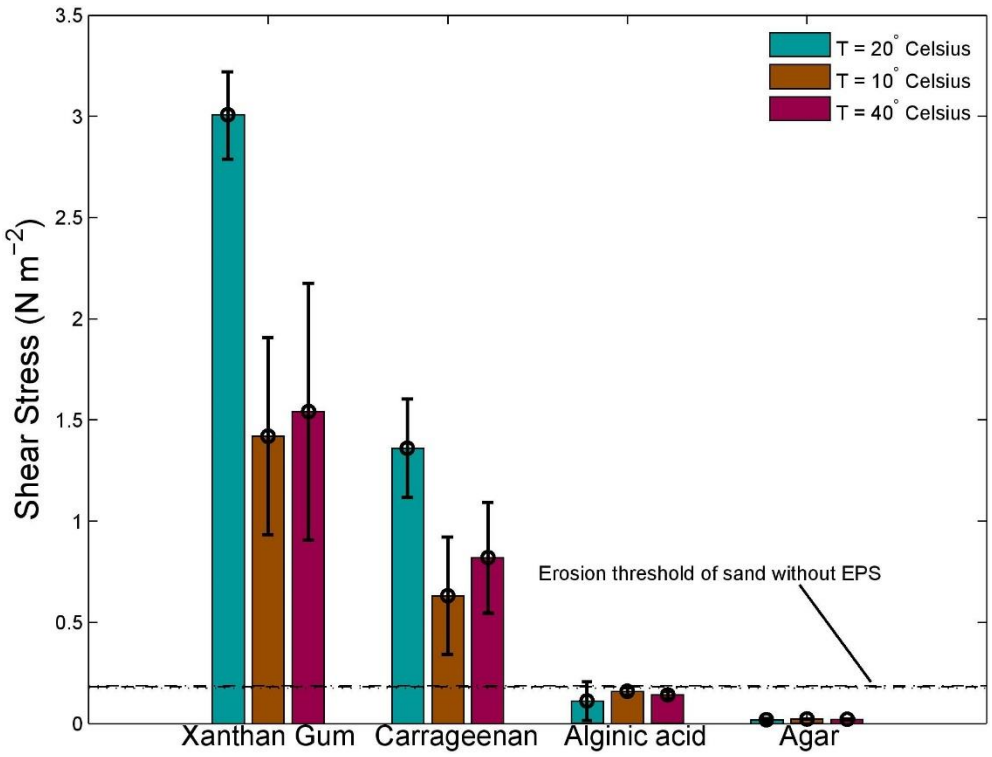

Figure 9. The erosion thresholds as a function of temperature for four ~~synthetic~~extracted EPS as measured with the CSM erosion device. The horizontal lines correspond to the erosion thresholds of sand without EPS for a temperature of 20° Celsius (dashed), a temperature of 10° Celsius (dotted), and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

3.2.7 Synthesis of the effects of ~~synthetic~~extracted EPS on sediment stability

In summary, ~~synthetic~~extracted EPS Xanthan Gum and Carrageenan increased the erosion threshold with higher EPS content (Table 1). For these two EPS, the relation between erosion threshold and EPS content was ~~linear~~linearly and predictable (Figure 4). In contrast, the ~~synthetic~~extracted EPS Alginic Acid and Agar did not increase the erosion threshold (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 the EPS Xanthan Gum and Carrageenan. A dry mixing procedure increased the erosion threshold while saline water, alkaline solutions and non-room temperature test conditions of ~~20~~10° Celsius and 40° Celsius decreased the erosion thresholds. The tests also showed that the effects of adding Xanthan Gum and Carrageenan on the erosion thresholds ceased to exist after about two weeks following initial application (Figure 6). These findings indicate that the effectiveness of ~~synthetic~~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 ~~synthetic~~extracted EPS Xanthan Gum and Carrageenan increases the critical erosion threshold, even at low EPS concentrations (Figure 4 and Table 1). The observation that the erosion threshold increased approximately linear with EPS content for Xanthan Gum is in agreement with the findings reported in ~~Tolhurst, Gust, and Paterson (2002).~~Tolhurst, Gust, and Paterson (2002). We find a similar linear increase in erosion threshold 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 across the measured range for Xanthan Gum and Carrageenan simplifies the prediction of biostabilisation effects due to ~~synthetic~~extracted EPS. Two

other ~~synthetic~~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 ~~synthetic~~extracted EPS Xanthan Gum and Carrageenan compares well (Table 2~~). We find a mean biostabilisation due to natural biofilm colonisation and development of almost four times that of the uncolonised sand. Such biostabilisation 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 (~~). 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 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). Biostabilisation due to synthetic 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 may be more appropriate to replicate the lower biostabilisation observations of natural biofilms due to the small effect on erosion thresholds for low concentrations.~~

). The presented cumulative probability distribution of critical 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). 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 may be more appropriate to replicate the lower biostabilisation observations of natural biofilms due to the small effect on erosion thresholds for low concentrations.

Table 2~~. Relative biostabilisation resulting from natural biofilm and Xanthan Gum and Carrageenan synthetic EPS as measured in this study. Biostabilisation is defined relative to the erosion threshold of sand without EPS.~~

. 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).

	Uncolonised	Median	Mean	Maximum
Biofilm	1	1.3	3.8	21.0

	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})	Dry mix	Saline	pH = 10	T = 10° Celsius
Xanthan Gum	27.6	15.2	10.3	7.8
Carrageenan	9.8	4.7	2.2	1.6

The concentrations of the EPS derived from the natural biofilm experiment (μg g^{-1}) are about three orders of magnitude lower than the applied ~~synthetic~~extracted EPS concentrations (mg g^{-1}) to achieve the same biostabilisation effect. Two reasons may explain these differences. First, the applied phenol-sulphuric acid assay measures a carbohydrate fraction of the total EPS, along with low-weight sugars that are extracted with the polymeric material ~~(Underwood, Paterson, and Parkes 1995).~~(Underwood, Paterson, and Parkes 1995). Along with the sensitivity of the EPS extraction methodology to a host of conditions ~~(Perkins et al. 2004)~~(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).~~(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.

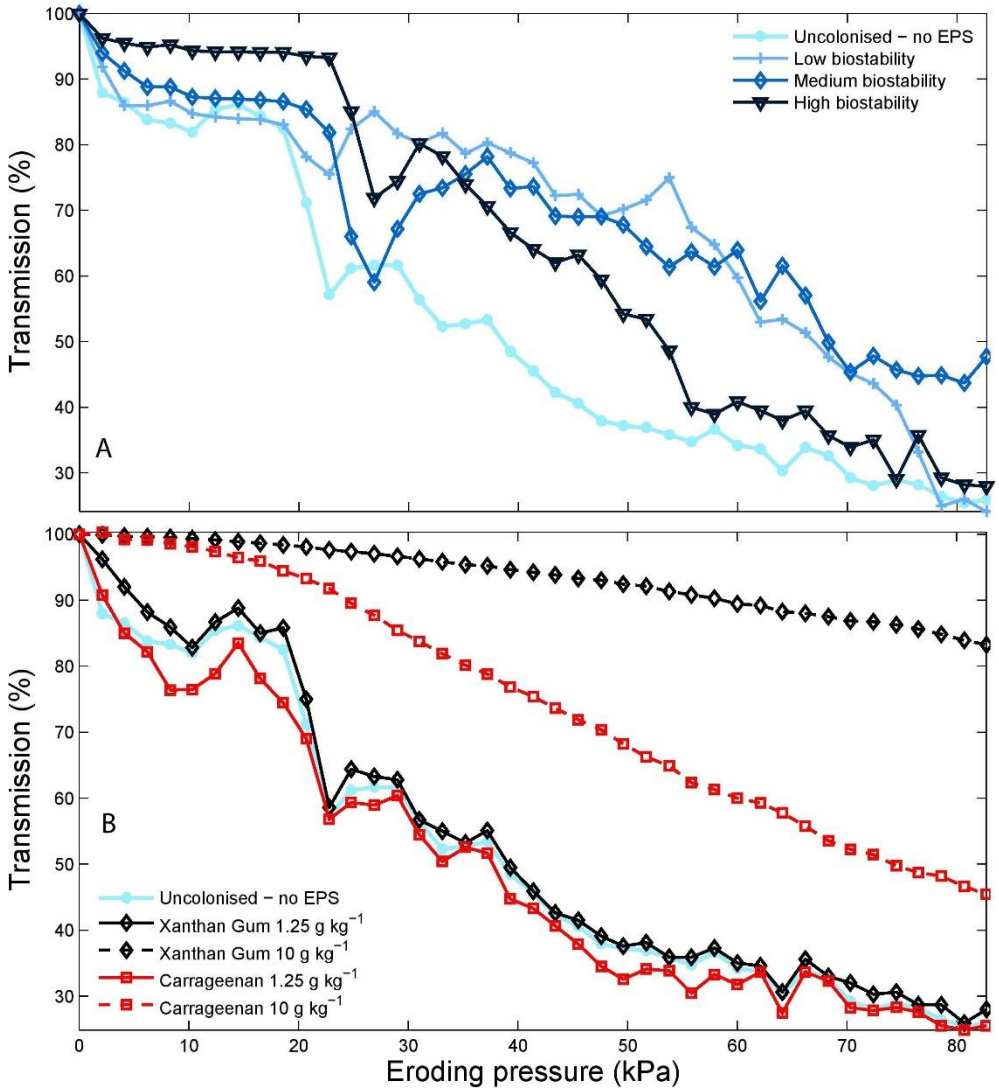

Figure 10. CSM erosion profiles for sediment with different degrees of biostability due to natural biofilm colonisation (A) and due to different Xanthan Gum and Carrageenan ~~synthetic~~extracted EPS contents (B).

Erosion profiles for low concentrations of ~~synthetic~~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)~~(Taylor and Paterson 1998), the ~~synthetic~~extracted EPS were mixed homogenously with depth in this study. As a consequence, the erosion rate for high concentrations of

~~synthetic~~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 ~~synthetic~~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 ~~synthetic~~extracted EPS behave linearly (Figure 4) and are therefore predictable. Different concentrations of these ~~synthetic~~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 ~~synthetic~~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 ~~synthetic~~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 synthetic 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 synthetic 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 synthetic EPS. Although this study has focused on replicating one aspect of natural biofilm behaviour only, future physical modelling studies employing synthetic 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~~

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 ~~synthetic~~extracted EPS for a range of conditions commonly used in physical modelling experiments. Four ~~synthetic~~extracted EPS were tested and addition of Xanthan Gum and Carrageenan increased the erosion threshold, while the addition of Alginic Acid and Agar did not increase the erosion threshold for all test conditions. Changes in erosion thresholds produced by the addition of Xanthan Gum and Carrageenan ~~synthetic~~extracted EPS compared well to measured erosion threshold resulting from natural biofilm colonisation of the same sandy substrate. The increase of the 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.

Acknowledgements. WL, SJM and DRP were supported by the European Community's Horizon 2020 Programme through the grant to the budget of the Integrated Infrastructure Initiative HYDRALAB+, Contract no. 654110. We thank Steve Darby and Chris Hackney from the University of Southampton for the generous loan of the CSM; Jane Reed for species ecology determination; Laura Jordan, Bas Bodewes and Robert Houseago for their help with laboratory work and Brendan Murphy and Mark Anderson for technical support. The authors contributed in the following proportions to conception and design, data collection, analysis and conclusions, and manuscript preparation: WL (40%, 80%, 70%, 70%), SJM (40%, 20%, 20%, 20%) and DRP (20%, 0%, 10%, 10%).

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