1.2.4 The identification and extraction of extracellular polymeric substances (EPS) from sediments

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ABSTRACT:
In the natural environment, biological polymers have been shown to cover sediment particles with a ‘glue-like’ coating. This biofilm can enhance sediment stability and strengthen resistance to hydrodynamic forcing. As a result, the erosion threshold of host environments and landforms may be significantly altered. Previous studies into the role of biological processes influencing sediment behaviour have partitioned biological processes into two competing camps: those which act as bio-stabilizers and those whose impact is as bio-
destabilizers. Bio-stabilizers (e.g., submerged aquatic vegetation (SAV)) may protect river and marine sediments, on the banks and bed, from erosion and resuspension by reducing the turbulence in close proximity to the bed. In contrast, the bio-
destabilizers (i.e., bio-
tubators) can increase the sediment erodibility, sediment water content, resuspension rate, and bed roughness. Recently focus has been paid to the importance of biological mucus, such as extracellular polymeric substrates (EPS), in stabilising sediment in geomorphic systems. Secreted by microorganisms, EPS has been shown to bond the surface of sediment particles together and strengthen the bonding structure between particles. This acts to increase erosion thresholds and encourage the growth of flocs in suspension that may subsequently settle out from the water column rapidly. At present, direct comparisons of differing EPS extraction and identification, methods have offered considerable variability in EPS concentration. Thus, our understanding of the role differing compositions and types of biological substances play on geomorphological processes is limited. As such caution should be taken when interpreting results and using these interpretations to understand modern process dynamics. Here, we focus on defining the different protocols which are currently used to identify EPS from natural sediments. We assess the suitability of their application to a range of natural environments and highlight their advantages and limitations. It is hoped that this comparison will facilitate more accurate, and comparable quantification of EPS concentrations, enabling a greater understanding of its role in modulating geomorphological processes.

KEYWORDS: EPS, sediment, phenol-sulphuric acid, Bradford assay, FTIR, extraction methods

1. Introduction
Traditionally, sedimentology and geomorphology have considered the interactions between physical, biological and chemical processes. Whilst the role of biology and ecology have been recognised as mechanisms controlling sediment stabilisation, focus has been largely placed on the role of vegetation as a stabilising agent (Roner et al., 2016). Studies have shown how the cohesiveness, or stability, of a landform may directly influence the rate of erodibility, and thus be shaped by the ambient environment. In recent years, increasing attention has been paid to the presence, and
role, of biologically produced microbial aggregates. When secreted into the surrounding sediment matrix, these extracellular polymeric substances (EPS) have been shown to possess complex, and highly effective stabilising properties (Tolhurst et al., 2002; Manning et al., 2013). In the natural environment, the concentration of EPS is highly variable, ranging from ~ 5% in biofilms to quantities in the region of ~ 0.01% - 0.1% in sandy-mud environments (Malarkey et al., 2015). The effective behaviour of EPS is remarkable, where minute concentrations have been shown to drive changes in sediment transport processes (Baas et al., 2016), scour patches associated with underwater infrastructure and bedform mobility (Schindler et al., 2015). For this reason, ignoring the role of these complex biological interactions could have critical implications as it suggests our current interpretation of sedimentary systems - and the mechanisms shaping them, may be wrong.

2. Identification of EPS in sediment

In the aquatic environment, the secretion of EPS by microorganisms can promote the construction of microbial aggregates such as biofilms, flocs and sludges. Commonly comprising, but not limited to, polysaccharides, proteins, and in smaller quantities, nucleic acids (Flemming and Wisgendedor, 2001), the EPS matrix has been shown to form a protective layer as metabolic products accumulate on the surface of a cell (Liu and Fang, 2002). This is shown where sand grains, which have been exposed to pervasive biological activity, have developed a cohesive bio-coating through the formation of ‘cage-like’ bridging structures (Figure 1), preventing the sand grains from moving independently (Malarkey et al., 2015).

In turn, these microbial assemblages provide fundamental functions to the aquatic ecosystem (Gerbersdorf and Wieprecht, 2015; Lagewag et al., 2017). For example, they have been shown to promote sediment particle aggregation, alter chemical properties to enable the release or adsorption of contaminants, and enhance a particle’s efficiency to remain in suspension within a flow through the stabilisation of sediment (van de Lageweg et al., 2017).

It is suggested that, at least in part, exopolymer concentration and associated sediment stability is largely determined by a combination of microbial production rate, extracellular carbohydrates, biomass and local environmental conditions (Yallop et al., 2001). These factors may vary considerably during alternating stages of biofilm development and maturation (Paterson, 1995; Laspidou and Rittmann, 2002). In recent years, the stabilising properties of EPS have been widely recognised both within the freshwater (Pedley and Rogerson, 2010), and intertidal zones (Schindler et al., 2015; Parsons et al., 2016) where production is largely determined by continuous fluid flow, and nutrient transfer acting between systems, promoting photosynthetic processes (Smith and Underwood, 1998). Furthermore, within these mixed sediment environments, cohesive EPS may also act in conjunction with benthic organisms to increase sedimentary stabilization via burrow formation and cast constructions (Tolhurst et al., 2002). In addition to verifying the adsorption of nutrients and pollution, high density binding sites of the EPS matrix secreted by benthic organisms, also provide important functions for the

![Figure 1: Bridging structures can be clearly distinguished, resulting in a ‘cage-like’ structure (Modified from: Parsons et al., 2016).](image-url)
connection of sediment particles, bridging the void space and enhancing sediment stability (Figure 2; Gerbersdorf and Wieprecht, 2015). As a result, this may explain why some studies have shown the erosion thresholds of both cohesive and non-cohesive sedimentary fractions to significantly increase in the presence of EPS (e.g., Tolhurst et al., 2002).

Although the importance of sediment type and location remains relatively unexplored, the relationship between photosynthetic processes and EPS production is consistent. Based on this, it is important to consider how EPS production may respond to subaerial sedimentary environments, which experience intermittent wetting and drying sequences. Studies by Major and Thomas (2011) have started to consider how exopolymers, secreted in dryland soils, may also increase the soil carbon pool through carbohydrates, as well as resisting erosion of the top soil. Studies such as these drive forward the possibility that EPS, secreted by microorganisms such as cyanobacteria, can exist within environments undergoing sequential wetting and drying events. Understanding the response to wetting and drying in more detail would ultimately offer a deeper insight into the controls on EPS production and enable a more concise application to geomorphic processes.

3. Overview of extraction methods

Before studies can be conducted into the more detailed functioning of EPS, attention must first be paid to the accurate identification, and quantification, of EPS content in the natural environment.

At present, there are two methods commonly associated with the extraction of EPS from fine-grained sediments: the Phenol-sulphuric acid (PSA) method (Dubois et al., 1956), and the Bradford assay method (Jachlewski et al., 2015). The phenol-sulfuric acid assay (Grant et al., 1986; Yallop et al., 1994), determines EPS content through the direct measurement of total carbohydrate concentration of the sample. Despite its popularity, conflicting views are proposed by Underwood et al. (1995) and Perkins et al. (2004), who deem this process invalid following comparative studies, where considerable dependence upon storage conditions, sample size, extraction media, and time, were found. They state that, whilst simple protocols such as these may evaluate the efficiency of EPS separation and allow for the measurement of EPS concentration between different sample types, they do not allow for the analysis of EPS concentrations were consistently lower than those were conditions promoted growth.

Whilst EPS in sediments is largely released by bacteria, microalgae and macrozoobenthos (Gerbersdorf et al., 2009), it is suggested that the composition of the EPS matrix is largely dependent upon the local environment. For example, studies have shown that phytoplankton are the primary producers of EPS in the pelagic zone, whilst in intertidal riverine settings, secretion via microalgae, mainly diatoms, dominate (Hanlon et al., 2006; Stal, 2011).

Notably, Gerbersdorf et al. (2009) suggest that the trends found in intertidal mudflats are the combined result of the locomotive ability characteristic of benthic diatoms, tidal cycles, and naturally occurring photosynthetic activity. For example, where light limitation, or turbidity in the overlying water column occurred, EPS concentrations were consistently lower than those were conditions promoted growth.

Figure 2. LTSEM (reproduced with permission of the SERG-Lab of Prof. D.M. Paterson) by Gerbersdorf and Wieprecht (2015). (A) Shows the glass beads embedded by the extracellular polymeric substances (EPS) matrix of naturally grown biofilm consisting mainly of heterotrophic bacteria and diatoms, and Inset: EPS strands and embedded microbes that surrounded the detached glass bead (empty space).
structure and composition (Flemming et al., 2000). This has resulted in an alternative such as Fourier-Transform infrared Spectroscopy (FTIR) and Low-temperature scanning electron microscopy (LTSEM) are used.

This section aims to compare the application of traditional sampling methods which have been shown to enable to separation of molecular subunits for the direct quantification of EPS content with modern techniques such as Fourier transform infrared spectroscopy (FTIR). As the modern integration of physical and biological cohesion advances rapidly, and the impact on erosional behaviour is recognised, the need for more realistic understanding of these complex cross-disciplinary boundaries is growing. Understanding these processes could have significant implications for coastal fringe and streambank stability (Black et al., 2002) and associated scouring around marine infrastructures (Black et al., 2002), among other factors.

3.1 Phenol-sulphuric acid

The phenol-sulphuric acid (PSA) method is an estimation of total carbohydrate content (Dubois et al., 1956). This is achieved through the application of sulphuric acid to the sample, to hydrolyse the carbohydrates into their monomeric (or molecular) subunits. It is recommended that, whilst sample size may be increased to optimize results, it is imperative that the ratio of reagents to volume is maintained (Masuko, 2005). Once the carbohydrate complex has been broken down, colorimetric analysis is conducted, whereby the reaction between phenol and sulphuric acid is determined with the aid of a colour reagent. If absorption is high, a strong reaction may be interpreted, implying the sample possesses a high carbohydrate concentration. If absorption is low, and a weak reaction occurs, the sample possesses low carbohydrate concentrations.

The PSA method remains one of the most commonly used techniques in the literature for determining EPS content (Flemming et al., 2000), due to its repeatability and ease. However, the precision of this method remains under question. Despite detecting almost all traces of carbohydrates, it is unable to identify variations within the absorptivity of different carbohydrates. Therefore, unless the sample is known to contain only a single carbohydrate, it cannot be assumed that the substances identified are directly related to EPS. This might be a problem where the characterisation of EPS is required (e.g. distinction between mat-forming bacteria and cyanobacteria, where the latter is shown to consist of more tightly-bound polymers; Underwood et al., 2003).

3.2 Bradford assay

The second, less commonly used technique is the Bradford assay method. Developed in 1976, this method determines EPS content by the concentration of total protein molecules (Jachlewski et al., 2015). An adaptation of the Lowry method, it can be used to determine the concentration of EPS based on the reaction of peptides with copper sulphate and Folin Ciocalteu reagent (Lowry et al., 1951).

Firstly, a standard should be developed, containing 2 mg/ml of the Bradford reagent (BSA), in 0.9% NaCl solution. This is achieved by dissolving approximately 60 mg of BSA in 30 mls of 0.9% NaCl. This can be stored frozen in aliquots.

Using this standard, Kruger (1994) recalls how between 10 and 100 μg of protein should be pipetted into a 100 μL test tube. Duplications of each sample (where sample concentration can vary) should also be made. For the calibration curve, these duplicates should consist of 10, 20, 40, 60, 80, and 100 μL of 1 mg/mL γ-globulin standard solution. These should all be made up to 100 μL. An additional 100 μL of distilled water should be pipetted into a test tube to provide a reagent blank.

If frozen, once the duplicates and blank have been made, the Bradford reagent should then be left to come to room temperature, before 50 μL of protein reagent are then added and mixed thoroughly by inversion or gentle vortex-mixing, with 1.5 mls of the Bradford reagent, into a 1 ml of disposable plastic cuvette. It is crucial to mix gently to avoid foaming, which may otherwise lead to poor reproducibility. Following this, the samples should be incubated at room temperature for between 5 to 45 minutes before being measured at 595 nm with a spectrometer. It is suggested that the reagent will remain stable for approximately 60 minutes. Similar methods
have been used by Kruger (1994), who proceeds to state that the $A_{595}$ (which refers to the state of absorbance at 595 nm) should be measured against the blank of the reagent 2 mins to 1 h after mixing. The value of the 100 μg should be approximately 0.4 at $A_{595}$. Note that the standard curve is often not linear (an example given below in Figure 3) with the level of curvature dependent upon the age of the assay reagent.

Figure 3: An example of the extraction of protein from soil samples using the Bradford assay method (Taken from Redmille-Gordon et al., 2013).

Unlike the Lowry method, the Bradford assay relies upon interactions acting between basic amino acid residues (e.g. arginine, lysine and histidine), rather than alkaline, and the Coomassie brilliant blue G-250 dye in an acidic matrix (Redmile-Gordon et al., 2013). The dye, commonly abbreviated to CBB, acts by binding the dye to proteins within the sample via Van der Waals forces (forces of repulsion and attraction) and hydrophobic interactions. This can be identified through a colour change, when the usually red form will shift to a blue form. This colour change is associated with the presence of certain basic amino commonly found within proteins. The extent of shift in colour indicates how many protein molecules bind to the CBB dye. An additional shift in adsorption maximum (from 465 nm to 595 nm) is also present.

Kruger (1994) suggests one of the primary drawbacks of the Bradford assay method is that the Coomassie Blue G250 used in the analysis appears to bind more readily to certain residues (e.g. arginyl and lysyl) over, for example, arginine and lysine, or peptides smaller than 3000 Da. For this reason, it seems reasonable to suggest that the Bradford method might be potentially unreliable when the proteins with specific components exist within the sample. Furthermore, studies by Redmile-Gordon et al. (2013) and Kirazov et al. (1993) suggest, from comparisons made between the Bradford assay and Lowry method, that whilst the former allows for the rapid quantification of proteins within soils, it also appeared to hold several limitations. The limitations found within these results, which include susceptibility to the concentration of polyphenol in the development of colour, contrast previous reports which suggest that the Lowry method allows for greater interference by substances (Redmile-Gordon et al., 2013).

The phenol-sulphuric acid (PSA) and Bradford assay methods have both been shown to provide an estimation of EPS concentration within a sample, based upon the total concentration of protein and polysaccharides. Whilst these techniques are considered cheap and repeatable, they both possess significant drawbacks. It seems that these drawbacks are greatest for the Bradford assay, which is only reliable when the type of protein is already known, whereas the PSA is transferable between all subunits of carbohydrates but is unable to distinguish different types.

3.3 Fourier Transform Infrared Spectroscopy

More recently, the application of Fourier Transform Infrared Spectroscopy (FTIR) to identify the presence of extracellular polymeric substances has been explored. FTIR spectroscopy has identified EPS in sludge aggregation (Guo et al., 2016) and the presence of organic matter in peatlands (Artz et al., 2008). This method has become increasingly common to identifying how vertical layers of the sediment matrix may vary with EPS content.

Through the vibrational characteristics within chemical bonds, FTIR spectroscopy has been shown to distinguish chemical classes such as carbohydrates, cellulose, lipids, proteins and fats without disturbance to the material. FTIR spectroscopy, which assumes Hooke’s law whereby the strain in an object is directly
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proportional to the stress applied, is comprised of four primary parts: the light source, interferometer, sample compartment, and detector (Figure 4).

![Figure 4: Schematic diagram of a Fourier Transform Infrared Spectrometer.](image)

As infrared energy is emitted from the light source in the form of the continuous radiation. It reaches the beam splitter where it is divided into two optical beams, one heading towards the fixed mirror, the second, towards the moving mirror. As these beams are reflected back to the beam splitter, an interference pattern is generated, representing the changes in the relative position of the moving mirror to the fixed mirror. The resulting beam subsequently passes through the sample, into the detector, where the final measurement is recorded. In laboratory studies, approximately 2 mg of the sample are first dried at a low temperature (approximately 50°C if the samples are frozen). This removes the potential for noise from the O-H absorbance and ensures that any high wavenumber ranges are coming from the EPS, not the sediment matrix. Once dried, the sample is ground until each particle size is unified and < 2mm. When testing for biological substances, the Attenuated Total Reflectance (Figure 5; ATR) technique is recommended over alternative sampling methods, because it measures the reflectance from the sample, rather than transmitting an infrared beam through it.

Once the sample is placed in good contact with the ATR crystal accessory, the beam is then directed towards the crystal. In regions where the sample absorbs some of this infrared energy, the evanescent wave generated by internal reflectance will be attenuated. In EPS extraction, the application of this technique enables the presence of different fractions, and their concentrations to be distinguished (Figure 6), a feature which is not applicable to the PSA and Bradford assay methods. The distinction of different fractions occurs from the variations in vibrational movements of the molecules generated in response to infrared absorption. These vibrations bands correlate to wavenumbers which are directly proportional to the amount of absorbed energy (Schmitt, 1998). Often referred to as the ‘fingerprint region’, the range of a particular group of molecules can be very specific for a substance. Schmitt (1998) suggests that a variation in signal, through the vibration of molecules, may be recognised between the cell wall, the membrane and the cytoplasm within the EPS structure. Nevertheless, these signals all remain within the region of 4000 and 500 cm⁻¹, where functional groups include peptide and proteins of polysaccharides, phospholipids and nucleic acids. As shown in Figure 6, clear structures, such as the C-O-C ring vibrations of oligo- and polysaccharide structures may be identified.

![Figure 5: Schematic diagram of an Attenuated Total Radiation (ATR) system in an FTIR.](image)

![Figure 6: Fourier transform infrared spectroscopy (FTIR) spectra showing soluble EPS (S-EPS; black), loosely bound EPS (LB-EPS; red) and tightly bound EPS (TB-EPS; blue) substances (Taken from Guo et al., 2016).](image)
4. Application to field experiments

The methods prescribed to the extraction of EPS may be determined by the location, concentration and nature of the biopolymer under study. For example, the majority of studies have focused on the mechanical protection derived from high concentrations of EPS in the form of surface biofilms, where surface scour is more likely than bedform development (Hagadorn and McDowell, 2012). Therefore, when applying them to geomorphic landscapes, it is important to consider the additional erosive and weathering processes acting on the system, e.g. exposure to wave action, or high-energy seasonal events.

However, EPS content may also be distributed at significantly lower concentrations (0.01-0.1%) throughout the sediment substratum (Lanuru et al., 2007). The variation of EPS content with depth has been considered in several studies where it has been suggested that concentrations are greater in the upper sediment layers (0-5 cm) where colloidal carbohydrates are prevalent (Gerbersdorf et al., 2009). With increasing depth, the photosynthetic processes, and other mechanisms (e.g. nutrient supply) break down, reducing the likelihood of EPS production. EPS on the surface of sediments, e.g. biofilms, can be analysed using any of the above methods, however when identifying EPS in the subsurface, FTIR spectroscopy may not be the most applicable because of the way the sample is deployed, it might not result in the uniform measurement. Under these conditions, simple protocols such as the phenol-sulphuric acid and Bradford assay method may be considered more appropriate. This is because EPS estimation can be made at different layers of the stratum, rather than the surface (Masuko, 2005). The following section will detail how the techniques mentioned above may be applied to the extraction of EPS from several contrasting environments, with variable concentrations.

4.1. Traditional protocols

4.1.1 Red cliff sand bar, Humber Estuary, NE England

Sediment cores, collected from the Red cliff sand bar, located in the upper Humber Estuary, NE England (shown in Figure 7), were collected in winter of 2014 and summer of 2015. The depth of these sediment cores varied from 160 mm to 300 mm, and were obtained from ripple crests and troughs, respectively. The granulometry of these samples suggests that they were mainly comprised of pure sand with median grain size of approximately 170 μm.

![Figure 7: The location of the Red cliff sand bar, upper Humber Estuary, UK.](image)

The EPS content of the bed sediment samples collected in the field survey were determined via carbohydrate analysis based on glucose equivalents and using the phenol sulphuric acid method (Dubois et al., 1956). First sub-samples, at a volume of 3 g, were extracted by evenly dividing the core into three parts. The EPS was then isolated from the sediment samples by adding 10 ml of 0.5 mol ml⁻¹ Ethylene Diamine Tetraacetic Acid (EDTA) solution before being placed in a water bath at 40 °C for approximately 30 mins and centrifuging at 6000g for 30 minutes. 35 ml of ethanol was then added to 15 mls of the supernatant prior to being left at 4 °C for 8 hours (or overnight) before repeat centrifuging at 6000g for 30 minutes. Following this, the final precipitate was dissolved in a total of 1 ml MilliQ water to enable the quantification of the amount of carbohydrate present in the sample. In addition, a set of standards dissolved by phenol and sulphuric acid, showing the known glucose concentration are necessary to establish a relationship between glucose content and spectrophotometer absorbance (shown in Figure 8). These standards were prepared by adding 10 ml of 0.5 mol ml⁻¹ Ethylene Diamine Tetraacetic Acid (EDTA) solution before being placed in a water bath at 40 °C for approximately 30 mins and centrifuging at 6000g for 30 minutes. Following this, the final precipitate was dissolved in a total of 1 ml MilliQ water to enable the quantification of the amount of carbohydrate present in the sample. In addition, a set of standards dissolved by phenol and sulphuric acid, showing the known glucose concentration are necessary to establish a relationship between glucose content and spectrophotometer absorbance (shown in Figure 8). These standards were prepared by adding 200 μl of phenol solution, 1 ml of concentrated sulphuric acid and 200 μl of dissolved precipitate to a 1.5 ml Eppendorf,
and mixing. These samples are compared to blank samples that are prepared in a similar fashion, before being measured for absorbance using a spectrophotometer at 490 nm. Finally, the actual EPS content for the sample can then be determined by converting measured absorbance through a simple arithmetic expression. Before the test samples were scaled, therefore allowing the calculation of percentage of EPS presence in the sample. Because the core was divided into three subsamples, the total EPS content is taken from the average of the three parts.

The percentage of estimated EPS sample is shown in figure 9. The results indicate that the EPS content in the winter was very low, at around 0.003%. In the summer 2015, however, the results stand at one order of a magnitude higher than in winter, reaching approximately 0.012%. The findings in this study were consistent with previous studies by Gerbersdorf et al. (2009) where significantly higher microorganism productivity were observed in the summer, during periods of high metabolic activity, and at sites where chlorophyll a values are high. It is suggested that the biomass and composition of the microbial community were determined by the hydrodynamic regime and season.

**Figure 8. Relationship between glucose content of sediment samples and spectrophotometer absorbance.**

| Table 1: Details of the set of experimental runs conducted, m is initial bed mud fraction (%) and e is initial bed mud EPS fraction (%) |
|-----------------|-----|----|
| m (%) | e (%) |
| A1 | 1.9 | 0 |
| A2 | 4.7 | 0 |
| A3 | 8.9 | 0 |
| A4 | 9.8 | 0 |
| A5 | 11.9 | 0 |
| A6 | 12.7 | 0 |
| A7 | 14.1 | 0 |
| B1 | 2.8 | 0.027 |
| B2 | 6.8 | 0.038 |
| B3 | 15.4 | 0.03 |
| C1 | 9.1 | 0.075 |
| C2 | 9.9 | 0.071 |
| C3 | 12 | 0.073 |
| C4 | 17.7 | 0.1 |
In order to investigate the relationship between EPS fraction within the substrate with bedform size and development, the initial and final depth-mean EPS contents were determined by applying the phenol-sulphuric acid assay (Dubois et al., 1956) method to millimetric slices taken from 100 mm depth, 10 mm diameter syringe core samples, whilst the bedforms were measured using Ultrasonic Ranging System (URS). Standard grain size analysis techniques based on laser diffraction techniques (Malvern 2000 particle size analyser) and optical methods (QICPIC particle size analyser) calculating the sediment size range, mean size) were used to quantify bed mud contents after each experiment. In part of the experimental runs, EPS was added at a low concentration (mean EPS content was 0.032 ± 0.006 %) in order to represent environments with low primary production rates (Figure 10: Lanuru et al., 2007). Three substrata with increasing mud content were made (2.8% < mud fraction < 15.4%). In series A (Table 1) only clay and sand substrates were used, with EPS added in series B to represent the winter season with low EPS production (~0.038 %). In series C, EPS content was set to approximately 3 times the concentrations in series B (mean = 0.086 ± 0.015%) to represent summer conditions characterised by high primary production rates (Lanuru et al., 2007). Four substrata with increasing mud content were made (9.1< mud fraction < 17.7%).

The results from this study (shown in Table 1), which have been presented in Parsons et al. (2016), suggest that even minute fractions of biologically cohesive EPS can have a considerable effect on bed form dimensions. This is due to surface bonding promoted by long chain molecular (polymeric) strands, which impose much greater strength than the van der Waals forces and inter-particle electrostatic bonding of clay particles (Mehta, 2004).

![Figure 10: LTSEM images, comparing initial substratum microstructure for selected runs in series A, B, and C. Top and bottom rows show low- and high-resolution images, respectively. Scale bar units are in micrometers.](image)

**Figure 10:** LTSEM images, comparing initial substratum microstructure for selected runs in series A, B, and C. Top and bottom rows show low- and high-resolution images, respectively. Scale bar units are in micrometers. (a) Run A2 (m= 4.7 %), with plated kaolin particle aggregates found predominantly between sand grains rather than on the exposed sand grain surfaces. (b) Run B2 (m= 6.8%; e= 0.038 %), showing kaolin-EPS aggregates dominated by EPS sheathes and partial coatings of sand grain surfaces. (c) Run C1 (m= 9.1 %; e= 0.075 %), showing EPS lining sand grain socket (top) and EPS strands and webs linking individual sand grains (bottom). Images obtained using procedures outlined in Tolhurst et al. (2002).

### 4.2 Modern techniques

#### 4.2.1 Biofilms

The application of Fourier Transform Infrared Spectroscopy (FTIR) to EPS extraction has been found in a range of environments.

In the identification of EPS from sediments, demonstrated here through the work of Jiao et al. (2010) is of particular interest. Here, FTIR spectroscopy techniques were used to understand the chemical composition of extracellular polymeric substances taken from two natural microbial pellicle biofilms of different growth were compared. Whilst the process is outlined fully in Jiao et al. (2010), for completeness in this article, a short overview of the methodology used has been provided.

Once the two biofilm samples (MDS for the mid developmental stage biofilm, and MB, for
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As previously mentioned, for the identification of EPS in sediments and fine powders, the ATR mode is commonly applied. Once the FTIR has been switched on and configured to a state where it is ready to analyse the material (or “spectra”) the crystal then must be prepared. Although it is common for some to remain, it is crucial that the crystal surface is cleaned of any residual sample. This should be approached with care and often involves the use of acetone in and around the crystal surface, in order to reduce the likelihood of scratches.

Once the crystal is cleaned, a background scan can be conducted. This will subtract any remaining residue. A small portion (15-20 mg) of the sample may then be added to the surface of the crystal, and pressure applied, crushing the sample.

Although dependant upon the FTIR used, the machine can then be calibrated to measure the spectra of the sample. In turn, a signal similar to those shown in Figure 11 will be produced.

In the study conducted by Jiao et al. (2010), the results, which are given in Figure 11, showed that more than double the quantity of EPS was identified from MB than from MDS (approx. 340 and 150 mg of EPS per g (dry weight)).

The results presented using the application of FTIR offer an interesting insight, in not only the geochemical response of EPS to the ambient environment, but also how the biochemical composition responds to the maturity of the biofilm, an issue which has rarely been considered in the present literature.

The work conducted by Jiao et al. (2010), previous and prior studies by Lima et al. (2008) offer excellent examples of where traditional sampling and extraction techniques have been used in combination with modern visualisation practises such as FTIR and low temperature scanning electron microscopy (LTSEM).

5. Conclusion

The secretion of microbial assemblages such as microphytobenthos, microbial mats and flocs can produce a self-formed ‘glue-like’ matrix otherwise known as extracellular polymeric substances (EPS). In addition to protecting the cells from ambient conditions, such as erosion, and stabilising the sediment grains, EPS has been shown to enhance the physiochemical properties of mud and clay particles. In the past, the physical mechanisms driving the erosion of geomorphic processes (e.g. wave action, sediment composition) have been heavily debated within the literature. Whilst additional studies have considered the role of biology in shaping geomorphology, the focus has remained almost entirely on that of vegetation. Recent technological advances mean we are now able to develop further our approaches to understanding the mechanisms driving geomorphology. However, for this to be successful, accurate quantification of the role each factor plays is necessary.

Here, we analysed some of the methods commonly used in the extraction of natural EPS from sediments. From the methods described, we came to the conclusion that traditional protocols, such as the phenol-sulphuric acid method offered a rapid and easy tool for identifying EPS, but the...
complexity in the genetic matrix meant that precise measurement was unlikely. In contrast, we favoured towards a combined approach, suggesting that using modern techniques such as FTIR might help to identify the physical structure of the EPS fabric. Based on this, it is recommended that we should not approach the use of 'traditional' sampling protocols and 'modern' visualisation techniques with an either/or pretence. Instead, these methods can, in fact, complement one another, and offer a more detailed description as we continue our quest to a) characterise, and b) quantify EPS in the natural environment. It is hoped that once the most appropriate methods can be sought to extract EPS from the natural environment, we can then begin to appreciate the role EPS plays both in shaping, and modulating the wider landscape.

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