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Time Evolution of Bacterial Extracellular Polymer Composition Studied with a Versatile Infrared Transmission Array Scanner

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Abstract

The chemical composition of extracellular polymeric substances (EPS) secreted by *E. Coli* K12 in solution was monitored as a function of time over various stages of bacterial growth. We have observed that the spectra become enhanced at 1132 cm^{-1} and 980 cm^{-1} , attributed to an increasing fraction of uronic and nucleic acids within the polymer matrix. Both of these acidic molecules have been shown to sequester cations from solution in order to neutralize their negative charge. We discuss the implication for this composition profile change in terms of previously observed restructuring of interfacial water molecules during the earliest stages of bacterial adhesion. To monitor each fraction of EPS under the same conditions, we illustrate the design and operation of a high throughput array scanner fitted to a Fourier-transform infrared spectrometer.

Introduction

Bacteria adhere to and colonize most natural and synthetic surfaces. As a result, bacterial adhesion is important in many diverse fields, including environmental, chemical and biomedical engineering, materials and environmental sciences, food processing, and marine science.¹⁻⁴ Initial adhesion of bacteria to a surface is the first step in the formation of a biofilm, a collection of surface-associated microbial cells that are enclosed within a matrix of extracellular polymeric substances (EPS).⁵ This EPS matrix provides protection against environmental stressors and aids in adhesion to the surface and cohesion among cells.⁶ Integral to biofilm formation are early interactions between the cells and substrate, since the bacteria must first reversibly and then irreversibly adhere onto the surface.⁷ Although there have been a number of studies on the growth, composition, and structure of mature biofilms,⁸⁻¹⁰ the details of initial cell adhesion and attachment remain unclear.

Recent studies using vibrational sum frequency generation (SFG) spectroscopy have shown that the interfacial water structure is a sensitive reporter of the microenvironment adjacent to a silica surface during adhesion of *E. Coli*.¹¹ Although the silica-water interface is sensitive to solution pH and ionic strength,¹² bulk solution pH and ionic strength measurements alone could not account for changes in the SFG signal intensity during cell growth and adhesion. However, it is known that the composition of the growth medium is altered as metabolites are excreted by growing bacteria, some of which have been found to aid in cell adhesion.¹³ Some excreted metabolites can settle onto surfaces to help condition them for initial cellular attachment. We therefore seek a cellular metabolomic method that can profile the growth medium and complement previous surface studies. Solution state ¹H nuclear magnetic resonance (NMR) has been extensively applied to metabolomics studies as it is amenable to high throughput and requires minimal sample preparation.¹⁴⁻¹⁶ Mass spectrometry (MS) techniques, combined with gas chromatography (GC) and liquid chromatography (LC), are also commonly employed for metabolomics.^{15,17} However, in LC-MS, ion suppression is known to hinder the detection of certain metabolites.¹⁷ Vibrational spectroscopic techniques such as Fourier transform infrared (FTIR) and Raman spectroscopy have been increasingly used for metabolomics.¹⁸⁻²¹ These techniques have the advantage of being

able to detect chemical signatures of metabolites in a non-invasive manner, thus facilitating on-line measurements. Although commercial accessories exist to enable high-throughput infrared transmission, internal or external specular reflectance, and diffuse reflectance measurements, they are costly and require dedicated substrates such as 96-well plates made of silicon or zinc selenide.

Here we demonstrate a simple modification to a standard FTIR spectrometer that can be placed in the sample compartment or in an external beam path. It is especially well-suited to accommodate IR-transparent substrates of any shape and size and partition the surface of each substrate into several wells. We have used this instrument to study changes in the EPS composition extracted from *E. Coli* K12 at various stages during their growth. We relate these changes to the growth curve obtained from light scattering measurements as well as our previous observation of interfacial water structure changes during early bacterial adhesion. Our findings suggest that an enhanced uronic and nucleic acid content may be responsible for creating a unique microenvironment at the silica surface consisting of charged polyelectrolytes and adhered cells.

FTIR Transmission Array Scanner

Optical bench and mechanical components

Our instrument is based on a Bruker Vertex 70 FTIR. Since our array scanner is too large to fit inside the sample compartment, we use the right exit port to send the beam outside the instrument over a distance of approximately 1 m in an acrylic box that we purge together with the FTIR interferometer compartment. The beam is focused to a diameter of 4 mm using ZnSe lenses, recollimated after the sample, and then focused onto an MCT detector (Kohlmair Technologies) with 1 mm² area, 25 ns rise time, and 20 MHz pre-amplifier. The detector output is split to allow simultaneous monitoring by the FTIR on-board electronics and a voltmeter (B&K 2831E) that is able to rapidly monitor the DC component of the signal. Our array scanner is controlled using stages with 100 mm of travel coupled to stepper motors (Standa 8MT167-100) and in-house code for fine control of motion in the *x/y* plane. Data acquisition is initiated by moving the desired sample well into the optical path; a

signal is then sent to the spectrometer to start a measurement. After the measurement is completed the sample stage will move onto the next spot, this allows for the automated collection of up-to 96 samples. Sample scanning and data acquisition code is available in the Supporting Information.

Sample array

Our sample array (Fig. 1a) was designed to house a 52 mm $\times$ 20 mm $\times$ 2 mm ATR crystal to serve as the IR transparent substrate (bypassing its intended reflection geometry), and partition the surface into 24 distinct wells. The base plate, crystal holder, spacer and cover were laser cut from 3 mm acrylic. A gasket laser cut from 1/32" high-temperature silicone rubber (Durometer 40A, McMaster-Carr) was placed between the substrate and the spacer layer to ensure contact, preventing leakage between the 4 mm diameter wells. Larger sample arrays were constructed to hold two IR-transparent crystals providing a total of 48 wells, and four crystals to create 96 wells (Fig. 1b). The time required to collect a single spectrum depends on the desired resolution (maximum interferometer path difference) and the number of interferograms collected for averaging. A typical set of parameters, collecting 64 spectra at a resolution of 4 cm^{-1} , results in a dwell time of 90 s per spot on the array. Accounting for a time of 4 s to move to the next spot (with a zig-zag raster configuration), 96 samples can be measured in a little over 2 h. This assumes that the same reference position is used for the calculation of transmittance for the remaining 95 spots, or that the reference spots are strategically placed along the raster path to avoid the need to return to those positions. These design principles may be modified to use any shape of IR transparent material. Schematics and files suitable for laser cutting and editing are included in the Supporting Information.

Spot-finding algorithm

One of the features of our design is the flexibility to accommodate various sample shapes and sizes, including different numbers of wells and in varying (x, y) locations. Once the desired substrates have been loaded into the holder, the software is provided with the approximate location of each

Figure 1: (a) Detail of a 24-well array in a 3×8 configuration. (b) Photo of a larger setup capable of holding four KRS-5 crystals to accommodate 96 wells in a single experiment.

well. An iterative process then occurs. In the first step, a series of 9 transmission measurements spaced 2 mm apart is acquired using the DC value of the interferogram. This data is fit to a two-dimensional Gaussian function

$$f(x, y) = A \exp \left[-\frac{(x - x_i)^2}{2\sigma_x^2} - \frac{(y - y_i)^2}{2\sigma_y^2} \right] \quad (1)$$

where A is the amplitude, (x_i, y_i) is the location of the best-fit centroid, σ_x and σ_y are the widths of the distribution, and the centroid is bounded within the limits of the measured area. If (x_i, y_i) coincides with the center of the 9 points (our current best guess), the center of the spot has been located, and data collection commences. Otherwise, a subsequent step with 9 new measurements is performed, but now spaced 1 mm apart and re-centered at (x_i, y_i) . In the final step, the spacing between the spots is reduced to 0.5 mm. This process is illustrated in Fig. 2, where the cross hairs represent the sought center of the well. The DC value is acquired in 1 s, and it takes approximately 1 s to move to each of the 9 positions. Therefore, 3 iterations would locate the center of the spot in just under 1 min. Although this adds 1.5 h to the total sample collection for 96 spots, this

Figure 2: Algorithm for finding the location of the sample spots. The initial 9 spots are based on a guess on the spot location. Transmitted intensity data is fit to a 2D Gaussian, whose centroid determines the location of the center spot for the next round, with reduced spread of the 9 points. This adds flexibility in enabling many different configurations of spots to be used with a variety of IR substrate shapes and sizes.

enables the use of arbitrarily shaped and positioned substrates to constitute the array. Furthermore, spot-finding may not be necessary for subsequent use of the same 96 spots provided the array is not disassembled and the IR substrates remain fixed in place.

Experimental

Bacterial growth. *Escherichia Coli* K12 MG1655 (ATCC700926) was stored at $-80\text{ }^{\circ}\text{C}$ in a solution of 50% (vol/vol) Luria-Bertani (LB) Broth and glycerol. The formulation of LB consisted tryptone (10 g/L), yeast extract (5 g/L) and sodium chloride (5 g/L). Liquid cultures were inoculated from agar plates in 100 mL of LB (20 g/L) and incubated at $37\text{ }^{\circ}\text{C}$ with aeration. Cells were cultivated for 25 h to allow growth to reach the stationary phase. All materials used in the growth,

including the solution of LB, were autoclaved before inoculation. Autoclaved LB growth medium was inoculated using a 1:100 dilution of a stationary phase liquid culture. During growth, 10 mL aliquots of the liquid culture were pipetted into 15 mL centrifuge tubes and stored at 4 °C to await further preparation. Cell growth was monitored by the optical density at 600 nm. Cells were removed via centrifugation at 3000 rpm for 30 min at 4 °C. The supernatant containing the soluble free EPS was decanted and the pellet containing the *E. Coli* cells was discarded. The supernatant was then passed through a 0.45 μm filter to remove residual cells, and stored at 4°C to await further preparation.

EPS extraction. EPS was isolated using an ethanol extraction method,²² precipitating from the supernatant with a 3:1 volume of cold ethanol and stored in the freezer overnight. Samples were centrifuged at 4000 rpm for 60 min and the supernatant was discarded. The pellet was dried overnight at 70 °C and then redissolved in 1 mL of 18.2 M Ω -cm water via sonication for 60 min.

IR spectra data collection. EPS composition was monitored by IR absorption using the instrument we have described above. 10 μL aliquots of each EPS sample were deposited onto a sample stage containing a germanium substrate. Each sample was spotted in triplicate and the deposited substrate was then dried at 70 °C for 40 min. The spectrometer and sample compartment were purged with dry air for 1 h before collecting spectra to reduce interference from atmospheric water stretching and carbon dioxide. Spectra in the range 4000–900 cm^{-1} were collected in transmission mode using the average of 64 scans at a resolution of 4 cm^{-1} . Spectra were background-corrected using the first column of each row of the array that were used as blanks.

Spectral preprocessing and normalization. Spectral artifacts can arise due to differences in the amount of sample deposited, and inconsistencies in the drying process that can distort the shape and surface of each spot, in addition to scattering affects and baseline offset. Extended multiplicative signal correction (EMSC) is a preprocessing method that allows for the separation of these phenomena.^{23,24} An EMSC model

$$Z(\omega) = A + B \cdot S_{\text{ref}} + C\omega + D\omega^2 + \epsilon(\omega) \quad (2)$$

was applied to the polysaccharide region $950\text{--}1300\text{ cm}^{-1}$ raw absorbance value Z to obtain the baseline shift A , a factor B that scales a reference spectrum S_{ref} , and a linear C and quadratic D wavelength-dependant term, and the sought residual $\epsilon(\omega)$.²⁵ In our case, S_{ref} was determined from the average of 21 spectra collected using 3 replicate spots for each of the 7 time points.

Results

Fig. 3 shows the optical density of the liquid culture measured at 600 nm as a function of time. The growth follows the expected logarithmic trend and can be divided into three phases: The lag phase ($t < 5$ h), the exponential phase ($5 < t < 12$ h), and the stationary phase ($t > 12$ h). This data can be described by a logarithmic model

$$f(t) = \frac{A}{1 + e^{-k(t-t_m)}} + b \quad (3)$$

where A is the amplitude, k is the growth rate, t_m is the mid-point of the exponential phase, and b is a baseline offset. Fitting the optical density data resulted in $k = 1.22\text{ h}^{-1}$ and $t_m = 8.2$ h, as indicated by the grey line in Fig. 3.

Figure 3: Cell culture population growth curve determined according to the optical density at 600 nm. The curve was obtained by a fit to Eq. 3.

Fig. 4 shows the infrared spectra collected from samples of EPS obtained at different stages of growth. The peaks located in the region of 2800 cm^{-1} to 3000 cm^{-1} are indicative of aliphatic

C–H stretching. For EPS, this region is dominated by the long hydrocarbon chains of fatty acids.²⁶ The Amide I, II, and III bands located at 1600–1690 cm^{-1} , 1480–1575 cm^{-1} , and 1230–1300 cm^{-1} respectively, are indicative of proteins.²⁷ These bands are sensitive to the conformational structure of the protein and can be used to determine their structural properties. However, the use of these peaks for structural determination requires high purity samples.²⁸ Thus, deconvolution of this peak is not possible in a complex mixture such as EPS. A large peak at 1400 cm^{-1} is observed in all spectra and is due to deprotonated C=O and C–O stretching of amino acid carboxylic groups.²⁵ The polysaccharide stretching region is observed around 1060 cm^{-1} . This region corresponds to the C–OH and C–O–C stretching of hydrated carbons.²⁹ A distinctive increase in a shoulder in the polysaccharide region was observed at 1132 cm^{-1} , which has often been assigned to uronic acids.³⁰ The band at 980 cm^{-1} can be attributed to the asymmetric stretch of O–P–O bonds in the phosphate backbone of DNA.³¹

EMSC normalized infrared spectra are plotted in Fig. 5a in the spectral region between 950–1300 cm^{-1} . A small increase in the nucleic acid content is observed at 980 cm^{-1} . This increase as a function of time is plotted in Fig. 5b. The grey line superimposed on the data is not a fit to the absorbance values, but a plot of the same logarithmic model with k and t_m determined from the growth curve. Only A and b have been adjusted to accommodate the scale of the absorbance data. This shows that the manner in which the increase in absorbance at 980 cm^{-1} is related to the trend in the population of cells from the light scattering data. A decrease in intensity is observed at 1240 cm^{-1} . This region contains spectral signatures from a variety of metabolites, including contributions from the Amide III band of proteins and the asymmetric stretch of P=O bonds in the phosphate backbone of DNA.²⁹ Since an increase in the asymmetric stretch of O–P–O in DNA is also observed at 980 cm^{-1} , it is likely that the peak at 1240 cm^{-1} is dominated by the amide III band. Polysaccharides have been extensively studied using IR spectroscopy and have been found to be composed of several different types of sugar molecules (monosaccharides), including glucose, fructose, and glucuronic acid.³² The overall decrease in the C–OH stretching mode at 1060 cm^{-1} could be indicative of the conversion of monosaccharides to polysaccharides.³³ Peaks

in the region $1100\text{--}1150\text{ cm}^{-1}$ have been attributed to the ester linkages within polysaccharides that contain uronic acid moieties.^{30,34,35} The absorbance at 1132 cm^{-1} as a function of time is plotted in Fig. 5c. Once again, the grey line corresponding to the growth curve is superimposed to show the relationship between the uronic acid content and the growth of *E. Coli* cells.

Figure 4: Absorbance data for each EPS fraction plotted between $900\text{--}4000\text{ cm}^{-1}$, and normalized to the Amide I band. Each individual spectrum is the average of all replicates and vertically offset for clarity.

Discussion

The time evolution of EPS composition revealed a distinct increase in uronic and nucleic acids. Both compounds lend structural stability to the biofilm matrix, helping with adhesion and cohesion among cells.⁶ They have also been linked to providing biofilms with their increased resistance to environmental stressors, such as desiccation and antibiotics.^{36,37} Furthermore, anionic portions of these EPS matrix components are known to associate with essential minerals and nutrients, as well as capture cations to promote mechanical stability of the biofilm.^{13,38,39}

Polysaccharides make up the majority of EPS.⁵ The presence of polyanionic polysaccharides in EPS, primarily due to uronic acids, confers a net negative charge to the biofilm matrix. In

Figure 5: (a) Normalized absorbance spectra with the vertical dash line highlighting signal changes 1132 cm^{-1} region. (b) Absorbance at 980 cm^{-1} , attributed to nucleic acids plotted as a function of time. (c) Absorbance at 1132 cm^{-1} , attributed to uronic acids plotted as a function of time. (d) Results from a previous surface spectroscopy study monitoring the water O–H stretching response. The grey lines in the bottom three panels are plotted using Eq. 3 with k and t_m determined by fitting the light scattering data from Fig. 3.

addition, uronic acid functionalities have been reported to form large fibril structures based on cationic bridging between carboxylic acid moieties and biological cations commonly found in growth media, such as Ca^{2+} , Na^{+} and Mg^{2+} .⁴⁰ The presence of these structures can increase the aggregation of bacteria and sequester ions from both the solution and the surface into the biofilm. The accumulation of acidic sugars in EPS matrix polysaccharides has been well documented in the literature for a wide variety of bacteria, including several strains of *E. Coli*.⁴¹ Anionic polysaccharides have also been reported to be important for cell signalling within the EPS matrix and play major roles in the virulence of bacterial biofilms.²⁶ The carboxylic acid functional groups in uronic acids are an important component of EPS as they are able to stabilize the glycosidic linkages between polysaccharides. This stabilization enables the biofilm to be more resistant to acid hydrolysis of the ester linkages between sugars.²⁶

Extracellular DNA (eDNA) has been known to play an important role in determining both the structure and properties of EPS, although the origin and function of these molecules are not as well understood.⁴² In recent years, eDNA has become a target for biofilm control due to its importance in initial adhesion events.³⁷ However, after maturation, degradation of a biofilm via DNase enzyme treatment becomes less efficient.⁴³ This highlights the importance of other EPS metabolites that may be necessary for the adhesive properties of eDNA, or perhaps supplement initial eDNA interactions with the surface.⁴⁴ In EPS, anionic phosphate groups can be found in the backbone of eDNA, in lipopolysaccharides, and in EPS-associated carbohydrates.⁴⁵ Moreover, near neutral pH, these phosphate functionalities would be the dominant ionic species present in solution. The increase in phosphate O–P–O stretching observed in IR spectra with cell growth is consistent with *E. coli* exposing a greater number of phosphate groups on their outer membrane at later stages of growth.⁴⁵ These negatively charged phosphate groups are stabilized in the EPS matrix by chelation with Ca^{2+} , Mg^{2+} or Mn^{2+} .⁴⁶

In a recent study by our group, we used vibrational sum frequency generation (SFG) spectroscopy to investigate adhesion and surface conditions at silica during the growth of *E. coli* K12.¹¹ A continual increase in the interfacial water O–H stretching was observed over a 24 h period as the

water signal reaches 53% of the neat silica–water signal (Fig. 5d). It was also demonstrated that this increase in SFG intensity exactly matched the population growth curve. To account for these trends, we proposed that a unique microenvironment is established at the surface as a result of metabolites and EPS accumulating on silica during cell growth, and a reduction in the local ionic strength as the EPS deplete ions from the region immediately adjacent to the surface, reducing screening of the negative surface charge on silica.⁴⁷ Bulk ionic strength measurements showed small changes that were not sufficient to account for the magnitude of the observed increase in SFG intensity.¹² However, the formation of a microenvironment at the surface composed of adhered cells, EPS, and metabolites, as well as the ions associated with them, could produce a local ionic strength that is much lower than in the bulk solution. The increasing fraction of uronic and nucleic acid moieties observed in the IR spectra support this hypothesis to explain the increase in SFG signal intensity during bacterial growth.

Silica surfaces in particular are sensitive to changes in solution pH and ionic strength. An increase in pH promotes the deprotonation of surface silanol groups, thereby increasing the negative surface charge density, while a decrease in the ionic strength of the solution results in an expansion of the electrical double layer, reducing screening of the surface charge by counterions.⁴⁷ Both of these processes work to increase the SFG response from interfacial water molecules, causing an increase in the signal intensity. An increase in uronic and nucleic acid secretion would enable more ions to be sequestered from the surrounding solution. As cations are attracted to the negatively charged silica, production of these EPS molecules near the surface would result in greater depletion of ions directly adjacent to the silica surface, consistent with the observed increase in SFG intensity. Moreover, when the same *E. coli* fractions were monitored with SFG spectroscopy, but with the cells removed, only samples from the late exponential and stationary phases showed an increase in interfacial water signal.¹¹ These fractions contain EPS with a higher uronic and nucleic acid content, and therefore have a greater capacity to sequester ions from the surface microenvironment. This lends further support to the possibility that EPS adsorption, specifically polysaccharides and eDNA, and their ability to alter the ionic strength at the interface, increases the structuring of

surface water molecules. However, although polysaccharides and eDNA make up the majority of EPS, other components, such as proteins and lipids, could also be contributing to changes in interfacial water structure.

Conclusions

We have described the design, construction, and operation of a versatile FTIR sample array scanner that can accommodate substrates of varying shape and size. Such instruments are particularly useful for the measurement of biological samples and associated replicates, as scans of many spots are required. We have demonstrated the operation of our instrument by studying the compositional changes of EPS extracted from *E. Coli* K12 at various stages of growth. Our finding that the EPS becomes enriched in uronic and nucleic acids over time, and in a manner that is commensurate with previous reports of changes in interfacial water structure over the same growth period provides evidence that illustrates the role of extracellular polymeric substances in altering the interfacial microenvironment prior to and during cell adhesion.

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Supporting Information Available

Editable files suitable for laser-cutting the acrylic and rubber components of the sample array, spot finding and data acquisition codes. This information is available free of charge via the Internet at <http://pubs.acs.org>

References

- (1) Dunne, W. M., Jr. Bacterial Adhesion: Seen Any Good Biofilms Lately? *Clin. Microbiol. Rev.* **2002**, *15*, 155–166.
- (2) Salta, M.; Wharton, J. A.; Blanche, Y.; Stokes, K. R.; Briand, J.-F. Marine Biofilms on Artificial Surfaces: Structure and Dynamics. *Environ. Microbiol.* **2013**, *15*, 2879–2893.
- (3) Carpentier, B.; Cerf, O. Biofilms and Their Consequences, with Particular Reference to Hygiene in the Food Industry. *J. Appl. Bacteriol.* **1993**, *75*, 499–511.
- (4) Costerton, J. W.; Lewandowski, Z.; Caldwell, D. E.; Korber, D. R.; Lappin-Scott, H. M. Microbial Biofilms. *Annu. Rev. Microbiol.* **1995**, *49*, 711–745.
- (5) Donlan, R. M. Biofilms: Microbial Life on Surfaces. *Emerg. Infect. Dis.* **2002**, *8*, 881–890.
- (6) Flemming, H.-C.; Wingender, J. The Biofilm Matrix. *Nature Rev.* **2010**, *8*, 623–633.
- (7) Hong, Y.; Brown, D. G. Variation in Bacterial ATP Level and Proton Motive Force Due to Adhesion to a Solid Surface. *Appl. Environ. Microbiol.* **2009**, *75*, 2346–2353.
- (8) Fletcher, M. The Effects of Proteins on Bacterial Attachment to Polystyrene. *J. Gen. Microbiol.* **1976**, *94*, 400–404.
- (9) Rosenberg, M.; Kjelleberg, S. Hydrophobic Interactions—Role in Bacterial Adhesion. *Adv. Microbial Ecol.* **1986**, *9*, 353–393.
- (10) Pätzold, R.; Keuntje, M.; Anders-von Ahlften, A. A New Approach to Non-Destructive Analysis of Biofilms by Confocal Raman Microscopy. *Anal. Bioanal. Chem.* **2006**, *386*, 286–292.
- (11) Jarisz, T. A.; Lane, S.; Gozdziński, L.; Hore, D. K. Ions, Metabolites, and Cells: Water as a Reporter of Surface Conditions During Bacterial Growth. *J. Chem. Phys.* **2018**, *148*, 222825.

- (12) Darlington, A. M.; Jarisz, T. A.; DeWalt-Kerian, E. L.; Roy, S.; Kim, S.; Azam, M. S.; Hore, D. K.; Gibbs, J. M. Separating the pH-Dependent Behavior of Water in the Stern and Diffuse Layers with Varying Salt Concentration. *J. Phys. Chem. C* **2017**, *121*, 20229–20241.
- (13) Vu, B.; Chen, M.; Crawford, R. J.; Ivanova, E. P. Bacterial Extracellular Polysaccharides Involved in Biofilm Formation. *Molecules* **2009**, *14*, 2535–2554.
- (14) Rochfort, S. Metabolomics Reviewed: A New “Omics” Platform Technology for Systems Biology and Implications for Natural Products Research. *J. Nat. Prod.* **2005**, *58*, 1813–1820.
- (15) Griffin, J. L. The Potential of Metabonomics in Drug Safety and Toxicology. *Drug Discovery Today: Technologies* **2004**, *1*, 285–293.
- (16) Griffin, J. L. Metabonomics: NMR Spectroscopy and Pattern Recognition Analysis of Body Fluids and Tissues for Characterisation of Xenobiotic Toxicity and Disease Diagnosis. *Cur. Op. Chem. Biol.* **2003**, *7*, 648–654.
- (17) Dunn, W. B.; Johnson, N. J. C. B. H. E. Measuring the Metabolome: Current Analytical Technologies. *Analyst* **2005**, *130*, 606–625.
- (18) Kunstar, A.; Leferink, A. M.; Okagbare, P. I.; Morris, M. D.; Roessler, B. J.; Otto, C.; Karperien, M.; van Blitterswijk, C. A.; Moroni, L.; van Apeldoorn, A. A. Label-Free Raman Monitoring of Extracellular Matrix Formation in Three-Dimensional Polymeric Scaffolds. *J. R. Soc. Interface* **2013**, *10*, 20130464.
- (19) Escoriza, M. F.; VanBriesen, J. M.; Stewart, S.; Maier, J. Raman Spectroscopic Discrimination of Cell Response to Chemical and Physical Inactivation. *Appl. Spectrosc.* **2007**, *61*, 812–823.
- (20) Quilés, F.; Humbert, F.; Delille, A. Analysis of Changes in Attenuated Total Reflection FTIR Fingerprints of *Pseudomonas fluorescens* from Planktonic State to Nascent Biofilm State. *Spectrochimica Acta Part A* **2010**, *75*, 610–616.

- (21) Humbert, F.; Quilès, F. In *Science Against Microbial Pathogens: Communicating Current Research and Techological Advances*; Méndez-Vilas, A., Ed.; Formatex Research Center, 2011; Chapter *In situ* Study of Early Stages of Biofilm Formation Under Different Environmental Stresses by ATR-FTIR Spectroscopy, pp 889–895.
- (22) Gong, A. S.; Bolster, C. H.; Benavides, M.; Walker, S. L. Extraction and Analysis of Extracellular Polymeric Substances: Comparison of Methods and Extracellular Polymeric Substance Levels in *Salmonella pullorum* SA 1685. *Environ. Eng. Sci.* **2009**, *26*, 1523–1532.
- (23) Afseth, N. K.; Kohler, A. Extended Multiplicative Signal Correction in Vibrational Spectroscopy, A Tutorial. *Chemometr. Intell. Lab.* **2012**, *117*, 92–99.
- (24) Martens, H.; Stark, E. Extended Multiplicative Signal Correction and Spectral Interference Subtraction: New Preprocessing Methods for Near Infrared Spectroscopy. *J. Pharm. Biomed. Analysis* **1991**, *9*, 625–635.
- (25) Kohler, A.; Bertrand, D.; Martens, H.; Hannesson, K.; Kirschner, C.; Ofstad, R. Multivariate Image Analysis of a Set of FTIR Microspectroscopy Images of Aged Bovine Muscle Tissue Combining Image and Design Information. *Anal. Bioanal. Chem.* **2007**, *389*, 1143–1153.
- (26) Bosch, A.; Serra, D.; Prieto, C.; Schmitt, J.; Naumann, D.; Yantorno, O. Characterization of *Bordetella pertussis* Growing as a Biofilm by Chemical Analysis of FT-IR Spectroscopy. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 736–747.
- (27) Kong, J.; Yu, S. Fourier Transform Infrared Spectroscopic Analysis of Protein Secondary Structure. *Acta Biochim. Biophys. Sinica* **2007**, *39*, 549–559.
- (28) Yang, H.; Yang, S.; Kong, J.; Dong, A.; Yu, S. Obtaining Information About Protein Secondary Structures in Aqueous Solution Using Fourier Transform IR Spectroscopy. *Nat. Protoc.* **2015**, *10*, 382–396.

- (29) Eboigbodin, K. E.; Biggs, C. A. Characterization of the Extracellular Polymeric Substances Produced by *Escherichia Coli* Using Infrared Spectroscopic, Proteomic, and Aggregation Studies. *Biomacromolecules* **2008**, *9*, 686–695.
- (30) Coimbra, M. A.; Barros, A.; Rutledge, D. N.; Delgadillo, I. FTIR Spectroscopy as a Tool for the Analysis of Olive Pulp Cell-Wall Polysaccharide Extracts. *Carbohydrate Research* **1999**, *317*, 145–154.
- (31) Badireddy, A. R.; Chellam, S.; Gassman, P. L.; Engelhard, M. H.; Lea, A. S.; Rosso, K. M. Role of Extracellular Polymeric Substances in Bioflocculation of Acativated Sludge Microorganisms Under Glucose-controlled Conditions. *Water Res.* **2010**, *44*, 4505–4516.
- (32) Grant, W. D.; Sutherland, I. W.; Wilkinson, J. F. Exopolysaccharide Colanic Acid and its Occurrence in the *Enterobacteriaceae*. *J. Bacteriol.* **1969**, *100*, 1187–1193.
- (33) Shingel, K. I. Determination of Structural Peculiarities of Dexran, Pullulan and γ -Irradiated Pullulan by Fourier-Transform IR Spectroscopy. *Carb. Res.* **2002**, *337*, 1445–1451.
- (34) Coimbra, M. A.; Barros, A.; Barros, M.; Rutledge, D. N.; Delgadillo, I. Multivariate Analysis of Uronic Acid and Neutral Sugars in Whole Pectic Samples by FT-IR Spectroscopy. *Carbohydrate Polymers* **1998**, *37*, 241–248.
- (35) Hori, R.; Sugiyama, J. A Combined FT-IR Microscopy and Principal Component Analysis on Softwood Cell Walls. *Carbohydrate Polymers* **2003**, *52*, 449–453.
- (36) Bramhachari, P. V.; Dubey, S. K. Isolation and Characterization of Exopolysaccharide Produced by *Vibrio harveyi* Strain VB23. *Lett. Appl. Microbiol.* **2006**, *43*, 571–577.
- (37) Das, T.; Sharma, P. K.; Busscher, H. J.; van der Mei, H. C.; Krom, B. P. Role of Extracellular DNA in Initial Bacterial Adhesion and Surface Aggregation. *Appl. Environ. Microbiol.* **2010**, *76*, 3405–3408.

- (38) Donota, F.; Fontana, A.; Baccou, J. C.; Schorr-Galindo, S. Microbial Exopolysaccharides: Main Examples of Synthesis, Excretion, Genetics and Extraction. *Carbohydrate Polymers* **2012**, *87*, 951–962.
- (39) Okshevsky, M.; Meyer, R. L. The Role of Extracellular DNA in the Establishment, Maintenance and Perpetuation of Bacterial Biofilms. *Crit. Rev. Microbiol.* **2015**, *41*, 341–352.
- (40) Vieira, A. A. H.; Ortolano, P. I. C.; Girollo, D.; Oliveira, M. J. D.; Bittar, T. B.; Lombardi, A. T.; Sartori, A. L.; Paulsen, B. S. Role of Hydrophobic Extracellular Polysaccharide of *Aulacoseira granulata* (Bacillariophyceae) on Aggregate Formation in a Turbulent and Hypereutrophic Reservoir. *Limnol. Oceanogr.* **2008**, *53*, 1887–1899.
- (41) Sutherland, I. W. Biosynthesis and Composition of Gram-Negative Bacterial Extracellular and Wall Polysaccharides. *Ann. Rev. Microbiol.* **1985**, *39*, 243–270.
- (42) Flemming, H.-C.; Neu, T. R.; Wozniak, D. J. The EPS Matrix: The “House of Biofilm Cells”. *J. Bacteriol.* **2007**, *189*, 7945–7947.
- (43) Schlafera, S.; Meyer, R. L.; Dige, I.; Regina, V. R. Extracellular DNA Contributes to Dental Biofilm Stability. *Caries Res.* **2017**, *51*, 436–442.
- (44) Okshevsky, M.; Regina, V. R.; Meyer, R. L. Extracellular DNA as a Target for Biofilm Control. *Curr. Opin. Biotechnol.* **2015**, *33*, 73–80.
- (45) Walker, S. L.; Hill, J. E.; Redman, J. A.; Elimelech, M. Influence of Growth Phase on Adhesion Kinetics of *Escherichia coli* D21g. *Appl. Environ. Microbiol.* **2005**, *71*, 3093–3099.
- (46) Mulcahy, H.; Charron-Mazenod, L.; Lewenza, S. Extracellular DNA Chelates Cations and Induces Antibiotic Resistance in *Pseudomonas aeruginosa* Biofilms. *PLoS Pathog.* **2008**, *4*, e1000213.
- (47) Dewan, S.; Yeganeh, M. S.; Borguet, E. Experimental Correlation between Interfacial Water Structure and Mineral Reactivity. *J. Phys. Chem. Lett.* **2013**, *4*, 1977–1982.

Graphical TOC Entry