Journal Pre-proof

Recent advances on the spectroscopic characterization of microbial biofilms: A critical review

Maria Chiara Sportelli, Christine Kranz, Boris Mizaikoff, Nicola Cioffi

PII: S0003-2670(22)00004-6

DOI: https://doi.org/10.1016/j.aca.2022.339433

Reference: ACA 339433

To appear in: *Analytica Chimica Acta*

Received Date: 13 July 2021

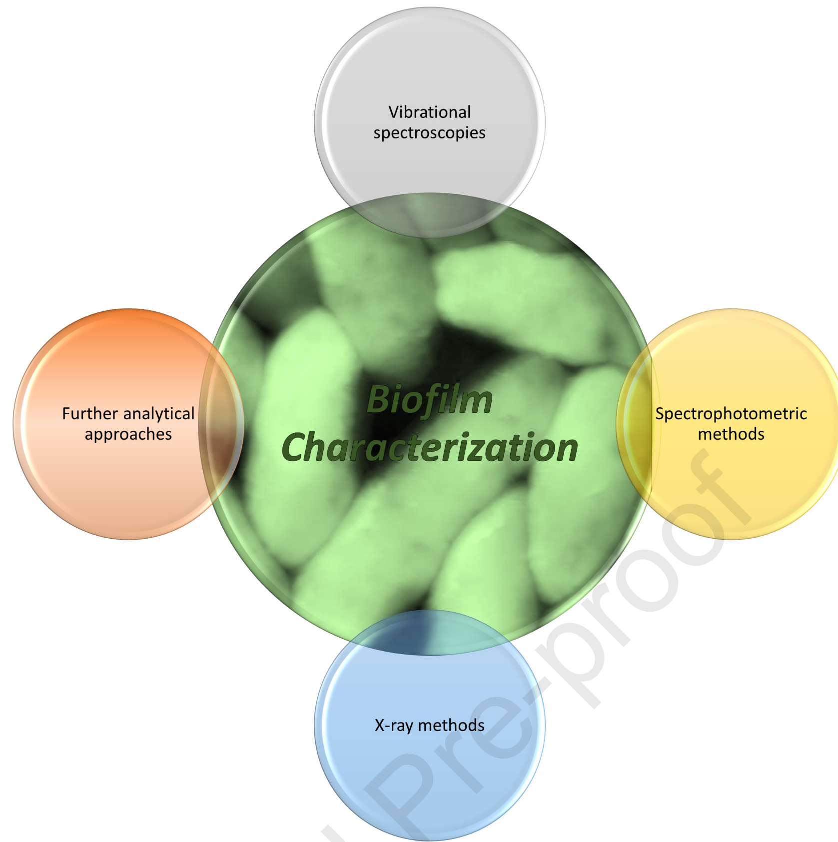
Revised Date: 30 December 2021

Accepted Date: 2 January 2022

Please cite this article as: M.C. Sportelli, C. Kranz, B. Mizaikoff, N. Cioffi, Recent advances on the spectroscopic characterization of microbial biofilms: A critical review, *Analytica Chimica Acta* (2022), doi: https://doi.org/10.1016/j.aca.2022.339433.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2022 Published by Elsevier B.V.



Journal Pre-proof

Journal Pre-proof

Recent advances on the spectroscopic characterization of microbial biofilms: a critical review

Maria Chiara Sportelli[1,2], Christine Kranz[3], Boris Mizaikoff[3], Nicola Cioffi[1,4*]

1. Chemistry Department, University of Bari “Aldo Moro”, V. Orabona, 4, 70126, Bari, Italy.
2. CNR, Istituto di Fotonica e Nanotecnologie UOS Bari, Physics Department, Via Amendola, 173, 70126 Bari, Italy.
3. Institute of Analytical and Bioanalytical Chemistry, Ulm University, Albert Einstein Allee, 11, 89081, Ulm, Germany.
4. CSGI Consortium, Bari Unit, University of Bari “Aldo Moro”, V. Orabona, 4, 70126, Bari, Italy

*Correspondence: N.C., nicola.cioffi@uniba.it; Tel.: +39 080 544 2020.

Abstract. Biofilms are a major cause of health and environmental issues. Bacteria organized in biofilms are much more resistant to biocides than their equivalents in the planktonic state. In this context, spectroscopic techniques have significantly contributed to a more fundamental understanding of biofilm formation, which is crucial to prevent and limit their generation, spreading, and maturation. In this review, recent progress on the main analytical approaches enabling the spectroscopic characterization of microbial biofilms is comparatively discussed. In addition, less commonly used techniques facilitating biofilm studies will be also presented. Advantages and drawbacks of each discussed technique will be underlined, thus providing an overview on spectroscopic approaches for studying biofilms.

Keywords. Biofilm; spectroscopic characterization, infrared attenuated total reflection, IR-ATR, Raman, antimicrobial, vibrational spectroscopy, X-ray photoelectron spectroscopy.

1. **Introduction**

Active biofilms are complex communities of bacteria embedded within a matrix composed of many different biomolecules (Fig. 1). A major challenge when studying biofilms resides in the temporally changing nature of this matrix, which correlates with the lifecycle of the microorganisms, and their response to environmental stimuli.

Different biofilm bacteria respond to their specific microenvironmental conditions with different growth models. Physiological cooperativity is a key factor in determining the biofilm structure and in founding the eventual collocations which make mature biofilms very efficient microbial

37 communities adherent to surfaces [1]. Protein structure and sequential transcription state the
38 elaborate structures of enzyme complexes; these molecular complexes are much more
39 efficient than randomly moving biomolecules. Analogously, strict organization of bacteria in
40 biofilms ensures higher efficiency in respect to planktonic state [2]. An intricate network of
41 molecular signaling, called quorum sensing, allows microbial communities embedded in a
42 biofilm to interact and cooperate [3]. A detailed description about the (bio)chemistry of biofilms
43 can be found elsewhere [4].

44 [FIG. 1 HERE]

45 *Figure 1: Schematic representation of biofilm components (a) and life cycle (b). (a) The mature*
46 *biofilm is built with a variety of compounds (DNA, RNA, proteins, lipids, enzymes, and extracellular*
47 *polysaccharides) called extracellular polymeric substances (EPSs). (b) Formation of biofilm starts*
48 *with attachment of planktonic cells to the surface. Next, bacteria start to form a monolayer and*
49 *produce the matrix which allows developing the mature biofilm. In the last stage, bacterial cells*
50 *multiplicate quickly, start to detach, and disperse. This process enables them to convert to motile*
51 *forms that can spread and colonize new surfaces. Reproduced from* [5], *© 2021 by the authors. Licensee MDPI,*
52 *Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the*
53 *Creative Commons Attribution (CC BY) license.*

54 Given their complexity, biofilm characterization strategies have developed as an
55 interdisciplinary research field involving a range of disciplines including biology, biochemistry,
56 analytical chemistry, physical chemistry, materials science, and others.
57 Surface colonization by microorganisms and the resulting development of microbial biofilms at
58 interfaces are frequently encountered in natural and artificial environments. Biofilms exist since
59 about 4 billion years, and are ubiquitous on earth [6]. Biofilm formation allows microorganisms
60 to survive at life-threatening environmental conditions, e.g., at extremely low or high
61 temperatures, across the entire pH range, and at pressures up to 100 MPa [7]. Moreover,
62 microbes embedded within biofilms show an increased resistance to antimicrobial agents [8].
63 This reduced antibiotic susceptibility [9] makes biofilm-related infections extremely harmful,
64 e.g., in clinical scenarios but also in food industry; the resistance mechanisms developed by
65 microbial communities within biofilms establishes a broad-spectrum defense [10], which
66 triggered extensive research to understand such defense mechanisms.
67 A plethora of techniques have been developed and are nowadays applied to study biofilms
68 and biofilm formation, ranging from molecular to atomic spectroscopic methods, microscopic
69 methods, sensing strategies, electrochemical approaches [11], mass spectrometry, etc. [12–
70 15].
71 Optical and high-resolution microscopies are historically relevant, as they were the first
72 techniques to be applied *in situ* [16–18], i.e., at living biofilms, and allowed gathering elaborate
73 information on bacterial spatial organization, effect of the substrate and substrate surface on
74 the colonization mechanisms, and biofilm rheological properties [15]. More recently,

75 fluorescence and confocal laser microscopies are becoming increasingly common to address
76 this aim [19–22]. These techniques enable to link the production of specific molecules inside
77 the biofilm to peculiar external conditions, contributing to a fundamental understanding of
78 biofilm formation and growth [23].
79 Although microscopy techniques have provided important information on biofilm and biofilm
80 formation, analytical methods giving access to molecular information on quorum sensing
81 molecules and changes in chemical signatures are a prerequisite for gaining fundamental
82 insight mechanistic studies. Mass spectrometry (MS) is exploited to obtain full metabolomic
83 assays of bacterial biofilms, giving information on regulatory mechanisms and examining
84 cellular and molecular heterogeneity [24–26]. In the case of complex biofilms and mixed
85 bacterial cultures, MS imaging (MSI) had a substantial impact in obtaining significant
86 knowledge in current microbiology, since it could be used to characterize bacteria at the
87 molecular level in three dimensions; specifically, it is mainly used to study intercellular
88 communication that mediates the formation of bacterial biofilms [27].
89 Spectrochemical characterization of biofilms is necessary for developing in-depth knowledge
90 on molecules involved during biofilm formation, and they are thus gaining importance (Fig. 2).
91 [FIG. 2 HERE]
92 *Figure 2: Summary of the spectroscopic techniques presented for the chemical characterization of biofilms.*
93 *Typical spatial resolution and/or penetration depth were expressed, when appropriate.*
94 From the pioneering papers dating back to the 1980ies [28], numerous papers were published
95 on the spectroscopic characterization of biofilms. Mainly vibrational spectroscopy is nowadays
96 used in this field, i.e., infrared and Raman spectroscopies, which give complementary
97 molecular information on both extracellular polymeric matrix and microorganism cell walls [29].
98 Besides, nuclear magnetic resonance (NMR) was implemented for NMR-based metabolomics
99 studies [30]. Information about spectrophotometric and turbidity measurements - which are
100 routinely used in biological laboratories to calculate bacterial concentration - will be provided
101 herein besides the application of to date less commonly used techniques such as X-ray
102 spectroscopic methods, photoacoustic spectroscopy, and combined or hyphenated
103 approaches.
104 We believe that this review complements previous reviews, which have mainly focused on
105 aspects such as biofilm formation [31,32], adhesion or detachment [33,34], biofilm
106 susceptibility to antibiotics [35–37], toxicity testing [38–40], and other biochemical subjects in
107 the field. Other approaches for biofilm characterization have been covered by different reviews
108 [11,13,15,41–46].
109

Journal Pre-proof

Journal Pre-proof

110 2. **Vibrational spectroscopy**
111 Radiation in the near-infrared and mid-infrared frequency regime is widely used in vibrational
112 spectroscopies to detect both planktonic and sessile microorganisms in aqueous
113 environments. The use of low-energy radiation ensures the absence of photodecomposition
114 and limits the degradation of biological molecules.
115 *2.1. Infrared spectroscopy*
116 The use of infrared spectroscopy for the characterization of biofilms can be tracked back to
117 1985 when Nichols et al. [47] published a seminal paper, which is to the best of our knowledge
118 the first example of infrared attenuated total reflectance (IR-ATR) analysis on biofilms. In this
119 pioneering paper, results obtained from diffuse reflectance infrared Fourier transform (DRIFT)
120 spectroscopy on freeze-dried microorganisms were compared to those obtained on hydrated
121 sessile bacteria. The use of IR-ATR spectroscopy allowed for the first time that the *in situ*
122 biofilm formation could be monitored. Since then, IR-ATR spectroscopy is regarded as a
123 powerful tool for understanding the interactions within adherent microbial consortia. Nichols et
124 al. provided useful reference information which was applied for studying biofilm structure
125 (where amide I and II, as well as carbohydrate bands are of crucial importance), along with the
126 health status of the microbial consortium. It should be noted that Nichols also hypothesized
127 that a detailed analysis of fingerprint band intensities could be helpful for understanding biofilm
128 metabolism.
129 This concept was furtherly evolved by Nivens et al. [48]. Fourier transform infrared (FT-IR)
130 spectroscopy enabled fast analyses via direct usage of interferograms with excellent signal-
131 to-noise ratio. Moreover, the increasing adoption of mercury-cadmium-tellurium (MCT)
132 detectors during the 1990ies allowed gathering improved wavenumber accuracy and spectral
133 sensitivity.
134 The first experiment on bacterial biofilms analyzed by IR-ATR spectroscopy with time
135 resolution and in fully hydrated state dates back to a pivotal work by Bremer and Gheesy [49]
136 in 1991. They reported the bio-colonization of a Germanium (Ge) internal reflection element
137 (IRE) enclosed in a flow cell, in which bacterial growth medium inoculated with a mixture of
138 bacteria was circulated. They compared the results generated by single- and double-beam
139 spectrometers, thereby demonstrating that a simultaneous background subtraction provided
140 by the double-beam measurement ensured a significant reduction of the chemical interference
141 from the bulk liquid phase, while the double-beam spectra yielded a stable baseline across the
142 entire mid-IR range. Time resolved monitoring of specific IR bands over a period of more than
143 a week provided information on the relative concentrations of metabolites that accumulate on
144 the solid surface at the base of the biofilm. A closer look at the graphical elements and specific
145 experiments reported in the work by Bremer and Gheesy, will help the reader to better
146 appreciate its influence on generations of similar studies.

147 Owing to the preconcentration at the IRE surface, it was possible to avoid any artefact due to
148 sample treatments (i.e., purification, isolation, extraction, etc.) and to obtain chemical
149 information on entire cells [7]. Mid-IR bands arise from the presence of proteins, nucleic acids,
150 lipids, polysaccharides within the biofilm. The identification of main IR bands for many
151 microorganisms is nowadays tabulated [50]. *In-vitro* analysis of biofilms by bioaccumulation at
152 the IRE can be considered as a "preconcentration" step of the biological molecules of interest,
153 which is specifically true for nascent biofilms that are less chemically multifaceted [51–54].
154 Specific molecules can be studied as well, focusing on specific spectral features; as an
155 example, spectrochemical and electrochemical properties of cytochrome C were analyzed
156 simultaneously by electrochemistry-coupled IR-ATR [55], on millimeter-sized interdigitated
157 microelectrode arrays (IDAs) serving as working electrodes and IRE components for
158 spectroscopy.
159 Besides bacterial characterization [56–58], FT-IR spectroscopy has been widely used for the
160 study of extracellular polymeric matrix (EPS). From the chemical point of view, EPS is a very
161 complex mixture of polysaccharides and proteins, DNA, lipids and humic substances. A
162 detailed review on the characterization of EPS by spectroscopic methods was published by
163 Zhang et al. discussing different analytical approaches to determine EPS binding properties of
164 inorganic species and consequent conformational changes [59]. FT-IR spectroscopy is
165 regarded as a way to distinguish among the various EPS biomolecules with each of them
166 related to specific IR bands [29,60]. In 2006, Bosch et al. proposed a first experimental
167 approach for the isolation and spectrochemical characterization of EPS [61]. *Bordetella*
168 *pertussis* biofilm was grown on polypropylene beads, and subsequently resuspended in pure
169 water, thus avoiding spectral interference from the growth medium. The supernatant,
170 containing EPS, was freeze-dried and analyzed with IR spectroscopy [62]. Lyophilized EPS
171 produced by cultures of two Gram negative bacteria (*Escherichia coli* and *Serratia*
172 *marcescens*) was investigated. This important contribution represents the first reported case
173 of using 2nd derivative IR-ATR spectroscopic analysis for a deeper understanding of the spatial
174 organization of biomolecules (i.e., secondary structure of proteins encoded in the amide I
175 band) [63]. Mathematical treatments on IR-ATR spectra can be difficult when there is high
176 overlapping of broad and weak signals: 2nd derivative can give rise to false features with
177 consequent signals misattributions. In order to overcome this intrinsic limitation, functionally
178 enhanced derivative spectroscopy (FEDS) has been recently introduced. Through a 1st
179 derivative of the inverse IR-ATR spectrum, Palencia et al. were able to discern with a single
180 analysis between different strains of *Helicobacter pylori* [64] and *Candida albicans* [65], which
181 spectra would have been superimposable with classical IR derivative analysis.
182 The analysis of EPS in fully hydrated conditions was only reported in 2012; such a delay is
183 comprehensibly due to the intricated chemical pathways, which are relevant to the production

184 of EPS via sessile bacteria especially in the first stages of biofilm formation promoting microbial
185 adhesion to surfaces [66]. Quilés et al. have used direct IRE colonization and flow-through IR-
186 ATR spectroscopy establishing first evidence and the first *in situ* proof of production and
187 structure determination of extracellular glycogen from *P. fluorescens* cells [67]. The same
188 group followed up with a study, probing spectrochemical properties of EPS with spatial
189 resolution, thanks to a combined use of IR-ATR spectroscopy and single-molecule force
190 microscopy [68], optical microscopy [43], or confocal microscopy coupled with epifluorescence
191 spectroscopy [69].
192 The highly hydrated nature of the EPS matrix (Fig. 3, right panel) makes the analysis of biofilm
193 quite difficult. In order to reduce interference arising from the aqueous matrix, approaches
194 based on micro-channels and lab-on-chip were developed only in recent years [51]. Quorum
195 sensing (QS) molecules (i.e., crucial in each step of biofilm development and aging) are easier
196 to detect in microfluidic small volumes; the limited diffusive dilution, peculiar of these systems,
197 allows a more rapid and facile detection by IR-ATR spectroscopy [70]. Kazarian firstly
198 developed a microfluidic chamber for biofilm analysis by infrared spectroscopic imaging using
199 a focal plane array detector in 2007 [71]. In this paper, the author combined FT-IR
200 spectroscopic imaging with a controlled-humidity microfluidic cell, thus targeting to study *in situ*
201 water adsorption by different sample areas, and biofilm behavior in a controlled environment.
202 A polydimethylsiloxane (PDMS) cell housing in combination with a large IRE crystal (i.e., ZnSe,
203 Ge or diamond) was used along with mini-channels self-adhering to the surface of the ATR
204 element [72]. However, this approach did not provide insight in fully-hydrated conditions, as
205 shown by Sharma et al. [66], yet, it paved the way for the introduction of FT-IR studies using
206 synchrotron radiation for analyzing biofilms.
207 Synchrotron radiation-based FT-IR (SR-FT-IR) spectroscopy can provide spatiotemporal
208 distributions and relative abundances of biomolecules in biofilms with unsurpassed resolution
209 [59]. The use of synchrotron radiation enables an improved signal-to-noise (s/n) ratio in
210 comparison to the conventional thermal IR sources. It is applicable to both Gram positive and
211 Gram negative bacterial biofilms, as well as to yeast colonies [73]. Due to the diffraction limit,
212 the radiation spot cannot be smaller than 2-10 μm, thus collecting information from small cell
213 clusters at a time [74,75], and penetration depth in the sub-millimeter range [76]. Until 2016,
214 mainly small humidified analysis chambers were used for SR-FT-IR, which did not allow
215 changing/refreshing of the growth medium, provoking degradation on the biological matter over
216 long-time analyses [77]. Microfluidics greatly assisted in overcoming this problem also allowing
217 for a fine-tuning of the liquid layer thickness above the biological sample. In the early stages,
218 closed channels were used for transmission experiments; CaF_2 was preferred as window
219 material although it caused toxicity to microbial cells [78]. Recently, open channel cells were
220 introduced, where one side of the liquid layer is exposed to air, and both liquid thickness and

221 flow are driven by capillary forces. Although humidity and temperature may impact the
222 measurements, its advantage is related to that the biofilm can be constantly supplied with fresh
223 growth medium [79].
224 As already outlined, IR-ATR spectroscopy is a powerful tool to study the interaction between
225 biofilms and antimicrobial agents, and the influence of external parameters on biofilm
226 development. For example, the effect of different concentrations of hydrocarbons on a nascent
227 biofilm of *P. fluorescens* was studied in real time [80]. The effect of antimicrobial peptides or
228 drugs added to the circulating growth medium in the flow-through IR-ATR system was studied
229 *in-vivo* and with temporal resolution for various biofilms [81–83]. Finally, the effect of culture
230 broth [84], nanoantimicrobials (Fig. 3, left panel) [85,86], and ZnSe crystal functionalization
231 [87,88] were investigated in the same way.
232 [FIG. 3 HERE]
233 *Figure 3: Left panel: Temporal evolution of relevant IR bands for biofilm formation. (a) Control IR-ATR spectra of a*
234 *P. fluorescens biofilm (arrows mark relevant IR bands) and (b) related integrated peak values (IPVs) as a function*
235 *of time. (c) IR-ATR spectra of P. fluorescens biofilm on antimicrobic-modified IRE (please note reversed time*
236 *scale for better illustration; the arrow indicates the decrease in IR bands associated to EPS); (d) related IPVs as a*
237 *function of time. Details of signal attributions are reported as Electronic supplementary material of* [86].
238 *Reproduced from* [86]*, Springer Nature, Copyright © 2017, under the terms of the Creative Commons CC BY*
239 *license. Right panel: Illustration of exopolymers typically found in the EPS of biofilms. Reprinted from* [23]*, with*
240 *permission from Elsevier.*
241 As a completion of the already mentioned techniques, it is worth mentioning surface-enhanced
242 infrared absorption-reflectance (SEIRA) spectroscopy, which has been used since the late
243 nineties for the characterization of biofilms [89]. The working concept of SEIRA lays on the use
244 of light and reflecting optics for selecting a surface area on the sample for infrared reflection-
245 absorption spectroscopic analysis. Changes in the chemical composition of *S. aureus* bacterial
246 membrane due to the action of antimicrobial agents were studied [90], along with responses
247 to environmental factors and signaling [91].
248
249 *2.2 Raman spectroscopy*
250 Compared to IR spectroscopy, Raman spectroscopy typically uses more energetic excitation
251 radiation, i.e., usually provided by a near IR, visible, or ultraviolet laser. IR signals are typically
252 much stronger than Raman signatures. Raman signals, while weaker, are usually not
253 obstructed by water. Based on the low polarizability and vibrational selection rules, water
254 bands are much less intense in Raman compared to those obtained in IR spectra. In general,
255 the bands observed in IR and Raman spectroscopy can be considered complementary given
256 the fundamentally different physical signal generation process, which renders them both
257 suitable for orchestrated studies on microbial biofilms using both methods. Raman was widely
258 explored in the 2000s for studying biofilm metabolism. Thanks to these vibrational techniques,

Journal Pre-proof

259 it was possible to access information of the molecular composition as well as of the surface
260 structure of living bacterial cells [52].
261 Micro-Raman spectroscopy [92] allows detecting few (i.e., below 50) microbial cells per time,
262 while *routine* IR is generally considered a “bulk” technique with a simultaneous sampling of
263 ~10^8 bacteria. Generally, visible-wavelength laser sources are used, which enable spatial
264 resolution studies, including spectral microscopy, up to the single-cell level and in three
265 dimensions [75,92]. In 2006, Quilès et al. proposed the use of micro-Raman for the analysis
266 of the shell of *Ascaris* eggs directly in their aqueous medium [93].
267 To overcome all problems related to low signal intensities in the biofilm analysis, conventional
268 Raman is, when possible, replaced by surface enhanced Raman scattering (SERS)
269 techniques. Three main ways have been developed for the preparation of biofilm samples for
270 SERS experiments [94]. The first approach consists in the simple mixing of bacteria with metal
271 colloids or ionic solutions, mainly composed of gold and silver; the solution is then drop cast
272 onto solid substrates. In the second approach, bacteria are allowed to colonize a surface
273 already modified with nanoparticles (NPs) or which is nanostructured itself; this is at present
274 the most diffused operational approach. Lastly, metal NPs can be synthesized directly on
275 bacterial surfaces by means of chemical reduction of precursor metal salts, by redox-active
276 molecules naturally present in many biofilms [95].
277 SERS requires that the used nanostructured material must have certain dielectric properties,
278 which are almost exclusively provided by noble metals, graphene and its oxides,
279 semiconductors [96]. The main drawback related to the use of metal nanoparticles for SERS
280 in biofilm characterization is the antimicrobial effect of some metals (especially Ag) on
281 microorganisms: for long experiments and high concentration of NPs, a significant decrease
282 of viable bacterial cells could be observed [97]. This phenomenon can be limited by using NPs
283 with sizes above 30 nm and by increasing the ratio between bacteria and NP concentrations
284 [97].
285 SERS signals are strongly dependent on the operating conditions relevant to sample
286 preparation (i.e. on NP morphology, their chemical composition and concentration, type of
287 liquid environment, chemical nature of the SERS substrate, etc.) [98,99]; hence, a wide
288 database is necessary for SERS signal attribution in biofilm study, along with standardized
289 approaches to the analysis[94]. Weiss et al. [100] pointed out that a fundamental knowledge
290 of the origin of Raman signal from microbes is crucial for reliable SERS analyses. They also
291 envisaged the strict correlation between SERS signals from single cells and their metabolic
292 activity.
293 The coupling of micro-Raman with optical microscopy allows for a detailed and 3D resolved
294 investigation of biofilm components separately [101], gathering information about the
295 distribution of carbohydrates, proteins, fatty acids, and nucleic acids in both spatially- and time-

Journal Pre-proof

296 resolved ways [59]. As an example, treatment of spectroscopic data by chemometrics tools
297 makes the information obtainable from a single measurement set particularly rich [102].
298 Indeed, mathematical pretreatments are required to enhance the information from the
299 investigated data and also decrease the influence of "side information" intrinsically included in
300 the spectra. Spectral pre-processing is considered mandatory, along with classical treatments
301 like normalizations, derivatives and smoothing, etc. [103].
302 In 2010, micro-Raman SPR imaging (SPR-i) [12] (Fig. 4) was firstly proposed for the imaging
303 of multicomponent biofilms from wastewater, with AgNPs as scattering enhancer [104].
304 [FIG. 4 HERE]
305 *Figure 4: Schematic of the setup for E. coli SPR-i (surface plasmon resonance imaging) experiments. A PDMS*
306 *chip containing two microchambers is reversibly sealed against the sensor surface. Reprinted from* [105]*, with the*
307 *permission of AIP Publishing.*
308 Up to that time, confocal laser scanning microscopy (CLSM) was among the few available
309 technique for 3D studying of biofilm structures. Differently from CLSM, micro-Raman SPR-i
310 does not require staining, and provides chemical information about complex biofilm matrices,
311 non-destructively, with molecular resolved information on bacteria [102] and microbial
312 constituents like EPS [29]. 2D and 3D structures of a *P. aeruginosa* biofilm were studied by
313 micro-Raman SERS up to 120 h; cultures were grown on biocompatible scaffolds to ensure
314 ordered 3D colonies. Effect of external stimuli was investigated, i.e. interaction with
315 doxorubicin-treated AgNPs; the latter served also as SPR enhancer [106]; the general
316 metabolic profile of *P. aeruginosa* was identified with SERS in their natural growth conditions.
317 A further development of micro-Raman SPR was given by Bodelon et al. [107]. Authors
318 focused on QS molecules involved in the formation of a *P. aeruginosa* biofilm, exploiting the
319 scattering properties of Au@SiO_2 nanorods (NRs). In particular, the expression of pyocyanin,
320 a heterocyclic nitrogen-based compound produced by *P. aeruginosa*, is strictly regulated by
321 the QS cycles. The detection of this molecules was performed by surface-enhanced resonance
322 Raman scattering (SERRS): in this approach, the frequency of the excitation laser is in
323 resonance with an electronic transition of the molecule. This way, a spatially resolved detection
324 of pyocyanin was achieved, giving a hint of spatial distribution in the QS molecules expression
325 at different location of the biofilm. Lab-on-chip and microfluidic systems, i.e., in analogy to
326 those described for IR spectroscopies, have been used in combination with Raman
327 spectroscopy as well [51].
328
329
330 3. **Spectrophotometric methods**
331 Spectrophotometric approaches are generally used for quality assessment and rapid detection
332 of biofilms: the amount of information obtainable form these techniques is much lower than the

Journal Pre-proof

333 one described above for infrared and Raman techniques. In fact, only one class of molecules
334 can be monitored or detected per measurement (polysaccharides, lipids, proteins/amino acids,
335 etc.) [108].
336 In 2005, Broschat proposed an inexpensive and nondestructive optical reflectance assay for
337 the measurement of biofilm formation [109]. Biofilm formation of *Enterococci* on numerous
338 opaque and nonopaque abiotic surfaces was studied with this semiquantitative method.
339 Plotting reflectance as a function of wavelength, the method could provide information on the
340 biofilm state and indicate if biofilm formation of the specific bacterial strain occurs.
341 Numerous biomolecules such as amino acids, photosynthetic pigments, riboflavin, tryptophan,
342 etc. display fluorescent quantum efficiencies which can be used for fluorescence
343 measurements [20]. Microorganisms typically exhibit fluorescence upon excitation, from
344 endogenous molecules, typically in the UV region of the electromagnetic spectrum.
345 Fluorescence spectra possess quantitative information, such as tryptophane content, as well
346 as some qualitative structural information like measurement of biomass for bacterial biofilms
347 grown in laminar flow chambers [110]. Fluorescence measurements have been used since the
348 nineties to monitor microbial changes, using fiber optic probes [19], or in biofilms grown on UV-
349 transparent quartz surfaces [7,48].
350 Besides, bacterial bioluminescence, although restricted to a small number of bacteria, can be
351 used to detect bacterial biomass (assuming constant light flux per cell), cellular activity (at a
352 given biomass), or gene expression [111]. The measurement of the emission at a specific
353 wavelength, typical of each microorganism, allows for the rapid monitoring of biomass
354 accumulation as a function of time [112].
355 Several different methods are available to assess the optical density of biofilms thus providing
356 information about film thickness and density [113]. Measuring of optical turbidity (or the
357 radiation intensity loss) is typically performed in a wavelength range between 600-1300 nm, in
358 order to minimize absorption by photodegradable molecules [20]. This near infrared (NIR)
359 window is also known as the “therapeutic window,” as it maximizes the penetration depth (30-
360 250 μm) into tissues and biofilms [114].
361
362
363 4. **Further analytical approaches**
364 Less frequently applied spectroscopic techniques such as photoacoustic spectroscopy (PAS),
365 which is based on the combination of light absorption and sound detection [7], can be used to
366 address specific analytical needs in the non-destructive characterization of biofilms. PAS
367 involves the absorption of an electromagnetic radiation within a biofilm, followed by its
368 conversion into heat, and biofilm thermal expansion [115]. The latter generates a pressure
369 wave, which is detected by microphones or piezoelectric crystals. The intensity of the

Journal Pre-proof

370 measured "sound" is a function of the optical absorption coefficient of the biofilm and its
371 thickness [116,117] (Fig. 5).
372 [FIG. 5 HERE]
373 *Figure 5: Photoacoustic sensor system (left) and flow channel with the three photoacoustic sensor heads (right).*
374 *Reprinted with permission from* [115]. *Copyright 2002 American Chemical Society.*

375 This technique allows optical absorption measurements even in strongly scattering or optical
376 opaque media [115]. PAS is used for the depth-resolved investigation of growth and
377 detachment processes of biofilms, when exposed to antimicrobial compounds or adverse
378 environment [118]. Schmid et al. proposed PAS (with pulsed radiation: PPAS) for the *in situ*
379 observation of the interaction with iron oxide particles on the outer and inner layers of the
380 biofilm [116].
381 Optical coherence tomography (OCT) is a high-resolution imaging technique which can
382 accomplish 2D and 3D characterization of biological and nonbiological structures in a manner
383 similar to PAS [119]. Because OCT uses near-infrared light rather than sound, imaging
384 resolution results to be 10 to 100 times higher. NIR wavelengths are used in OCT imaging to
385 increase imaging penetration through highly scattering structures: it is possible to achieve a
386 penetration depth in the range of centimeters for transparent samples, and of few millimeters
387 in highly scattering species [120]. To the best of our knowledge, the first attempt to biofilm
388 imaging through OCT dates back in 2006, when Xi et al. obtained the *in situ* imaging of a *P.*
389 *aeruginosa* biofilm developed in a capillary flow cell [121]. The further development of
390 mathematical models for improved settings of experimental parameters made the analysis
391 more straightforward [122]. In combination with other techniques (like X-ray based ones), OCT
392 ensures a detailed time-resolved characterization of biofilm structure and density under
393 different conditions [123,124]. OCT was used in the last years to study biofilm response to
394 shear stress and consequent dynamic deformation [125], as well as colonies response to
395 antibiotics [126] and antimicrobial substances like graphene oxide [127].
396 Also x-ray based spectroscopic techniques are employed in biofilm studies [29]. X-ray
397 photoelectron spectroscopy (XPS) was used to determine the elemental composition of
398 biofilms, along with relative atomic percentages of specific chemical environments [128].
399 Although destructive, XPS can provide semi-quantitative details on the yield of membranes
400 oxidation due to the presence of reactive oxygen species (ROS), amino acids esterification
401 induced by apoptosis markers, etc. [7].
402 X-ray based techniques are rarely used for biofilm characterization: high-energy radiation can,
403 in fact, damage biological matter, and many precautions are needed. Among them, small angle
404 x-ray scattering (SAXS) was used to study EPS, from a molecular and structural point of view.
405 Traditionally used to analyze proteins in crystals or suspension, SAXS can be also used to

406 analyze interactions within specimens in complex mixtures [12,129]. Even though the
407 achievable resolution is significantly lower compared to other techniques, SAXS has great
408 potential to retrieve information on the structural properties of EPS in biofilms [59,130,131].
409 Dogsa et al. used SAXS to characterize EPS structures at different pH values, demonstrating
410 that pH variation causes major rearrangements of EPS structure [132]. Trainor et al. applied
411 grazing incidence X-ray fluorescence (GIXRF) to the investigation of the distribution of heavy
412 metals on wet environmental interfaces (like biofilms) [133]. Similarly, total reflection X-ray
413 fluorescence spectrometry (TXRF), a highly sensitive method for determining trace elements
414 down to the ppb range, was used to quantify metal accumulation in aquatic biofilms [134,135].
415 NMR spectroscopy is used in biofilm research to determine the metabolic properties of
416 prokaryotic and eukaryotic cells. ^{1}H and ^{13}C NMR, specifically, allow for the direct, time-
417 resolved, and non-invasive monitoring of metabolic pathways of living bacterial suspensions
418 or bacterial biofilms on porous substrates [7,136]. Moreover, solid-state NMR (generally
419 associated with imaging, MRI) method has been used to study the chemical composition [137]
420 and molecular mobility of EPS [75], and to generate 2D and 3D maps of *S. oneidensis* with
421 molecular resolution [138]. MRI, also called magnetic resonance tomography (MRT), is
422 however quite expensive and time-consuming, and the high number of molecules present in
423 the sample during *in situ* analysis (i.e. without purification or isolation steps of specific biofilm
424 components) often requires adding paramagnetic relaxation agents (such as lanthanide ions)
425 for achieving a sufficient image contrast [139].
426 Among the many different technologies available for the fast monitoring of biofilm growth,
427 optical sensors are the most promising, as they afford direct imaging of biofilm growth on
428 surfaces, with high sensitivity and selectivity towards different biological species. Biofilm
429 formation is extremely sensitive to various growth and environmental parameters, resulting in
430 the high variability in biofilms between repeated experiments. Experimental repeatability can
431 be affected by this biofilm mutability. Sensors and miniaturized devices can aid in the non-
432 invasive characterization of bacterial biofilms with minimum alteration of the biofilm
433 surrounding [41]. As an interesting practical example, nanosensors find application for the
434 monitoring of food-derived biofilms in industry: bioassays based on multifunctional optical
435 nanosensors are promising to ensure and promote food safety and quality [140]. Surface
436 sensitive sensors for biofilm monitoring were reviewed by Fischer et al., in 2016 [20]. These
437 sensors exploit the total internal reflection (TIR) principle, which generates an evanescent field
438 of reflected light, interacting with the biofilm. These systems, generally composed by an optical
439 fiber coupled with a reflecting crystal, allow reducing H_2O interferences in resulting spectra
440 [141]. Alternatively, they are based on surface plasmon resonance (SPR), which uses the
441 differences in refractive indexes at the biofilm-environmental interface [142]. SPR is a surface
442 sensitive technique which sampling depth typically does not exceed a few hundred

443 nanometers, decaying exponentially with the distance from the metal layer at the sensor
444 surface. To increase the sampling depth (biofilms thickness can vary between >1 μm up to
445 hundreds of microns), reverse-symmetry waveguides are frequently used [143].
446 Among laser-ablation-based analytical techniques, we must cite mass spectrometry (MS).
447 Despite not a spectroscopic approach, the development of MS in atmospheric pressure
448 enabled the direct living cell analysis [51], thus giving a great burst to the characterization of
449 biofilms. Desorption electrospray ionization (DESI) MS and the direct analysis in real time
450 (DART) were used by Watrous et al. [144] for monitoring the exchange of secondary
451 metabolites between *Bacillus subtilis* and *Streptomyces coelicolor*. Analogously, laser ablation
452 electrospray ionization (LAESI) was used to characterize distribution of metabolites in bacterial
453 biofilms or mixed-specimen biofilms [145–148]. Because of the absence of chemical species
454 amplification in MS approaches, biofilm analysis and/or imaging is challenging. Dozens of
455 chemical compounds can be detected simultaneously, and their identification can be
456 challenging when unexpected fragmentations or rearrangements have to be considered [45].
457
458
459 5. **Concluding remarks**
460 Bacterial biofilms are living communities characterized by fast changes in their chemical and
461 biological properties; they can respond and react actively to a wide variety of environmental
462 stimuli and cues. Therefore, the analytical characterization and identification of these changes
463 represents a great challenge. This review has outlined how spectroscopic techniques
464 contribute to the understanding of biofilms, identify constituents, understand antibiotic
465 resistance mechanisms, locate specific compounds with imaging techniques. These analytical
466 tools can provide a plethora of information, both from the spectrochemical and the
467 morphological/spatial point of view. In this paper, we reviewed the literature for spectroscopic
468 studies of bacterial biofilms, with a focus on the past and future paths of all the different
469 spectroscopic approaches. Our intent was not a comprehensive listing of all the existing
470 studies on this topic; we intended, instead, to present selected examples elucidating which
471 technique could be more suitable for a precise case of study, or to address a specific analytical
472 problem. Different analytical spectroscopic techniques can be combined to achieve information
473 on biofilm structural, chemical, surface, and metabolic properties.
474 Analytical instrumental developments and improvements give access to detect biofilm-related
475 infections *in situ*. A comprehensive understanding based on improved measurement
476 technologies may help to develop new antibiotic-free therapies. Macro-sized approaches are
477 currently used routinely for study biofilms: they principally provide an end-point
478 characterization at a laboratory step, which is usually “invasive” in nature and destroys or alters
479 the biofilm. However, these methods allow for the analysis of large areas and biofilm portions

Journal Pre-proof

480 with minimum analysis time. Miniaturized devices offer advantages such as the ability to
481 perform the analysis in a sensitive and non-invasive way, providing temporal and lateral
482 resolution. These systems also help in the advancement of new treatments for biofilm fighting,
483 by monitoring antimicrobial-biofilm interaction directly, with contained reagents and equipment
484 costs. These emerging technologies have the potential to support the establishment of
485 univocal practices for biofilm characterization and treatment. To us, appears clear that an
486 effective biofilm detection and consequent fighting mainly requires low-cost, easily producible,
487 portable devices requiring minimal maintenance. Addressing these tasks will bring new
488 technologies for bio-safer devices in healthcare, food, and other industrial fields.
489 Hence, in the next decade, biofilm studies likely will move towards *in situ* and multi-modal
490 characterization via high-throughput analysis modes, involving spectroscopic approaches as
491 they are highly suitable for such multimodal measurements (i.e., 2D correlation of Raman and
492 IR). In combination with chemometric tools for analytical data evaluation, this may significantly
493 contribute to a comprehensive understanding of complex processes in biofilms.
494

Journal Pre-proof

Journal Pre-proof

495 **Author Contributions:** M.C.S performed bibliographic search and wrote the first draft of the
496 paper. C.K., B.M and N.C. took part in scientific discussions, refined the different sections of
497 this review, and coordinated the study.

498 **Acknowledgments:** Partial financial support is acknowledged from the European Union's
499 Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Grant
500 Agreement No. 813439, and from the Italian MIUR project “E-Design” ARS01_01158.
501 M.C.S. acknowledges the financial support from Fondo Sociale Europeo "Research for
502 Innovation (REFIN)"; project n° 435A866B.

503 **Conflicts of Interest:** The authors declare no conflict of interest.

Journal Pre-proof

504 **References:**

505 [1] J.W. Costerton, Z. Lewandowski, D. DeBeer, D. Caldwell, D. Korber, G. James, Biofilms,
506 the customized microniche., J. Bacteriol. 176 (1994) 2137–2142.
507 https://doi.org/10.1128/jb.176.8.2137-2142.1994.
508 [2] M.E. Shirtliff, J.T. Mader, A.K. Camper, Molecular Interactions in Biofilms, Chemistry &
509 Biology. 9 (2002) 859–871. https://doi.org/10.1016/S1074-5521(02)00198-9.
510 [3] C.D. Nadell, J.B. Xavier, S.A. Levin, K.R. Foster, The Evolution of Quorum Sensing in
511 Bacterial Biofilms, PLOS Biology. 6 (2008) e14.
512 https://doi.org/10.1371/journal.pbio.0060014.
513 [4] J.S. Dickschat, Quorum sensing and bacterial biofilms, Nat. Prod. Rep. 27 (2010) 343–
514 369. https://doi.org/10.1039/B804469B.
515 [5] G. Topka-Bielecka, A. Dydecka, A. Necel, S. Bloch, B. Nejman-Faleńczyk, G. Węgrzyn, A.
516 Węgrzyn, Bacteriophage-Derived Depolymerases against Bacterial Biofilm, Antibiotics. 10
517 (2021) 175. https://doi.org/10.3390/antibiotics10020175.
518 [6] J. Azeredo, N.F. Azevedo, R. Briandet, N. Cerca, T. Coenye, A.R. Costa, M. Desvaux,
519 G.D. Bonaventura, M. Hébraud, Z. Jaglic, M. Kačániová, S. Knøchel, A. Lourenço, F.
520 Mergulhão, R.L. Meyer, G. Nychas, M. Simões, O. Tresse, C. Sternberg, Critical review
521 on biofilm methods, Critical Rev. Microbiol. 43 (2017) 313–351.
522 https://doi.org/10.1080/1040841X.2016.1208146.
523 [7] E. Denkhaus, S. Meisen, U. Telgheder, J. Wingender, Chemical and physical methods for
524 characterisation of biofilms, Microchim. Acta. 158 (2007) 1–27.
525 https://doi.org/10.1007/s00604-006-0688-5.
526 [8] M.C. Sportelli, R.A. Picca, N. Cioffi, Recent advances in the synthesis and characterization
527 of nano-antimicrobials, TrAC, Trends Anal. Chem. 84, part A (2016) 131–138.
528 https://doi.org/10.1016/j.trac.2016.05.002.
529 [9] N. Rodis, Resistance mechanisms in bacterial biofilm formations - Review Article, Journal
530 of Medical Research and Health Education. 0 (2020).
531 https://www.imedpub.com/abstract/resistance-mechanisms-in-bacterial-biofilm-
532 formations--review-article-30913.html (accessed April 23, 2021).
533 [10] P.S. Stewart, Mechanisms of antibiotic resistance in bacterial biofilms, International
534 Journal of Medical Microbiology. 292 (2002) 107–113. https://doi.org/10.1078/1438-4221-
535 00196.
536 [11] G. Caniglia, C. Kranz, Scanning electrochemical microscopy and its potential for studying
537 biofilms and antimicrobial coatings, Anal Bioanal Chem. 412 (2020) 6133–6148.
538 https://doi.org/10.1007/s00216-020-02782-7.
539 [12] C. Wilson, R. Lukowicz, S. Merchant, H. Valquier-Flynn, J. Caballero, J. Sandoval, M.
540 Okuom, C. Huber, T.D. Brooks, E. Wilson, B. Clement, C.D. Wentworth, A.E. Holmes,
541 Quantitative and Qualitative Assessment Methods for Biofilm Growth: A Mini-review, Res
542 Rev J Eng Technol. 6 (2017). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6133255/
543 (accessed April 17, 2020).
544 [13] R.V. Linares, L. Fortunato, N.M. Farhat, S.S. Bucs, M. Staal, E.O. Fridjonsson, M.L. Johns,
545 J.S. Vrouwenvelder, T. Leiknes, Mini-review: novel non-destructive in situ biofilm
546 characterization techniques in membrane systems, Desalin. Water Treat. 57 (2016)
547 22894–22901. https://doi.org/10.1080/19443994.2016.1180483.
548 [14] S. Keleştemur, E. Avci, M. Çulha, Raman and Surface-Enhanced Raman Scattering for
549 Biofilm Characterization, Chemosensors. 6 (2018) 5.
550 https://doi.org/10.3390/chemosensors6010005.
551 [15] H. Boudarel, J.-D. Mathias, B. Blaysat, M. Grédiac, Towards standardized mechanical
552 characterization of microbial biofilms: analysis and critical review, Npj Biofilms and
553 Microbiomes. 4 (2018) 1–15. https://doi.org/10.1038/s41522-018-0062-5.
554 [16] M. Relucenti, G. Familiari, O. Donfrancesco, M. Taurino, X. Li, R. Chen, M. Artini, R. Papa,
555 L. Selan, Microscopy Methods for Biofilm Imaging: Focus on SEM and VP-SEM Pros and
556 Cons, Biology. 10 (2021) 51. https://doi.org/10.3390/biology10010051.

Journal Pre-proof

557 [17]T.R. Neu, B. Manz, F. Volke, J.J. Dynes, A.P. Hitchcock, J.R. Lawrence, Advanced
558 imaging techniques for assessment of structure, composition and function in biofilm
559 systems, FEMS Microbiology Ecology. 72 (2010) 1–21. https://doi.org/10.1111/j.1574-
560 6941.2010.00837.x.
561 [18]S. Schlafer, R.L. Meyer, Confocal microscopy imaging of the biofilm matrix, Journal of
562 Microbiological Methods. 138 (2017) 50–59. https://doi.org/10.1016/j.mimet.2016.03.002.
563 [19]J.C. Garcia-Betancur, A. Yepes, J. Schneider, D. Lopez, Single-cell Analysis of Bacillus
564 subtilis Biofilms Using Fluorescence Microscopy and Flow Cytometry, JoVE (Journal of
565 Visualized Experiments). (2012) e3796. https://doi.org/10.3791/3796.
566 [20]M. Fischer, G.J. Triggs, T.F. Krauss, Optical Sensing of Microbial Life on Surfaces, Appl.
567 Environ. Microbiol. 82 (2016) 1362–1371. https://doi.org/10.1128/AEM.03001-15.
568 [21]M. Kuehn, M. Hausner, H.-J. Bungartz, M. Wagner, P.A. Wilderer, S. Wuertz, Automated
569 Confocal Laser Scanning Microscopy and Semiautomated Image Processing for Analysis
570 of Biofilms, Appl. Environ. Microbiol. 64 (1998) 4115–4127.
571 https://doi.org/10.1128/AEM.64.11.4115-4127.1998.
572 [22]S.R. Wood, J. Kirkham, P.D. Marsh, R.C. Shore, B. Nattress, C. Robinson, Architecture of
573 Intact Natural Human Plaque Biofilms Studied by Confocal Laser Scanning Microscopy, J
574 Dent Res. 79 (2000) 21–27. https://doi.org/10.1177/00220345000790010201.
575 [23]T. Seviour, N. Derlon, M.S. Dueholm, H.-C. Flemming, E. Girbal-Neuhauser, H. Horn, S.
576 Kjelleberg, M.C.M. van Loosdrecht, T. Lotti, M.F. Malpei, R. Nerenberg, T.R. Neu, E. Paul,
577 H. Yu, Y. Lin, Extracellular polymeric substances of biofilms: Suffering from an identity
578 crisis, Water Research. 151 (2019) 1–7. https://doi.org/10.1016/j.watres.2018.11.020.
579 [24]N. Takahashi, J. Washio, G. Mayanagi, Metabolomic approach to oral biofilm
580 characterization—A future direction of biofilm research, Journal of Oral Biosciences. 54
581 (2012) 138–143. https://doi.org/10.1016/j.job.2012.02.005.
582 [25]R. Guo, X. Luo, J. Liu, H. Lu, Mass spectrometry based targeted metabolomics precisely
583 characterized new functional metabolites that regulate biofilm formation in Escherichia coli,
584 Analytica Chimica Acta. 1145 (2021) 26–36. https://doi.org/10.1016/j.aca.2020.12.021.
585 [26]T. Si, B. Li, K. Zhang, Y. Xu, H. Zhao, J.V. Sweedler, Characterization of Bacillus subtilis
586 Colony Biofilms via Mass Spectrometry and Fluorescence Imaging, J. Proteome Res. 15
587 (2016) 1955–1962. https://doi.org/10.1021/acs.jproteome.6b00127.
588 [27]J.D. Watrous, P.C. Dorrestein, Imaging mass spectrometry in microbiology, Nature
589 Reviews Microbiology. 9 (2011) 683–694. https://doi.org/10.1038/nrmicro2634.
590 [28]D.C. White, Environmental effects testing with quantitative microbial analysis: Chemical
591 signatures correlated with in situ biofilm analysis by FT/IR, Toxicity Assessment. 1 (1986)
592 315–338. https://doi.org/10.1002/tox.2540010305.
593 [29]E. Karunakaran, J. Mukherjee, B. Ramalingam, C.A. Biggs, “Biofilmology”: a
594 multidisciplinary review of the study of microbial biofilms, Appl Microbiol Biotechnol. 90
595 (2011) 1869–1881. https://doi.org/10.1007/s00253-011-3293-4.
596 [30]B. Zhang, R. Powers, Analysis of bacterial biofilms using NMR-based metabolomics,
597 Future Med Chem. 4 (2012) 1273–1306. https://doi.org/10.4155/fmc.12.59.
598 [31]C.R. Arciola, D. Campoccia, P. Speziale, L. Montanaro, J.W. Costerton, Biofilm formation
599 in Staphylococcus implant infections. A review of molecular mechanisms and implications
600 for biofilm-resistant materials, Biomaterials. 33 (2012) 5967–5982.
601 https://doi.org/10.1016/j.biomaterials.2012.05.031.
602 [32]S. Pérez-Rodríguez, J.M. García-Aznar, J. Gonzalo-Asensio, Microfluidic devices for
603 studying bacterial taxis, drug testing and biofilm formation, Microbial Biotechnology. n/a
604 (n.d.). https://doi.org/10.1111/1751-7915.13775.
605 [33]B.P. Singh, S. Ghosh, A. Chauhan, Development, dynamics and control of antimicrobial-
606 resistant bacterial biofilms: a review, Environ Chem Lett. (2021).
607 https://doi.org/10.1007/s10311-020-01169-5.
608 [34]A. Tilahun, S. Haddis, A. Teshale, T. Hadush, Review on Biofilm and Microbial Adhesion,
609 International Journal of Microbiological Research. 3 (2016) 11.
610 https://doi.org/10.5829/idosi.ijmr.2016.63.73.

611 [35]J.J. Harrison, H. Ceri, C.A. Stremick, R.J. Turner, Biofilm susceptibility to metal toxicity,
612 Environmental Microbiology. 6 (2004) 1220–1227. https://doi.org/10.1111/j.1462-
613 2920.2004.00656.x.
614 [36]P. Gilbert, J. Das, I. Foley, Biofilm Susceptibility to Antimicrobials, Adv Dent Res. 11 (1997)
615 160–167. https://doi.org/10.1177/08959374970110010701.
616 [37]D.L. Williams, S.R. Smith, B.R. Peterson, G. Allyn, L. Cadenas, R.T. Epperson, R.E.
617 Looper, Growth substrate may influence biofilm susceptibility to antibiotics, PLOS ONE.
618 14 (2019) e0206774. https://doi.org/10.1371/journal.pone.0206774.
619 [38]S.S.I. Abdalla, H. Katas, F. Azmi, M.F.M. Busra, Antibacterial and Anti-Biofilm
620 Biosynthesised Silver and Gold Nanoparticles for Medical Applications: Mechanism of
621 Action, Toxicity and Current Status, Current Drug Delivery. 17 (2020) 88–100.
622 https://doi.org/10.2174/1567201817666191227094334.
623 [39]L. Barral-Fraga, M.T. Barral, K.L. MacNeill, D. Martiñá-Prieto, S. Morin, M.C. Rodríguez-
624 Castro, B.-A. Tuulaikhuu, H. Guasch, Biotic and Abiotic Factors Influencing Arsenic
625 Biogeochemistry and Toxicity in Fluvial Ecosystems: A Review, International Journal of
626 Environmental Research and Public Health. 17 (2020) 2331.
627 https://doi.org/10.3390/ijerph17072331.
628 [40]G.G. Pircalabioru, M.-C. Chifiriuc, Nanoparticulate drug-delivery systems for fighting
629 microbial biofilms: from bench to bedside, Future Microbiology. 15 (2020) 679–698.
630 https://doi.org/10.2217/fmb-2019-0251.
631 [41]S. Subramanian, R.C. Huiszoon, S. Chu, W.E. Bentley, R. Ghodssi, Microsystems for
632 biofilm characterization and sensing – A review, Biofilm. 2 (2020) 100015.
633 https://doi.org/10.1016/j.bioflm.2019.100015.
634 [42]D. Chirman, N. Pleshko, Characterization of bacterial biofilm infections with Fourier
635 transform infrared spectroscopy: a review, Applied Spectroscopy Reviews. 0 (2021) 1–29.
636 https://doi.org/10.1080/05704928.2020.1864392.
637 [43]B. Gieroba, M. Krysa, K. Wojtowicz, A. Wiater, M. Pleszczyńska, M. Tomczyk, A. Sroka-
638 Bartnicka, The FT-IR and Raman Spectroscopies as Tools for Biofilm Characterization
639 Created by Cariogenic Streptococci, International Journal of Molecular Sciences. 21
640 (2020) 3811. https://doi.org/10.3390/ijms21113811.
641 [44]J.W. Simkins, S. Schuhmann, G. Guthausen, M. Heijnen, S.L. Codd, J.D. Seymour,
642 Characterization of biofilm distribution in hollow fiber membranes using Compressed
643 Sensing Magnetic Resonance Imaging, Journal of Membrane Science. 594 (2020)
644 117437. https://doi.org/10.1016/j.memsci.2019.117437.
645 [45]Y. Xu, Y. Dhaouadi, P. Stoodley, D. Ren, Sensing the unreachable: challenges and
646 opportunities in biofilm detection, Current Opinion in Biotechnology. 64 (2020) 79–84.
647 https://doi.org/10.1016/j.copbio.2019.10.009.
648 [46]Y. Salama, M. Chennaoui, A. Sylla, M. Mountadar, M. Rihani, O. Assobhei,
649 Characterization, structure, and function of extracellular polymeric substances (EPS) of
650 microbial biofilm in biological wastewater treatment systems: a review, Desalination and
651 Water Treatment. 57 (2016) 16220–16237.
652 https://doi.org/10.1080/19443994.2015.1077739.
653 [47]P.D. Nichols, J. Michael Henson, J.B. Guckert, D.E. Nivens, D.C. White, Fourier transform-
654 infrared spectroscopic methods for microbial ecology: analysis of bacteria, bacteri-polymer
655 mixtures and biofilms, Journal of Microbiological Methods. 4 (1985) 79–94.
656 https://doi.org/10.1016/0167-7012(85)90023-5.
657 [48]D.E. Nivens, R.J. Palmer, D.C. White, Continuous nondestructive monitoring of microbial
658 biofilms: A review of analytical techniques, Journal of Industrial Microbiology. 15 (1995)
659 263–276. https://doi.org/10.1007/BF01569979.
660 [49]P.J. Bremer, G.G. Geesey, An evaluation of biofilm development utilizing non-destructive
661 attenuated total reflectance Fourier transform infrared spectroscopy, Biofouling. 3 (1991)
662 89–100. https://doi.org/10.1080/08927019109378165.
663 [50]M. Consumi, K. Jankowska, G. Leone, C. Rossi, A. Pardini, E. Robles, K. Wright, A.
664 Brooker, A. Magnani, Non-Destructive Monitoring of P. fluorescens and S. epidermidis
665 Biofilm under Different Media by Fourier Transform Infrared Spectroscopy and Other

666 Corroborative Techniques, Coatings. 10 (2020) 930.
667 https://doi.org/10.3390/coatings10100930.
668 [51] A. Vertes, V. Hitchins, K.S. Phillips, Analytical Challenges of Microbial Biofilms on Medical
669 Devices, Anal. Chem. 84 (2012) 3858–3866. https://doi.org/10.1021/ac2029997.
670 [52] U. Neugebauer, U. Schmid, K. Baumann, W. Ziebuhr, S. Kozitskaya, V. Deckert, M.
671 Schmitt, J. Popp, Towards a Detailed Understanding of Bacterial Metabolism—
672 Spectroscopic Characterization of Staphylococcus Epidermidis, ChemPhysChem. 8
673 (2007) 124–137. https://doi.org/10.1002/cphc.200600507.
674 [53] F. Quilès, F. Humbert, A. Delille, Analysis of changes in attenuated total reflection FTIR
675 fingerprints of Pseudomonas fluorescens from planktonic state to nascent biofilm state,
676 Spectrochim. Acta, Part A. 75 (2010) 610–616. https://doi.org/10.1016/j.saa.2009.11.026.
677 [54] F.F. Veiga, L.V. de Castro-Hoshino, F. Sato, M.L. Baesso, S. Silva, M. Negri, T.I.E.
678 Svidzinski, Characterization of a biofilm formed by Fusarium oxysporum on the human
679 nails, International Journal of Dermatology. In press (2021).
680 https://doi.org/10.1111/ijd.15747.
681 [55] X. Liu, J. Zhan, L. Liu, F. Gan, J. Ye, K.H. Nealson, C. Rensing, S. Zhou, In Situ
682 Spectroelectrochemical Characterization Reveals Cytochrome-Mediated Electric
683 Syntrophy in Geobacter Coculture, Environ. Sci. Technol. 55 (2021) 10142–10151.
684 https://doi.org/10.1021/acs.est.1c00356.
685 [56] P. Stenclova, S. Freisinger, H. Barth, A. Kromka, B. Mizaikoff, Cyclic Changes in the Amide
686 Bands Within Escherichia coli Biofilms Monitored Using Real-Time Infrared Attenuated
687 Total Reflection Spectroscopy (IR-ATR), Appl. Spectrosc. (2019) 0003702819829081.
688 https://doi.org/10.1177/0003702819829081.
689 [57] G.S. Lorite, A.A. de Souza, D. Neubauer, B. Mizaikoff, C. Kranz, M.A. Cotta, On the role
690 of extracellular polymeric substances during early stages of Xylella fastidiosa biofilm
691 formation, Colloids Surf., B. 102 (2013) 519–525.
692 https://doi.org/10.1016/j.colsurfb.2012.08.027.
693 [58] G.S. Lorite, C.M. Rodrigues, A.A. de Souza, C. Kranz, B. Mizaikoff, M.A. Cotta, The role
694 of conditioning film formation and surface chemical changes on Xylella fastidiosa adhesion
695 and biofilm evolution, Journal of Colloid and Interface Science. 359 (2011) 289–295.
696 https://doi.org/10.1016/j.jcis.2011.03.066.
697 [59] P. Zhang, B. Feng, Y.-P. Chen, Y.-Z. Dai, J.-S. Guo, In situ characterizations for EPS-
698 involved microprocesses in biological wastewater treatment systems, Critical Reviews in
699 Environmental Science and Technology. 49 (2019) 917–946.
700 https://doi.org/10.1080/10643389.2018.1477416.
701 [60] N.N. Wickramasinghe, M.M. Hlaing, J.T. Ravensdale, R. Coorey, P.S. Chandry, G.A.
702 Dykes, Characterization of the biofilm matrix composition of psychrotrophic, meat spoilage
703 pseudomonads, Sci Rep. 10 (2020) 16457. https://doi.org/10.1038/s41598-020-73612-0.
704 [61] A. Bosch, D. Serra, C. Prieto, J. Schmitt, D. Naumann, O. Yantorno, Characterization of
705 Bordetella pertussis growing as biofilm by chemical analysis and FT-IR spectroscopy, Appl
706 Microbiol Biotechnol. 71 (2006) 736–747. https://doi.org/10.1007/s00253-005-0202-8.
707 [62] C. Yin, F. Meng, G.-H. Chen, Spectroscopic characterization of extracellular polymeric
708 substances from a mixed culture dominated by ammonia-oxidizing bacteria, Water
709 Research. 68 (2015) 740–749. https://doi.org/10.1016/j.watres.2014.10.046.
710 [63] A.R. Badireddy, B.R. Korpol, S. Chellam, P.L. Gassman, M.H. Engelhard, A.S. Lea, K.M.
711 Rosso, Spectroscopic Characterization of Extracellular Polymeric Substances from
712 Escherichia coli and Serratia marcescens: Suppression Using Sub-Inhibitory
713 Concentrations of Bismuth Thiols, Biomacromolecules. 9 (2008) 3079–3089.
714 https://doi.org/10.1021/bm800600p.
715 [64] S.L. Palencia, A. García, M. Palencia, S.L. Palencia, A. García, M. Palencia,
716 VIBRATIONAL SPECTRUM CHARACTERIZATION OF OUTER SURFACE OF
717 HELICOBACTER PYLORI BIOFILMS BY FUNCTIONALLY-ENHANCED DERIVATIVE
718 SPECTROSCOPY (FEDS), Journal of the Chilean Chemical Society. 65 (2020) 5015–
719 5022. https://doi.org/10.4067/S0717-97072020000405015.

720 [65] S.L. Palencia, A. García, M. Palencia, Mid-Infrared Vibrational Spectrum Characterization of the Outer Surface of Candida albicans by Functionally Enhanced Derivative Spectroscopy, J Appl Spectrosc. 88 (2021) 166–180. https://doi.org/10.1007/s10812-021-01155-x.
724 [66] G. Sharma, A. Prakash, COMBINED USE OF FOURIER TRANSFORM INFRARED AND RAMAN SPECTROSCOPY TO STUDY PLANKTONIC AND BIOFILM CELLS OF CRONOBACTER SAKAZAKII, Journal of Microbiology, Biotechnology and Food Sciences. 9 (2014) 310–314.
728 [67] F. Quilès, P. Polyakov, F. Humbert, G. Francius, Production of Extracellular Glycogen by Pseudomonas fluorescens: Spectroscopic Evidence and Conformational Analysis by Biomolecular Recognition, Biomacromolecules. 13 (2012) 2118–2127. https://doi.org/10.1021/bm300497c.
732 [68] A. Fahs, F. Quilès, D. Jamal, F. Humbert, G. Francius, In Situ Analysis of Bacterial Extracellular Polymeric Substances from a Pseudomonas fluorescens Biofilm by Combined Vibrational and Single Molecule Force Spectroscopies, J. Phys. Chem. B. 118 (2014) 6702–6713. https://doi.org/10.1021/jp5030872.
736 [69] F. Quilès, F. Humbert, On the production of glycogen by Pseudomonas fluorescens during biofilm development: an in situ study by attenuated total reflection-infrared with chemometrics, Biofouling. 30 (2014) 709–718. https://doi.org/10.1080/08927014.2014.915956.
740 [70] V. Janakiraman, D. Englert, A. Jayaraman, H. Baskaran, Modeling Growth and Quorum Sensing in Biofilms Grown in Microfluidic Chambers, Ann Biomed Eng. 37 (2009) 1206–1216. https://doi.org/10.1007/s10439-009-9671-8.
743 [71] S.G. Kazarian, Enhancing high-throughput technology and microfluidics with FTIR spectroscopic imaging, Anal Bioanal Chem. 388 (2007) 529–532. https://doi.org/10.1007/s00216-007-1193-3.
746 [72] K.L.A. Chan, S. Gulati, J.B. Edel, A.J. de Mello, S.G. Kazarian, Chemical imaging of microfluidic flows using ATR-FTIR spectroscopy, Lab Chip. 9 (2009) 2909–2913. https://doi.org/10.1039/B909573J.
749 [73] S. Cheeseman, Z.L. Shaw, J. Vongsvivut, R.J. Crawford, M.F. Dupont, K.J. Boyce, S. Gangadoo, S.J. Bryant, G. Bryant, D. Cozzolino, J. Chapman, A. Elbourne, V.K. Truong, Analysis of Pathogenic Bacterial and Yeast Biofilms Using the Combination of Synchrotron ATR-FTIR Microspectroscopy and Chemometric Approaches, Molecules. 26 (2021) 3890. https://doi.org/10.3390/molecules26133890.
754 [74] H.-Y.N. Holman, K. Bjornstad, M.P. McNamara, M.C. Martin, W.R. McKinney, E.A. Blakely, Synchrotron infrared spectromicroscopy as a novel bioanalytical microprobe for individual living cells: cytotoxicity considerations, JBO. 7 (2002) 417–424. https://doi.org/10.1117/1.1485299.
758 [75] M. Pousti, M.P. Zarabadi, M.A. Amirdehi, F. Paquet-Mercier, J. Greener, Microfluidic bioanalytical flow cells for biofilm studies: a review, Analyst. 144 (2018) 68–86. https://doi.org/10.1039/C8AN01526K.
761 [76] L. Monico, L. Cartechini, F. Rosi, W. De Nolf, M. Cotte, R. Vivani, C. Maurich, C. Miliani, Synchrotron radiation Ca K-edge 2D-XANES spectroscopy for studying the stratigraphic distribution of calcium-based consolidants applied in limestones, Sci Rep. 10 (2020) 14337. https://doi.org/10.1038/s41598-020-71105-8.
765 [77] K. Loutherback, G. Birarda, L. Chen, H.-Y.N. Holman, Microfluidic Approaches to Synchrotron Radiation-Based Fourier Transform Infrared (SR-FTIR) Spectral Microscopy of Living Biosystems, Protein Pept Lett. 23 (2016) 273–282. https://doi.org/10.2174/0929866523666160106154035.
769 [78] P. Hinsmann, J. Frank, P. Svasek, M. Harasek, B. Lendl, Design, simulation and application of a new micromixing device for time resolved infrared spectroscopy of chemical reactions in solution, Lab Chip. 1 (2001) 16–21. https://doi.org/10.1039/B104391A.
773 [79] H.-Y.N. Holman, R. Miles, Z. Hao, E. Wozei, L.M. Anderson, H. Yang, Real-Time Chemical Imaging of Bacterial Activity in Biofilms Using Open-Channel Microfluidics and Synchrotron

775 FTIR Spectromicroscopy, Anal. Chem. 81 (2009) 8564–8570.
776 https://doi.org/10.1021/ac9015424.
777 [80] A. Delille, F. Quilès, F. Humbert, In Situ Monitoring of the Nascent Pseudomonas
778 fluorescens Biofilm Response to Variations in the Dissolved Organic Carbon Level in Low-
779 Nutrient Water by Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy,
780 Appl. Environ. Microbiol. 73 (2007) 5782–5788. https://doi.org/10.1128/AEM.00838-07.
781 [81] F. Humbert, S. Saadi, F. Quilès, K. Hani, In situ assessment of antibacterial activity of
782 dermaseptine S4 derivatives against Pseudomonas fluorescens nascent biofilms by using
783 ATR-FTIR spectroscopy, in: Microbes in Applied Research, WORLD SCIENTIFIC, 2012:
784 pp. 547–550. https://doi.org/10.1142/9789814405041_0111.
785 [82] F. Quilès, S. Saadi, G. Francius, J. Bacharouche, F. Humbert, In situ and real time
786 investigation of the evolution of a Pseudomonas fluorescens nascent biofilm in the
787 presence of an antimicrobial peptide, Biochim. Biophys. Acta, BBA. 1858 (2016) 75–84.
788 https://doi.org/10.1016/j.bbamem.2015.10.015.
789 [83] F. Quilès, I. Accoceberry, C. Couzigou, G. Francius, T. Noël, S. El-Kirat-Chatel, AFM
790 combined to ATR-FTIR reveals Candida cell wall changes under caspofungin treatment,
791 Nanoscale. 9 (2017) 13731–13738. https://doi.org/10.1039/C7NR02170D.
792 [84] E. Yunda, F. Quilès, In situ spectroscopic analysis of Lactobacillus rhamnosus GG flow on
793 an abiotic surface reveals a role for nutrients in biofilm development, Biofouling. 35 (2019)
794 494–507. https://doi.org/10.1080/08927014.2019.1617279.
795 [85] J.J. Ojeda, M.E. Romero-Gonzalez, H.M. Pouran, S.A. Banwart, In situ monitoring of the
796 biofilm formation of Pseudomonas putida on hematite using flow-cell ATR-FTIR
797 spectroscopy to investigate the formation of inner-sphere bonds between the bacteria and
798 the mineral, Mineralogical Magazine. 72 (2008) 101–106.
799 https://doi.org/10.1180/minmag.2008.072.1.101.
800 [86] M.C. Sportelli, E. Tütüncü, R.A. Picca, M. Valentini, A. Valentini, C. Kranz, B. Mizaikoff, H.
801 Barth, N. Cioffi, Inhibiting P. fluorescens biofilms with fluoropolymer-embedded silver
802 nanoparticles: an in-situ spectroscopic study, Sci. Rep. 7 (2017) 11870.
803 https://doi.org/10.1038/s41598-017-12088-x.
804 [87] E. Yunda, H. Alem, G. Francius, R. Gago, F. Quilès, Chemical Functionalization of the Zinc
805 Selenide Surface and Its Impact on Lactobacillus rhamnosus GG Biofilms, ACS Appl.
806 Mater. Interfaces. 12 (2020) 14933–14945. https://doi.org/10.1021/acsami.0c01335.
807 [88] M.C. Sportelli, R. Quarto, R.A. Picca, G. Caniglia, C. Kranz, A. Valentini, H. Barth, B.
808 Mizaikoff, N. Cioffi, CANNIBALISMO NELL'INTERAZIONE BIOFILM-ANTIMICROBICI, La
809 Chimica e l'Industria. 102 (2020) 48. https://doi.org/10.17374/CI.2020.102.6.48.
810 [89] H.-Y.N. Holman, D.L. Perry, J.C. Hunter-Cevera, Surface-enhanced infrared absorption-
811 reflectance (SEIRA) microspectroscopy for bacteria localization on geologic material
812 surfaces, Journal of Microbiological Methods. 34 (1998) 59–71.
813 https://doi.org/10.1016/S0167-7012(98)00069-4.
814 [90] S.I. Kudryashov, A.A. Nastulyavichus, E.R. Tolordava, A.N. Kirichenko, I.N. Saraeva, A.A.
815 Rudenko, Y.M. Romanova, A.Y. Panarin, A.A. Ionin, T.E. Itina, Surface-Enhanced IR-
816 Absorption Microscopy of Staphylococcus aureus Bacteria on Bactericidal Nanostructured
817 Si Surfaces, Molecules. 24 (2019) 4488. https://doi.org/10.3390/molecules24244488.
818 [91] A.A. Kamnev, FTIR spectroscopic studies of bacterial cellular responses to environmental
819 factors, plant-bacterial interactions and signalling, Spectroscopy. 22 (2008) 83–95.
820 https://doi.org/10.3233/SPE-2008-0329.
821 [92] N. Pradhan, S.K. Pradhan, B.B. Nayak, P.S. Mukherjee, L.B. Sukla, B.K. Mishra, Micro-
822 Raman analysis and AFM imaging of Acidithiobacillus ferrooxidans biofilm grown on
823 uranium ore, Research in Microbiology. 159 (2008) 557–561.
824 https://doi.org/10.1016/j.resmic.2008.06.006.
825 [93] F. Quilès, J.-Y. Balandier, S. Capizzi-Banas, In situ characterisation of a microorganism
826 surface by Raman microspectroscopy: the shell of Ascaris eggs, Anal Bioanal Chem. 386
827 (2006) 249–255. https://doi.org/10.1007/s00216-006-0638-4.

828 [94] L. Cui, D. Zhang, K. Yang, X. Zhang, Y.-G. Zhu, Perspective on Surface-Enhanced Raman
829 Spectroscopic Investigation of Microbial World, Anal. Chem. 91 (2019) 15345–15354.
830 https://doi.org/10.1021/acs.analchem.9b03996.
831 [95] H. Zhou, D. Yang, N.P. Ivleva, N.E. Mircescu, R. Niessner, C. Haisch, SERS Detection of
832 Bacteria in Water by in Situ Coating with Ag Nanoparticles, Anal. Chem. 86 (2014) 1525–
833 1533. https://doi.org/10.1021/ac402935p.
834 [96] Y. Hu, Á.I. López-Lorente, B. Mizaikoff, Versatile Analytical Platform Based on Graphene-
835 Enhanced Infrared Attenuated Total Reflection Spectroscopy, ACS Photonics. 5 (2018)
836 2160–2167. https://doi.org/10.1021/acsphotonics.8b00028.
837 [97] L. Cui, S. Chen, K. Zhang, Effect of toxicity of Ag nanoparticles on SERS spectral variance
838 of bacteria, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 137
839 (2015) 1061–1066. https://doi.org/10.1016/j.saa.2014.08.155.
840 [98] C.N. Kotanen, L. Martinez, R. Alvarez, J.W. Simecek, Surface enhanced Raman scattering
841 spectroscopy for detection and identification of microbial pathogens isolated from human
842 serum, Sensing and Bio-Sensing Research. 8 (2016) 20–26.
843 https://doi.org/10.1016/j.sbsr.2016.03.002.
844 [99] E. Witkowska, K. Niciński, D. Korsak, T. Szymborski, A. Kamińska, Sources of variability
845 in SERS spectra of bacteria: comprehensive analysis of interactions between selected
846 bacteria and plasmonic nanostructures, Anal Bioanal Chem. 411 (2019) 2001–2017.
847 https://doi.org/10.1007/s00216-019-01609-4.
848 [100] R. Weiss, M. Palatinszky, M. Wagner, R. Niessner, M. Elsner, M. Seidel, N.P. Ivleva,
849 Surface-enhanced Raman spectroscopy of microorganisms: limitations and applicability
850 on the single-cell level, Analyst. 144 (2019) 943–953.
851 https://doi.org/10.1039/C8AN02177E.
852 [101] N.P. Ivleva, M. Wagner, H. Horn, R. Niessner, C. Haisch, Raman microscopy and
853 surface-enhanced Raman scattering (SERS) for in situ analysis of biofilms, Journal of
854 Biophotonics. 3 (2010) 548–556. https://doi.org/10.1002/jbio.201000025.
855 [102] T. Ramirez-Mora, C. Dávila-Pérez, F. Torres-Méndez, G. Valle-Bourrouet, Raman
856 Spectroscopic Characterization of Endodontic Biofilm Matrices, Journal of Spectroscopy.
857 2019 (2019) e1307397. https://doi.org/10.1155/2019/1307397.
858 [103] Y. Roggo, P. Chalus, L. Maurer, C. Lema-Martinez, A. Edmond, N. Jent, A review of
859 near infrared spectroscopy and chemometrics in pharmaceutical technologies, Journal of
860 Pharmaceutical and Biomedical Analysis. 44 (2007) 683–700.
861 https://doi.org/10.1016/j.jpba.2007.03.023.
862 [104] H. Zhou, D. Yang, N.P. Ivleva, N.E. Mircescu, S. Schubert, R. Niessner, A. Wieser, C.
863 Haisch, Label-Free in Situ Discrimination of Live and Dead Bacteria by Surface-Enhanced
864 Raman Scattering, Anal. Chem. 87 (2015) 6553–6561.
865 https://doi.org/10.1021/acs.analchem.5b01271.
866 [105] P.N. Abadian, N. Tandogan, J.J. Jamieson, E.D. Goluch, Using surface plasmon
867 resonance imaging to study bacterial biofilms, Biomicrofluidics. 8 (2014).
868 https://doi.org/10.1063/1.4867739.
869 [106] Mine Altunbek, Seda Kelestemur, Mustafa Culha, In situ monitoring of biomolecular
870 processes in living systems using surface-enhanced Raman scattering, in: 2015.
871 https://doi.org/10.1117/12.2207321.
872 [107] G. Bodelón, V. Montes-García, V. López-Puente, E.H. Hill, C. Hamon, M.N. Sanz-Ortiz,
873 S. Rodal-Cedeira, C. Costas, S. Celiksoy, I. Pérez-Juste, L. Scarabelli, A.L. Porta, J.
874 Pérez-Juste, I. Pastoriza-Santos, L.M. Liz-Marzán, Detection and imaging of quorum
875 sensing in Pseudomonas aeruginosa biofilm communities by surface-enhanced resonance
876 Raman scattering, Nat Mater. 15 (2016) 1203–1211. https://doi.org/10.1038/nmat4720.
877 [108] V. Reipa, J. Almeida, K.D. Cole, Long-term monitoring of biofilm growth and disinfection
878 using a quartz crystal microbalance and reflectance measurements, Journal of
879 Microbiological Methods. 66 (2006) 449–459.
880 https://doi.org/10.1016/j.mimet.2006.01.016.

881 [109] S.L. Broschat, F.J. Loge, J.D. Peppin, D. White, D.R. Call, E. Kuhn, Optical reflectance
882 assay for the detection of biofilm formation, JBO. 10 (2005) 044027.
883 https://doi.org/10.1117/1.1953347.
884 [110] P. Angell, A.A. Arrage, M.W. Mittelman, D.C. White, On line, non-destructive biomass
885 determiantion of bacterial biofilms by fluorometry, Journal of Microbiological Methods. 18
886 (1993) 317–327. https://doi.org/10.1016/0167-7012(93)90013-8.
887 [111] J.L. Kadurugamuwa, K.P. Francis, Bioluminescent Imaging of Bacterial Biofilm
888 Infections In Vivo, in: F.R. DeLeo, M. Otto (Eds.), Bacterial Pathogenesis: Methods and
889 Protocols, Humana Press, Totowa, NJ, 2008: pp. 225–239. https://doi.org/10.1007/978-1-
890 60327-032-8_18.
891 [112] W.H. Wallace, J.T. Fleming, D.C. White, G.S. Sayler, An algD-bioluminescent reporter
892 plasmid to monitor alginate production in biofilms, Microb Ecol. 27 (1994) 225–239.
893 https://doi.org/10.1007/BF00182407.
894 [113] R. Bakke, R. Kommedal, S. Kalvenes, Quantification of biofilm accumulation by an
895 optical approach, Journal of Microbiological Methods. 44 (2001) 13–26.
896 https://doi.org/10.1016/S0167-7012(00)00236-0.
897 [114] P. Tinham, T.R. Bott, Biofouling assessment using an infrared monitor, Water Sci.
898 Technol. 47 (2003) 39–43.
899 [115] T. Schmid, U. Panne, C. Haisch, M. Hausner, R. Niessner, A Photoacoustic Technique
900 for Depth-Resolved In Situ Monitoring of Biofilms, Environ. Sci. Technol. 36 (2002) 4135–
901 4141. https://doi.org/10.1021/es0158657.
902 [116] T. Schmid, C. Helmbrecht, U. Panne, C. Haisch, R. Niessner, Process analysis of
903 biofilms by photoacoustic spectroscopy, Anal Bioanal Chem. 375 (2003) 1124–1129.
904 https://doi.org/10.1007/s00216-002-1690-3.
905 [117] T. Schmid, U. Panne, C. Haisch, R. Niessner, Photoacoustic absorption spectra of
906 biofilms, Review of Scientific Instruments. 74 (2003) 755–757.
907 https://doi.org/10.1063/1.1512766.
908 [118] T. Schmid, U. Panne, J. Adams, R. Niessner, Investigation of biocide efficacy by
909 photoacoustic biofilm monitoring, Water Research. 38 (2004) 1189–1196.
910 https://doi.org/10.1016/j.watres.2003.10.057.
911 [119] M. Wagner, H. Horn, Optical coherence tomography in biofilm research: A
912 comprehensive review, Biotechnology and Bioengineering. 114 (2017) 1386–1402.
913 https://doi.org/10.1002/bit.26283.
914 [120] D. Huang, E.A. Swanson, C.P. Lin, J.S. Schuman, W.G. Stinson, W. Chang, M.R. Hee,
915 T. Flotte, K. Gregory, C.A. Puliafito, J.G. Fujimoto, Optical Coherence Tomography,
916 Science. 254 (1991) 1178–1181.
917 [121] C. Xi, D.L. Marks, S. Schlachter, W. Luo, S.A.B. M.d, High-resolution three-dimensional
918 imaging of biofilm development using optical coherence tomography, JBO. 11 (2006)
919 034001. https://doi.org/10.1117/1.2209962.
920 [122] M. Mohan, V.K. Nigam, R. Poddar, Towards characterization of bacterial colonies and
921 biofilms: An approach based on swept source optical coherence tomography, Optik. 185
922 (2019) 592–598. https://doi.org/10.1016/j.ijleo.2019.03.135.
923 [123] S. Schaefer, J. Walther, D. Strieth, R. Ulber, U. Bröckel, Insights into the Development
924 of Phototrophic Biofilms in a Bioreactor by a Combination of X-ray Microtomography and
925 Optical Coherence Tomography, Microorganisms. 9 (2021) 1743.
926 https://doi.org/10.3390/microorganisms9081743.
927 [124] J. Hou, C. Wang, R.T. Rozenbaum, N. Gusnaniar, E.D. de Jong, W. Woudstra, G.I.
928 Geertsema-Doornbusch, J. Atema-Smit, J. Sjollema, Y. Ren, H.J. Busscher, H.C. van der
929 Mei, Bacterial Density and Biofilm Structure Determined by Optical Coherence
930 Tomography, Sci Rep. 9 (2019) 9794. https://doi.org/10.1038/s41598-019-46196-7.
931 [125] F. Blauert, H. Horn, M. Wagner, Time-resolved biofilm deformation measurements
932 using optical coherence tomography, Biotechnology and Bioengineering. 112 (2015)
933 1893–1905. https://doi.org/10.1002/bit.25590.
934 [126] J. Won, W. Hong, P. Khampang, D.R. Spillman, S. Marshall, K. Yan, R.G. Porter, M.A.
935 Novak, J.E. Kerschner, S.A. Boppart, Longitudinal optical coherence tomography to

Journal Pre-proof

Journal Pre-proof

936 visualize the in vivo response of middle ear biofilms to antibiotic therapy, Sci Rep. 11
937 (2021) 5176. https://doi.org/10.1038/s41598-021-84543-9.
938 [127] M.U. Farid, J. Guo, A.K. An, Bacterial inactivation and in situ monitoring of biofilm
939 development on graphene oxide membrane using optical coherence tomography, Journal
940 of Membrane Science. 564 (2018) 22–34. https://doi.org/10.1016/j.memsci.2018.06.061.
941 [128] T. Bj, XPS and SIMS studies of surfaces important in biofilm formation. Three case
942 studies., Ann N Y Acad Sci. 831 (1997) 114–126. https://doi.org/10.1111/j.1749-
943 6632.1997.tb52189.x.
944 [129] A.R. von Gundlach, V.M. Garamus, T.M. Willey, J. Ilavsky, K. Hilpert, A. Rosenhahn,
945 Use of small-angle X-ray scattering to resolve intracellular structure changes of
946 Escherichia coli cells induced by antibiotic treatment, J Appl Crystallogr. 49 (2016) 2210–
947 2216. https://doi.org/10.1107/S1600576716018562.
948 [130] R.P. Rambo, J.A. Tainer, Super-Resolution in Solution X-Ray Scattering and Its
949 Applications to Structural Systems Biology, Annual Review of Biophysics. 42 (2013) 415–
950 441. https://doi.org/10.1146/annurev-biophys-083012-130301.
951 [131] K.B. Solmaz, Y. Ozcan, N. Mercan Dogan, O. Bozkaya, S. Ide, Characterization and
952 Production of Extracellular Polysaccharides (EPS) by Bacillus Pseudomycoides U10,
953 Environments. 5 (2018) 63. https://doi.org/10.3390/environments5060063.
954 [132] I. Dogsa, M. Kriechbaum, D. Stopar, P. Laggner, Structure of Bacterial Extracellular
955 Polymeric Substances at Different pH Values as Determined by SAXS, Biophysical
956 Journal. 89 (2005) 2711–2720. https://doi.org/10.1529/biophysj.105.061648.
957 [133] T.P. Trainor, A.S. Templeton, P.J. Eng, Structure and reactivity of environmental
958 interfaces: Application of grazing angle X-ray spectroscopy and long-period X-ray standing
959 waves, Journal of Electron Spectroscopy and Related Phenomena. 150 (2006) 66–85.
960 https://doi.org/10.1016/j.elspec.2005.04.011.
961 [134] K. Friese, M. Mages, K. Wendt-Potthoff, T.R. Neu, Determination of heavy metals in
962 biofilms from the River Elbe by total-reflection X-ray fluorescence spectrometry1This paper
963 was presented at the 6th Conference on "Total Reflection X-Ray Fluorescence Analysis
964 and Related Methods" (TXRF '96) held in two parts in Eindhoven (The Netherlands) and
965 Dortmund (Germany) in June 1996, and is published in the Special Issue of
966 Spectrochimica Acta, Part B, dedicated to that Conference.1, Spectrochimica Acta Part B:
967 Atomic Spectroscopy. 52 (1997) 1019–1025. https://doi.org/10.1016/S0584-
968 8547(96)01633-3.
969 [135] N. Zhang, C.E.L. Thompson, I.H. Townend, K.E. Rankin, D.M. Paterson, A.J. Manning,
970 Nondestructive 3D Imaging and Quantification of Hydrated Biofilm-Sediment Aggregates
971 Using X-ray Microcomputed Tomography, Environ. Sci. Technol. 52 (2018) 13306–13313.
972 https://doi.org/10.1021/acs.est.8b03997.
973 [136] M.P. Herrling, S. Lackner, H. Nirschl, H. Horn, G. Guthausen, Chapter Four - Recent
974 NMR/MRI studies of biofilm structures and dynamics, in: G.A. Webb (Ed.), Annual Reports
975 on NMR Spectroscopy, Academic Press, 2019: pp. 163–213.
976 https://doi.org/10.1016/bs.arnmr.2019.02.001.
977 [137] Z.U. Rehman, J.S. Vrouwenvelder, P.E. Saikaly, Physicochemical Properties of
978 Extracellular Polymeric Substances Produced by Three Bacterial Isolates From Biofouled
979 Reverse Osmosis Membranes, Frontiers in Microbiology. 12 (2021) 1763.
980 https://doi.org/10.3389/fmicb.2021.668761.
981 [138] R.S. Renslow, M.J. Marshall, A.E. Tucker, W.B. Chrisler, X.-Y. Yu, In situ nuclear
982 magnetic resonance microimaging of live biofilms in a microchannel, Analyst. 142 (2017)
983 2363–2371. https://doi.org/10.1039/C7AN00078B.
984 [139] G. Guthausen, J.R. Machado, B. Luy, A. Baniodeh, A.K. Powell, S. Krämer, F.
985 Ranzinger, M.P. Herrling, S. Lackner, H. Horn, Characterisation and application of ultra-
986 high spin clusters as magnetic resonance relaxation agents, Dalton Trans. 44 (2015)
987 5032–5040. https://doi.org/10.1039/C4DT02916J.
988 [140] H. Pu, Y. Xu, D.-W. Sun, Q. Wei, X. Li, Optical nanosensors for biofilm detection in the
989 food industry: principles, applications and challenges, Critical Reviews in Food Science
990 and Nutrition. 61 (2021) 2107–2124. https://doi.org/10.1080/10408398.2020.1808877.

Journal Pre-proof

991 [141] M. Fischer, M. Wahl, G. Friedrichs, Design and field application of a UV-LED based
992 optical fiber biofilm sensor, Biosensors and Bioelectronics. 33 (2012) 172–178.
993 https://doi.org/10.1016/j.bios.2011.12.048.
994 [142] J.S. Kee, S.Y. Lim, A.P. Perera, Y. Zhang, M.K. Park, Plasmonic nanohole arrays for
995 monitoring growth of bacteria and antibiotic susceptibility test, Sensors and Actuators B:
996 Chemical. 182 (2013) 576–583. https://doi.org/10.1016/j.snb.2013.03.053.
997 [143] R. Horváth, L.R. Lindvold, N.B. Larsen, Reverse-symmetry waveguides: theory and
998 fabrication, Appl Phys B. 74 (2002) 383–393. https://doi.org/10.1007/s003400200823.
999 [144] J. Watrous, N. Hendricks, M. Meehan, P.C. Dorrestein, Capturing Bacterial Metabolic
1000 Exchange Using Thin Film Desorption Electrospray Ionization-Imaging Mass
1001 Spectrometry, Anal. Chem. 82 (2010) 1598–1600. https://doi.org/10.1021/ac9027388.
1002 [145] S.N. Dean, C. Walsh, H. Goodman, M.L. van Hoek, Analysis of mixed biofilm
1003 (Staphylococcus aureus and Pseudomonas aeruginosa) by laser ablation electrospray
1004 ionization mass spectrometry, Biofouling. 31 (2015) 151–161.
1005 https://doi.org/10.1080/08927014.2015.1011067.
1006 [146] C.W. Bacon, D.M. Hinton, T.R. Mitchell, Screening of Bacillus mojavensis biofilms and
1007 biosurfactants using laser ablation electrospray ionization mass spectroscopy, Journal of
1008 Applied Microbiology. 125 (2018) 867–875. https://doi.org/10.1111/jam.13905.
1009 [147] L. Yuan, M.F. Hansen, H.L. Røder, N. Wang, M. Burmølle, G. He, Mixed-species
1010 biofilms in the food industry: Current knowledge and novel control strategies, Critical
1011 Reviews in Food Science and Nutrition. 60 (2020) 2277–2293.
1012 https://doi.org/10.1080/10408398.2019.1632790.
1013 [148] D. Parrot, S. Papazian, D. Foil, D. Tasdemir, Imaging the Unimaginable: Desorption
1014 Electrospray Ionization – Imaging Mass Spectrometry (DESI-IMS) in Natural Product
1015 Research, Planta Med. 84 (2018) 584–593. https://doi.org/10.1055/s-0044-100188.
1016

Journal Pre-proof

Journal Pre-proof

Highlights

- Analytical Spectroscopy can significantly contribute to biofilm characterization.
- Progress on the main spectroscopic approaches to biofilm analysis is discussed.
- Advantages and drawbacks of different techniques are comprehensively presented.

Journal Pre-proof