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How microbial biofilms impact the interactions of Quantum Dots with mineral surfaces?

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35 Abstract

36 The increasing use of Quantum Dots (QDs) - nanoparticles exhibiting unique optical
37 properties – and their incorporation in multiple engineering products is likely to result in the
38 release of this new class of contaminants into natural systems. In soils, bacterial biofilms and
39 mineral surfaces form highly reactive interfaces, which may control QDs' environmental fate.
40 However, little is known regarding QDs' stability in, and modes of interactions with,
41 biofilm/mineral interfaces. This study examines the interactions, distributions and stability of
42 thioglycolic acid-capped CdSe/ZnS QDs at the corundum (α -Al₂O₃)/*Shewanella oneidensis*
43 MR-1 interface, for exposure times ranging between 1h to 24h. Long Period – X-ray Standing
44 Wave – Fluorescence Yield spectroscopy and Grazing Incidence – X-ray Absorption
45 Spectroscopy were used. Results indicate increases in Zn and Se concentrations within the
46 biofilm/crystal system with time, demonstrating its high accumulation capacity over 24h. In
47 addition, dissolution of a part of the ZnS shell occurs within 1h, highlighting the potential
48 degradation of QDs when exposed to the biofilm/crystal compartment. Once released, Zn(II)
49 migrates toward the biofilm-crystal interface and interacts preferentially with the crystal
50 surface. In contrast, the remaining CdSe core is mostly preserved, and stays within the
51 biofilm thickness. However, at 24h, Se and Zn present similar distribution profiles indicating a
52 general reduction in ZnS shell dissolution at this longer exposure time.

53

54 Keywords: Biofilm, Dissolution, Quantum Dots, Mineral, Interactions

55

56 Abbreviations: QD/QDs= Quantum Dots, LP-XSW-FY= Long Period – X-ray Standing Wave
57 – Fluorescence Yield, GI-XAS= Grazing Incidence – X-ray Absorption Spectroscopy,
58 NP/NPs= Nanoparticles, EPS= exopolymeric substances, TGA= thioglycolic acid, ICP-QMS=
59 inductively coupled plasma-quadrupole mass spectrometry, SEM= Scanning electron
60 microscopy, LCF= linear combination fitting, ROS= reactive oxygen species, EPM=
61 electrophoretic mobility, DLS= Dynamic Light Scattering

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72 I. Introduction

73

74 Quantum Dots (QDs) are fluorescent semiconductor nanocrystals which present unique
75 optical and electronic size-dependent properties, such as electroluminescence. QDs exhibit
76 sizes between 2 and 10 nm, which place them in the most reactive class of NPs (Auffan et
77 al., 2009a). They are incorporated into solar cells to increase efficiency (Lin et al., 2014), are
78 used in medicine for *in-vivo* and *in-vitro* diagnosis (Aldeek et al., 2011; Liu et al., 2012), and
79 are at the center of intensive research for innovative low-energy applications in imaging
80 technologies and for designing novel solution-processed functional optoelectronic materials
81 (Kagan et al., 2016). These nanoparticles (NPs) usually have a core-shell structure, with the
82 core composed of CdSe, InP, PbSe, or ZnSe, surrounded by a shell of wider band-gap
83 material such as ZnS or CdS (Chen et al., 2017). For potential industrial uses (Hardman,
84 2005), to allow QDs dispersion in aqueous phases and to enhance their biological
85 compatibility or their stability, they are capped by organic or inorganic ligands (Breus et al.,
86 2015). Nevertheless, given their high reactivity, these engineered materials are sometimes
87 considered as potential contaminants, and substantial evidence of NP and QD toxicity to
88 microorganisms have been reported (Brayner et al., 2006; Fabrega et al., 2011; Mahendra et
89 al., 2008).

90

91 The recent global increase in NP production volumes has raised societal and
92 environmental concerns. During their life-cycles, NP-containing materials can experience
93 abrasion or leaching that could result in significant release to the environment (Mueller and
94 Nowack, 2008) where NPs are expected to accumulate in soils, sediments and landfills,
95 according to probabilistic modeling results (Keller et al., 2013; Wang and Nowack, 2018). In
96 those compartments, QDs are likely to undergo various physico-chemical processes such as
97 homo- or hetero-aggregation, complexation with organic matter or biomass, chemical
98 transformations, and partial or complete dissolution, all of which affect the reactivity, toxicity,
99 transport and fate of QDs in natural systems (Lowry et al., 2012). Some of these processes
100 have already been investigated under environmentally relevant conditions, highlighting the
101 importance of pH (Kaur and Tripathi, 2014), ionic strength (Slaveykova and Startchev, 2009;
102 Zhang et al., 2008) and the presence of natural organic matter (Navarro et al., 2009) on QDs'
103 stability in different types of aqueous conditions (Chen et al., 2017; Slaveykova and
104 Startchev, 2009). Nevertheless, given the complexity of natural systems and the multiplicity
105 of the associated physico-chemical processes, many questions remain open regarding the
106 fate of released QDs, especially with regard to interactions with microorganisms.

107

108 In soils and sediments, one of the most reactive compartments is composed of microbial
109 biofilms growing at the surfaces of minerals (Costerton et al., 1987). By far the main
110 microbial organization modes are biofilms (Flemming and Wuertz, 2019), structures
111 composed of cells encased in a complex three-dimensional organic matrix of exopolymeric
112 substances (EPS), and found in virtually all subsurface environments on Earth (Ménez et al.,
113 2012). These structures exhibit heterogeneity in compositions, hydrophobic microdomains
114 (Aldeek et al., 2011), pH (Hidalgo et al., 2009), redox conditions (Babauta et al., 2012),
115 thickness, spatial organization, etc. depending on parameters such as microbial strains or
116 nutrient availability (Allison, 2003; Sutherland, 2001). Thus, biofilms are highly reactive
117 dynamic systems (Sutherland, 2001), exhibiting elevated specific surface areas, high site
118 densities (Borrok et al., 2005) and reactive microenvironments (Stewart, 2003). Bacterial
119 cells generally present an overall negative surface charge at neutral pH due to the presence
120 of carboxyl (pK_a : 3-4.5) or phosphoryl (pK_a : 7-8) groups (Ha et al., 2010; Palmer et al., 2007).
121 In addition, despite their small concentrations at the surface of *S. oneidensis*, sulfhydryl
122 groups seem to play an important role on metal sorption (Yu and Fein, 2015). The negative
123 surface charge of bacteria at neutral pH indicates that biofilms can be viewed as negatively
124 charged entities, since EPS add supplementary functional sites that are also negatively
125 charged (Tourney and Ngwenya, 2014). Functional groups and surface charge can partly
126 control the interactions and speciation of metals (Wang et al., 2016b), metalloids (Templeton
127 et al., 2003) and NPs (Golmohamadi et al., 2013) within biofilm thicknesses. However,
128 studies suggest that attractive forces (hydrophobic or van der Waals forces) may
129 overwhelmed the electrostatic forces when the NPs have penetrated the diffuse layer. For
130 example, we showed in our previous work that hydrophobic interactions controlled the
131 transport of silver NPs coated with polyvinylpyrrolidone at the biofilm/mineral interface
132 (Desmau et al., 2018). Similarly, Lerner et al. (2012) demonstrated that the increase in
133 coating hydrophobicity favor the retention of NPs within biofilm. Thus, the attractive forces
134 need to be considered to explain the interactions between NPs and biofilm (Fulaz et al.,
135 2019; Mitzel et al., 2016). Besides, the transport of solutes, antibacterial agents, metal(loid)s
136 and NPs within biofilms are also controlled by the density, the organization and the overall
137 specific characteristics of the matrix such as the size of the water channels and fluid voids or
138 the development of chemical gradients and microenvironments within biofilms (Allison, 2003;
139 Choi et al., 2010; Couason et al., 2019; Dranguet et al., 2017; Peulen and Wilkinson, 2011;
140 Stewart and Costerton, 2001). In addition to the biofilm's reactivity, the mineral surfaces
141 where they developed are often also highly reactive and drive numerous processes in soil
142 (Brown, 2001; Brown et al., 1999) such as the sorption of metals or surface precipitation.
143 Nevertheless, to the best of our knowledge, the role of the biofilm/mineral interface on the

144 transport and transformation of NPs has been under-investigated, despite its elevated
145 reactivity and known impact on metal speciation and mobility (Wang et al., 2016a).

146

147 To predict QD fluxes, fate and potential impacts to ecosystems, it is critical to understand
148 the behavior and physico-chemical transformations undergone by QDs when exposed to the
149 biofilm/mineral compartment, as highlighted by Saleh et al. (2015). However, the associated
150 mechanisms remain poorly constrained and need to be investigated. For instance, the
151 ecotoxicological potential of some QDs, whose deleterious impact toward bacteria has been
152 demonstrated, is intimately related to their stabilities, with aged-QDs being more toxic than
153 fresh ones (Mahendra et al., 2008). Due to the high reactivity of the biofilm/mineral interface,
154 the high site densities and the presence of microenvironments within the biofilm, the stability
155 of the QDs and the speciation of the constituting elements are likely to evolve.

156 Finally, working with QDs presents the additional advantage of enabling study of a NP
157 composed of four different elements. By monitoring the fate of each individual element, it is
158 possible to precisely monitor the core and shell behavior independently, and thus to track
159 specific processes such as dissolution. Consequently, QDs can be used as a model NP in
160 order to more accurately constrain the general mechanisms associated with NPs' biosorption
161 and transformation at biofilm/mineral interfaces.

162

163 The current study investigates QD interactions with the biofilm/mineral interface, by
164 quantifying the evolution of their partitioning and physico-chemical transformations over the
165 course of 24h. The system used here is composed of a well-defined corundum ($\alpha\text{-Al}_2\text{O}_3$)
166 mineral surface coated with an axenic biofilm of *S. oneidensis* MR-1. These model gram-
167 negative bacteria are commonly found in soils, sediments and aquifers, and represent an
168 appropriate model microorganism. Interactions between QDs and the interface are monitored
169 using two synchrotron related techniques. Long Period-X-ray Standing Wave-Fluorescence
170 Yield (LP-XSW-FY) spectroscopy allows *in-situ* determination of elements' distribution at the
171 biofilm/crystal interface. This technique was previously used to investigate the distribution of
172 silver NPs (Desmau et al., 2018), Zn(II) and Pb(II) (Templeton et al., 2001; Wang et al.,
173 2016b) in biofilm/crystal systems. In addition, Grazing Incidence-X-Ray Absorption
174 Spectroscopy (GI-XAS) measurements provide information on speciation of elements, giving
175 an overall view of QDs' physico-chemical transformations at the biofilm/crystal interface.

176

177 II. Materials and Methods

178

179 1. Quantum Dots

180

181 QDs were obtained in a single-step synthesis procedure described by Bae et al. (2008). The
182 QDs present a chemical composition gradient with a CdS transition between the CdSe core
183 and the ZnS shell (see Fig. 1 for a schematic representation of the QD). To allow their
184 transfer to aqueous solution, they were functionalized with a layer of thioglycolic acid (TGA)
185 ligand, following a protocol previously established by Supiandi et al. (2019). To isolate and
186 purify QDs from the reaction medium, several cycles of concentration-dispersion steps in
187 borate buffer and milli-Q water were performed. QDs at the end of synthesis were placed in
188 milli-Q water at pH=10 to ensure their chemical stability during storage. This solution was
189 green fluorescent under 312 nm UV light, characteristic of QDs with a size around 7 nm (Bae
190 et al., 2008), and a core size of 3.4 nm as determined by absorbance measurements
191 between 400 and 650nm, and according to the core diameter to wavelength relationship
192 (Jasieniak et al., 2009). According to Faucher et al. (2018), the CdSe core measures 3 nm,
193 and the total diameter, with the ZnS shell, is ~6-7 nm based on STEM Electron Energy Loss
194 Spectroscopy performed on the same QDs. Finally, the ZnS shell is not homogeneous and
195 presents occasional holes in its structure (Fig. 1) (Faucher et al., 2018).

196 In QD stock solutions, the initial concentrations of Cd, Se and Zn were measured by
197 inductively coupled plasma atomic emission spectroscopy. The average concentrations of
198 four syntheses are $38.5 \pm 4.9 \mu\text{M}$, $8.1 \pm 0.8 \mu\text{M}$ and $4.6 \pm 0.5 \mu\text{M}$ for Zn, Cd and Se, respectively
199 with an average molar ratio between elements of 0.2 ± 0.1 , 1.9 ± 1.3 and 9.0 ± 6.1 , for Cd/Zn,
200 Cd/Se and Zn/Se, respectively. The calculated molar ratio of Cd/(Cd+Zn) is around 25%, in
201 agreement with Bae et al. (2008). The electrophoretic mobility (EPM) and zeta potential
202 (ZetaSizer, Malvern) of QDs functionalized with TGA were measured in 5mM NaNO₃ at pH
203 ranging from 2 to 10. The hydrodynamic diameter was also assessed by Dynamic Light
204 Scattering (DLS) at neutral pH (Dynapro Nanostar, Wyatt Technology, California, USA).

205

206 2. Sample preparation

207

208 The biofilm/crystal system and the biofilm growth protocol were previously
209 described in Desmau et al. (2018). Briefly, the system is composed of a highly polished,
210 cleaned and oriented single crystal substrate of $\alpha\text{-Al}_2\text{O}_3(1\text{-}102)$ with a surface roughness of
211 3\AA (commercial Pi-KEM Ltd.) adequate for LP-XSW-FY spectroscopy. *S. oneidensis* MR-1
212 biofilms were grown for 10 days on substrates placed in a sterilized flow-through bottle,
213 following the protocol established by Wang et al. (2016b). First, a tripticase soy broth
214 (BioMérieux, 51019) suspension inoculated with *S. oneidensis* MR-1, in a reproducible way
215 (Desmau et al., 2018), was left to settle for 1 hour in the bottle. Then, sterile synthetic growth
216 medium (table S1) at pH 7.0 was pumped continuously through the bottle for 10 days at

217 ambient temperature. After 10 days, the biofilm-coated surfaces were then gently placed in
218 10 mL Falcon centrifuge tubes containing the same growth medium, and were stored at 4°C
219 for 1 week before measurement. The number of colony-forming units was similar before and
220 after 1 week at 4°C (data not shown). Prior to measurement, the biofilm/crystal systems were
221 rinsed to remove excess nutrients, then exposed to a QD suspension in 5 mM NaNO₃
222 solution at ambient temperature. This background electrolyte can be considered an ideal soil
223 solution which minimizes particle aggregation (Chen et al., 2017). The experiments were
224 performed with a Cd concentration of 137±2 nM and a Zn concentration of 748±60 nM.
225 Solution pH was adjusted to 7.0±0.1, if necessary, in the course of experiments to ensure
226 acceptable living conditions for *S. oneidensis* MR-1, using 0.1 M HNO₃ or 0.1 M NaOH.

227 A new biofilm/crystal system was used for each exposure time (1h, 3h, 10h and
228 24h) and technique, with measurements conducted in either duplicate or triplicate as detailed
229 below. The 24h maximum exposure time was chosen to minimize biofilm structure alteration
230 due to QD toxicity (Dumas et al., 2010).

231 Samples were gently immersed in 21 mL of fresh QD solutions, with the biofilm side facing
232 down to ensure the study of QD transport. All tubes were shielded from light using aluminum
233 foil to avoid QD degradation (Li et al., 2012), and were gently shaken at 20 rpm. For LP-
234 XSW-FY measurements, samples were placed in Kapton-covered sample holders, mounted
235 vertically, and purged with humid He gas. For GI-XAS measurements, the fluorescence
236 detector was mounted perpendicular to the sample surface. In order to obtain a detailed
237 characterization of QD fate in the system, control experiments, mass balance measurements
238 and SEM imaging were performed. Cd and Zn uptake were estimated by biofilm digestion in
239 2% HNO₃, after 1h, 3h and 24h of exposure, in triplicate. To obtain concentration in mg per
240 gram of biofilm, four biofilms were weighed just after growth, and after 12h oven drying at
241 450°C. The average biofilm dry weight was 0.30±0.02 mg per sample. As Cd and Zn are
242 naturally present within biofilms, originating from impurities present in nutrient solutions
243 during growth, their concentrations in biofilms that were not exposed to QDs were also
244 measured in triplicate by the same method. The amount of Cd and Zn in solution, and sorbed
245 onto the Falcon tube walls, were also measured using an Agilent 7900 ICP-QMS (see
246 appendix 1, SI), in triplicate. The Falcon tubes were rinsed with 2% HNO₃ after the
247 experiments to assess the quantity of QDs sorbed onto the tube walls. The amounts of
248 dissolved species in the supernatant were estimated by QD removal by centrifugal
249 ultrafiltration (3 kDa, Amicon®, Millipore) and measured by ICP-QMS. Control experiments
250 were performed with biofilm/crystal systems exposed to Cd(II) (36.1±0.2 nM) and Zn(II)
251 (324±6 nM) ions for 24h in triplicate. Samples were imaged using a Zeiss Ultra Device SEM

252 with field emission gun at 15 keV, using the protocol presented by Desmau et al. (2018).
253 Please refer to appendix 2 and Fig. S1 and S2 (see SI) to see protocols and results.

254

255 3. LP-XSW-FY measurements and data analysis

256

257 LP-XSW-FY (Long Period – X-ray Standing Wave – Fluorescence Yield) spectroscopy
258 enables the measurement of elemental depth profiles at nanoscale resolution. Compared to
259 other techniques used to study the transport of NPs in biofilms, this technique enables the
260 study of the distribution of NPs within the whole interfacial region (biofilm+mineral), without
261 modification of the system (Desmau et al., 2018), and thus allow us to obtain an overall
262 understanding of the NPs' fate. However, LP-XSW-FY does not permit the monitoring of
263 specific mechanisms that could occur in some parts of the biofilm due to the local presence
264 of microenvironments, for example. Indeed, a surface area of 1 mm x 1 cm was probed for
265 each location.

266 LP-XSW-FY measurements were conducted at beamline 13-ID-C at
267 GeoSoilEnvironCARS (GSECARS) at the Advanced Photon Source (APS). The LP-XSW-FY
268 set-up, data analysis and modeling is similar to the protocol described in Desmau et al.
269 (2018) and is detailed in appendix 3 (see SI). More information on LP-XSW-FY principles
270 and applications can be found in Trainor et al. (2006). The limitations of using this technique
271 in such systems (biofilm/crystal interface + NPs) are presented in Desmau et al. (2018) and
272 in appendix 3 (SI). Briefly, the technique is highly dependent on the roughness and the
273 thickness of the sample. The high heterogeneity of the biofilm associated with the presence
274 of dense nano-metric objects are likely to impact the formation of the standing waves. Thus,
275 the modeling can be quite complicated. These parameters have been considered in our
276 models, allowing the semi-quantification of the distribution of Zn and Se, although the
277 interpretation has to be performed carefully. The monochromatic 13.3 keV X-ray beam was
278 collimated using a pair of 1 meter, Rh coated Si mirrors in Kirkpatrick-Baez geometry. The
279 final beam profile of 1000 μm vertical by 10 μm horizontal was defined by slits. X-ray
280 reflectivity measurements were performed by scanning X-ray incidence angle between 0.0°
281 and 0.5° while monitoring the intensities of the incident (I_0) and reflected (I_1) X-ray beams
282 using N₂-filled gas ionization chambers. Zn K α and Se K α fluorescence yield data were
283 collected using a 4-element silicon drift detector (SII NanoTechnology, Vortex-ME4) in two or
284 more locations for each sample to verify reproducibility.

285 The reflectivity and the fluorescence yield are modeled to obtain a semi-quantitative
286 distribution of the elements of interest (here Zn and Se). The full description of the physical
287 model is presented in the SI (appendix 3). In this study, the biofilm/crystal system was
288 divided into three compartments: the crystal surface-biofilm interface (labeled C₁), the biofilm

289 thickness (C_2) and the biofilm-gas interface (C_3) (Fig. S4). The model (see appendix 3, SI)
290 enables estimation of the distribution of the elements across the compartments. Goodness of
291 the fit were estimated by performing a χ^2 test and confidence interval were estimated using a
292 Student's test, as we did it in our previous work (Desmau et al., 2018) (see appendix 3, SI).

293

294 4. GI-XAS measurements and data analysis

295

296 GI-XAS (Grazing Incidence – X-ray Absorption Spectroscopy) allows the determination of
297 elemental speciation at different depths within the biofilm/crystal system. We estimate a
298 minimum detection limit of 15% by weight. Thus, local phenomena resulting in small
299 quantities of certain species would not be detected. EXAFS spectra were collected at Zn K-
300 edge (~9.6 keV) and XANES spectra were collected at Se K-edge (~12.6 keV) at the crystal
301 surface-biofilm interface (C_1), and within the biofilm thickness (C_2). Measurements were
302 performed at beamline 11-2 at the Stanford Synchrotron Radiation Lightsource (SSRL) using
303 a grazing-incidence spectrometer in vertical scattering geometry. GI-XAS spectra were
304 collected at room temperature in fluorescence mode using a Canberra 100-pixel Ge solid-
305 state monolith pixel detector. The incident beam energies were selected using a LN₂ cooled
306 Si (220) monochromator and collimated using a pair of 1 m-long Rh-coated Si mirrors. Zn
307 and Se metal foils were used during experiments for energy calibration. In order to
308 specifically probe the two compartments of interest, Zn K-edge and Se K-edge fluorescence
309 data were collected at incidence angles of 0.30° and 0.25° to interrogate the crystal surface-
310 biofilm interface (C_1), and at 0.18° and 0.10° for the biofilm thickness (C_2), respectively. Note
311 that for the crystal surface-biofilm interface, it is not possible to only probe the speciation of
312 Zn and Se at the surface of the crystal. Indeed, part of the signal originated also from the first
313 nanometers of the biofilm, meaning that the signal from the surface could be partially
314 “contaminated” by the signal from the biofilm. For XAS data analysis, three or four scans
315 were averaged, background subtracted, and fitted using the SIXPack interface (Webb, 2005)
316 and the IFEFFIT XAFS analysis package (Ravel and Newville, 2005). Linear combination
317 fitting (LCF) was used to quantify the presence of several possible species. Additional
318 species were considered only when they improved the goodness-of-fit by at least 15%.

319

320 III. Results

321

322 1. Characterization of QDs

323

324 The surface charge of the QDs is negative over the entire measured pH range, with an
325 increase in the negativity of the surface charge for pH>8 (Fig. S5). At pH=6.80±0.05 and

326 8.01±0.05, the EPMs values are -1.3±0.3 and -0.8±0.7 $\mu\text{m cm/Vsec}$, respectively. The
327 corresponding zeta potentials are -16.6±3.1 mV and -9.8±0.7 mV, respectively. The EPM
328 measured at pH 8 present an uncertainty higher than for the other pH studied (Fig. S5),
329 which could indicate that those points are outliers. When pH decreases, pH 8 could also
330 correspond to the pH where the carboxylates could start slowly to protonate (carboxylate pK_a
331 on surfaces can be higher than in solution (Chen et al., 2000)), favoring the presence of
332 different particles (individual particles, dimmers, trimers...). In addition, at pH lower than 8,
333 surface charge is lower which would probably favor aggregation of the particles due to the
334 increase of the protonation, and the EPM, or zeta potential, would be estimated for
335 aggregates and not for a unique QD. The partial homo-aggregation of QDs at neutral pH
336 seems to be confirmed by the average hydrodynamic diameter measured by DLS (41.7 nm).
337 In addition, more than 60% of the particles present a diameter less than 30 nm (with size
338 ranging from 5 to 200 nm, DLS results), validating the hypothesis of different types of
339 particles in solution. This has been considered for the discussion of results.
340 Nevertheless, the surface charge of the QDs, or their agglomerates, at pH 7 is regarded as
341 negative, considering the whole analysis.

342

343 2. Zn and Se distributions

344

345 All LP-XSW-FY profiles collected from different locations on a given sample were similar,
346 and these profiles were thus averaged. The measured critical angle is located at
347 $0.167\pm 0.004^\circ$ (Fig. 2, marked with black arrows), in agreement with the theoretical value of
348 0.17° at 13.3 keV. All profiles are presented with their fit depicted as a continuous line (Fig.
349 2). In general, for each exposure time, Zn and Se FY data present a first peak located
350 between 0.03° - 0.07° corresponding to the biofilm-gas interface, and a second one around
351 0.17° corresponding to the crystal surface-biofilm interface (Fig. 2). At 1h and 3h of
352 exposure, the most intense Se FY peaks are located at small incidence angle, while Zn FY
353 peaks are located around 0.04° and 0.17° (Fig. 2). However, at 10h and 24h, the FY profiles
354 are quite similar between Zn and Se, with a broad distribution between 0.05° and 0.17° (Fig.
355 2). Normalized FY intensities increase with time of exposure, from 1h to 24h (Fig. 3).

356 The Zn and Se distributions determined by LP-XSW-FY profile modeling are presented in
357 Fig. 4. After 1h of exposure, most of the Zn ($80\pm 3.5\%$) and Se ($60\pm 4.5\%$) are located in the
358 biofilm (C_2). The remaining Zn is mostly located at the crystal surface-biofilm interface (C_1 ,
359 $17\pm 3.5\%$), whereas the remaining Se is detected at the biofilm-gas interface (C_3 , $40\pm 4.5\%$),
360 indicating a difference in Zn and Se distribution. This distribution is further pronounced at 3h,
361 Se distribution is similar to the one obtained at 1h, whereas $72\pm 5.5\%$ of Zn is now located at

362 the crystal surface-biofilm interface (C_1). At 10h and 24h, identical distributions are observed
363 for Se and Zn, with ~25% at the biofilm-gas interface (C_3), ~70% in the biofilm (C_2), and ~5%
364 at the crystal surface-biofilm interface (C_1) (see Fig. 4 for estimated confidence interval).
365 One could note here the high percentage of Zn located at the mineral surface (C_1) at 3h
366 compared to the percentage of Zn at 1h, even if the shape the shape of the curves
367 describing normalized fluorescence intensity appears to be relatively similar. The higher
368 percentage of Zn obtained at 3h could be explained by the larger peak of the modeled data
369 at 3h, at high incident angle, that could increase the percentage of Zn at the mineral surface,
370 at the expense of the percentage of Zn present in the biofilm thickness (C_2). In addition,
371 compared to the others samples, the confidence interval of the Zn-3h sample is a little bit
372 larger (see appendix 3, SI), and the value of the χ^2 test is smaller for the Zn-sample at 1h
373 compared to the one at 3h, which could indicate a better fit for the data at 1h. Thus, the
374 percentage of Zn at the mineral surface could be overestimated and this spectrum could be
375 considered as an outlier. Nevertheless, we considered that, even if the percentage of Zn at
376 the mineral surface is overestimated, the observed tendency seems to be similar to the one
377 at 1h with Zn present at the mineral surface, while Se is absent.

378

379 3. Zn and Se speciation in the different compartments

380

381 Zn-EXAFS and Se-XANES spectra for reference compounds and samples exposed to
382 QDs are presented along with their fits in Fig. 5 and S6, respectively. LCF and χ^2 results are
383 reported in Fig. S6 for Se, and in Table S2 for Zn. The Zn reference compounds used to
384 perform LCF are aqueous Zn(II) (from dissolved $Zn(NO_3)_2$) exposed to a *S. oneidensis*
385 biofilm for 3h, and native Zn-QDs. Other reference compounds were tested to improve the fit
386 quality, such as Zn-acetate (Zn associated with carboxyl functional groups), Zn-phosphate
387 (Zn complexation with phosphoryl groups), and Zn-cysteine (Zn complexation with thiol
388 groups). None of those references improved the fit quality by at least 15%. Wang et al.
389 (2016b) identified the first neighbor of Zn(II) exposed to *S. oneidensis* biofilm as an oxygen
390 atom at a distance of 1.98 ± 0.01 Å (fourfold oxygen coordination). In our Zn-QDs reference
391 compound, the first neighbor is sulfur with a distance of 2.34 ± 0.02 Å consistent with the Zn-S
392 distance in Wurtzite (Chukavin et al., 2017). For our samples, only Zn linked to S as first
393 neighbor is detected within the biofilm thickness (C_2) for all exposure times. At the crystal
394 surface (C_1), all Zn is linked to S at 24h of exposure, while for shorter times (1h and 3h) 79 to
395 85% Zn is linked to S and the remaining fraction is associated with O in the first shell. For
396 these last two conditions, inclusion of a Zn-O bond in LCFs improves the goodness-of-fit
397 values (χ^2 reduced from 3.2 to 2.3 and from 1.9 to 0.9).

398

399 As Se is supposed to be associated with Cd in QDs, its speciation is expected to be Se(-
400 II) with a K-edge energy of 12658 eV (Ryser, 1999). However, the K-edge energy of the Se-
401 QD reference compound is measured at a slightly higher energy, 12659.9 eV, which is
402 between that of Se(-II) and Se(IV) (i.e. 12662 eV (Ryser, 1999)). Thus, in these QDs, Se is
403 not only present as Se(-II) but some of it is oxidized. The study of Faucher et al. (2018) by
404 STEM Electron Energy Loss Spectroscopy on QDs using the same synthesis showed that
405 the ZnS shell thickness is not homogeneous, and parts of the CdSe core could be directly
406 exposed to the solution and therefore undergo oxidation. After 1h and 3h of exposure, within
407 both the biofilm thickness (C_2) and at the crystal surface (C_1), the Se redox state is similar to
408 the QDs reference compound. However, at 24h, a small fraction of Se appears to be more
409 oxidized since a Se(IV) reference compound is now required for the fitting procedure (χ^2
410 reduced from 0.33 to 0.15 and from 0.41 to 0.13; Fig. S6).

411

412 4. Elemental sequestration in the biofilm/crystal system

413

414 The amounts of Cd and Zn remaining in the supernatant, trapped in the biofilm, and
415 sorbed on tube walls were measured by ICP-QMS. In solution, the total concentrations of Zn
416 and Cd decreased (from 748 ± 60 nM to 280 ± 59 nM and from 137 ± 2 nM to 41 ± 12 nM,
417 respectively), whereas they increased within the biofilm (from 2.2 ± 1.4 to 10.3 ± 1.8 mg/g_{biofilm}
418 for Zn and from 7 ± 5 μ g/g_{biofilm} to 2.6 ± 0.7 mg/g_{biofilm} for Cd) and on tube walls (less than 1 nM
419 at the beginning of the experiment to 287 ± 6 nM for Zn and 112 ± 3 nM for Cd). Regarding Zn
420 and Cd distribution in the whole experimental system at 24h of exposure, 46.5% and 26% of
421 total Zn and Cd, respectively, remained in solution while a much larger fraction was sorbed
422 onto tube walls (47.5% of Zn and 70% of Cd). Within the biofilm thickness, 6% of total Zn and
423 4% of total Cd are trapped (Fig. S7).

424 The percentage of dissolved Zn, compared to total Zn in the experiment, in the supernatant
425 as a function of time of exposure remains constant (around 10%), corresponding to an
426 average concentration of 81 ± 25 nM (Fig. S8a). The fraction of dissolved Cd is closer to zero
427 (around 1% in average), corresponding to a concentration of 2.5 ± 2.9 nM (Fig. S8b). As
428 dissolved concentrations of Zn and Cd in supernatant remain constant over time (Fig. S8),
429 the presence of those dissolved species is likely to result from an initial presence of
430 dissolved Zn and Cd in the experiments. Indeed, ZnS is known to be stable in water (Priadi
431 et al., 2012) so no dissolution in solution is expected during the course of experiments. Note
432 that the mass balance is conserved in all experiments (Fig. S9).

433

434 Control experiments performed with Cd(II) and Zn(II) ions at similar concentrations
435 show that most Zn and Cd remain in solution, 96.5% and 96% respectively, while only 5% Zn
436 and 1.5% Cd are sorbed onto tube walls. In addition, 2.5% of the Cd is found in the biofilm
437 while no additional Zn is detected in this compartment (Fig. S7).

438

439 IV. Discussion

440

441 1. QD sequestration at the biofilm/crystal interface

442

443 When the samples are exposed to QDs, most of the Zn and Cd is found associated with
444 the tube walls, highlighting a high affinity of QDs for this type of plastic. Despite exhibiting a
445 lower surface area compared to tube walls (estimated at 2.4 cm² for the biofilm/crystal
446 system vs. 50 cm² for the tube), the biofilm/crystal system traps QDs in appreciable amounts,
447 with 13% of Zn and 15% of Cd being present in the biofilm relative to the solution after 24h.
448 In addition, the increase in Zn, Se and Cd in this compartment with time, as measured by
449 ICP-QMS and fluorescence intensity (Fig. 3), indicates continuous accumulation in the
450 biofilm/crystal system over time (Fig. 6-1). As described in the introduction, the overall
451 surface charge of *S. oneidensis* at pH=7 is negative (Ha et al., 2010) meaning that the
452 interactions with negatively charged QDs would not be favored. Nevertheless, the interaction
453 between negatively charged NPs and *S. oneidensis* MR-1 biofilm has been previously
454 observed with 60 nm silver NPs coated with polyvinylpyrrolidone (Desmau et al., 2018). In
455 this previous study, we highlighted the role of other parameters, such as NP size and
456 hydrophobicity, on the interactions between NPs and biofilms, when both of them are
457 negatively charged. For example, smaller particles are able to diffuse into all parts of the
458 biofilm. In the present study, the relatively small size of the QDs (60% have a hydrodynamic
459 diameter less than 30 nm) could explain their ability to strongly accumulate in the biofilm. In
460 addition, the strong interactions between negatively charged QDs and *S. oneidensis* biofilm
461 have also been explained in the past by the presence of hydrophobic microdomains within
462 the biofilm thickness (Aldeek et al., 2011; Aldeek et al., 2013), validating the role of
463 hydrophobic interactions in NP-biofilm interactions.

464

465 2. QD fast dissolution within the biofilm

466

467 A semi-quantitative distribution of Zn and Se in the biofilm/crystal system can be obtained
468 by modelling LP-XSW-FY data, using the 3 compartments previously described (Fig. 4).
469 Interestingly, at short time of exposure (i.e. 1h and to a certain extend at 3h), an absence of
470 co-localization between Zn and Se at the interfaces (C₁ and C₃) is observed. This lack of co-

471 localization can be explained by the presence of dissolved Zn(II) that interacts differently with
472 the biofilm and crystal surface than QDs (Fig. 6-2). Zn in solution could originate either from
473 (i) dissolved Zn(II) present in the supernatant (~10%), or (ii) from dissolution of QDs in the
474 biofilm matrix. Regarding (i), when exposed to free Zn(II) at 899 nM, the amount of Zn
475 trapped in the biofilm/crystal system is minimal compared to QD exposure experiments (0 vs.
476 6 %, see III.3 and Fig. S7). As a result, the 10% of Zn(II), ~96 nM, present in the supernatant
477 could not fully explain the Zn detected at the crystal surface in QD experiments by LP-XSW-
478 FY at 1 and 3h. In addition, Wang et al. (2016b) studied the distribution of dissolved Zn(II) in
479 the *S. oneidensis* MR-1 biofilm/ α -Al₂O₃(1-102) crystal system and showed that for all
480 concentrations (10⁻⁴ to 10⁻⁷M) and exposure times investigated, Zn(II) was mostly associated
481 with the biofilm and did not interact with the crystal surface. Dissolved Zn(II) present in the
482 supernatant in our study is expected to behave the same and would partition mostly to the
483 biofilm. Since this is not observed, the involvement of dissolved Zn from the supernatant can
484 thus be discarded.

485 Therefore, Zn at the crystal surface is likely to originate from a partial QD dissolution
486 in the biofilm thickness (ii). The fast dissolution of ZnS nanoparticles (Dehner et al., 2010), in
487 contact with bacteria has been observed before, as has their dissolution in other systems
488 such as organic wastes (Le Bars et al., 2018). In our study, the Zn(II) from the ZnS shell
489 would be released much closer to the crystal surface, and interact more easily with the highly
490 reactive functional sites from the crystal (Wang et al., 2016b). The local dissolution of the
491 ZnS shell in the biofilm is further supported by the GI-XAS results at 1h and 3h of exposure
492 (Fig. 5, Table S2). Within the biofilm (C₂), Zn remains associated with QDs, as indicated by
493 the presence of S in the first coordination shell. However, at the crystal surface-biofilm
494 interface (C₁), a fraction of Zn (15-21%) shows O in the first-coordination shell. This indicates
495 the partial dissolution of the ZnS shell and the interaction of the released Zn(II) with O-
496 bearing reactive sites located at the bottom of the biofilm and the crystal surface.
497 Unfortunately, to the best of our knowledge, no accurate binding constant is available for
498 Zn(II) adsorbed onto α -Al₂O₃ (1-102). Nevertheless, studies conducted on Zn(II) sorption
499 onto Al-oxide surface coatings on aquifer sand (Coston et al., 1995) and at the
500 alginate/alumina interface (Wang et al., 2013), point out the relatively high affinity of Zn for
501 the alumina surface. We infer that in our system, the partial dissolution of the ZnS layer of
502 the QDs occurs first in the biofilm microenvironments (C₂), then the released Zn(II) migrates
503 toward the crystal surface (C₁) where it is partially sorbed. Unlike the results of Wang et al.
504 (2016b) , in which Zn was found mostly located in the biofilm, QD dissolution occurs close
505 enough to the surface (Fig. 6-2) to allow Zn to reach the crystal without being trapped or
506 inhibited by interactions with biofilm functional groups during its transport.

507 Dissolution of the ZnS shell exposes the QD core, and could favor ionic Cd and Se
508 release. However, according to our GI-XANES measurements (Fig. S6), no modification of
509 Se speciation is detected after 1 and 3h of exposure, indicating a higher stability of CdSe
510 cores compared to ZnS shells. A more stable CdSe core has already been observed in
511 oxidative environments (Metz et al., 2009), and when QDs are in contact with algae
512 (Slaveykova and Startchev, 2009). At 1h and 3h of exposure, Se is present at the surface of
513 the biofilm-gas interface (C₃, 40-50%) and within the biofilm (C₂, 50-60%), indicating that
514 CdSe cores, stable over time, have a preferential interaction with the biofilm compartment for
515 short term exposure.

516

517 NP dissolution when in contact with bacteria has been observed before (Auffan et al.,
518 2009b), specifically in toxicity studies, and can occur from three different processes: it can be
519 ligand-mediated (Wirth et al., 2012), redox-mediated (Kroll et al., 2014) or due to change in
520 the local physico-chemical conditions (Dehner et al., 2010). The high site density of
521 functional groups at the bacteria surfaces and in the EPS matrix, along with the presence of
522 extracellular organic ligands in the biofilm pores (Ha et al., 2010; Morel and Price, 2003;
523 Wang et al., 2016b) favor ligand-mediated dissolution. The complexation of metal(loid)s in
524 the system can limit the concentration of free species (a change in local physico-chemical
525 conditions) and thus, trigger dissolution: when the ion activity product is less than the
526 solubility product, dissolution is enhanced. Finally, the production of reactive oxygen species
527 (ROS), by bacteria such as *S. oneidensis* (Diaz et al., 2013), is a well-known phenomenon
528 that could be amplified in the biofilm (Wan et al., 2017) and in the presence of NPs (Lu et al.,
529 2008). Production of ROS would enhance the redox-mediated dissolution mechanism, as
530 has been proposed by Zhang et al. (2012) for QDs exposed to EPS.

531

532 3. Crystal surface as a driver of QD accumulation with time

533

534 At longer exposure times (i.e. after 10h), FY profiles of Zn and Se become similar (Fig.
535 2). This is either indicative of ZnS shells that are not dissolving anymore, or that crystal sites
536 are saturated and unable to attract any additional free Zn while the biofilm/crystal system
537 continues to accumulate QDs.

538 The relative increase in Zn and Se FY over time (Fig. 3) indicate that the total uptake of the
539 QDs and their products of dissolution continue over 24 hours, which means that the whole
540 interface does not reach saturation (Templeton et al., 2001). Nevertheless, over time the
541 relative amount of Zn in the crystal surface compartment (C₁) compared with the biofilm
542 compartments (C₂ and C₃) decreases (Fig. 4), which could indicate that some fraction of the
543 crystal sites reach saturation within the 24 hours (Templeton et al., 2001). Thus, it is likely

544 that partial saturation of the crystal surface with respect to Zn(II) occurs in the present
545 system. On the other hand, the relative amount of Se increases a little bit at the crystal
546 surface (C_1), from 0% at 1h to $6\pm 2.5\%$ at 24h which could indicate the presence of sites still
547 available. This migration toward the biofilm/crystal interface (C_1) could indicate that the
548 transport of QDs or CdSe cores is partially driven by their interaction with the crystal surface,
549 with the biofilm slowing their progression. Indeed, the accumulation of QDs over time would
550 favor the partial saturation of the functional sites of the biofilm, advancing from the top
551 (where the QDs enter the system) to the bottom. The attraction of negatively charged NPs by
552 $\alpha\text{-Al}_2\text{O}_3$ surfaces has been previously reported for silver NPs coated with polyvinylpyrrolidone
553 (Desmau et al., 2018). For QDs, their small size, and the chemical interactions they establish
554 with the biofilm's functional groups and microdomains, explain their relatively slow transport
555 rate toward the crystal, while this surface remains attractive to NPs at longer exposures
556 times (Fig. 6-3).

557

558 In addition to the partial saturation of the surface sites, slowing of ZnS dissolution
559 after 10h of exposure could explain the observed results. The fact that, at 24h, Zn is mostly
560 linked to S as first-shell neighbor, even at the crystal surface-biofilm interface (C_1) (Table
561 S2), in contrast with the Zn speciation observed at 1h and 3h, where 15-21% of Zn was
562 associated with O as first-shell neighbor, suggests a decrease in dissolution rate of the ZnS
563 shell. This decrease could be the result of changes in physico-chemical conditions in the
564 biofilm microenvironments, with fast consumption of extracellular organic chelatants during
565 the first hours of exposure leading to a slowing of ligand-mediated dissolution processes.
566 The saturation of the biofilm's functional sites over time would also impact this type of
567 dissolution.

568

569 Finally, minor oxidation of CdSe was observed at 24h in both the biofilm thickness
570 (C_2) and at the crystal surface-biofilm interface (C_1), shown by a slight increase in Se
571 oxidation state from Se(-II) to Se(IV) (Fig. S6). This late oxidation originates from the
572 exposure of Cd and Se from the core to oxidative environments (Derfus et al., 2004), and
573 suggests that oxidative conditions are presented within the biofilm. Thus, the oxidation of the
574 CdSe cores would be favored by the presence of holes in the ZnS shell as observed after
575 QD synthesis (Faucher et al., 2018), or because of partial ZnS dissolution in the
576 biofilm/crystal system, occurring in the first hours of exposure increasing the ZnS shell
577 porosity.

578

579 Similarities among Zn and Se distributions in the whole biofilm/crystal system indicate
580 first the accumulation of QDs, and later, the control exerted by the mineral surfaces over QD,

581 CdSe core and Zn(II) distributions. The accumulation of elements within the biofilm/mineral
582 system at long exposure times masks the details of processes such as dissolution and
583 oxidation that we were able to observe at shorter exposure times.

584

585 V. Conclusion

586

587 The present study provides an overall view of the fate of QDs when these NPs are
588 exposed to the biofilm/crystal interface (Fig. 6), a widespread environmental compartment.
589 Even in a system that minimizes the biofilm to solution volume ratio, the high accumulation
590 potential of this interface is manifest. Most importantly, the fast dissolution of the ZnS shell
591 shortly after QD exposure constitutes a potential key process when regarding the fate of QDs
592 in the environment, by promoting the fast degradation of these NPs and thus by limiting their
593 persistence. This process is likely to be promoted at lower concentration, as in natural
594 environments, and thus has to be taken into account when studying the environmental fate of
595 QDs. Here, ZnS dissolution is likely to occur in the biofilm thickness as a consequence of
596 high functional site densities as well as local oxidative conditions, and is followed by the
597 migration of dissolved Zn(II) toward the crystal surface. The CdSe cores, however, remain
598 mostly intact in the biofilm thickness. At longer exposure times, a general partitioning closer
599 to the crystal surface is observed, highlighting the importance of α -Al₂O₃ in the whole system
600 reactivity. ZnS shell dissolution is not discernible, partly masked by the accumulation of QDs,
601 but also likely occurs as a result of biofilm functional site saturation. Oxidative conditions in
602 the biofilm thickness seem to be partly preserved with evidence of slight Se oxidation.
603 Further studies are needed, particularly in order to investigate the involvement of bacterial
604 metabolic activity in ZnS dissolution processes for potential use of bacterial biofilms as
605 remediation tools in QD polluted environments.

606

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629 **Conflicts of interest**

630 There are no conflicts to declare.

631

632 **References**

633

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