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

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Abstract

The increasing use of Quantum Dots (QDs) - nanoparticles exhibiting unique optical properties – and their incorporation in multiple engineering products is likely to result in the release of this new class of contaminants into natural systems. In soils, bacterial biofilms and mineral surfaces form highly reactive interfaces, which may control QDs' environmental fate. However, little is known regarding QDs' stability in, and modes of interactions with, biofilm/mineral interfaces. This study examines the interactions, distributions and stability of thioglycolic acid-capped CdSe/ZnS QDs at the corundum (α-Al_2O_3)/*Shewanella oneidensis* MR-1 interface, for exposure times ranging between 1h to 24h. Long Period – X-ray Standing Wave – Fluorescence Yield spectroscopy and Grazing Incidence – X-ray Absorption Spectroscopy were used. Results indicate increases in Zn and Se concentrations within the biofilm/crystal system with time, demonstrating its high accumulation capacity over 24h. In addition, dissolution of a part of the ZnS shell occurs within 1h, highlighting the potential degradation of QDs when exposed to the biofilm/crystal compartment. Once released, Zn(II) migrates toward the biofilm-crystal interface and interacts preferentially with the crystal surface. In contrast, the remaining CdSe core is mostly preserved, and stays within the biofilm thickness. However, at 24h, Se and Zn present similar distribution profiles indicating a general reduction in ZnS shell dissolution at this longer exposure time.

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

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

I. Introduction

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

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

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

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

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

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

The current study investigates QD interactions with the biofilm/mineral interface, by quantifying the evolution of their partitioning and physico-chemical transformations over the course of 24h. The system used here is composed of a well-defined corundum (α-Al_2O_3) mineral surface coated with an axenic biofilm of *S. oneidensis* MR-1. These model gram-negative bacteria are commonly found in soils, sediments and aquifers, and represent an appropriate model microorganism. Interactions between QDs and the interface are monitored using two synchrotron related techniques. Long Period-X-ray Standing Wave-Fluorescence Yield (LP-XSW-FY) spectroscopy allows *in-situ* determination of elements' distribution at the biofilm/crystal interface. This technique was previously used to investigate the distribution of silver NPs (Desmau et al., 2018), Zn(II) and Pb(II) (Templeton et al., 2001; Wang et al., 2016b) in biofilm/crystal systems. In addition, Grazing Incidence-X-Ray Absorption Spectrocopy (GI-XAS) measurements provide information on speciation of elements, giving an overall view of QDs' physico-chemical transformations at the biofilm/crystal interface.

II. Materials and Methods

1. Quantum Dots

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

In QD stock solutions, the initial concentrations of Cd, Se and Zn were measured by inductively coupled plasma atomic emission spectroscopy. The average concentrations of four syntheses are 38.5±4.9 μM, 8.1±0.8 μM and 4.6±0.5 μM for Zn, Cd and Se, respectively with an average molar ratio between elements of 0.2±0.1, 1.9±1.3 and 9.0±6.1, for Cd/Zn, Cd/Se and Zn/Se, respectively. The calculated molar ratio of Cd/(Cd+Zn) is around 25%, in agreement with Bae et al. (2008). The electrophoretic mobility (EPM) and zeta potential (ZetaSizer, Malvern) of QDs functionalized with TGA were measured in 5mM $NaNO_3$ at pH ranging from 2 to 10. The hydrodynamic diameter was also assessed by Dynamic Light Scattering (DLS) at neutral pH (Dynapro Nanostar, Wyatt Technology, California, USA).

2. Sample preparation

The biofilm/crystal system and the biofilm growth protocol were previously described in Desmau et al. (2018). Briefly, the system is composed of a highly polished, cleaned and oriented single crystal substrate of α-Al_2O_3(1-102) with a surface roughness of 3Å (commercial Pi-KEM Ltd.) adequate for LP-XSW-FY spectroscopy. *S. oneidensis* MR-1 biofilms were grown for 10 days on substrates placed in a sterilized flow-through bottle, following the protocol established by Wang et al. (2016b). First, a tripticase soy broth (BioMérieux, 51019) suspension inoculated with *S. oneidensis* MR-1, in a reproducible way (Desmau et al., 2018), was left to settle for 1 hour in the bottle. Then, sterile synthetic growth medium (table S1) at pH 7.0 was pumped continuously through the bottle for 10 days at

ambient temperature. After 10 days, the biofilm-coated surfaces were then gently placed in 10 mL Falcon centrifuge tubes containing the same growth medium, and were stored at 4°C for 1 week before measurement. The number of colony-forming units was similar before and after 1 week at 4°C (data not shown). Prior to measurement, the biofilm/crystal systems were rinsed to remove excess nutrients, then exposed to a QD suspension in 5 mM $NaNO_3$ solution at ambient temperature. This background electrolyte can be considered an ideal soil solution which minimizes particle aggregation (Chen et al., 2017). The experiments were performed with a Cd concentration of 137±2 nM and a Zn concentration of 748±60 nM. Solution pH was adjusted to 7.0±0.1, if necessary, in the course of experiments to ensure acceptable living conditions for *S. oneidensis* MR-1, using 0.1 M HNO_3 or 0.1 M NaOH.

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

Samples were gently immersed in 21 mL of fresh QD solutions, with the biofilm side facing down to ensure the study of QD transport. All tubes were shielded from light using aluminum foil to avoid QD degradation (Li et al., 2012), and were gently shaken at 20 rpm. For LP-XSW-FY measurements, samples were placed in Kapton-covered sample holders, mounted vertically, and purged with humid He gas. For GI-XAS measurements, the fluorescence detector was mounted perpendicular to the sample surface. In order to obtain a detailed characterization of QD fate in the system, control experiments, mass balance measurements and SEM imaging were performed. Cd and Zn uptake were estimated by biofilm digestion in 2% HNO_3, after 1h, 3h and 24h of exposure, in triplicate. To obtain concentration in mg per gram of biofilm, four biofilms were weighed just after growth, and after 12h oven drying at 450°C. The average biofilm dry weight was 0.30±0.02 mg per sample. As Cd and Zn are naturally present within biofilms, originating from impurities present in nutrient solutions during growth, their concentrations in biofilms that were not exposed to QDs were also measured in triplicate by the same method. The amount of Cd and Zn in solution, and sorbed onto the Falcon tube walls, were also measured using an Agilent 7900 ICP-QMS (see appendix 1, SI), in triplicate. The Falcon tubes were rinsed with 2% HNO_3 after the experiments to assess the quantity of QDs sorbed onto the tube walls. The amounts of dissolved species in the supernatant were estimated by QD removal by centrifugal ultrafiltration (3 kDa, Amicon®, Millipore) and measured by ICP-QMS. Control experiments were performed with biofilm/crystal systems exposed to Cd(II) (36.1±0.2 nM) and Zn(II) (324±6 nM) ions for 24h in triplicate. Samples were imaged using a Zeiss Ultra Device SEM

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

3. LP-XSW-FY measurements and data analysis

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

LP-XSW-FY measurements were conducted at beamline 13-ID-C at GeoSoilEnvironCARS (GSECARS) at the Advanced Photon Source (APS). The LP-XSW-FY set-up, data analysis and modeling is similar to the protocol described in Desmau et al. (2018) and is detailed in appendix 3 (see SI). More information on LP-XSW-FY principles and applications can be found in Trainor et al. (2006). The limitations of using this technique in such systems (biofilm/crystal interface + NPs) are presented in Desmau et al. (2018) and in appendix 3 (SI). Briefly, the technique is highly dependent on the roughness and the thickness of the sample. The high heterogeneity of the biofilm associated with the presence of dense nano-metric objects are likely to impact the formation of the standing waves. Thus, the modeling can be quite complicated. These parameters have been considered in our models, allowing the semi-quantification of the distribution of Zn and Se, although the interpretation has to be performed carefully. The monochromatic 13.3 keV X-ray beam was collimated using a pair of 1 meter, Rh coated Si mirrors in Kirkpatrick-Baez geometry. The final beam profile of 1000 μm vertical by 10 μm horizontal was defined by slits. X-ray reflectivity measurements were performed by scanning X-ray incidence angle between 0.0° and 0.5° while monitoring the intensities of the incident (I_0) and reflected (I_1) X-ray beams using N_2-filled gas ionization chambers. Zn Kα and Se Kα fluorescence yield data were collected using a 4-element silicon drift detector (SII NanoTechnology, Vortex-ME4) in two or more locations for each sample to verify reproducibility.

The reflectivity and the fluorescence yield are modeled to obtain a semi-quantitative distribution of the elements of interest (here Zn and Se). The full description of the physical model is presented in the SI (appendix 3). In this study, the biofilm/crystal system was divided into three compartments: the crystal surface-biofilm interface (labeled C_1), the biofilm

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

4. GI-XAS measurements and data analysis

GI-XAS (Grazing Incidence – X-ray Absorption Spectroscopy) allows the determination of elemental speciation at different depths within the biofilm/crystal system. We estimate a minimum detection limit of 15% by weight. Thus, local phenomena resulting in small quantities of certain species would not be detected. EXAFS spectra were collected at Zn K-edge (~9.6 keV) and XANES spectra were collected at Se K-edge (~12.6 keV) at the crystal surface-biofilm interface (C_1), and within the biofilm thickness (C_2). Measurements were performed at beamline 11-2 at the Stanford Synchrotron Radiation Lightsource (SSRL) using a grazing-incidence spectrometer in vertical scattering geometry. GI-XAS spectra were collected at room temperature in fluorescence mode using a Canberra 100-pixel Ge solid-state monolith pixel detector. The incident beam energies were selected using a LN_2 cooled Si (220) monochromator and collimated using a pair of 1 m-long Rh-coated Si mirrors. Zn and Se metal foils were used during experiments for energy calibration. In order to specifically probe the two compartments of interest, Zn K-edge and Se K-edge fluorescence data were collected at incidence angles of 0.30° and 0.25° to interrogate the crystal surface-biofilm interface (C_1), and at 0.18° and 0.10° for the biofilm thickness (C_2), respectively. Note that for the crystal surface-biofilm interface, it is not possible to only probe the speciation of Zn and Se at the surface of the crystal. Indeed, part of the signal originated also from the first nanometers of the biofilm, meaning that the signal from the surface could be partially "contaminated" by the signal from the biofilm. For XAS data analysis, three or four scans were averaged, background subtracted, and fitted using the SIXPack interface (Webb, 2005) and the IFEFFIT XAFS analysis package (Ravel and Newville, 2005). Linear combination fitting (LCF) was used to quantify the presence of several possible species. Additional species were considered only when they improved the goodness-of-fit by at least 15%.

III. Results

1. Characterization of QDs

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

8.01±0.05, the EPMs values are -1.3±0.3 and -0.8±0.7 μm cm/Vsec, respectively. The corresponding zeta potentials are -16.6±3.1 mV and -9.8±0.7 mV, respectively. The EPM measured at pH 8 present an uncertainty higher than for the other pH studied (Fig. S5), which could indicate that those points are outliers. When pH decreases, pH 8 could also correspond to the pH where the carboxylates could start slowly to protonate (carboxylate pK_a on surfaces can be higher than in solution (Chen et al., 2000)), favoring the presence of different particles (individual particles, dimmers, trimers…). In addition, at pH lower than 8, surface charge is lower which would probably favor aggregation of the particles due to the increase of the protonation, and the EPM, or zeta potential, would be estimated for aggregates and not for a unique QD. The partial homo-aggregation of QDs at neutral pH seems to be confirmed by the average hydrodynamic diameter measured by DLS (41.7 nm). In addition, more than 60% of the particles present a diameter less than 30 nm (with size ranging from 5 to 200 nm, DLS results), validating the hypothesis of different types of particles in solution. This has been considered for the discussion of results.

Nevertheless, the surface charge of the QDs, or their agglomerates, at pH 7 is regarded as negative, considering the whole analysis.

2. Zn and Se distributions

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

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

the crystal surface-biofilm interface (C_1). At 10h and 24h, identical distributions are observed for Se and Zn, with ~25% at the biofilm-gas interface (C_3), ~70% in the biofilm (C_2), and ~5% at the crystal surface-biofilm interface (C_1) (see Fig. 4 for estimated confidence interval).

One could note here the high percentage of Zn located at the mineral surface (C_1) at 3h compared to the percentage of Zn at 1h, even if the shape the shape of the curves describing normalized fluorescence intensity appears to be relatively similar. The higher percentage of Zn obtained at 3h could be explained by the larger peak of the modeled data at 3h, at high incident angle, that could increase the percentage of Zn at the mineral surface, at the expense of the percentage of Zn present in the biofilm thickness (C_2). In addition, compared to the others samples, the confidence interval of the Zn-3h sample is a little bit larger (see appendix 3, SI), and the value of the χ^2 test is smaller for the Zn-sample at 1h compared to the one at 3h, which could indicate a better fit for the data at 1h. Thus, the percentage of Zn at the mineral surface could be overestimated and this spectrum could be considered as an outlier. Nevertheless, we considered that, even if the percentage of Zn at the mineral surface is overestimated, the observed tendency seems to be similar to the one at 1h with Zn present at the mineral surface, while Se is absent.

3. Zn and Se speciation in the different compartments

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

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

4. Elemental sequestration in the biofilm/crystal system

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

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

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

IV. Discussion

1. QD sequestration at the biofilm/crystal interface

When the samples are exposed to QDs, most of the Zn and Cd is found associated with the tube walls, highlighting a high affinity of QDs for this type of plastic. Despite exhibiting a lower surface area compared to tube walls (estimated at 2.4 cm^2 for the biofilm/crystal system vs. 50 cm^2 for the tube), the biofilm/crystal system traps QDs in appreciable amounts, with 13% of Zn and 15% of Cd being present in the biofilm relative to the solution after 24h. In addition, the increase in Zn, Se and Cd in this compartment with time, as measured by ICP-QMS and fluorescence intensity (Fig. 3), indicates continuous accumulation in the biofilm/crystal system over time (Fig. 6-1). As described in the introduction, the overall surface charge of *S. oneidensis* at pH=7 is negative (Ha et al., 2010) meaning that the interactions with negatively charged QDs would not be favored. Nevertheless, the interaction between negatively charged NPs and *S. oneidensis* MR-1 biofilm has been previously observed with 60 nm silver NPs coated with polyvinylpyrrolidone (Desmau et al., 2018). In this previous study, we highlighted the role of other parameters, such as NP size and hydrophobicity, on the interactions between NPs and biofilms, when both of them are negatively charged. For example, smaller particles are able to diffuse into all parts of the biofilm. In the present study, the relatively small size of the QDs (60% have a hydrodynamic diameter less than 30 nm) could explain their ability to strongly accumulate in the biofilm. In addition, the strong interactions between negatively charged QDs and *S. oneidensis* biofilm have also been explained in the past by the presence of hydrophobic microdomains within the biofilm thickness (Aldeek et al., 2011; Aldeek et al., 2013), validating the role of hydrophobic interactions in NP-biofilm interactions.

2. QD fast dissolution within the biofilm

A semi-quantitative distribution of Zn and Se in the biofilm/crystal system can be obtained by modelling LP-XSW-FY data, using the 3 compartments previously described (Fig. 4). Interestingly, at short time of exposure (i.e. 1h and to a certain extend at 3h), an absence of co-localization between Zn and Se at the interfaces (C_1 and C_3) is observed. This lack of co-

localization can be explained by the presence of dissolved Zn(II) that interacts differently with the biofilm and crystal surface than QDs (Fig. 6-2). Zn in solution could originate either from (i) dissolved Zn(II) present in the supernatant (~10%), or (ii) from dissolution of QDs in the biofilm matrix. Regarding (i), when exposed to free Zn(II) at 899 nM, the amount of Zn trapped in the biofilm/crystal system is minimal compared to QD exposure experiments (0 vs. 6 %, see III.3 and Fig. S7). As a result, the 10% of Zn(II), ~96 nM, present in the supernatant could not fully explain the Zn detected at the crystal surface in QD experiments by LP-XSW-FY at 1 and 3h. In addition, Wang et al. (2016b) studied the distribution of dissolved Zn(II) in the *S. oneidensis* MR-1 biofilm/α-Al_2O_3(1-102) crystal system and showed that for all concentrations (10^{-4} to 10^{-7}M) and exposure times investigated, Zn(II) was mostly associated with the biofilm and did not interact with the crystal surface. Dissolved Zn(II) present in the supernatant in our study is expected to behave the same and would partition mostly to the biofilm. Since this is not observed, the involvement of dissolved Zn from the supernatant can thus be discarded.

Therefore, Zn at the crystal surface is likely to originate from a partial QD dissolution in the biofilm thickness (ii). The fast dissolution of ZnS nanoparticles (Dehner et al., 2010), in contact with bacteria has been observed before, as has their dissolution in other systems such as organic wastes (Le Bars et al., 2018). In our study, the Zn(II) from the ZnS shell would be released much closer to the crystal surface, and interact more easily with the highly reactive functional sites from the crystal (Wang et al., 2016b). The local dissolution of the ZnS shell in the biofilm is further supported by the GI-XAS results at 1h and 3h of exposure (Fig. 5, Table S2). Within the biofilm (C_2), Zn remains associated with QDs, as indicated by the presence of S in the first coordination shell. However, at the crystal surface-biofilm interface (C_1), a fraction of Zn (15-21%) shows O in the first-coordination shell. This indicates the partial dissolution of the ZnS shell and the interaction of the released Zn(II) with O-bearing reactive sites located at the bottom of the biofilm and the crystal surface. Unfortunately, to the best of our knowledge, no accurate binding constant is available for Zn(II) adsorbed onto α-Al_2O_3 (1-102). Nevertheless, studies conducted on Zn(II) sorption onto Al-oxide surface coatings on aquifer sand (Coston et al., 1995) and at the alginate/alumina interface (Wang et al., 2013), point out the relatively high affinity of Zn for the alumina surface. We infer that in our system, the partial dissolution of the ZnS layer of the QDs occurs first in the biofilm microenvironments (C_2), then the released Zn(II) migrates toward the crystal surface (C_1) where it is partially sorbed. Unlike the results of Wang et al. (2016b) , in which Zn was found mostly located in the biofilm, QD dissolution occurs close enough to the surface (Fig. 6-2) to allow Zn to reach the crystal without being trapped or inhibited by interactions with biofilm functional groups during its transport.

Dissolution of the ZnS shell exposes the QD core, and could favor ionic Cd and Se release. However, according to our GI-XANES measurements (Fig. S6), no modification of Se speciation is detected after 1 and 3h of exposure, indicating a higher stability of CdSe cores compared to ZnS shells. A more stable CdSe core has already been observed in oxidative environments (Metz et al., 2009), and when QDs are in contact with algae (Slaveykova and Startchev, 2009). At 1h and 3h of exposure, Se is present at the surface of the biofilm-gas interface (C_3, 40-50%) and within the biofilm (C_2, 50-60%), indicating that CdSe cores, stable over time, have a preferential interaction with the biofilm compartment for short term exposure.

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

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

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

The relative increase in Zn and Se FY over time (Fig. 3) indicate that the total uptake of the QDs and their products of dissolution continue over 24 hours, which means that the whole interface does not reach saturation (Templeton et al., 2001). Nevertheless, over time the relative amount of Zn in the crystal surface compartment (C_1) compared with the biofilm compartments (C_2 and C_3) decreases (Fig. 4), which could indicate that some fraction of the crystal sites reach saturation within the 24 hours (Templeton et al., 2001). Thus, it is likely

that partial saturation of the crystal surface with respect to Zn(II) occurs in the present system. On the other hand, the relative amount of Se increases a little bit at the crystal surface (C_1), from 0% at 1h to 6±2.5% at 24h which could indicate the presence of sites still available. This migration toward the biofilm/crystal interface (C_1) could indicate that the transport of QDs or CdSe cores is partially driven by their interaction with the crystal surface, with the biofilm slowing their progression. Indeed, the accumulation of QDs over time would favor the partial saturation of the functional sites of the biofilm, advancing from the top (where the QDs enter the system) to the bottom. The attraction of negatively charged NPs by α-Al_2O_3 surfaces has been previously reported for silver NPs coated with polyvinylpirrolidone (Desmau et al., 2018). For QDs, their small size, and the chemical interactions they establish with the biofilm's functional groups and microdomains, explain their relatively slow transport rate toward the crystal, while this surface remains attractive to NPs at longer exposures times (Fig. 6-3).

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

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

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

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

V. Conclusion

The present study provides an overall view of the fate of QDs when these NPs are exposed to the biofilm/crystal interface (Fig. 6), a widespread environmental compartment. Even in a system that minimizes the biofilm to solution volume ratio, the high accumulation potential of this interface is manifest. Most importantly, the fast dissolution of the ZnS shell shortly after QD exposure constitutes a potential key process when regarding the fate of QDs in the environment, by promoting the fast degradation of these NPs and thus by limiting their persistence. This process is likely to be promoted at lower concentration, as in natural environments, and thus has to be taken into account when studying the environmental fate of QDs. Here, ZnS dissolution is likely to occur in the biofilm thickness as a consequence of high functional site densities as well as local oxidative conditions, and is followed by the migration of dissolved Zn(II) toward the crystal surface. The CdSe cores, however, remain mostly intact in the biofilm thickness. At longer exposure times, a general partitioning closer to the crystal surface is observed, highlighting the importance of α-Al_2O_3 in the whole system reactivity. ZnS shell dissolution is not discernible, partly masked by the accumulation of QDs, but also likely occurs as a result of biofilm functional site saturation. Oxidative conditions in the biofilm thickness seem to be partly preserved with evidence of slight Se oxidation.
Further studies are needed, particularly in order to investigate the involvement of bacterial metabolic activity in ZnS dissolution processes for potential use of bacterial biofilms as remediation tools in QD polluted environments.

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Conflicts of interest

There are no conflicts to declare.

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