This article was downloaded by: [Pearce, Carolyn I.]

On: 1 December 2009

Access details: Access Details: [subscription number 915893145]

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Environmental Technology

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t791546829>

Investigating different mechanisms for biogenic selenite transformations: *Geobacter sulfurreducens*, *Shewanella oneidensis* and *Veillonella atypica*

Carolyn I. Pearce ^a; Richard A. D. Patrick ^a; Nicholas Law ^a; John M. Charnock ^a; Victoria S. Coker ^a; Jon W. Fellowes ^a; Ronald S. Oremland ^b; Jonathan R. Lloyd ^a

^a School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester, UK ^b School of Earth Sciences, US Geological Survey, Water Resources Division, California, USA

To cite this Article Pearce, Carolyn I., Patrick, Richard A. D., Law, Nicholas, Charnock, John M., Coker, Victoria S., Fellowes, Jon W., Oremland, Ronald S. and Lloyd, Jonathan R. 'Investigating different mechanisms for biogenic selenite transformations: *Geobacter sulfurreducens*, *Shewanella oneidensis* and *Veillonella atypica*', *Environmental Technology*, 30: 12, 1313 – 1326

To link to this Article: DOI: 10.1080/09593330902984751

URL: <http://dx.doi.org/10.1080/09593330902984751>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Investigating different mechanisms for biogenic selenite transformations: *Geobacter sulfurreducens*, *Shewanella oneidensis* and *Veillonella atypica*

Carolyn I. Pearce^{a*}, Richard A.D. Patrick^a, Nicholas Law^a, John. M. Charnock^a, Victoria. S. Coker^a,
Jon W. Fellowes^a, Ronald S. Oremland^b and Jonathan R. Lloyd^a

^a*School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester, UK;* ^b*School of Earth Sciences, US Geological Survey, Water Resources Division, Menlo Park, California, USA*

(Received 19 December 2008; Accepted 21 April 2009)

The metal-reducing bacteria *Geobacter sulfurreducens*, *Shewanella oneidensis* and *Veillonella atypica*, use different mechanisms to transform toxic, bioavailable sodium selenite to less toxic, non-mobile elemental selenium and then to selenide in anaerobic environments, offering the potential for *in situ* and *ex situ* bioremediation of contaminated soils, sediments, industrial effluents, and agricultural drainage waters. The products of these reductive transformations depend on both the organism involved and the reduction conditions employed, in terms of electron donor and exogenous extracellular redox mediator. The intermediary phase involves the precipitation of elemental selenium nanospheres and the potential role of proteins in the formation of these structures is discussed. The bionanomineral phases produced during these transformations, including both elemental selenium nanospheres and metal selenide nanoparticles, have catalytic, semiconducting and light-emitting properties, which may have unique applications in the realm of nanophotonics. This research offers the potential to combine remediation of contaminants with the development of environmentally friendly manufacturing pathways for novel bionanominerals.

Keywords: bionanominerals; bioreduction; selenium; metal-reducing bacteria; microbial biogeochemistry

1. Introduction

Selenium occurs in one of four oxidation states, namely the oxyanions selenate [Se(VI), SeO_4^{2-}] and selenite [Se(IV), SeO_3^{2-}], in elemental form [Se(0)] and as selenide [Se(-II)]; the latter is found in chalcogen selenides such as FeSe, in methylated compounds such as dimethylselenide and in the chalcogen-bearing proteins, selenocysteine and selenomethionine [1–4]. Elemental Se can exist in a range of structures either as α -, β - and γ -monoclinic red Se comprising Se_8 rings, as trigonal trapezohedral (hexagonal) black Se comprised of ordered helical [-Se- $_n$] chains, or as amorphous Se [5].

Se is found in pyritiferous and/or organic-rich lithologies and it can be released into the environment through the weathering of these materials. Seleniferous rocks, containing in excess of 100 ppm Se, outcrop in the United States, Ireland, India and China, and are often accompanied by occurrences of Se toxicity in wildfowl, cattle and the local population [6–11].

Bioavailable Se is present in the environment at very low concentrations in the form of selenate and selenite, but it can reach toxic micromolar concentrations through a combination of natural environmental

processes and subsequent human activity [12]. Se pollution is also associated directly with waste materials from a broad spectrum of anthropogenic operations, including mining, agricultural, petrochemical, and industrial manufacturing operations. The problems associated with Se pollution in the aquatic environment are exacerbated through agricultural irrigation practices, particularly in arid/semi-arid regions. Shallow subsurface layers of clay impede the downward movement of irrigation water, resulting in the subsequent build up of soil trace elements as excess water evaporates from the soil surface [13]. The application of irrigation water can also solubilize and leach out naturally occurring Se in the soil, in the alkaline, oxidizing conditions prevalent in arid climates. Se present in the contaminated surface water can then enter the aquatic food chain and is bioaccumulated, in the form of proteinaceous Se, to toxic levels, particularly in fish and rice. High concentrations of bioaccumulated Se lead to deformity and death in livestock and wild fowl. In humans, Se has the narrowest range between nutritional requirements and toxicity of any essential element [14], and thus chronic toxicity is not readily predictable, with effects of Se deficiency leading to

*Corresponding author. Email: carolyn.pearce@manchester.ac.uk

cancer, severe skin lesions, heart and liver failure, while toxicity can lead to serious hair and nail disorders, gastrointestinal symptoms, ulceration and a variety of nervous disorders [15–17]. Deficiency is relatively easy to treat using dietary supplements, but an increasing recognition of Se toxicity has led to the emergence of Se as an important environmental contaminant, with the increasing use of manufactured Se nanoparticles presenting an as yet undefined risk.

Microbial processes in the environment result in Se transformations, including changes in valence or chemical form [18]. These processes can be used to develop stabilization strategies, in which toxic, bioavailable Se oxyanions are reduced to less available elemental Se. The products of these reductive transformations include a range of functional bionanominerals with interesting physical properties of potential technological significance [19–22]. In this study, different mechanisms for the biotransformation of the Se oxyanion, sodium selenite, Se(IV), involving the medical bacterial isolate *Veillonella atypica*, which has been shown to exhibit high activity in terms of selenide, Se(-II), production [20] and the subsurface metal reducing bacteria *Geobacter sulfurreducens* and *Shewanella oneidensis*, are assessed in terms of the effect of alternative electron donors and the addition of an exogenous extracellular redox mediator, anthraquinone disulfonic acid (AQDS). Se is readily metabolized by microbes, and is involved in a range of metabolic processes including assimilation, methylation, detoxification, and anaerobic respiration [2,23]. For example, in *Bacillus selenitireducens*, the reduction mechanism involves energy conservation by oxidation of lactate coupled to growth via respiratory reduction of Se(IV) using Se-specific dissimilatory enzymes [24]. Fe(III) reducers such as *S. oneidensis* [25] and *G. sulfurreducens* can also reduce Se(IV), with c-type cytochromes implicated in electron transfer to the metalloid. Unlike *Shewanella* and *Geobacter* species, *V. atypica* is not reported to possess high concentrations of cytochromes [26] and is thought to produce Se(0) nanospheres from Se(IV) via a hydrogenase-coupled reduction, mediated by ferredoxin. This research shows that the reduction rate and the nature of the bionanomineral phases produced can be controlled by changing reduction conditions and by selecting bacteria with particular attributes. The Se bionanomineral phases are composite materials in which biomolecules such as proteins and/or polysaccharides act as a template to direct nanoparticle nucleation and growth [27]. Identifying microbial proteins involved in environmental nanoscale biomineralization processes will shed light on the form and fate of aqueous and nanoparticulate metals/metalloids in natural waters and sediments [28], and will also offer the potential for synthesizing specific peptide sequences to template

nanomaterials for technological and environmental applications. In this study, specific proteins implicated in playing a role in the formation of Se(0) nanospheres by *G. sulfurreducens* have been identified.

2. Experimental details

All chemicals were of analytical grade and obtained from Sigma-Aldrich (Dorset, UK), unless otherwise stated. All organisms were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ).

2.1. Selenite reduction experiments with resting cells of *G. sulfurreducens*, *S. oneidensis* MR-1 and *V. atypica*

Late-exponential-phase cultures of (i) *G. sulfurreducens* (ATCC 51573), grown anaerobically at 30°C in defined medium with 25 mmol. l⁻¹ sodium acetate and 40 mmol. l⁻¹ sodium fumarate as the electron donor and acceptor, respectively [29,30]; (ii) *S. oneidensis* MR-1 (ATCC 70050) grown anaerobically at 30°C in tryptone soy broth; and (iii) *V. atypica* (ATCC 14894) grown anaerobically in defined medium by fermentation of 50 mmol. l⁻¹ lactate [20,31] were isolated by centrifugation (4000 × g for 20 min). The cells were washed and re-suspended in anaerobic 3-(N-morpholino)propane-sulfonic acid (MOPS) buffer (20 mmol. l⁻¹, pH 7.5) to an OD_{600nm} of ~1.0. These 'resting' cells were then challenged with Se(IV) as Na₂SeO₃ (6.5 mmol. l⁻¹) in MOPS buffer (20 mmol. l⁻¹, pH 7.5) with a total volume of 20 ml. To determine the effect of an exogenous extracellular electron shuttle on Se(IV) reduction, anthraquinone-2, 6-disulphonate (disodium salt, AQDS) was added at a final concentration of 100 µM. When sodium acetate was used as a potential electron donor for reduction by *G. sulfurreducens*, it was added at a final concentration of 10 mmol. l⁻¹. The bottles were sealed with butyl rubber stoppers and de-gassed with N₂. When hydrogen was used as a potential electron donor, the headspace was then filled with hydrogen. The bottles containing *G. sulfurreducens* and *S. oneidensis* cultures were placed in a 30°C incubator, and the bottles containing *V. atypica* cultures were placed in a 37°C incubator, without shaking, for 500 h. Samples were removed for protein analysis using a bicinchoninic acid assay kit (Sigma), as described by the method of Smith *et al.* [32]. In order to calculate the amount of biomass from the protein analysis, it was assumed that the biomass contained approximately 55% protein by dry weight [33]. All experiments were done at least twice, in triplicate, to ensure reproducibility, and representative data are presented in this study.

2.2. Ion chromatography (IC) and inductively coupled plasma–atomic emission spectrometry (ICP–AES)

Aliquots were removed from experimental cultures under an anaerobic atmosphere, using a sterile needle, passed through a 0.22 µm filter and flash frozen in liquid nitrogen for storage at –80°C. The total SeO_3^{2-} concentration was determined by IC (Dionex, UK) and the total Se concentration in solution by ICP–AES (Horizon, V.G. elemental, UK). Se(0) was calculated by subtracting total Se in solution from the total Se starting concentration (6.5 mmol. l^{-1}) and Se(–II) was calculated by subtracting concentration of Se(IV) in solution from total Se in solution. ICP–AES was carried out under an anaerobic atmosphere to prevent oxidation of reduced Se species. For IC, a Dionex DX600 system with GP50 gradient pump and CD20 conductivity detector was used, with an injection loop of 50 µL and wide-bore (4 mm) columns. The mobile phase was isocratic 3.6 mmol. l^{-1} Na_2CO_3 /3.4 mmol. l^{-1} NaHCO_3 with a flow rate of 1.4 mL/min. The system backpressure was 2600–2800 psi. For ICP–AES analysis of Se a wavelength of 196 nm was used. For both techniques, calibration standards of 0, 12.5, 50 and 100 mmol. l^{-1} Na_2SeO_3 were prepared by dilution of concentrated reference element stock solutions. Calibration blocks were placed at intervals throughout each analytical run in order to correct for instrument drift. The average analytical error was <3% for IC data and ± 0.103 ($3 \times \sigma$) for ICP–AES. Figures 1 to 4 show the initial change in concentration with time and a measurement after reduction for 500 h in the presence of the organisms is quoted as the end point in the text.

2.3. X-ray absorption spectroscopy (XAS)

XAS data were collected at the STFC Daresbury SRS synchrotron, on Wiggler beam line 16.5, with the storage ring operating at 2 GeV and a current of 100–200 mA. A pre-monochromator 1.2 m uncoated mirror was used, bent to provide vertical collimation, and to remove some higher energy photons. A Si(220) double crystal monochromator was employed, detuned to 70% transmission to minimize any residual harmonics. Energy calibration was performed using a standard sample of red elemental selenium, ground and diluted with boron nitride. Samples were measured in a 1 mm thick aluminium frame sample holder with Mylar windows at ca. 77 K using a cold-finger stage. Spectra were collected at the Se K-edge in fluorescence-yield mode over the energy range 12,400–13,300 eV, with the sample cell at 45° to the incident X-ray beam, using a Canberra 30-element solid state detector. The structural environment was determined using extended X-ray absorption fine structure (EXAFS) and X-ray absorption near edge structure (XANES) analysis. Background subtracted EXAFS

spectra were analysed in EXCURV98 using full-curved-wave theory [34]. Phase shifts were derived in the programs from *ab initio* calculations using Hedin-Lundqvist potentials and von Barth ground states [35]. Fourier transforms of the EXAFS spectra were used to obtain an approximate radial distribution function around the central Se atom (the absorber atom); the peaks of the Fourier transform can be related to shells of surrounding back-scattering atoms characterized by atom type, number of atoms in the shell, the absorber–scatterer distance and the Debye–Waller factor, $2\sigma^2$ (a measure of both the thermal motion between the absorber and scatterer and of the static disorder or range of absorber–scatterer distances). The data were fitted for each sample by defining a theoretical model and comparing the calculated EXAFS spectrum with the experimental data. Shells of back-scatterers were added around the selenium and by refining an energy correction E_F (the Fermi energy), the absorber–scatterer distance and Debye–Waller factor for each shell, a least squares residual (the *R*-factor [36]) was minimized. The coordination numbers were refined for oxygen atoms from water molecules for hydrated HSe^- (hydrogen atoms are too weak as scatterers to be detected by XAS).

2.4. Transmission electron microscopy

Transmission electron microscopy (TEM) was used to examine the structure of the washed cells and accumulation of selenium minerals after exposure to Se(IV) for 500 h. Cells were collected and washed three times in anaerobic 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) buffer (10 mmol. l^{-1} , pH 7), then fixed with 2.5% glutaraldehyde. Whole mounts of bacteria were placed on carbon-coated Cr 200-mesh copper grids. Samples were examined using a FEI Tecnai 12 Biotwin transmission electron microscope (FEI, Eindhoven, The Netherlands) at 100 kV. EDX was also performed at 100 kV by using a spot size of 200 nm, a current of 25 mA, and a (live) counting time of 100 s.

2.5. X-ray diffraction

X-ray diffraction (XRD) measurements were acquired over a 2θ range of 20°–60° with a step size of 0.02° and a scan time of 2 s per step, using a Bruker D8 Advance X-ray diffractometer (Bruker AXS Ltd, Coventry, UK) with Cu $K\alpha 1$ source. The samples were dried to form a uniform film on a glass slide, which was transferred to the instrument immediately after preparation.

2.6. Identification of proteins associated with Se(0) nanospheres produced by *G. sulfurreducens*

To identify proteins associated with Se(0) nanospheres produced by *G. sulfurreducens*, cultures

grown anaerobically at 30°C for one week, in defined medium supplemented with Na_2SeO_3 (1 mmol. l^{-1}), were homogenized by syringing through a sterile 21-gauge syringe needle and pelleted by centrifugation. The Se(0) nanospheres were fractionated from cells and media using a density-based approach involving iterative centrifugation through a chemically inert solution, which preserves any organic biomolecules by avoiding the use of surfactants or solvents [28]. The pellet was re-suspended in lithium polytungstate solution (LST) of density $\sim 2.8 \text{ g cm}^{-3}$ (Geoliquids, Prospect Heights, IL, USA), and then subjected to further low-speed centrifugation (5 min at 1000 rpm) in autoclaved Eppendorf tubes. The Se(0) nanosphere fraction ($\rho \sim 4.8 \text{ g cm}^{-3}$) was easily pelleted at the bottom of LST-filled tubes during centrifugation, while the cell biomass fraction rose to the top of the LST solution. The separated fractions were washed several times in deionized water to remove LST. The nanosphere-rich and nanosphere-poor fractions from the separation process were heated at 110°C for 30 min in reducing Laemmli sample buffer (2% sodium dodecyl sulphate (SDS), 3% 2-mercaptoethanol, 40 μL) to denature any proteins. The separate fractions were then centrifuged ($13000 \times g$ for 5 min) to remove the Se(0) nanospheres prior to SDS polyacrylamide gel electrophoresis. The fractions (25 μL) were run in an 18 % Tris-HCl gel with Tris/glycine/SDS running buffer. Kaleidoscope (BioRad) pre-stained molecular weight ladders were run on both sides of the gel and gel-separated protein bands were detected using Imperial Protein Stain. Bands of interest were excised under sterile conditions for trypsin digestion. Tryptic peptides were analysed using liquid chromatography-based high-resolution tandem mass spectrometry (LC-MS/MS). Proteins of interest were identified by matching MS/MS spectra against a database constructed using predicted peptides from the *Geobacter sulfurreducens* PCA genome [37] using the SEQUEST algorithm [38] and filtered with BioWorks and DTASelect [39] at the peptide level using conservative filters [Xcorr of at least 1.8 (+1), 2.2 (+2) 3.5 (+3)]. Only proteins identified with two fully tryptic peptides at conservative filters were considered for further biological study.

3. Results and discussion

3.1. Mechanisms and kinetics of Se(IV) reduction

3.1.1 Se(IV) reduction by *G. sulfurreducens* using acetate or hydrogen as the electron donor

For *G. sulfurreducens* with acetate as an electron donor (Figure 1 A and B), IC showed that the Se(IV) reduction rate was increased by a factor of three as a result of the

addition of AQDS (1.1 mmol. l^{-1} $\text{Na}_2\text{SeO}_3 \text{ g}^{-1}$ (biomass) h^{-1} with AQDS versus 0.4 mmol. l^{-1} $\text{Na}_2\text{SeO}_3 \text{ g}^{-1}$ (biomass) h^{-1} without). ICP–AES showed that this reduction in Se(IV) corresponded to a decrease in total soluble Se, presumably owing to the formation of insoluble Se(0) (noted as a red precipitate). A significant concentration (2.5 mmol. l^{-1}) of Se(IV) remained in solution after reduction by *G. sulfurreducens* for 500 h without AQDS, with the majority of the remaining Se present as Se(0), and a minor proportion ($\sim 15\%$) reduced to the soluble Se(-II) phase. In the presence of AQDS, no Se(IV) remained after 500 h reduction and Se(0) was the dominant phase, with approximately a third of the total Se further reduced to Se(-II), as indicated by the concentration of total Se in solution from ICP–AES. There was a slight decrease in the amount of Se(0) corresponding to formation of Se(-II) after 16 h, even when a significant concentration of Se(IV) remained in solution, suggesting that the reduction mechanism was not biphasic and that Se(0) was reduced continuously to Se(-II) (Figure 1 B). Figure 1 C shows the formation of Se(0) nanospheres (50–100 nm) on the surface of the rod-shaped *G. sulfurreducens* cells after 24 h reduction and is representative of samples both with and without AQDS. This spherical morphology has been reported previously for extracellular Se precipitates [22,25,27]. Figures 1 D and E show the Se(0) nanophases formed after 500 h reduction without and with AQDS, respectively. The composition of the Se(0) nanophases produced during the reduction of Se(IV) by *G. sulfurreducens* was confirmed by EDX and a representative spectrum of the particles in Figure 1 C to E is provided in Figure 1 F. A large difference in the size and morphology of the particles can be observed between Figures 1 D and E, highlighting the significance of the redox mediator in the reduction mechanism. Without AQDS, the end product was predominantly red amorphous Se(0), comprising disordered $[-\text{Se}-]_n$ chains and Se_8 -rings (Figure 1 D). Diffraction data (Figure 1 G), obtained only from the acicular structures of 1–2 μm in length, visible in Figure 1 D, show weak Bragg peaks at 23.5 and 29.7, corresponding to the (100) and (101) reflections of Se(0), respectively [5]. It has been reported that amorphous Se(0) is unstable at ambient temperatures, and in heating experiments undergoes a glass transition in the range 30–47°C, crystallization (to the trigonal form) in the range 120–150°C and melting $\sim 222^\circ\text{C}$ [40,41]; the activation energy for the crystallization is 40–60 kJ/mol [42]. Under the conditions described here, the red amorphous phase was still present after several months, suggesting that organic material associated with the *G. sulfurreducens* cells may play a role in stabilizing the Se(0) nanospheres. Kessi *et al.* [27] also reported that the amorphous Se allotrope produced by *Rhodospirillum rubrum* was stable for

Figure 1. Graphs to show reduction of Se(IV) (◆) and the formation of Se(0) (■) followed by Se(-II) (▲) using *G. sulfurreducens* with acetate, without AQDS (A) and with AQDS (B). TEM (whole mount) of *G. sulfurreducens* showing Se precipitates after 24 h Se(IV) reduction (C) and after 500 h Se(IV) reduction without AQDS (D) and with AQDS (E). EDX of Se precipitates (F). X-ray powder diffraction data for Se precipitates after 500 h Se(IV) reduction (G).

months in the presence of the cultures. The Se(0) produced in the presence of AQDS was predominantly in the form of stubby trigonal crystallites comprising ordered helical $[-\text{Se}_n]$ chains linked together by inter-chain van der Waals forces [43], as confirmed by the strong, sharp (100) and (101) reflections in Figure 1 G.

Reduction of Se(IV) by *G. sulfurreducens* using hydrogen as the electron donor occurs at a faster rate than that observed with acetate (Figure 2). Again, the reduction rate was improved by the addition of AQDS ($1.1 \text{ mmol. l}^{-1} \text{ Na}_2\text{SeO}_3 \text{ g}^{-1} \text{ (biomass) h}^{-1}$ with AQDS versus $0.8 \text{ mmol. l}^{-1} \text{ Na}_2\text{SeO}_3 \text{ g}^{-1} \text{ (biomass) h}^{-1}$ without), as indicated by IC. ICP–AES showed that reduction of Se(IV) corresponded to a decrease in total soluble Se, attributable presumably to the rapid formation of red insoluble Se(0), confirmed visually (Figures 2 A and B). No detectable Se(IV) was present in solution after reduction for 500 h both with and without AQDS. The dominant Se phase after 500 h reduction was soluble Se(-II), as confirmed by XAS and total Se in solution measured using ICP–AES, with 4.5 mmol. l^{-1} and 4.0 mmol. l^{-1} present in the solutions with and without AQDS, respectively. Again, the presence of both Se(IV) and Se(-II) at the same time is indicative of a continuous reduction mechanism from Se(IV) to Se(-II) (Figures 2 A and B). Formation of Se(0) nanospheres (50–100 nm) on the surface of the *G. sulfurreducens* cells after 24 h reduction is shown in Figure 2 C. Figure 2 D shows bright field and dark field TEM images of the stubby trigonal Se(0) crystallites formed after 500 h reduction, and is representative of the samples both with and without AQDS. The composition of the Se(0) nanophases produced during the reduction of Se(IV) by *G. sulfurreducens* was confirmed by EDX and a representative spectrum of the particles in Figures 2 C and D is provided in Figure 2 E. The crystal structure of the Se(0) after 500 h was confirmed by the diffraction data shown in Figure 2 F. The shape and size of crystallites formed by *G. sulfurreducens* using hydrogen as electron donor (Figure 2 D) and using acetate as the electron donor with AQDS (Figure 1 E) were very similar, but much less precipitated material was present in the samples with hydrogen owing to further reduction to the soluble Se(-II) phase. The soluble Se-bearing phase was analysed using XAS at the Se K-edge (Figures 2 G, H and I). The magnitude of the EXAFS oscillations (Figure 2 G) was very small indicating that Se was surrounded by weak scatterers. The best fit for the Fourier transform (Figure 2 H) gave no clear indication of any neighbouring atoms closer than five oxygens at $3.34 \text{ \AA}$, suggesting that the selenium had completely dissociated and was surrounded by water molecules (Table 1). This long distance reflects the attraction of the δ^+ hydrogen atoms towards the negatively charged Se(-II), so that the water molecules are orientated with the oxygens further away.

Hydrogen is a very weak scatterer and is only detectable by EXAFS in high-concentration solutions with very well-ordered water molecules around the dissolved ion [44]. The XANES (Figure 2 I) is distinct from that for Se(VI), Se(IV) or Se(0) and the lower edge position indicates that it is a more reduced species.

3.1.2 Se(IV) reduction by *S. oneidensis* using hydrogen as the electron donor

S. oneidensis is unable to metabolize acetate as an electron donor under anaerobic conditions [45]. Thus, hydrogen was used as the sole electron donor for reduction of Se(IV). IC showed that the Se(IV) reduction rate more than doubled upon addition of AQDS ($0.5 \text{ mmol. l}^{-1} \text{ Na}_2\text{SeO}_3 \text{ g}^{-1} \text{ (biomass) h}^{-1}$ with AQDS versus $0.2 \text{ mmol. l}^{-1} \text{ Na}_2\text{SeO}_3 \text{ g}^{-1} \text{ (biomass) h}^{-1}$ without). Although they were considerably less efficient than *G. sulfurreducens* cells, the *S. oneidensis* cells also employed a continuous mechanism to reduce Se(IV), through Se(0), down to Se(-II) (Figures 1 to 3). A significant concentration of Se(IV) (2.6 mmol. l^{-1}) remained in solution after 500 h reduction without AQDS, and overall very little Se(-II) was produced with or without AQDS. Figure 3 C shows Se(0) nanospheres on the surface of the rod-shaped *S. oneidensis* cells after 24 h reduction without AQDS. These are slightly larger nanospheres (100–250 nm) than those observed previously (Figures 1C and 2C) and those formed by *S. oneidensis* with AQDS (data not shown), which could be a result of the slower rate of reaction [46]. Klonowska *et al.* [25] also recorded spherical deposits of Se(0) attached to *S. oneidensis* cells and suggested that Se(IV) reduction occurs at the surface of the cell as it is related to the electron transfer capacity of cytochrome *c* on the outer membrane. Figure 2 D shows Se(0) nanospheres formed after 500 h reduction, and is representative of the samples both with and without AQDS. It can be seen that, after this longer period of reduction, three different sizes (~200, 100 and 50 nm) of Se(0) nanospheres are present, but it is unclear why these particular particle sizes appear to predominate. The composition of the Se(0) nanophases produced during the reduction of Se(IV) by *S. oneidensis* was confirmed by EDX and a representative spectrum of the particles in Figures 3 C and D is provided in Figure 3 E. The Se(0) nanospheres produced by *S. oneidensis* without AQDS were amorphous and no Bragg peaks were visible in the XRD data. However, with AQDS, the diffraction data (Figure 3 F) show weak Bragg peaks corresponding to the (100) and (101) reflections of Se(0). This sample was also a darker shade of red, suggesting the formation of a more (but poorly) crystalline product when AQDS was added.

Figure 2. Graphs to show reduction of Se(IV) (♦) and the formation of Se(0) (■) followed by Se(-II) (▲) using *G. sulfurreducens* with hydrogen, without AQDS (A) and with AQDS (B). TEM (whole mount) of *G. sulfurreducens* showing Se precipitates after 24 h Se(IV) reduction (C), and bright field and dark field TEM after 500 h Se(IV) reduction (D). EDX of Se precipitates (E). X-ray powder diffraction data for Se precipitates after 500 h Se(IV) reduction (F). Se K-edge (G) k^3 -weighted EXAFS spectra (H) Fourier transforms and (I) XANES spectra of soluble Se phase produced after 500 h Se(IV) reduction using *G. sulfurreducens* with hydrogen.

Table 1. Results of XAS data analysis for the soluble Se phase.

Organism	No. and type of atom	Shell radius (Å)	Debye–Waller factor (2Å ²)	R value/data range (k)	Suggested phase
<i>G. sulfurreducens</i>	5 O	3.34	0.035	30.5/3–8	HSe [−]
<i>V. atypica</i>	4 O	3.32	0.036	38.2/3–8	HSe [−]

Figure 3. Graphs to show reduction of Se(IV) (♦) and the formation of Se(0) (■) followed by Se(-II) (▲) using *S. oneidensis* with hydrogen, without AQDS (A) and with AQDS (B). TEM (whole mount) of *S. oneidensis* showing Se precipitates after 24 h Se(IV) reduction without AQDS (C) and after 500 h Se(IV) reduction (D). EDX of Se precipitates (E). X-ray powder diffraction data for Se precipitates after 500 h Se(IV) reduction (F).

3.1.3 *Se(IV) reduction by V. atypica using hydrogen as the electron donor*

The ability of *V. atypica* to reduce Se(IV) was assessed with hydrogen as the sole electron donor, because acetate did not support Se(IV) reduction under anaerobic conditions [20]. Figures 4 A and B show a vast difference in the ability of this organism to reduce Se(IV) with and without AQDS. The initial Se(IV) reduction rate for *V. atypica* cells without AQDS was $0.2 \text{ mmol. l}^{-1} \text{ Na}_2\text{SeO}_3 \text{ g}^{-1} (\text{biomass}) \text{ h}^{-1}$, and the dominant phase remained as Se(IV) after 500 h reduction (3.7 mmol. l^{-1}). With AQDS, the *V. atypica* cells were able to reduce Se(IV) at a rate of $1.1 \text{ mmol. l}^{-1} \text{ Na}_2\text{SeO}_3 \text{ g}^{-1} (\text{biomass}) \text{ h}^{-1}$, five times faster than without AQDS, and the soluble Se(-II) phase accounted for 100% of the Se after 500 h reduction. Figures 4 C and D show nanospheres on the surface of the *V. atypica* micrococci and intracellular nanospheres respectively, after 24 h reduction with AQDS. EDX suggested that the particles were Se-rich, in keeping with formation of elemental Se (Figure 4 E). The relatively small size of the nanospheres ($\sim 50 \text{ nm}$) could be a result of a faster rate of reduction [46]. After 500 h reduction without AQDS, the Se(0) precipitates remained as red nanospheres and the lack of Bragg peaks in the diffraction data confirmed that it was completely amorphous. The soluble Se(-II) phase produced after Se(IV) reduction for 500 h by *V. atypica* cells, with AQDS, was analysed using XAS at the Se K-edge (Figures 4 F, G and H). The weak EXAFS oscillations (Figure 4 F) were again indicative of weak scatterers surrounding the central Se atom. The best fit for the Fourier transform (Figure 4 G) indicated four oxygens at a very similar distance ($3.32 \text{ \AA}$) to that found for the Se(-II) produced by *G. sulfurreducens* suggesting that the selenium was present as dissociated HSe^- surrounded by water molecules (Table 1). The difference between the coordination numbers (N) in Table 1 is probably not significant as N in EXAFS can often have errors of 20%, especially in analysis of dilute solutions [47]. The XANES (Figure 4 H) is also very similar to that for the Se(-II) produced by *G. sulfurreducens* (Figure 2 I). It has been shown that the biogenic Se(-II) solution, produced by *G. sulfurreducens* and *V. atypica* under ambient conditions, as opposed to the hazardous, expensive production of NaHSe from Al_2Se_3 , can be employed in an aqueous-based, wet chemical synthesis for the fabrication of CdSe/ZnSe quantum dots [20,48].

3.1.4 *Comparison of mechanisms and kinetics of Se(IV) reduction by the different organisms*

The graphs showing the rate of Se(IV) reduction and the formation of Se(0) followed by Se(-II), with hydrogen and AQDS, using *V. atypica* (Figure 4 B), *S.*

oneidensis (Figure 3 B) and *G. sulfurreducens* (Figure 2 B), suggests that these organisms may employ different reduction mechanisms. For *V. atypica*, the production of Se(-II) occurred only after 100% of the Se(IV) had been reduced to Se(0) and was, therefore, a biphasic reaction. This type of biphasic reduction reaction for the production of Se(-II) has been reported previously for the Se(IV)-respiring Gram-positive haloalkaliphile, *B. selenitireducens* [49]. For *S. oneidensis* cells, production of Se(-II) was limited, but for *G. sulfurreducens* cells, a substantial amount of Se(-II) was produced by the continuous reduction of Se(IV) through Se(0) to Se(-II). These differences may be explained by considering the way in which these organisms potentially interact with electron acceptors. Zannoni *et al.* [2] proposed a general biochemical mechanism for producing Se(0) in bacterial cultures, involving the reaction between Se(IV) and reduced thiols, which are regenerated by the action of glutathione reductase and/or thioredoxin reductase; all of the organisms in this study may employ this intracellular mechanism to some degree. Indeed, the annotation for the sequenced organisms lists glutathione reductase and thioredoxin reductase for *S. oneidensis* and glutathione reductase for *G. sulfurreducens*. However, *G. sulfurreducens* and *S. oneidensis* can potentially use a different mechanism, involving *c*-type cytochromes on the outer membrane, which are implicated in the reduction of a wide range of metals, including Fe(III) oxides [50]. *S. oneidensis* has also been shown to release flavins as electron-shuttling compounds [51] and, although *G. sulfurreducens* has not been shown to synthesize electron-shuttling compounds, both of these organisms are able to use exogenous extracellular electron shuttles, such as AQDS, to transfer electrons from the cell to extracellular electron acceptors [52]. Unlike *Shewanella* and *Geobacter* species, *V. atypica* is not reported to possess high concentrations of cytochromes [26] and it is proposed that the production of reduced selenium species is achieved via a hydrogenase-coupled reduction, mediated by ferredoxin [53]. It is possible that *S. oneidensis* and *G. sulfurreducens* are better equipped to deal with both the soluble Se(IV) and the insoluble Se(0) as electron acceptors in the extracellular environment, owing to their extensive array of outer membrane cytochromes [37]. The difference in the ability of *V. atypica* to reduce Se(IV) with and without AQDS also supports this hypothesis. Without AQDS, *V. atypica* may only be able to transfer electrons to Se(IV) via the intracellular ferredoxin-mediated pathway. With a redox mediator, *V. atypica* has the potential to transfer reducing equivalents to extracellular Se(IV), generating both intracellular and extracellular Se(0) precipitates, which can be reduced subsequently to Se(-II).

Figure 4. Graphs to show reduction of Se(IV) (♦) and the formation of Se(0) (■) followed by Se(-II) (▲) using *V. atypica* with hydrogen, without AQDS (A) and with AQDS (B). TEM (whole mount) of *V. atypica* after 24 h Se(IV) reduction with AQDS showing extracellular Se precipitates (C), and bright field and dark field TEM showing intracellular Se precipitates (D). EDX of Se precipitates (E). Se K-edge (F) k^3 -weighted EXAFS spectra (G) Fourier transforms and (H) XANES spectra of soluble Se phase produced after 500 h Se(IV) reduction using *V. atypica* with hydrogen.

3.2. Identification of proteins associated with Se(0) nanospheres produced by *G. sulfurreducens*

Kessi *et al.* [27] reported that a protein content of about 20 mg of protein/mmol. I^{-1} of selenium was found in suspensions of Se(0) particles isolated from culture media after growth in the presence of Se(IV) and suggested the existence of a selenium–protein complex. However, successful identification of the proteins involved in these complexes has not, as yet, been achieved. To this end, biogenic Se(0) nanospheres produced during growth of *G. sulfurreducens* were isolated using a density-based separation technique. Figure 5 shows an SDS PAGE image of the nanoparticle-poor (A) and nanoparticle-rich (B) fractions obtained. The gel band (highlighted) produced by proteins associated with Se(0) nanospheres when reacted with a protein-specific stain was excised and the proteins identified by LC–MS/MS. The results suggest that the Se(0) nanoparticles produced by *G. sulfurreducens* were associated with a 15 kDa *c*-type cytochrome. Peptides were detected matching this protein with a sequence coverage of 31.5% and the location in the gel (Figure 5) matched the predicted molecular mass of the annotated protein. The *c*-type cytochromes have also been shown to be essential for the formation of extracellular UO_2 nanoparticles by *S. oneidensis* MR-13, and implicated in the reduction of a wide range of other metals [54]. This protein has not been characterized previously but analysis of the gene

sequence by the SignalP program [55] suggests post-translational secretion of the cytochrome, consistent with its association with extracellular Se nanospheres.

Proteolytic fragments of the redox-active, iron–sulphur protein ferredoxin oxido-reductase were also identified in the *G. sulfurreducens* band, suggesting that this organism may employ an intracellular ferredoxin-mediated pathway for the reduction of Se(IV) similar to that observed for *V. atypica*, followed by secretion or release of the Se nanospheres through cell lysis.

4. Conclusions

Anaerobic bioreactor systems have been shown to efficiently remove Se oxyanions from aqueous waste streams [56]. When evaluating Se biotreatment systems, it is essential to study the underlying biological mechanisms of reduction. The reduction rate and the nature of the selenium phases produced is strongly influenced by the nature of the organisms and by the reducing conditions employed. While this may present problems in terms of the formation of a dissolved Se(-II) species, rather than insoluble Se(0) that can be retained in the bioreactor, it does offer the potential to link bioremediation strategies with production of functional bionanomaterials. The products of Se(IV) biotransformations include: (i) red amorphous Se(0) nanospheres which act as an efficient sorbents for problematic vaporous contaminants, such as mercury [57], as well as having useful photo-optical and semiconducting properties for application in photocopiers, microelectronic circuits and solar cells [22]; (ii) black crystalline trigonal Se(0) which is a *p*-type extrinsic semiconductor and is used in producing photocells, photographic exposure meters, pressure sensors and electrical rectifiers because of its high photoconductivity [58]; and (iii) Se(-II), which can be used in the synthesis of fluorescent, semiconductor CdSe/ZnSe quantum dots with applications in biomedicine [59], electronics [60] and chemoselective photocatalysis [61].

It is also important to consider the potential impact of microbial processes on Se speciation in sediments. For example, in sediments dominated by *Geobacter* species, Se may well be fully reduced to the Se(-II) form where it could accumulate as FeSe [49], particularly if humics are present to act as redox mediators, whereas Se(0) would potentially be the major sink for Se oxyanions in sediments dominated by *Shewanella* species. However, careful examination of the total Se budget in sediment systems, including the impact of competing bio-methylation reactions [62], is required to fully assess the impact of different microbial communities.

This research represents the first attempt to identify the proteins present in protein–nanoparticle complexes, with the discovery that *c*-type cytochromes and ferredoxin are associated with the post reduction mineral

Figure 5. SDS-PAGE (gel) image of Se(0) nanoparticle-poor (A) and nanoparticle-rich (B) extractions from *G. sulfurreducens*. Molecular weight standards are far left. Box in (B) highlights protein band 15 kDa in size associated with biogenic Se(0) nanoparticles.

phases and may therefore be involved in the formation of Se(0) nanospheres by *G. sulfurreducens*. These protein–nanoparticle complexes could play a key role in bionanomineral synthesis by bacteria and a systematic study of the role of microbial proteins in nanoscale biomineralization processes will provide a valuable insight into the fate of nanoparticulate metals/metalloids in natural waters, as well as potentially facilitating the discovery of specific peptide sequences to template nanomaterials for technological applications.

Acknowledgements

The authors are greatly indebted to P. Lens for his organization of the G16 conference. The financial support from RCUK is acknowledged. The authors thank A. Bewsher for the IC analysis, P. Lythgoe for the ICP–AES and J. Waters for his assistance with XRD analysis. STFC Daresbury Laboratory are acknowledged for the provision of beamtime (grant no. 50242) and R. Bilsborrow is acknowledged for his assistance on 16.5. Portions of this work were performed at the Molecular Foundry, Lawrence Berkeley National Laboratory, which is supported by the Office of Science, Office of Basic Energy Sciences, US Department of Energy, under contract no. DE-AC02-05CH11231, with special thanks to R. Zuckermann (Biological Nanostructures Facility). Mass spectrometry was performed by L. Kohlstaedt at the Vincent J. Coates Proteomics Mass Spectrometry Laboratory, UC Berkeley and the authors also thank M. Wilkins for his advice in this area. The authors are grateful to J. Moreau for his assistance with density-based separation techniques and T. Prakash for invaluable discussions on the production of Se-based nanomaterials.

References

- [1] A. Bock, K. Forchhammer, J. Heider, W. Leinfelder, G. Sawers, B. Veprék, and F. Zinoni, *Selenocysteine – the 21st amino-acid*, Mol. Microbiol. 5 (1991), pp. 515–520.
- [2] D. Zannoni, F. Borsetti, J.J. Harrison, and R.J. Turner, *The bacterial response to the chalcogen metalloids Se and Te*, Advan. Microbial Physiol. 53 (2008), pp. 1–72.
- [3] J.B. Jones, G.L. Dilworth, and T.C. Stadtman, *Occurrence of selenocysteine in the selenium-dependent formate dehydrogenase of Methanococcus-Vannielii*, Arch. Biochem. Biophys. 195 (1979), pp. 255–260.
- [4] J.E. Stolz, P. Basu, J.M. Santini, and R.S. Oremland, *Arsenic and selenium in microbial metabolism*, Ann. Rev. Microbiol. 60 (2006), pp. 107–130.
- [5] P. Cherin and P. Unger, *The crystal structure of trigonal selenium*, Inorg. Chem. 6 (1967), pp. 1589–1591.
- [6] P.A.M. Rogers, S.P. Arora, G.A. Fleming, R.A.P. Crinion, and J.G. McLaughlin, *Selenium toxicity in farm-animals – treatment and prevention*, Ir. Vet. J. 43 (1990), pp. 151–153.
- [7] S.K. Dhillon and K.S. Dhillon, *Pools of selenium in some Indian soils at field capacity and submerged moisture regimes*, Aust. J. Soil Res., 42 (2004), pp. 247–257.
- [8] K.S. Dhillon and S.K. Dhillon, *Accumulation of selenium in sugarcane (Sachharum-Officinarum Linn) in seleniferous areas of Punjab, India*, Environ. Geochem. Health, 13 (1991), pp. 165–170.
- [9] R.A.P. Crinion and J.P. O'Connor, *Selenium intoxication in horses*, Ir. Vet. J. 35 (1978), pp. 81–86.
- [10] R.A.P. Crinion, *Distribution of selenium intoxication in Co Meath – a retrospective study*, Ir. Vet. J. 34 (1980), pp. 123–127.
- [11] G.Q. Yang, S.Z. Wang, R.H. Zhou, and S.Z. Sun, *Endemic selenium intoxication of humans in China*, Am. J. Clin. Nutri. 37 (1983), pp. 872–881.
- [12] R.S. Oremland, N.A. Steinberg, A.S. Maest, L.G. Miller, and J.T. Hollibaugh, *Measurement of in situ rates of selenate removal by dissimilatory bacterial reduction in sediments*, Environ. Sci. Technol. 24 (1990), pp. 1157–1164.
- [13] A.D. Lemly, *Aquatic selenium pollution is a global environmental safety issue*, Ecotoxicol. Environ. Safety, 59 (2004), pp. 44–56.
- [14] T.K. Raab, *Towards and ecosystem approach to remediation in the Great Basin*, in *Bioremediation of Contaminated Soils*, D.L. Wise, D.J. Trantolo and E.J. Cichon, eds, CRC Press, Boca Raton, 2000, pp. 1–22.
- [15] C.G. Wilber, *Selenium: A Potential Environmental Poison and a Necessary Food Constituent*, Charles C. Thomas Publisher, Springfield, 1983.
- [16] L.D. Koller and J.H. Exon, *The 2 faces of selenium – deficiency and toxicity – are similar in animals and man*, Can. Rev. Vet. Res., 50 (1986), pp. 297–306.
- [17] Y. Saito, Y. Yoshida, and T. Akazawa, *Cell death caused by selenium deficiency and protective effect of antioxidants*, J. Biol. Chem. 278 (2003), pp. 39, 28–439.
- [18] A. Shrift, *Selenium cycle in nature*, Nature 201 (1964), pp. 1304.
- [19] D.C. Miller and T.J. Webster, *Nanoparticulate selenium for anticancer orthopedic implants*, in *Cancer Nanotechnology*, H.S. Nalwa and T.J. Webster, eds, American Scientific Publishers, 2007, pp. 307–316.
- [20] C.I. Pearce, V.S. Coker, J.M. Charnock, R.A.D. Patrick, J.F.W. Mosselmans, N. Law, T. Beveridge, and J.R. Lloyd, *Microbial manufacture of chalcogenide-based nanoparticles via the reduction of selenite using Veillonella atypica: an in situ EXAFS study*, Nanotechnol. 19 (2008), pp. 1–13.
- [21] N. Ralston, *Nano-selenium captures mercury*, Nat. Nanotechnol. 3 (2008), pp. 527–528.
- [22] R.S. Oremland, H.J. Mitchell, J. Switzer Blum, S. Langley, T.J. Beveridge, P.M. Ajayan, M. Pulickel, T. Sutto, A.V. Ellis, and S. Curran, *Structural and spectral features of selenium nanospheres produced by Se-respiring bacteria*, AEM 70 (2004), pp. 52–60.
- [23] R.S. Dugan and W.T. Frankenberger, Jr, *Microbial transformations of selenium and the bioremediation of seleniferous environments*, Bioremediation J. 3 (1999), pp. 171–188.
- [24] J. Switzer Blum, A.B. Bindi, J. Buzzelli, J.F. Stolz, and R.S. Oremland, *Bacillus arsenicoselenatis, sp. nov. and Bacillus selenitireducens, sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic*, Arch. Microbiol. 171 (1998), pp. 19–30.
- [25] A. Klonowska, S.T. Heulin, and A. Vermeglio, *Selenite and tellurite reduction by Shewanella oneidensis*, AEM 71 (2005), pp. 5607–5609.
- [26] A.T. Johns, *Cytochrome b and bacterial succinic dehydrogenase*, Biochem. J. 49 (1951), pp. 559–560.
- [27] J. Kessi, M. Ramuz, E. Wehrli, M. Spycher, and R. Bachofen, *Reduction of selenite and detoxification of elemental selenium by the phototrophic*

- bacterium Rhodospirillum rubrum*, AEM 65 (1999), pp. 4734–4740.
- [28] J.W. Moreau, P.K. Weber, M.C. Martin, B. Gilbert, I.D. Hutcheon, and J.F. Banfield, *Extracellular proteins limit the dispersal of biogenic nanoparticles*, Science, 316 (2007), pp. 1600–1603.
- [29] J.R. Lloyd, C. Leang, A.L.H. Myerson, M.V. Coppi, S. Cuifo, B. Methe, S.J. Sandler, and D.R. Lovely, *Biochemical and genetic characterization of PpcA, a periplasmic c-type cytochrome in Geobacter sulfurreducens*, Biochem. J. 369 (2003), pp. 153–161.
- [30] N. Law, S. Ansari, F.R. Livens, J.C. Renshaw, and J.R. Lloyd, *Formation of nanoscale elemental silver particles via enzymatic reduction by Geobacter sulfurreducens*, AEM, 74 (2008), pp. 7090–7093.
- [31] E. Foubert Jr and H. Douglas, *Studies on the anaerobic Micrococci. II. The fermentation of lactate by Micrococcus lactilyticus*, J. Bacteriol. 56 (1948), pp. 35–36.
- [32] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, and D.C. Klenk, *Measurement of protein using bicinchoninic acid*, Anal. Biochem. 150 (1985), pp. 76–85.
- [33] F.C. Neidhardt, J.L. Ingraham, and M. Schaechter, *Physiology of the Bacterial Cell: A Molecular Approach*, Sinauer Associates, Sunderland, MA, 1990.
- [34] S.J. Gurman, N. Binsted, and I. Ross, *A rapid, exact curved-wave theory for EXAFS calculations*, J. Phys. C 17 (1984), pp. 143–151.
- [35] L. Hedin and S. Lundqvist, *Effects of electron–electron and electron–phonon interactions on the one-electron states of solids*, Solid State Phys. 23 (1969), pp. 1–181.
- [36] N. Binsted, R.W. Strange, and S.S. Hasnain, *Constrained and restrained refinement in EXAFS data analysis with curved wave theory*, Biochemistry 31 (1992), pp. 12,117–12,125.
- [37] B.A. Methé, K.E. Nelson, J.A. Eisen, I.T. Paulsen, W. Nelson, J.F. Heidelberg, D. Wu, M. Wu, N. Ward, M.J. Beanan, R.J. Dodson, R. Madupu, L.M. Brinkac, S.C. Daugherty, R.T. DeBoy, A.S. Durkin, M. Gwinn, J.F. Kolonay, S.A. Sullivan, D.H. Haft, J. Selengut, T.M. Davidsen, N. Zafar, O. White, B. Tran, C. Romero, H.A. Forberger, J. Weidman, H. Khouri, T.V. Feldblyum, T.R. Utterback, S.E. Van Aken, D.R. Lovley, and C.M. Fraser, *Genome of Geobacter sulfurreducens: metal reduction in subsurface environments*, Science 302 (2003), pp. 1967–1969.
- [38] J.K. Eng, A.L. McCormack, and A.L. Yates III JR, *An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database*, JASMS 5 (1994), pp. 976–989.
- [39] D.L. Tabb, W.H. McDonald, and J.R. Yates III, *DTASelect and Contrast: Tools for assembling and comparing protein identifications from shotgun proteomics*, J. Proteome Res. 1 (2002), pp. 21–26.
- [40] A. Peled and E. Hadziioannou, *Thermal characterization of amorphous selenium films obtained by low-temperature photodeposition*, J. Mater. Sci. 26 (1991), pp. 1769–1774.
- [41] S.O. Kasap and S. Yannacopoulos, *Kinetics of structural relaxations in the glassy semiconductor amorphous selenium*, Can. J. Mat. Res. 4 (1989), pp. 893–905.
- [42] H.Y. Zhang, Z.Q. Uhu, and K. Lu, *Transformation from the amorphous to the nanocrystalline state in pure selenium*, Nanostructured Mater. 5 (1995), pp. 41–52.
- [43] B.W. Corb, W.D. Wei, and B.L. Averbach, *Atomic models of amorphous selenium*, J. Non-Cryst. Solids, 53 (1982), pp. 29–42.
- [44] P. D’Angelo, V. Barone, G. Chillemi, N. Sanna, W. Meyer-Klaucke, and N.V. Pavel, *Hydrogen and higher shell contributions in Zn²⁺, Ni²⁺, and Co²⁺ aqueous solutions: an x-ray absorption fine structure and molecular dynamics study*, JACS 124 (2002), pp. 1958–1967.
- [45] K.H. Nealson and D.A. Saffarini, *Iron and manganese in anaerobic respiration: environmental significance, physiology and regulation*, Annu. Rev. Microbiol. 148 (1994), pp. 311–343.
- [46] K.D. Kim and H.T. Kim, *Comparison of the effect of reaction parameters on particle size in the formation of SiO₂, TiO₂, and ZrO₂ nanoparticles*, Mater. Lett. 57 (2003), pp. 3211–3216.
- [47] J.F.W. Mosselmans, G.R. Helz, R.A.D. Patrick, J.M. Charnock, and D.J. Vaughan, *Antimony speciation in sulfidic solutions determined by XAS*, Appl. Geochem. 15 (2000), pp. 879–889.
- [48] A.J. Rogach, A. Kornowski, M. Gao, A. Eychmüller, and H. Weller, *Synthesis and characterization of a size series of extremely small thiol-stabilized CdSe nanocrystals*, J. Phys. Chem. B 103 (1999), pp. 3065–3069.
- [49] M.J. Herbel, J.S. Blum, R.S. Oremland, and S.E. Borglin, *Reduction of elemental selenium to selenide: experiments with anoxic sediments and bacteria that respire Se-oxyanions*, Geomicrobiol. J. 20 (2003), pp. 587–602.
- [50] V.S. Coker, R.A.D. Patrick, G. van der Laan, and J.R. Lloyd, *Formation of magnetic minerals by non-magnetotactic prokaryotes*, in *Magnetoreception and Magnetosomes in Bacteria*, Series Microbiology Monographs, D. Schüller, ed., Springer, Heidelberg 2006, pp. 275–300.
- [51] H. von Canstein, J. Ogawa, S. Shimizu, J.R. Lloyd, *Secretion of flavins by Shewanella species and their role in extracellular electron transfer*, AEM 74 (2008), pp. 615–623.
- [52] V.S. Coker, A.M.T. Bell, C.I. Pearce, R.A.D. Patrick, G. van der Laan, and J.R. Lloyd, *Time-resolved synchrotron powder X-ray diffraction study of magnetite formation by the Fe(III)-reducing bacterium Geobacter sulfurreducens*, Am. Mineral. 93 (2008), pp. 540–547.
- [53] C.A. Woolfolk and H.R. Whitely, *Reduction of inorganic compounds with molecular hydrogen by Micrococcus lactilytus 1. Stoichiometry with compounds of arsenic, selenium, tellurium, transition and other elements*, J. Bacteriol. 84 (1962), pp. 647–658.
- [54] M.J. Marshall, S. Alexander, A.S. Beliaev, A.C. Dohnalkova, D.W. Kennedy, L. Shi, Z. Wang, M.I. Boyanov, B. Lai, K. Barry, K.M. Kemner, J.S. McLean, S.B. Reed, D.E. Culley, V.L. Bailey, C.J. Simonson, D.A. Saffarini, M.F. Romine, J.M. Zachara, and J.K. Fredrickson, *c-type cytochrome-dependent formation of U(IV) nanoparticles by Shewanella oneidensis*, PLOS Biol. 4 (2006), pp. 1324–1333.
- [55] J.D. Bendtsen, H. Nielsen, G. von Heijne, and S. Brunak, *Improved prediction of signal peptides: SignalP 3.0.*, J. Mol. Biol. 340 (2004), pp. 783–795.

- [56] M. Lenz, E.D. Van Hullebusch, G. Hommes, P.F.X. Corvini, and P.N.L. Lens, *Selenate removal in methanogenic and sulfate-reducing upflow anaerobic sludge bed reactors*, *Water Res.* 42 (2008), pp. 2184–2194.
- [57] N.C. Johnson, S. Manchester, L. Sarin, Y. Gao, I. Kulaots, and R.H. Hurt, *Mercury vapor release from broken compact fluorescent lamps and in situ capture by new nanomaterial sorbents*, *Environ. Sci. Technol.* 42 (2008), pp. 5772–5778.
- [58] H. Yin, Z. Xu, H. Bao, J. Bai, and Y. Zheng, *Single crystal trigonal selenium nanoplates converted from selenium nanoparticles*, *Chem. Lett.* 43 (2005), pp. 122–123.
- [59] M. Bruchez, Jr, M. Moronne, P. Gin, S. Weiss, and A.P. Alivisatos, *Semiconductor nanocrystals as fluorescent biological labels*, *Science* 281 (1998), pp. 2013–2016.
- [60] T. Trindade, P. O'Brien, and N.L. Pickett, *Nanocrystalline semiconductors: synthesis, properties, and perspectives*, *Chem. Mater.* 13 (2001), pp. 3843–3858.
- [61] M. Warrier, M.K.F. Lo, H. Monbouquette, and M.A. Garcia-Garibay, *Photocatalytic reduction of aromatic azides to amines using CdS and CdSe nanoparticles*, *Photochem. Photobiol. Sci.* 3 (2004), pp. 859–863.
- [62] G.M. Gadd, *Microbial formation and transformation of organometallic and organometalloid compounds*, *FEMS Microbiol. Rev.* 11 (1993), pp. 297–316.