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Developments in the Study and Applications of Bacterial Transformations of Selenium Species

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Abstract

Microbial bio-transformations of the essential trace element selenium are now recognised to occur among a wide variety of microorganisms. These transformations are used to convert the element into its assimilated form of selenocysteine, which is at the active centre of a number of key enzymes, and to produce selenium nanoparticles, quantum dots, metal selenides and methylated selenium species that are indispensable for biotechnological and bioremediation applications. The focus of this review is to present the state-of-the-art of all aspects of the investigations into the bacterial transformations of selenium species, and to consider the characterization and biotechnological uses of these transformations and their products.

Keywords

selenium species, bacterial selenium bio-transformation, selenium nanoparticles, selenides, selenium-containing quantum dots, methylated selenium species

Introduction

The phylogenetical diversity and distribution of bacterial Se bio-transformations are now recognised to be widespread. (1, 2) A variety of methods and techniques have been used in a bid to elucidate the different mechanisms that are involved in the microbial transformation of selenium species. The emphasis in most studies has been to demonstrate that selenite or selenate is transformed by the bacterium or bacterial consortia. Invariably, the products from such reactions are selenium nanoparticles (SeNPs), metal selenide and quantum dots (3), or the methylated selenium species concomitantly produced in the headspace and solution medium. (4-6) In other investigations, the focus was to localize where the biotransformation reactions are occurring in the cells (see **Scheme 1**).The experiments were conducted assuming that the detected selenium species are produced solely by the biochemical reactions that take place in the microorganisms under the incubation conditions. However, this may be a simplified interpretation of what is likely to be occurring. Until recently, complex interactions between bacterium cells forming biofilms, and the probability of abiotic reactions involving selenium-containing reactants generated by the biotic processes have been given scant attention. (4, 7, 8)

The aim of this review is to critically appraise information from recent literature on the microbial transformations of selenium species, their characterization, and to examine the developments and potential biotechnological uses of bacterial inspired selenium-containing products and related processes.

Outline of mechanisms of bacterial transformation of selenium species

Over the last decade, to the best of our knowledge, there have been no reports of the direct oxidation of reduced selenium compounds by microorganisms. Solubilization of elemental selenium (Se^0) can be mediated by microbial release of reactive sulfur compounds such as sulfite (SO_3^{2-}), sulfide (S^{2-}) and thiosulfate ($S_2O_3^{2-}$) via the formation of soluble selenosulfur complexes, as has recently been reported by Goff et al. for a *Bacillus* sp., presenting an example of "bio-induced" chemical weathering of Se^0. (9) Thus from the applied microbiology and biotechnology view point the reduction reactions of selenium oxyanions producing Se^0 or selenides Se^{2-}, which ultimately form nanostructures, and volatile selenium species, are of particular interest.

The oxyanion, selenate (SeO_4^{2-}) can be reduced by microorganisms during the course of anaerobic respiration, where it acts as the ultimate electron acceptor, and the process is mediated by selenate reductases. This has been shown for bacteria such as *Salmonella enterica* (10) and *E. coli*. (11) For *Thauera selenatis*, its selenate reductase was shown to be very similar to thermostable nitrate reductases (pNAR) found in hyperthermophilic archaea. (12) Other anaerobic methane-oxidizing bacteria have been recently shown to be capable of coupling methane oxidation to selenate reduction (13), suggesting a possible link between the biogeochemical cycles of selenium and methane. Subedi et al. have reported the simultaneous selenate reduction and denitrification by a consortium of bacteria from a mine-impacted natural marsh sediment.(14) Tan and co-workers have demonstrated a competitive reduction between SeO_4^{2-} and structurally similar sulfate (SO_4^{2-}) for the obligate aerobic bacterium *Comamonas testosterone*. When the genes responsible for the reduction of SO_4^{2-} ions are deleted, the reduction of SeO_4^{2-} ions to red Se^0 was not observed indicating that the reduction of selenate was catalysed by enzymes of the sulfate reduction pathway. (15)

The pathways of the more common SeO_3^{2-} reduction by different microorganisms include: (i) the so-called Painter-type reactions involving thiol groups (16); (ii) processes involving the thioredoxin – thioredoxin reductase system; (iii) siderophore-mediated reduction; (iv) sulfide-mediated reduction, and (v) dissimilatory reduction. Details of these mechanisms can be found in (1). According to Rauschenbach et al. (17) selenite reductases have not been characterized thus far, and investigators have failed to identify any for *Desulfurispirillum indicum* strain S5, a novel obligate anaerobe belonging to the phylum *Chrysiogenetes*, a dissimilatory selenate-, selenite-, arsenate-, nitrate- and nitrite-reducing bacterium. For *Rhizobium selenitireducens*, besides nitrite reductase involved in SeO_3^{2-} reduction, another protein showing selenate reductase activity was characterized. (18) It was shown to be a member of a protein family termed old-yellow-enzymes (OYE); the latter are often involved in protecting cells from oxidative stress and are generally active on a wide variety of substrates. Furthermore, a novel aerobic selenite reductase (CsrF) was identified in *Alishewanella*

sp. WH16-1, a facultative anaerobic bacterium isolated from mining soil capable of reducing SeO_3^{2-} to Se^0 nanoparticles as well as chromate (VI). (19) Recently, a selenite reductase in *Bacillus selenitireducens* specific for SeO_3^{2-} but not SeO_4^{2-}, AsO_4^{3-} or $S_2O_3^{2-}$ has been identified. (20)

A generalized scheme of the biotransformation of selenium compounds in a bacterial cell is shown in Scheme 1. Selenite is reduced to Se^0 mainly in reactions involving thiol-containing molecules and various oxidoreductases, while other proteins may also be involved in the reduction of both oxyanions. (16) Selenium oxyanions reduction results in the formation of amorphous red and other allotropic Se forms. The formation of intra- or extracellular SeNPs has been shown for the commonly studied *T. selenatis* (21); the plant-growth-promoting rhizobacterium *Azospirillum brasilense,* (16) methane-oxidising bacteria *Methylococcus capsulatus* and *Methylosinus trichosporus* (22) and many others. Information on the types of microorganisms (bacteria and fungi) involved in the reduction of selenium oxyanions has been published. (1-3, 23)

Volatile methylated species have been identified during Se biotransformation and these include: dimethyl selenide (CH_3–Se–CH_3), dimethyl diselenide (CH_3–Se–Se–CH_3) and dimethyl selenenyl sulfide (CH_3–Se–S–CH_3). (24) Interestingly, while the methane-oxidizing bacterium *Methylosinus trichosporium* was found to produce dimethyl diselenide and dimethyl selenenyl sulfide only, another methane-oxidizing bacterium, *Methylococcus capsulatus*, produced five volatile Se-containing substances. Besides the three dimethylated forms mentioned above, methyl selenol (CH_3–Se–H) and methylselenoacetate (CH_3–Se–C (=O) CH_3) were detected in the headspace (22). Reduction of organic forms of Se can result in the formation of volatile and highly toxic H_2Se, although ultimate microbial dissimilatory reduction of selenium species to selenides is limited in environmental microorganisms. (25)

Selenium oxyanions reduction mechanisms have been relatively well studied and reported in a number of articles and reviews (see for example: (1, 2, 16)).However, the formation of SeNPs (i.e., their assembly from precursors), and the factors regulating this process are yet to be elucidated. Processes for SeNPs formation inside cells with their subsequent release, as well as the removal of Se^0 precursors after the intracellular reduction of selenium oxyanions may involve unknown transport systems.(26-30) Tugarova et al. (31, 32), have shown that proton-dependent transport is involved in SeO_3^{2-} reduction. Inhibition of proton-dependent transport resulted in Se^0 accumulated as intracellular crystallites without formation of extracellular SeNPs.(32)

It has been proposed that SeNPs formation can proceed via Ostwald ripening. (26-27) However, biogenic SeNPs in contrast to chemically synthesized ones are always capped by various biomacromolecules, mainly proteins, polysaccharides and lipids (see for example (16,31,33-36), indicating that SeNPs formation is more complex than the Ostwald ripening process would suggest. A recent proposal is that the precursor for the Se^0 formation in methane-oxidizing bacteria is methyl selenol, and that the semi-

volatile methylated Se species polymerise to form particulate selenium allotropes (4). Lampis et al. proposed a possible biosynthetic mechanism of selenite reduction with the formation of SeNPs by the bacterium *Stenotrophomonas maltophilia*. They also identified an alcohol dehydrogenase homologue, possibly associated both with the biogenic synthesis of SeNPs and also involved in their stabilization. (27)

Cell-surface-bound SeNPs formation may have another role in addition to detoxification and that is to protect the microbial cells from high level of harmful effects of UV radiation via light absorption and/or scattering. Similar action of intracellular granules of polyhydroxyalkanoates (PHA; carbon and energy storage materials biosynthesized and accumulated by many prokaryotes) have been reported recently. (37, 38) Noteworthy is that both biogenic SeNPs (see (22, 32, 39, 40)) and chemically synthesized analogues (41, 42) have similar optical spectra of their aqueous suspensions, including their absorption in the UV region.Understanding the processes involved in the synthesis of SeNPs could be useful in the study of the biogeochemical origins of individual selenium -containing mineral deposits. Indeed, study of the genetic bases and diversity of the reduction processes will no doubt result in predictable and efficient production of useful industrial materials. These aspects are discussed below.

Diversity and distribution of selenium transforming organisms (gene analysis and culture-independent metagenomics)

The study of the diversity and speciation of selenium transforming microorganisms and communities by means of the metagenomic approach using high throughput sequencing analyses has been poorly represented when compared to studies based on culture dependent methods. In a majority of investigations, the focus was on highly speciated microbial cenoses inside specific conditioned environments, such as Se-amended bioreactors intended for the biosynthesis of valuable end-products, or in granular sludge from wastewater treatment plants. However, sparse information is available on the assessment of microbial communities in soil or plant rhizosphere.

Bai and co-workers reported changes in the microbial community structure found in a bioreactor designed for the oxidation of methane coupled to selenite reduction by bacteria. (43) There was a remarkable shift in the makeup of the denitrifying anaerobic methane oxidation (DAMO) community when selenite replaced nitrate as the electron acceptor after prolonged nitrate reduction. Alpha-, Beta- and Gammaproteobacteria as well as Igavibacteria increased in the presence of selenite, whereas Methanomicrobia and Nitrospira significantly decreased when compared to the composition of the community in the presence of nitrate. At genus level, *Methylococcus*, *Lautropia*, *Verribacter* and *Denitratisoma* – all belonging to Beta- and Gammaproteobacteria – were the most abundant in the presence of SeO_3^{2-}.

A metagenomic approach was also chosen in order to understand the composition of the microbial community selected after exposure to SeO_3^{2-} in anaerobic granular sludge from a fullscale reactor treating brewery wastewater. (44) High-throughput sequencing

of 16S rRNA gene showed that Negativicutes, Gammaproteobacteria and Clostridia were the most abundant classes in SeO_3^{2-} reducing microbial aggregates, with *Veillonellaceae* (ca. 20%) and *Pseudomonadaceae* (ca.10%) as the main families represented.

High-resolution phylogenetic analysis of anoxic contaminated soil amended with selenate revealed that the relative frequency of an operational taxonomic unit (OTU) from the genus *Dechloromonas* increased markedly from 0.2% to 36%. Multiple OTUs representing less abundant microorganisms from the *Rhodocyclaceae* and *Comamonadaceae* showed significant increases as well. (45) In a study of the rhizomicrobiome of Se hyperaccumulator and non-hyperaccumulator plants grown on seleniferous soil, Cochran and co-workers investigated the effect of selenium-hyperaccumulator plants on the diversity and composition of rhizosphere microbiomes. They found higher diversity of the OTUs in the rhizosphere of hyperaccumulator plants when compared to non-accumulators and the bulk soil.(46)The microbiome of the seleniferous soil was composed of taxa belonging mainly to *Crenarchaeota* (Archea), *Acidobacteria* and *Actinobacteria*, in contrast to hyperaccumulator plant rhizospheres in which *Acidobacteria*, *Crenarchaeota* (Archea) and *Proteobacteria* were dominant.

There are few examples of the exploitation of mixed microbial cultures for selenium species biotransformation. A consortium of four selenium tolerant rhizosphere aerobic bacteria belonging to *Bacillus* spp. was used to remove the element from Se enriched natural soils. (47) The strains were isolated from Se contaminated soils in the region of Punjab, India, by culture enrichment, and the consortium developed was tested on SeO_3^{2-} or SeO_4^{2-} spiked soils. While complete removal of Se was observed in SeO_3^{2-} augmented soils, 72% removal was recorded for the SeO_4^{2-} contaminated soils after 120 days. A methanogenic granular sludge from a bioreactor used for the treatment of paper waste streams has been shown to produce selenium sulfide (SeS_2) in a new process to recover Se from $SeO_4^{2-}$and SeO_3^{2-} polluted streams, where the former is reduced first to the latter which in turn reacts with sulfide to form SeS_2. (48) (See also the discussion on biofilms below.)

The recent reduction in the cost of high throughput sequencing analyses will allow the accumulation of a wide range and variety of sequencing data of microbial communities involved in selenium tranformation in different environmental matrices. The information will enable better understanding of the biogeochemical cycle of selenium in the environment and will probably furnish interesting information on the microbial species involved in the biotransformation of the element. At the same time, the information would be useful in identifying appropriate cultural conditions to apply in order to obtain new microbial isolates in axenic cultures for biotechnological exploitation.

The role of biofilms in the biotransformation of selenium species

Selenium biotransformation has been extensively described for planktonic cells; however, in the environment, microorganisms are commonly found as biofilms (49) where resistance to toxic metals is up to 600 times higher than in planktonic forms. (50) Moreover, bacteria at any stage of biofilm development are generally believed to be physiologically distinct from those in the planktonic state. (51)

As with planktonic cells, selenium also undergoes biotransformation into less bioavailable species in biofilms. (8, 52,53) The presence of Se altered the microbial diversity and induced structural changes in the biofilms. (8,53,54) Yang et al. (53) observed that a multispecies biofilm consisting of selenium-resistant *Rhodococcus* sp., *Pseudomonas* sp., *Bacillus* sp. and *Arthrobacter* sp., incubated aerobically in the presence of selenate or selenite transformed the selenium oxyanions into SeNPs, with SeO_3^{2-} more readily reduced than SeO_4^{2-}. The results showed that specific regional communities within the biofilms were responsible for selenium detoxification, as indicated by the localised distribution of reduced selenium species within the biofilm structure. The formation of SeNPs (size range 50−700 nm) was observed inside the bacterial cells and also shown to be associated with proteins and polysaccharides from the extracellular polymeric substances (EPS). Bioaccumulation of Se has also been observed in more complex, heterogeneous biofilms containing not only bacteria, but also diatoms and filamentous algae. Interestingly, in the more heterogeneous biofilm community, Se partitioned differently into the various components of the biofilm, with diatoms containing approximately two-thirds of the Se. Also, density-separated algae fractions from the biofilms showed that the concentration of Se was significantly higher in the fraction not containing filamentous green algae compared to the filamentous green algal fraction. (55)

The immobilization of selenium has also been observed under anaerobic conditions. A recent study by Tan et al. (8), using biofilms from an anaerobic sludge inoculum in the presence of SeO_4^{2-}, revealed that colloidal SeNPs were formed by microbial reduction within the biofilm matrix, and retained in the biofilm system. The study also addressed how the biofilm structure was affected, not only by the presence of SeO_4^{2-}, but also by the presence of other electron acceptors such as NO_3^- and SO_4^{2-}. Relatively thin and compact biofilms were formed in the presence of SeO_4^{2-} alone, while thicker biofilms occurred in the presence of NO_3^- or SO_4^{2-}. The thicker biofilms in the presence of NO_3^- or SO_4^{2-} revealed gas pockets within the biofilm matrix, likely to be due to the microbial production of gases. With respect to Se removal, the presence of NO_3^- did not have a stimulating effect showing similar removal efficiency to that grown in the presence of SeO_4^{2-} only. In contrast, the presence of SO_4^{2-} showed higher removal efficiencies and greater biomass growth when compared to SO_4^{2-} free treatments. A possible explanation for the increase in Se removal in the presence of SO_4^{2-} could be related to abiotic reactions possibly occurring between Se-containing species and S compounds within the biofilm matrix. (8, 56)

In biofilm-mediated biotransformation the biogenic elemental Se formed is retained in the biofilm matrix. In contrast, when using planktonic cultures, one major drawback is that the biogenic Se^0 remains in suspension as SeNPs for prolonged periods. (57-59) Under these conditions, further treatment such as electrocoagulation or precipitation is required to remove the SeNPs. (1,60, 61) The study of biofilms has provided evidence that selenium is immobilised in the biofilm matrix, thus modifying both its stability and bioavailability in the environment. (53) In addition, biofilms are to be preferred for effective and reliable biotransformation and sequestration of selenium.

Since diet is the primary route of Se exposure and uptake in vertebrates, Se bioaccumulation in biofilms, as the base of the food chain, could serve as the primary food source for benthic invertebrates and higher trophic organisms. (62) Moreover, differences in the proportions of bacteria, filamentous algae and/or diatoms in naturally occurring biofilms could lead to variations in Se accumulation in these ecosystems, as observed by Arnold et al. (55) Depending on how Se partitions between these various components, Se exposure via ingestion by higher organisms could vary, because these organisms may preferentially feed on specific biofilm components and, thus, be exposed to different concentrations of Se. (55,62) The use of biofilms for Se sequestration represents an important and viable means of Se-laden wastewater treatment and bioremediation of selenium-contaminated areas such as mine-impacted sites. (52, 53, 63)

Selenium immobilisation by biofilms is a complex phenomenon and has distinct dynamics and controlling factors. The composition of the microbial communities is a major determining factor in Se uptake and biotransformation by biofilms, and therefore the behaviour of each would be different.While Yang et al. (53) used a multispecies biofilm consisting of selenium-resistant bacteria, and Tan et al. (64) studied inocula from a reactor treating Se-laden wastewater, other biofilm communities may be severely affected by the presence of Se. Recently it was shown how SeNPs disrupted the quorum sensing signalling system of *Pseudomonas aeruginosa*, provoking a reduction of 80% in the volume of the bacterial biofilm, and demonstrating the potential use of SeNPs as effective antibacterial agents. (65) Physicochemical and environmental factors affect the growth of EPS-producing cells, influence the structure and composition of the biofilm matrix, and its role in Se uptake. (66) As described by Tan et al., (64) the presence of other electron acceptors (or, in general, other reducing or oxidizing species) may also affect the efficiency of Se uptake by biofilms. Aerobic or anaerobic conditions, maturity of the biofilm, duration of the interactions are parameters which determine the extent of Se uptake and thus biotransformation. Therefore, close monitoring and regulation of the experimental conditions is recommended in order to yield maximum Se removal. (66)

It is envisaged that the use of multispecies biofilms rather than isolated planktonic microorganisms for the remediation of Se-compounds in water reservoirs, the development of more efficient biofilm-based reactors (8,64, 67,68), the use of such

bioreactors for selenium removal from wastewater (69) and the exploitation of the biofilm microbes for the manufacture of biogenic Se nanospheres and nanorods will be the focus of future research. (69, 70) It is still unclear how biofilms are affected or modified in response to the stress caused by exposure to high levels of Se oxyanions, and what effects these changes have on the metabolic pathways of the element. In addition, the effects of the presence of selenium resistant microorganisms on the composition and overall behaviour of a mixed culture are poorly understood. More importantly, the impact on molecular level mechanisms describing quorum sensing signalling processes of transcription and translation of enzyme genes are yet to be elucidated. Studies aimed at reducing the knowledge gaps and to expand our understanding of the natural microbial interactions, dynamics and ecology in these bacterial communities, will greatly enhance the advantages of the use of biofilms for the biotransformation and immobilization of selenium. Developments in the knowledge underpinning the behaviour of biofilms will lead to the production of engineered synthetic microbial consortia with increased robustness, featuring communities able to compartmentalize functions, with simultaneous execution of multiple tasks and metabolic division-of-labour. (71)

Multidisciplinary approach for the characterization of selenium speciation in bacterial transformations

Over the years, a suite of complementary microscopic, spectroscopic, chromatography-mass spectrometric and synchrotron-based techniques have emerged for the characterization of the physical (size, morphology, structure, crystallography, etc.) and chemical (oxidation state, elemental composition, local coordination, chemical speciation, etc.) properties of selenium biotransformation products (22, 31-34, 72-75). A list of the techniques and the information they provide are summarized in Table 1.

The characterization of Se-containing particulates by Raman spectroscopy and Raman microscopy have been used to determine their size, morphology (76, 77), and to obtain structural data. (4, 22, 31, 32) Raman spectroscopic measurements can be used to differentiate between the various Se allotropes. The Se–Se stretching vibration mode in Raman spectra can be used to identify the structure of Se. Amorphous SeNPs exhibit a broadened Se–Se band at ~250 cm^{-1} as reported for SeNPs biosynthesized by azospirilla. (31, 32) Raman peaks corresponding to the symmetric stretching mode of trigonal Se occurs at 234 cm^{-1}, (72) the corresponding peak for monoclinic Se is located at 264 cm^{-1}, (78) while covalently bound sulfur can be revealed by the Se–S band around 352–377 cm^{-1}. (32, 73)

The nature of the organic matter (lipids, proteins, polysaccharides) associated with biogenic SeNPs has been investigated by infrared (IR) spectroscopy. (4, 22, 31, 34) IR spectroscopy has enabled the identification of the presence of polymeric materials surrounding the NPs and demonstrated their role in increasing the thermodynamic stability of biogenic SeNPs. (33) Amorphous Se (a-Se) is thermodynamically unstable

and undergoes transformation to trigonal Se at increased temperatures. Monoclinic Se (m-Se) is metastable and could also eventually undergo conversion to the trigonal form (t-Se). (79) Transformation of SeNPs from monoclinic nanospheres to t-Se nanorods by the cells of *Pseudomonas alcaliphila* was revealed by the use of a combination of TEM and Raman spectroscopy. (74) Ho et al. (80) described the process of transformation of a-Se nanospheres produced by *Shewanella* to t-Se nanostructures (e.g. nanowires, nanoribbons, nanorods, etc.) where organic solvents such as DMSO play a major role. In addition, the anaerobic biotransformation of a-Se nanospheres to t-Se nanorods has been shown for microbial granular activated sludge at a high temperature (55 °C). (75) Results from time-dependent SeNP experiments have shown that the cells of the strain *Stenotrophomonas bentonitica* and their proteins are able to transform amorphous Se^0 nanospheres to one-dimensional (1D) t-Se nanostructures (hexagons, polygons and nanowires) under mesophilic conditions.

Recently, modern spectroscopic and imaging techniques based on synchrotron radiation have been used to investigate the biotransformation of selenium by multispecies biofilms avoiding damage to the sensitive samples. (53) Information from the Se K-edge EXAFS analysis was used to demonstrate the ability of the biofilm to reduce selenite to SeNPs. In addition, nanoscale Se L_{III} edge Scanning Transmission X-ray Microscopy (STXM) showed the co-localization of elemental Se with microbial cells, EPS and lipids using the carbon K-edge. Structural and chemical data from the reaction products can be used to investigate Se biotransformation mechanisms (oxidation, reduction, etc.), to study the stability of the products and to inform the development of strategies for Se remediation.

Beside measurements on the bacterial material, samples from the headspace and medium should be included as a matter of course. The information produced by these measurements will serve to fill in the gaps in our understanding of the metabolic and non-metabolic processes that are involved in the biotransformation of selenium-containing species. Recently, Eswayah et al. have shown that it is possible using sorptive extraction followed by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) to investigate both the volatile and semi-volatile selenium species produced during the biotransformation steps, and based on their findings have proposed the mechanisms for the formation of SeNPs. (4)

All the above mentioned bulk spectroscopic and microscopic techniques are useful for the investigation of the chemical speciation and physicochemical properties of biogenic SeNPs. However, the heterogeneity that exists in SeNPs generated by complex biological systems (e.g. biofilms, granular activated sludge, microbial consortia) often makes it difficult to interpret chemical speciation and structure data by means of bulk techniques such as EXAFS spectroscopy. In recent years, the development of microscopic resolved synchrotron radiation using micro- or nano-focused based techniques (for example: micro (μ)EXAFS/XANES, μXRD, μinfrared spectroscopy, etc.) has created new opportunities for the investigation of the speciation and spatial

heterogeneity of the chemical elements associated with the selenium species (see, e.g. (81,82) for detailed discussion of some of these techniques). Other techniques which could provide information on the distribution of selenium species in bacteria include laser ablation-inductively coupled plasma-mass spectrometry and matrix assisted laser desorption ionisation-MS which can be used to localize and identify selenium-containing species and biomolecules associated with the selenium particulates, respectively.

Both the quantitative and qualitative distribution of the different Se species, and structures within complex biological/environmental samples can now be studied. The information from *in-situ* kinetic and thermodynamic properties of the biotransformations of SeNPs using synchrotron based techniques would provide the basis for comprehensive understanding of the processes which control the size and structure of the selenium-containing particulates. It is particularly so, since their environmental stability and industrial applications are intimately linked to their structural characteristics.

Bioremediation of selenium contamination

Remediation technologies involving microorganisms (bioremediation) offer an environment-friendly approach for the clean-up of pollution. (2, 8, 52, 83-85) Bioremediation of selenium in various environmental niches results in the reduction of selenium oxyanions and precipitation of solid Se^0 (SeNPs), together with the formation of volatile methylated selenium compounds (2, 22, 24, 25) thus reducing the total Se burden in the immediate vicinity of the pollution source.

In an approach developed by Barlow et al. (86) the selenite-reducing bacteria (*Bacillus subtilis*) were encapsulated in semi-permeable biodegradable polymeric membranes (polymersomes) to rapidly reduce dissolved SeO_3^{2-}. The bacteria remained viable throughout the synthesis of the polymersomes followed by proliferation when the incubation temperature was raised to 37 ºC, with rapid formation of biofilms and the conversion of soluble selenite (3 mM) to individual and clustered spherical SeNPs (~200–350 nm). The SeNPs remained entrapped in the membrane and as a result they were easily retrieved from the solution.

A new *Cronobacter* sp. isolated and enriched from domestic waste water was found to grow heterotrophically, using organic substrates such as acetate, lactate, propionate or butyrate as the electron donor, and to reduce selenite to SeNPs under microaerobic conditions. (87) The latter conditions were favourable for its growth and resulted in several-fold increased SeO_3^{2-} removal when lactate was used as the electron donor. In a different study, a UASB reactor was successfully used for ex situ bioremediation, where Se-rich soil was leached with water, followed by treatment of the leachate in which 90% of the Se was removed at a rate of ca. 44 µg Se per gram of granular sludge. (88) It has been shown that it is possible to remove selenite (20–100 mg L^{-1}) from high-salinity (70 g·L^{-1}) artificial waste water with removal efficiency of up to 98% using

aerobic sequencing batch reactors with activated sludge derived from a municipal wastewater treatment plant. (89) Mass balance analysis showed that bio-volatilization was the main route of selenium removal. A similar sequencing batch reactor with activated sludge under oxygen-limiting conditions has been successfully used to reductively remove up to 98% SeO_4^{2-} (1 mM) from waste water in the presence of 3% NaCl, with most of selenium accumulating in the sludge as micrometer-sized particles. (90)

Recently, biofilm of selenate-reducing bacteria was utilized in a model of a membrane biofilm reactor with H_2 as the electron donor, for simultaneous reduction and removal of SeO_4^{2-} (maximum removal efficiency up to ca. 50–61% depending on the conditions applied) and nitrate (up to 97–99.9%) from aqueous solutions.(91) It is generally accepted that microorganisms isolated from selenium-contaminated environments are more tolerant of Se compounds, and therefore more suited for selenium bioremediation. An example is the use of two *Lysinibacillus* spp. (*L. xylanilyticus* and *L. macrolides*) isolated from a Se-rich soil and shown to be capable of using both SeO_4^{2-} and SeO_3^{2-} as electron acceptors to produce Se^0 nanospheres (80–200 nm). (92)

The reduction of selenite to Se^{2-} by *E. coli* resulting in the formation of insoluble and thus much less toxic metal selenides, makes selenite-reducing microorganisms possible candidates for bioremediation of not only selenium-polluted lands, but also when mercury is presnt. (93) Mercury immobilization (Hg^0 is formed when Hg^{2+} is reduced) by biogenic SeNPs can be improved in the presence of soil-borne dissolved organic matter (DOM). DOM enhances the stability of the SeNPs resulting in up to 99% Hg immobilization. (94) The extent to which toxic methylmercury is formed in the presence of methylated selenium species and their effect on plant growth is of interest. (95)

Soil bacteria with phytostimulating properties and tolerance for selenium oxyanions can be used for the dual purpose of soil bioremediation and the promotion of plant growth. Several strains of bacteria of the widely studied genus *Azospirillum*, many of which display plant-growth-promoting traits, have been shown to be relatively tolerant to SeO_3^{2-} and to efficiently reduce it to SeNPs (31,32, 34, 35, 96, 97) and also to selenium–sulfur mixed NPs ($Se_{8-n}S_n$) in the presence of both selenite and high concentrations of sulfate (~0.8 g L^{-1}). (73) Recently, a *Herbaspirillum* sp., a plant-growth-promoting endophyte specific to the tea plant *Camellia sinensis* (L.), has been shown to be capable of reducing selenate (via selenite) to SeNPs in culture medium. Indeed, more than two-fold higher Se content was found in the plant leaves grown on selenate-spiked soil compared to the control plants. (36) The combined utilization of selenium oxyanion conversions to Se^0 and possibly other Se species that are relatively non-toxic and bioavailable to plants in addition to their plant growth-promotion traits are definitely of potential agricultural and agrobiotechnological significance.

Bacterial transformations in the production of biotechnologically useful products

Examples of biotechnologically useful selenium-containing products are summarized in Table 2. (29,30,32,40,48,73,77,87,99,100–116)

Se^{2-} ions can form largely insoluble metal selenides in the presence of appropriate heavy metal species, such as Hg^{2+}, Cd^{2+}, Cu^{+} or Cu^{2+}, etc. Microorganisms such as *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Saccharomyces cerevisiae* have been shown to reduce SeO_3^{2-} in the presence of the corresponding cations to form cadmium and zinc selenides (98–101). Incubation of the plant pathogenic fungus *Helminthosporum solani* in aqueous solution with $CdCl_2$ and $SeCl_4$ has been shown to produce small nanospheres of CdSe. (102) The Gram-negative bacterium *Pantoea agglomerans* was found to form Cu^{2+}- and Cu^{+}-containing black nanocrystallites ($Cu_{2-x}Se$) in the presence of Cu^{2+}–EDTA and SeO_3^{2-}, (103) exhibiting the ability to simultaneously reduce copper(II) to copper(I) and SeO_3^{2-} to Se^{2-}.

The first complete genome data have been recently reported for *B. cereus* (strain CC-1 isolated from marine sediments), a selenite/selenate-reducing and metal selenide-producing bacterium. (104) The putative genes involved in selenate/selenite reduction as well as in salt and metal resistance were identified, and the bacterium was shown to be capable of producing SeNPs (in the absence of heavy metal ions) or photoluminescent Bi_2Se_3, PbSe and Ag_2Se NPs when Bi^{3+}, Pb^{2+} or Ag^{+} nitrates, respectively, are present. The addition of 5 mM glutathione (GSH) significantly inhibited the formation of cell-bound Bi_2Se_3 nanosheet-like particles and instead SeNPs were formed. (105) Hence it was proposed that specific enzymes, instead of thiols, were responsible for the formation of metal selenides in this bacterium. In contrast, *Lysinibacillus* sp. was found to synthesize both extra- and intracellular Bi_2Se_3 nanosheets, formation of which was faster when 5 mM GSH was added indicating the existence of different mechanisms of biogenic nano-Bi_2Se_3 formation. (105)

Recently, there have been reports on the applications of microbial synthesized Se-containing NPs in chemotherapy, drug delivery, as well as in cancer diagnostics, prevention and treatment. (117–118) Biogenic SeNPs have been shown to exhibit antioxidant and anti-tumour activity, immunostimulatory and anti-inflammatory effects in animal models (106); for recent reviews, see (118,119–121). Investigations into the antimicrobial and antibiofilm activities of microbial synthesized SeNPs have shown that the surface bioorganic layers characteristic of biogenic nanostructures play important roles in their biochemical behaviour. (122)

Bacterial selenoproteins and selenoproteomes

Although the focus of this review has been on the visible changes in the chemical speciation of selenium species in the presence of bacteria, and the uses of the products of the biotransformation reactions, it is important to note that selenium is an essential element for bacteria. It is incorporated in a variety of prokaryotic selenoproteins, which are involved in biochemical redox functions. The mechanism and the genes responsible for the synthesis and insertion of selenocysteine, the amino acid at the

active centre of these proteins, have been described.(123-126) The unique genetic signature of this mechanism has provided researchers with the information that has enabled them to easily establish if a particular bacterium has the ability to synthesize selenoproteins from the examination of its complete sequenced genome.(127-129) Over 70 prokaryotic selenoprotein families have so far been identified but the biochemical roles of some are yet to be elucidated.(130) The variety of the selenoproteomes in each bacterium presents clues as to the extent to which it utilizes the element in it metabolism and its ability to tolerate exposure to high levels of selenium species. The deployment of the genomic approach for the screening and selection of suitable selenium-tolerant bacteria and to the study of selenium-rich environmental niches will yield information on how bacteria have evolved to use the element. In addition, it is probable that bacteria with the desirable characteristics, which can be harnessed to produce useful biotechnological products, will be identified.

Concluding remarks and future directions

The complexity of bacterial biotransformation of selenium species has only recently began to emerge. It is now clear that selenium biotransformation is widespread in diverse prokaryotes, some anaerobes, and certain clostridial species, while the focus of current research has been on planktonic microorganisms and their ability to convert selenium species to reduced selenium anions, elemental selenium, metal-selenide and quantum dots, methylated volatile and semi-volatile compounds. A holistic approach is therefore now required in order to gain a better understanding of the types of reactions that are not only occurring on the surfaces and inside bacterial cells but also in the culture medium and to characterize the products of such reactions. There have been few studies which replicate the conditions in selenium-rich environmental niches in which the microorganisms thrive by interacting with each other to form biofilms, and utilize selenium oxyanions in order to conserve energy. The application of functional gene analysis and metagenomics to the study of these microbial niches will provide a better understanding of how selenium biogeochemical cycle interacts with those of other elements leading to the identification of the key factors which influence, determine and underpin selenium biotransformation. These developments will enable the discovery and introduction of innovative biotechnological applications of the products thereof.

Compliance with Standards

Conflicts of interest There are no conflicts of interest to declare

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Table 1
Microscopic and spectroscopic techniques used to investigate the speciation of selenium and the structure of SeNPs produced by microorganisms

Technique	Information provided
X-ray Absorption Spectroscopy, XAS: (X-ray Absorption Near Edge Structure, XANES*; Extended X-Ray Absorption Fine Structure, EXAFS**)	Element specific technique Determination of local coordination of Se: *Oxidation state; VI, IV, 0, -II **Structural parameters of biogenic Se species: number and chemical identities of near neighbours atoms and the average interatomic distances up to 5-6 Å.
X-ray Photoelectron Spectroscopy	Surface chemistry of purified biogenic SeNPs (oxidation state, nature of functional groups of organic matter adsorbed to SeNPs surfaces, etc.) Elemental composition of surface-bound Se NPs of whole cells (outermost 10 nm of the cell wall)
X-Ray Diffraction	Determination of size and phase of SeNPs (amorphous, monoclinic, trigonal)
Infrared Spectroscopy	Compositional data: nature of organic matter (lipids, proteins, polysaccharides) associated with biogenic SeNPs Monitoring molecular-level changes in the structure and composition of cellular macrocomponents involved in the interactions with SeNPs.
Raman Spectroscopy	Sensitive to differences in various allotropic changes (amorphous, monoclinic, trigonal) and crystallinity of Se in SeNPs Composition of SeNPs (presence of Se-S, etc.)
Scanning Transmission Electron Microscopy (STEM) coupled with a High Angle Annular Dark-Field (HAADF)	Cellular localization of the biogenic SeNPs Elemental composition (S, Se, P, etc.) Crystallographic properties of the SeNPs
Variable Pressure Field Emission Scanning Electron Microscope (VP-FESEM)	Determination of size and chemical composition of SeNPs (interactions with organic matter including proteins, EPS, etc.)
Dynamic light scattering and zeta potential analysis	Particle size and surface charge

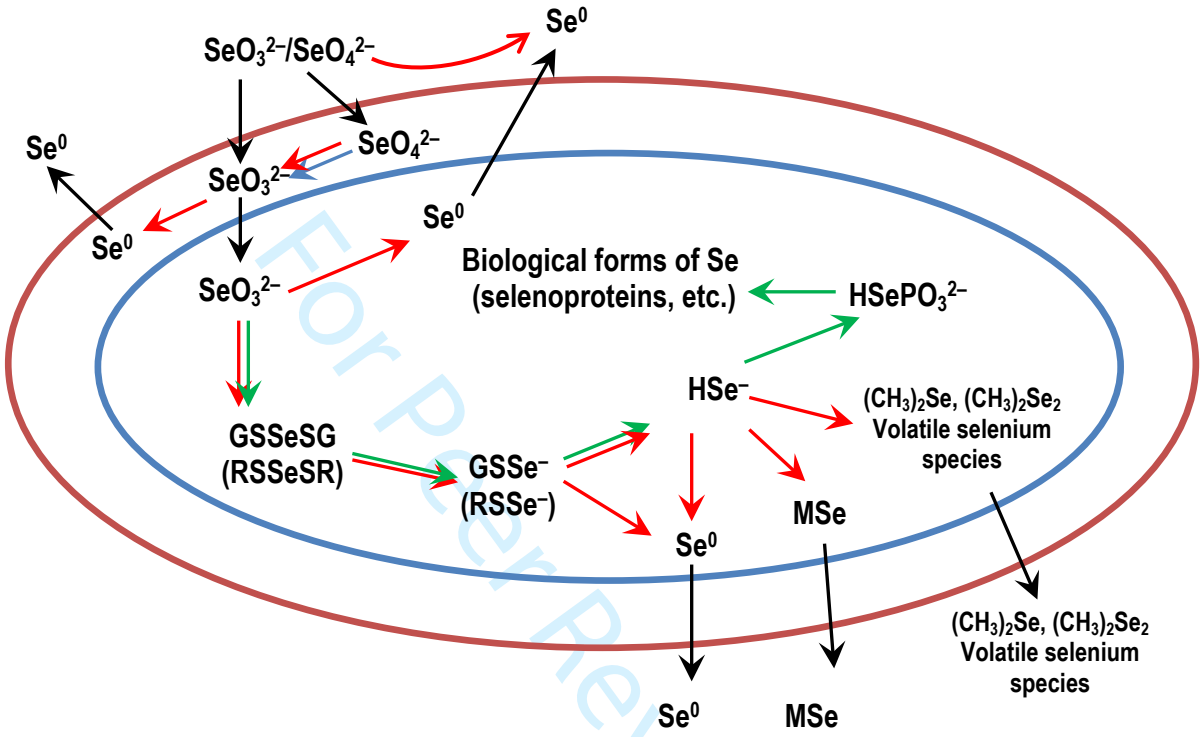
Table 2
Biotechnologically useful selenium-containing nano-sized products of microbial origin and conditions of their biogenic synthesis*

Compo-sition	Micro-organisms	Electron donors (medium) / electron acceptors	Conditions	Localisation, properties, morphology, size	Notes	Refer ences
Se^0	*Cronobacter* sp.	Acetate, lactate, propionate or butyrate / selenite	Microaerobic	Extracellular (aggregates)	Selenite bioreduction rates 0.10–0.24 $mM \cdot d^{-1}$	(87)
Se^0	*Cronobacter* sp.	Graphite felt electrode / selenite	Anaerobic electrotrophic bioreduction (at –0.3 V vs. SHE)	NPs (50 to 300 nm) attached to the electrode	Selenite bioelectroreduction rate 0.03 $mM \cdot d^{-1}$	(87)
Se^0	*Pseudomonas putida*	LB broth / selenite	Aerobic	Extracellular spherical NPs and aggregates (100–500 nm)	High selenite bioreduction rate (0.444 $mM \cdot h^{-1}$)	(107)
Se^0	*Pseudomonas aeruginosa*	Peptone nutrient broth / selenite	Aerobic	Extracellular (cell surface-bound), spherical, amorphous (~47–165 nm; average size ~96 nm)	Covered with a bioorganic layer (NPs characterised by a range of instrumental techniques)	(77)
Se^0	*Tetrahymena thermophila*	Proteose peptone medium / selenite	Aerobic	Intracellular amorphous spherical (50–500 nm), with irregular NPs	Covered with a bioorganic layer (including proteins); NPs characterised by a range of instrumental techniques)	(108)
Se^0	*Staphylococcus carnosus*	LB culture medium / selenite	Aerobic	Intracellular (isolated by cell disruption and separated); spherical (average sizes ~440–525 nm)	Associated with proteins. NPs showed considerable anti-nematode and antimicrobial activities	(109)
Se^0	A microbial community of anaerobic sludge	Lactic acid / selenate; selenium sulphide (SeS_2)	Anaerobic bioreduction of selenate or SeS_2 (precipitated during reduction of selenite by sulphide)	Amorphous nanospheres; hexagonal acicular crystallites (not attached to biomass)	Higher pH and temperatures are favourable for obtaining crystals (without a bioorganic 'coating')	(40,48)
Se^0	*Escherichia coli* (weakly virulent α-hemolytic	Culture broth / selenite	Aerobic	Intracellular spherical or ovoid NPs; 30–120 nm	Promising as an adjuvant (for the immunisation of livestock and poultry against	(110)

	strain B-5)				colibacillosis)	
Se^0	*Escherichia coli* (selenite reductase CsrF overexpressing strain)	LB culture medium / selenite	Aerobic	Intra- and extracellular irregular nanospheres (60–105 nm)	Covered with a bioorganic layer. High potential for adsorption and removal of dyes	(111)
Se^0	*Lactobacillus casei*	MRS culture broth (Sigma) / selenite	Anaerobic	Intracellular spherical NPs; 50–80 nm	Promising as a probiotic	(106)
Se^0	*Azospirillum brasilense*	Autotrophic (in physiological solution) / selenite	Microaerobic	Extracellular, spherical (~50–100 nm), amorphous	Covered with a bioorganic layer	(32)
$Se_{8-n}S_n$	*Azospirillum brasilense*	Malate-containing salt medium + 1 $g \cdot L^{-1}$ $(NH_4)_2SO_4$ / selenite	Aerobic (selenite reduction in the presence of an increased concentration of sulphates)	Extracellular, spherical (~400 nm), amoprhous	Covered with a bioorganic layer (NPs characterised by a range of instrumental techniques)	(73)
Se^0	*Mariannaea* sp.	Modified Martin medium with 1 $g \cdot L^{-1}$ glucose / SeO_2	Aerobic (at varying SeO_2 concentrations and pH 5–12)	Intracellular (~45 nm) or extracellular (~212 nm) crystalline spherical NPs	Extracellular localisation of NPs at alkaline pH. NPs associated with proteins	(30)
Se^0, Se^0–Te^0	Microbial community of methanogenic granular sludge	Anaerobic granular sludge (with lactate) / selenite + tellurite	Anaerobic (simultaneous reduction of selenite and tellurite)	EPS-entrapped crystalline Se^0, Te^0 and mixed Se^0–Te^0 irregular anisotropic nanostructures	First demonstration of mixed Se^0–Te^0 NPs formed by anaerobic microorgaisms	(112)
CdSe	*Veillonella atypica*	H_2 / selenite (with 0.1 mM AQDS as an electron shuttling compound)	Anaerobic (with further filtering the Se^{2-}-containing culture and adding Cd^{II}–GSH solution)	Fluorescent QDs; 2.3–3.6 (± 1.2) nm	Associated with a range of proteins and GSH as a capping agent	(100)
CdSe	*Helminthosporum solani*	Incubation in aqueous solution of $CdCl_2$ / $SeCl_4$	Aerobic (ambient conditions)	Extracellular monodisperse spheres (QDs; mean diameter 5.5 ± 2 nm)	Characterised by a range of instrumental techniques	(102)
$CdS_{0.5}Se_{0.5}$	*Staphylococcus aureus*	GSH / selenite	Aerobic; intracellular reduction (further interaction with Cd^{2+})	Intracellular uniform monodisperse nanocrystals (1.8 ± 0.5 nm; fluorescent QDs)	Low crystallinity; possible presence of a capping protein/peptide layer	(113)
CdSe	*Bacillus subtilis*	LB culture medium / selenite	Aerobic; intracellular reduction (further	Blocks of intracellular nanocrystals with angular shape	No isolation and chemical analysis of CdSe was performed	(99)

			interaction with Cd^{2+})	(fluorescent QDs)		
CdSe	*Saccharomyces cerevisiae*	Sterilised yeast extract peptone medium / selenite	Aerobic; Se^{IV}-exposed cells (in fresh medium) added to $CdCl_2$ solution	Intracellular QDs (isolated by cell lysis and homogenisation with further separation); ~2.8 nm	Biosynthetic protocol optimized by concentrations and times of exposure	(101)
CdSe	*Shewanella oneidensis*	LB medium / selenite	Anaerobic (incubation with selenite followed by $CdCl_2$ addition)	Intracellular high-purity uniform fluorescent QDs (~3.3± 0.6 nm)	Highest CdSe bioproduction rates. (Extracellular Se^0 NPs also obtained)	(29)
CdSe; CdSe/CdS	A methanogenic microbial consortium	Anaerobic granular sludge (with lactate) / selenite	Anaerobic (selenite reduction in the presence of Cd^{2+}–NTA complex)	Extracellular fluorescent CdSe and CdSe/CdS core-shell NPs (10–190 nm)	CdSe NPs capped by extracellular polymeric substances (contain impurities of Se^0 NPs)	(114)
CdSe	*Pseudomonas stutzeri*	GSH / selenite	Aerobic (selenite reduction in the presence of Cd^{2+})	Intracellular fluorescent QDs (isolated by cell disruption and separated); < 10 nm	Covered with a bioorganic layer (QDs characterised by a range of instrumental techniques)	(115)
$CdS_{1-x}Se_x$	*Tetrahymena pyriformis*	Proteose peptone medium / selenite	Aerobic (incubation with selenite followed by $CdCl_2$ addition)	Intracellular fluorescent QDs (isolated by cell lysis and disruption, separated and purified); 8.3 ± 0.8 nm	Optimised biosynthetic protocol; QDs characterised by a range of instrumental techniques	(116)
$Cu_{2-x}Se$	*Pantoea agglomerans*	Glucose-containing salt medium (with EDTA–Cu^{2+}) / selenite	Anaerobic	Extracellular uniform crystallites (~80 nm)	Capped by proteins (NPs characterised by a range of instrumental techniques)	(103)
Bi_2Se_3	*Lysinibacillus sp.*	Tryptic soy broth / selenite	Aerobic (selenite reduction in the presence of Bi^{3+} nitrate)	Extracellular (also intracellular) crystalline nanosheets (~60 nm; average thickness 5–6 nm)	Covered with a bioorganic layer (proteins). Promising for photothermal therapy against cancer cells	(105)
Se^0, Bi_2Se_3, PbSe, Ag_2Se	*Bacillus cereus*	Tryptic soy broth / selenite	Aerobic (selenite reduction to Se^0 or, in the presence of either of metal ions, to metal selenides)	Extra- and intracellular trigonal Se^0 NPs (without metal ions); extracellular crystalline photoluminescent PbSe and Ag_2Se, cell-bound Bi_2Se_3 (~10–50 nm)	Adding 1% PVP to the culture medium changed the size and morphology of Bi_2Se_3 and PbSe NPs	(104)

* Abbreviations: AQDS, anthraquinone-2,6-disulphonate ; EPS, extracellular polymeric substances; GSH, reduced glutathione; LB, liquid Luria-Bertani broth; NPs, nanoparticles; NTA, nitrilotriacetic acid; PVP, polyvinyl pyrrolidone; QDs, quantum dots; SHE, standard hydrogen electrode



Arrows indicate different processes:

- Anaerobic respiration
- Detoxification
- Assimilation
- Transport

G – glutathione;
R = thiol-containing proteins such as thioredoxin, bacillithiol;
M = metal (Cd, Cu, Pb, Hg).