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

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

2 Species

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21   **Abstract**

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

31   **Keywords**

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

34   **Introduction**

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

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

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Outline of mechanisms of bacterial transformation of selenium species

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

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

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

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97   sp. WH16-1, a facultative anaerobic bacterium isolated from mining soil capable of
98   reducing SeO_3^{2-} to Se^0 nanoparticles as well as chromate (VI). (19) Recently, a selenite
99   reductase in *Bacillus selenitireducens* specific for SeO_3^{2-} but not SeO_4^{2-}, AsO_4^{3-} or
100 $S_2O_3^{2-}$ has been identified. (20)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

215 **The role of biofilms in the biotransformation of selenium species**

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

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

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

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

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

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

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

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

314   **Multidisciplinary approach for the characterization of selenium speciation in**
315   **bacterial transformations**

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

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

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

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

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

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

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

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378 heterogeneity of the chemical elements associated with the selenium species (see, e.g.
379 (81,82) for detailed discussion of some of these techniques). Other techniques which
380 could provide information on the distribution of selenium species in bacteria include
381 laser ablation-inductively coupled plasma-mass spectrometry and matrix assisted laser
382 desorption ionisation-MS which can be used to localize and identify selenium-
383 containing species and biomolecules associated with the selenium particulates,
384 respectively.

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

Bioremediation of selenium contamination

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

400 In an approach developed by Barlow et al. (86) the selenite-reducing bacteria (*Bacillus*
401 *subtilis*) were encapsulated in semi-permeable biodegradable polymeric membranes
402 (polymersomes) to rapidly reduce dissolved SeO_3^{2-}. The bacteria remained viable
403 throughout the synthesis of the polymersomes followed by proliferation when the
404 incubation temperature was raised to 37 ºC, with rapid formation of biofilms and the
405 conversion of soluble selenite (3 mM) to individual and clustered spherical SeNPs
406 (~200–350 nm). The SeNPs remained entrapped in the membrane and as a result they
407 were easily retrieved from the solution.

408 A new *Cronobacter* sp. isolated and enriched from domestic waste water was found to
409 grow heterotrophically, using organic substrates such as acetate, lactate, propionate or
410 butyrate as the electron donor, and to reduce selenite to SeNPs under microaerobic
411 conditions. (87) The latter conditions were favourable for its growth and resulted in
412 several-fold increased SeO_3^{2-} removal when lactate was used as the electron donor. In a
413 different study, a UASB reactor was successfully used for ex situ bioremediation, where
414 Se-rich soil was leached with water, followed by treatment of the leachate in which
415 90% of the Se was removed at a rate of ca. 44 µg Se per gram of granular sludge. (88)
416 It has been shown that it is possible to remove selenite (20–100 mg L^{-1}) from high-
417 salinity (70 g·L^{-1}) artificial waste water with removal efficiency of up to 98% using

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418 aerobic sequencing batch reactors with activated sludge derived from a municipal
419 wastewater treatment plant. (89) Mass balance analysis showed that bio-volatilization
420 was the main route of selenium removal. A similar sequencing batch reactor with
421 activated sludge under oxygen-limiting conditions has been successfully used to
422 reductively remove up to 98% SeO_4^{2-} (1 mM) from waste water in the presence of 3%
423 NaCl, with most of selenium accumulating in the sludge as micrometer-sized particles.
424 (90)

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

434 The reduction of selenite to Se^{2-} by *E. coli* resulting in the formation of insoluble and
435 thus much less toxic metal selenides, makes selenite-reducing microorganisms possible
436 candidates for bioremediation of not only selenium-polluted lands, but also when
437 mercury is presnt. (93) Mercury immobilization (Hg^0 is formed when Hg^{2+} is reduced)
438 by biogenic SeNPs can be improved in the presence of soil-borne dissolved organic
439 matter (DOM). DOM enhances the stability of the SeNPs resulting in up to 99% Hg
440 immobilization. (94) The extent to which toxic methylmercury is formed in the
441 presence of methylated selenium species and their effect on plant growth is of interest.
442 (95)

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

457 **Bacterial transformations in the production of biotechnologically useful products**

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458   Examples of biotechnologically useful selenium-containing products are summarized

459   in Table 2. (29,30,32,40,48,73,77,87,99,100–116)

460   Se^{2-} ions can form largely insoluble metal selenides in the presence of appropriate

461   heavy metal species, such as Hg^{2+}, Cd^{2+}, Cu^+ or Cu^{2+}, etc. Microorganisms such as

462   *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Saccharomyces cerevisiae* have been

463   shown to reduce SeO_3^{2-} in the presence of the corresponding cations to form cadmium

464   and zinc selenides (98–101). Incubation of the plant pathogenic fungus

465   *Helminthosporum solani* in aqueous solution with $CdCl_2$ and $SeCl_4$ has been shown to

466   produce small nanospheres of CdSe. (102) The Gram-negative bacterium *Pantoea*

467   *agglomerans* was found to form Cu^{2+}- and Cu^+-containing black nanocrystallites ($Cu_{2-x}Se$)

468    $_x$Se) in the presence of Cu^{2+}–EDTA and SeO_3^{2-}, (103) exhibiting the ability to

469   simultaneously reduce copper(II) to copper(I) and SeO_3^{2-} to Se^{2-}.

470   The first complete genome data have been recently reported for *B. cereus* (strain CC-1

471   isolated from marine sediments), a selenite/selenate-reducing and metal selenide-

472   producing bacterium. (104) The putative genes involved in selenate/selenite reduction

473   as well as in salt and metal resistance were identified, and the bacterium was shown to

474   be capable of producing SeNPs (in the absence of heavy metal ions) or

475   photoluminescent Bi_2Se_3, PbSe and Ag_2Se NPs when Bi^{3+}, Pb^{2+} or Ag^+ nitrates,

476   respectively, are present. The addition of 5 mM glutathione (GSH) significantly

477   inhibited the formation of cell-bound Bi_2Se_3 nanosheet-like particles and instead SeNPs

478   were formed. (105) Hence it was proposed that specific enzymes, instead of thiols, were

479   responsible for the formation of metal selenides in this bacterium. In contrast,

480   *Lysinibacillus* sp. was found to synthesize both extra- and intracellular Bi_2Se_3

481   nanosheets, formation of which was faster when 5 mM GSH was added indicating the

482   existence of different mechanisms of biogenic nano-Bi_2Se_3 formation. (105)

483   Recently, there have been reports on the applications of microbial synthesized Se-

484   containing NPs in chemotherapy, drug delivery, as well as in cancer diagnostics,

485   prevention and treatment. (117–118) Biogenic SeNPs have been shown to exhibit

486   antioxidant and anti-tumour activity, immunostimulatory and anti-inflammatory

487   effects in animal models (106); for recent reviews, see (118,119–121). Investigations

488   into the antimicrobial and antibiofilm activities of microbial synthesized SeNPs have

489   shown that the surface bioorganic layers characteristic of biogenic nanostructures play

490   important roles in their biochemical behaviour. (122)

491   **Bacterial selenoproteins and selenoproteomes**

492   Although the focus of this review has been on the visible changes in the chemical

493   speciation of selenium species in the presence of bacteria, and the uses of the products

494   of the biotransformation reactions, it is important to note that selenium is an essential

495   element for bacteria. It is incorporated in a variety of prokaryotic selenoproteins,

496   which are involved in biochemical redox functions. The mechanism and the genes

497   responsible for the synthesis and insertion of selenocysteine, the amino acid at the

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

Concluding remarks and future directions

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

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Compliance with Standards

533 **Conflicts of interest** There are no conflicts of interest to declare

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536 **Ethical approval**

537 This article does not contain any studies with human participants or animals
538 performed by any of the authors.

539

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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

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Table 2
Biotechnologically useful selenium-containing nano-sized products of microbial origin and conditions of their biogenic synthesis*
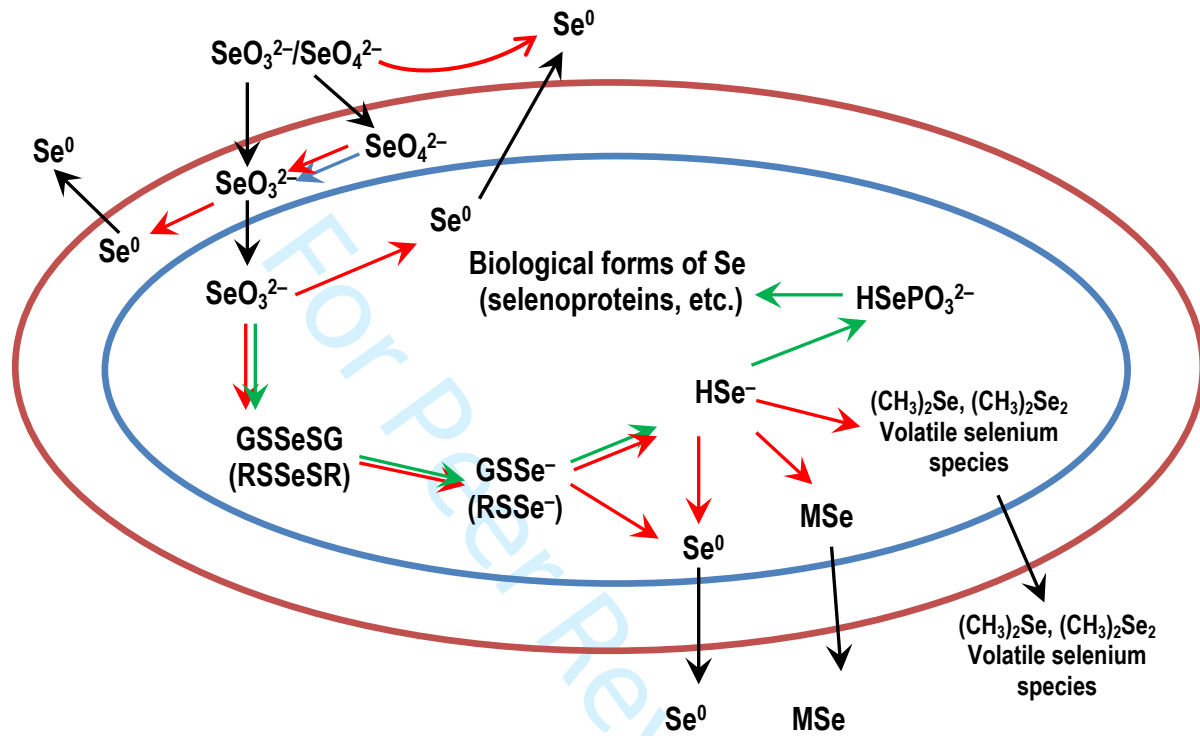
Composition	Micro-organisms	Electron donors (medium) / electron acceptors	Conditions	Localisation, properties, morphology, size	Notes	References
Se^0	*Cronobacter* sp.	Acetate, lactate, propionate or butyrate / selenite	Microaerobic	Extracellular (aggregates)	Selenite bioreduction rates 0.10–0.24 mM·d^{-1}	(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·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·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)

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	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 $g·L^{-1}$ $(NH_4)_2SO_4$ / selenite	Aerobic (selenite reduction in the presence of an increased concentration of sulphates)	Extracellular, spherical (~400 nm), amorphous	Covered with a bioorganic layer (NPs characterised by a range of instrumental techniques)	(73)
Se^0	*Mariannaea* sp.	Modified Martin medium with 1 $g·L^{-1}$ 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)

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			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)

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* 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

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Se^0

SeO_3^{2-}/SeO_4^{2-}

SeO_4^{2-}

Se^0

SeO_3^{2-}

Se^0

Se^0

Se^0

SeO_3^{2-}

Biological forms of Se (selenoproteins, etc.)

$HSePO_3^{2-}$

HSe^-

$(CH_3)_2Se, (CH_3)_2Se_2$ Volatile selenium species

GSSeSG (RSSeSR)

GSSe$^-$ (RSSe$^-$)

Se^0

MSe

Se^0

MSe

$(CH_3)_2Se, (CH_3)_2Se_2$ Volatile selenium species

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).