# Bacterial Metabolism of Selenium—For Survival or Profit

**Lucian C. Staicu and Larry L. Barton**

**Abstract** Selenium (Se) is transformed by phylogenetically diverse bacteria following several basic strategies which include: (1) satisfying a trace element requirement for bacterial synthetic machinery (*assimilatory* metabolism), (2) cellular energy production coupled to oxidation/reduction reactions (*dissimilatory* metabolism), and (3) detoxification processes. Some bacteria can use Se for respiration under limiting anaerobic conditions, generating energy to sustain growth. Under aerobic conditions, Se behaves as a toxicant and bacteria have evolved different strategies to counteract it. An important detoxification mechanism involves the formation of Se nanoparticles with a diminished toxic potential, but the cells have to properly manage these products in order to maintain their integrity. The bacterial metabolism of Se can be regarded as a survival mechanism when Se compounds prove to be highly toxic. Secondly, selenium is used to obtain energy in a nutrient-depleted environment, therefore allowing to specialized bacterial species to prevail over competitors that cannot exploit this resource. To achieve the Se metabolic activities, numerous unique enzymes are employed. While some enzymes have been isolated and are markedly specific for Se, many of the Se enzymes remain to be isolated. The formation of Se nanoparticles inside bacteria and the transportation mechanisms to the extracellular environment are still under debate. Se nanoparticles do not appear to play a nutritional (energy storage) or ecological function for bacteria, being by-products of bacterial metabolism. However, from a biotechnological standpoint, these conversions could be used to (1) clean up industrial effluents rich in Se and (2) to produce biomaterials with industrial applications (*biofactory*).

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**Abbreviations**

| | |
|---|---|
| DSeR | Dissimilatory selenate reduction |
| GSH | Glutathione |
| M | Molar |
| $NAD^+$ | Nicotinamide adenine dinucleotide |
| NP | Nanoparticle |
| QD | Quantum dots |
| ROS | Reactive oxygen species |
| Se | Selenium |
| $Se^0$ | Elemental selenium (zero valence state) |
| Se(IV) | Selenite, $SeO_3^{2-}$ |
| Se(VI) | Selenate, $SeO_4^{2-}$ |
| Sec | Selenocysteine |
| SefA | Selenium factor A |
| SeMet | Selenomethionine |
| $SeO_x$ | Selenium oxyanions (selenite and selenate) |
| SerABC | Selenate reductase isolated from *Thauera selenatis* |
| SOD | Superoxide dismutase |
| SRB | Sulfate-reducing bacteria |

## 1 Introduction

Microbial metabolism of selenium (Se) has only been studied marginally until the late 1980s mainly due to analytical limitations. What galvanized the research on this topic was a series of environmental pollution events having Se oxyanions, selenate and selenite, as the causative agents. The first major case occurred in North Carolina (USA) during the mid-1970s, when Se leached from the coal ash deposited in the vicinity of Lake Belews eliminated 19 out of 20 fish species (Lemly 2002). The second event took place in California (USA) as a result of extensive irrigation systems that led to the leaching of Se from seleniferous soils to Kesterson Reservoir. The high levels of bioaccumulated Se have been linked to deformities and death observed in the waterfowl and fish populations of the reservoir, triggering environmental actions (Presser and Ohlendorf 1987; Ohlendorf 1989). These environmental disasters prompted scientists to explore in more detail the microbial transformations of Se and their biogeochemical implications.

A major finding was the ability of some bacteria to use selenium for anaerobic respiration. This discovery shed light on the biogeochemistry of selenium, and the major contribution played by bacteria in the cycling of this element. The first

reductase with high affinity for selenium was identified in the periplasmic compartment of *Thauera selenatis*. Apart from their use as terminal electron acceptors, selenium compounds can behave as powerful toxicants, but bacteria have evolved different strategies to counteract their impact. A major strategy is the production of solid nanoparticles with a significantly lower toxicity. The scientific merits of investigating the bacterial metabolism of selenium consist not only in the elucidation of fundamental biogeochemical aspects, but also in the applied side of environmental research (e.g., the biological treatment of industrial effluents and the production of functional biomaterials).

This chapter discusses the central role of selenate and selenite in the selenium biogeochemical cycle with the formation of Se (nano)particles, both as a detoxification process and as a residual product of the energy generation process. We examine the toxicity of Se for bacteria and different avenues employed by bacteria in its detoxification. This review includes an overview of selenium dissimilatory reduction, the transmembrane movement of selenium, selenium stress response of bacteria, and regulatory processes associated with selenium metabolism. This chapter also presents several biotechnological applications founded on bacterial metabolism.

## 2 Selenium Biogeochemical Cycle

As part of the chalcogen elements (group 16 of the periodic table), selenium shares common properties with sulfur (S) and tellurium (Te). Unlike S that is abundant in the Earth's crust, Se is present in nano- to micromolar amounts and it rarely occurs in its native state (Kabata-Pendias 2000). In nature, Se is associated with metal-sulfide minerals (e.g., pyrite and chalcopyrite) and biolites/sedimentary rocks of biologic origin (e.g., coal, oil, and bituminous shales), but can also be enriched in seleniferous soils (Winkel et al. 2011).

Selenium has four oxidation states, (+VI), (+IV), (0), and (−II), that are commonly observed in biology. A biogeochemical cycle of Se comprising inorganic and organic forms that are transferred through different environmental compartments was first proposed by Shrift (1964). Following this seminal article, bacteria were later found to be involved in most transformations undergone by Se (Fig. 1).

Similar to S and Te, Se hydrolyzes in aqueous solutions to form oxyanions ($SeO_x$), selenate (Se[+VI], $SeO_4^{2-}$), and selenite (Se[+IV], $SeO_3^{2-}$). Both Se oxyanions are water-soluble, bioavailable, and toxic (Hamilton 2004). Selenium oxyanions are environmentally persistent as a consequence of their pH-independent solubility and limited interaction with cations (Chapman et al. 2010). The mechanism of toxicity is related to the incorporation of Se in sulfur-rich proteins and protein structures (e.g., sulfur-to-sulfur linkages) due to the chemical similarity between the two elements, which results in dysfunctional biomolecules (Stadtman 1974). In addition, selenium poisoning has also been linked to oxidative stress (Hoffman 2002).

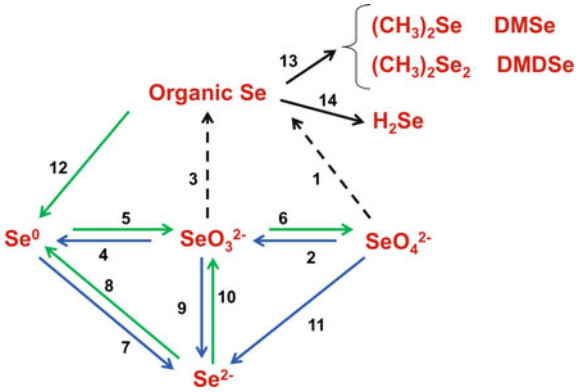


**Fig. 1** Selenium biochemical cycle. (*1*) Assimilatory selenate reduction, (*2*) selenate reduction (SerABC), (*3*) assimilatory selenite reduction, (*4*) selenite reduction to $Se^0$, (*5*) oxidation of $Se^0$, (*6*) selenite oxidation, (*7*) reduction of elemental selenium to selenide, (*8*) oxidation of selenide to $Se^0$, (*9*) selenite reduction to selenide, (*10*) selenide oxidation by aldehyde ferredoxin oxidoreductase, nitrite reductase, and peroxiredoxin, (*11*) selenate reduction where selenite does not accumulate as an intermediate, (*12*) thiol reductases, (*13*) methylation reactions by soil bacteria, (*14*) Se-cysteine lyase. *Dashed lines* (*1* and *3*) indicate assimilatory selenium reduction. *Green lines* (*5*, *6*, *8*, *10*, and *12*) indicate Se oxidation reactions, *blue lines* (*2*, *4*, *7*, *9*, and *11*) show oxidation reaction, and *solid black lines* represent methylation reactions (*13*) and production of $H_2Se$ (*14*), respectively

In contrast, elemental selenium, $Se^0$, is solid and insoluble in water, displaying a lower toxicological potential (Fernandez-Martinez and Charlet 2009). However, biogenic $Se^0$ (i.e., resulted through microbial metabolism) is bioavailable to filter-feeding mollusks (e.g., clams) and fish (Luoma et al. 1992; Schlekat et al. 2000). Other concerns have been raised with regard to its increased colloidal properties that make it stable in the water column and prone to long distance transport in aquatic ecosystems (Buchs et al. 2013; Staicu et al. 2015a).

In its most reduced valence state, selenide, Se(−II), selenium is unstable and reacts readily with metal cations by forming metal selenides (e.g., CdSe, ZnSe). Selenides can also be found as inorganic gaseous hydrogen selenide, $H_2Se$, or integrated in a variety of organic compounds including dimethylselenide, DMSe, dimethyldiselenide, DMDSe, two amino acids (selenocysteine and selenomethionine), and selenoproteins (Fernandez-Martinez and Charlet 2009).

Figure 1 shows the biogeochemical transformations of selenium in nature, stressing the importance of bacterial transformations in the cycling of this chalcogen element. Bacteria can reduce $SeO_4^{2-}$ either in an assimilatory manner (**1**), for its synthetic machinery (building blocks for amino acids and proteins), or in a dissimilatory manner (**2**), involving selenate reductases for energy generation under anaerobic conditions. $SeO_3^{2-}$ can be reduced by bacteria to generate organic Se (**3**) and $Se^0$ (**4**). On the oxidative side, $Se^0$ was documented to be bacterially oxidized to $SeO_3^{2-}$ (**5**) and $SeO_4^{2-}$ (**6**), although the reaction rates of these transformations are considerably slower than the reductive side of the cycle. The

generation and depletion of selenides proceed via $Se^0$ (**7**, **8**, and **12**) or directly from $SeO_3^{2-}$ (**9** and **10**) and $SeO_4^{2-}$ (**11**), without the transitory accumulation of $SeO_3^{2-}$. Apart from inorganic selenides, the organic lower valence states of Se are also a source of volatile organic Se (e.g., DMSe and DMDSe) (**13**) and volatile inorganic Se, $H_2Se$ (**14**) (Doran and Alexander 1977; Dugan and Frankenberger 2001). All these bacterial transformations will be discussed in detail in this chapter.

## 3 Dissimilatory and Assimilatory Selenium Reduction

The basic metabolic pathways for selenate could reflect the bacterial activities with other inorganic compounds, and the metabolism of sulfate and nitrate can be used as a reference. The assimilatory reduction of sulfate and the assimilatory reduction of nitrate produce sulfide and ammonia, respectively, for the synthesis of amino acids. Conversely, the dissimilatory reduction of sulfate and the dissimilatory reduction of nitrate produce hydrogen sulfide and $N_2$, respectively. In the dissimilatory reactions, sulfate or nitrate is the final electron acceptors and bacteria couple growth to the electron transfer reactions. Thus, it should be considered that there are two fundamentally distinct metabolic pathways for microbial cells to use selenate.

One pathway, which can be designated as *assimilatory* reduction, is the uptake and reduction of selenate for the synthesis of selenomethionine and selenocysteine to be used in selenium-containing enzymes and selenocompounds as cofactors in several enzymes (Fig. 2). Assimilatory selenate reduction would be used by both aerobes and anaerobes, and since few selenium-containing amino acids are required, a small quantity of selenate is required. In selenoproteins, selenium has structural and enzymatic roles, being an effective antioxidant and a key player in the thyroid hormone metabolism (Rayman 2000). Selenocysteine (Sec), the 21st proteinogenic amino acid, is encoded by the UGA codon that normally encodes the termination of protein translation. In general, Sec is located in the selenoenzyme's active sites. Selenoenzymes serve oxidoreductase functions arising from their ability to deplete reactive oxygen species (ROS). Additionally, various other functions have been documented including hormone activation and deactivation, protein folding, micronutrient delivery to internal organs, protein repair, and others (Labunskyy et al. 2014). More than 50 distinct selenoprotein families are currently known, and although their distribution varies greatly among species, they are present in all three domains of life (Labunskyy et al. 2014). Several examples of selenoenzymes include glutathione peroxidase (GPx), thioredoxin reductase, tetraiodothyronine deiodinase, selenophosphate synthetase, and selenoprotein P. The other selenoaminoacid, selenomethionine (SeMet), is randomly incorporated into proteins in place of methionine (Met) because $tRNA^{Met}$ has a limited capacity to discriminate between Met and SeMet (Schrauzer 2000). SeMet is considered a major natural food form of Se, being present in whole wheat grains, soybean, Brazil nuts, and seafood. Other organic forms of Se, DMSe and DMDSe, result from bacterial methylation processes.

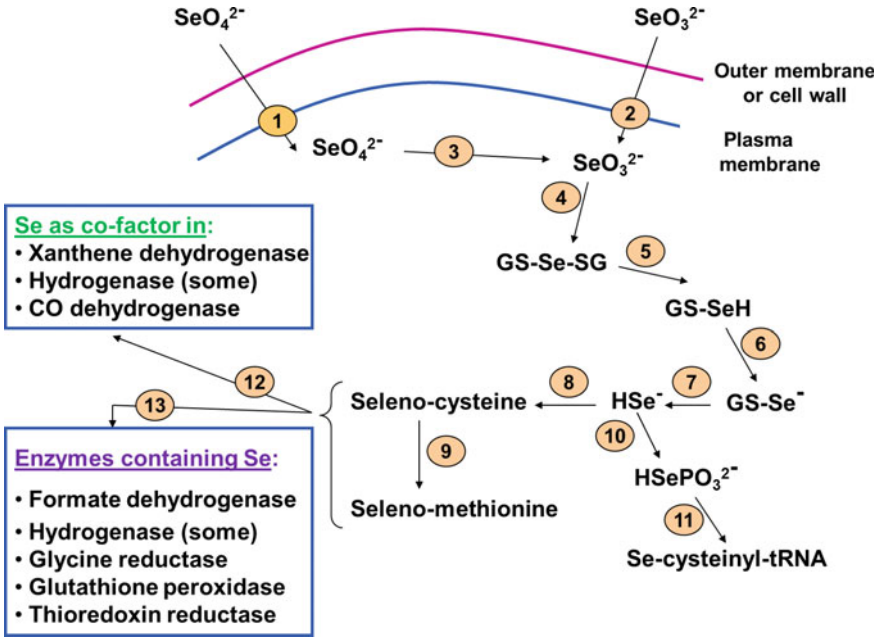


**Fig. 2** Assimilatory selenate and selenite reduction in prokaryotes. Designations are as follows: (*1*) sulfate or selenate uptake transporter; (*2*) selenite uptake transporter; (*3*) selenate reduction may use ATP sulfurylase; (*4–7*) reactions involving glutathione; *8* O-acetylserine thiol lyase; *9* methionine synthase; *10* selenophosphate synthetase; *11* L-selenocysteinyl-tRNA synthesis; *12* synthesis of selenium cofactors; *13* incorporation of Se-containing amino acids into enzymes

The second pathway, *dissimilatory* reduction, is the use of selenate as the final electron acceptor in metabolism with $Se^0$ as the commonly occurring product, while in some cases Se(−II) is generated (Fig. 3). In order to energize the plasma membrane of bacteria using dissimilatory selenate reduction, a considerable quantity of selenate would be reduced and this would result in appreciable levels of $Se^0$ as the end product. A difference in end products may be used to distinguish assimilatory selenate reduction from dissimilatory selenate reduction (see Fig. 2). Dissimilatory selenate reduction would result in copious quantities of $Se^0$ as a respiratory product, while detoxifying of selenate by the assimilatory selenate reduction pathway results in alkyl (methyl) selenide with minimal levels of $Se^0$. The dissimilatory selenate reduction process occurs in a few known anaerobic bacteria (Table 1) and the best characterized are *T. selenatis, Bacillus selenitireducens, Enterobacter cloacae* SLD1a-1, and *Sulfurospirillum barnesii. Cupriavidus* (formally classified as *Ralstonia*) *metallidurans* CH34 growing in the presence of selenate produces both alkyl selenide and $Se^0$ by a process primarily associated with assimilatory selenate reduction (Sarret et al. 2005). Bebien et al. (2002a) propose that in *Escherichia coli*, selenate reductase is located in cytoplasm which

suggests that the putative reductase has a physiological role in selenium assimilation rather than in respiration. Detoxification of selenate by bacteria that lack the selenate dissimilatory reduction may be primarily attributed to production of alkyl selenide and secondarily to $Se^0$ production.

Selenite reduction by bacteria may be by an assimilatory selenite reduction system and a detoxifying process (Fig. 3). At this time, there is no robust evidence for bacteria coupling selenite respiration to cell growth. Exposure of *Cupriavidus* (*Ralstonia*) *metallidurans* CH34 to selenite initially results in a slow uptake of selenite with similar quantities of alkyl selenide and $Se^0$ produced, but with continued exposure to selenite, high levels of $Se^0$ are produced leading Sarret and coworkers (2005) to conclude that a selenite uptake system was induced. An additional selenium pathway would be the reduction of selenate and selenite to $Se^0$ without coupling electron flow to the plasma membrane with the result that these reactions do not energize bacterial growth. The function of these reactions may be for detoxification.

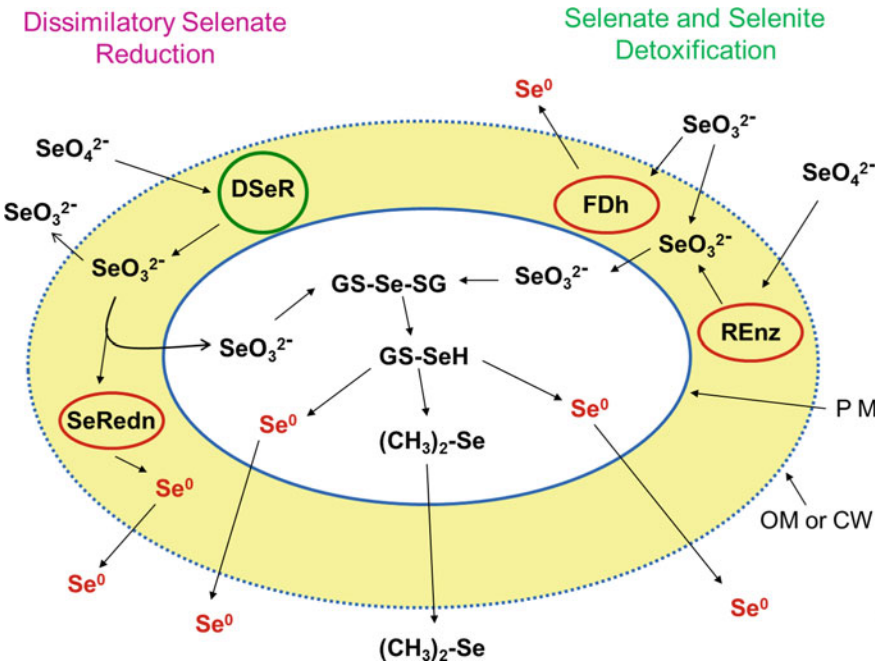


**Fig. 3** Model of selenium detoxification reactions and dissimilatory selenate reduction in bacteria. Designations are as follows: *CW* cell wall; $(CH_3)_2$-*Se* dimethyl selenide; *DSeR* dissimilatory selenate reductase; *FDh* fumarate reductase; *GS-Se-SG* selenodiglutathione; *GS-SeH* selenoglutathione; *OM* outer membrane; *PM* plasma membrane; *REnz* reductase enzyme; *SeRedn* selenite reductase

## 4 Bacterial Utilization of Selenate

Apart from oxygen, other electron acceptors (e.g., $NO_3^-$, $SO_4^{2-}$, $S^0$, Fe(III), etc.) can be utilized by bacteria for respiration as the final step of the electron transport chain. Because of their scarcity and the need for dedicated analytic tools for measurement, Se compounds have only been investigated as terminal electron acceptors by the end of the 1980s. The first selenate-respiring bacterium identified and fully characterized was *T. selenatis* (see **Box**). Table 1 provides abridged timeline of reports concerning selenate-respiring bacteria.

### Box. *Thauera selenatis*. Novel type of anaerobic respiration

*Thauera selenatis* was the first bacterium described to carry out anaerobic respiration on selenate, being isolated by Joan Macy and coworkers in 1989 in California from a bioreactor treating Se-rich effluents. Initially identified as a *Pseudomonas* sp. AX, four years later the taxonomic position was emended based on chemotaxonomic and ribosomal RNA sequencing to *T. selenatis*, a new genus within the beta subclass of Proteobacteria (Macy et al. 1993). The species is motile, Gram-negative, and rod-shaped with a single polar flagellum. The name of the genus was given in the honor of Rudolf K. Thauer, a German biochemist renowned for his work on

**Table 1** Chronology of selenate-respiring bacteria identification

| Isolate | Year of report[1] | $O_2$ requirements | DSeR[a] | Taxonomic affiliation | References |
|---|---|---|---|---|---|
| Strain SES-1* | 1989 | n/a | n/a | n/a | Oremland et al. (1989) |
| *Pseudomonas* sp. AX** | 1989 | Facultative anaerobe | Yes | | Macy et al. (1989) |
| *Thauera selenatis*** | 1993 | Facultative anaerobe | Yes | β-Proteobacteria | Macy et al. (1993) |
| Strain SES-3*** | 1994 | Strict anaerobe | Yes | | Oremland et al. (1994) |
| *Aeromonas hydrophila* | 1998 | Anaerobe | Yes | γ-Proteobacteria | Knight and Blakemoore (1998) |
| *Bacillus selenitireducens* | 1998 | Anaerobe | Yes | Bacilli | Switzer Blum et al. (2001) |
| *Bacillus arsenicoselenatis* | 1998 | Anaerobe | Yes | Bacilli | Switzer Blum et al. (2001) |
| *Sulfurospirillum barnesii* SES-3*** | 1999 | Strict anaerobe | Yes | ε-Proteobacteria | Stolz et al. (1999) |
| *Selenihalanaerobacter shriftii* | 2001 | Strict anaerobe | Yes | Clostridia | Switzer Blum et al. (2001) |

[a]Dissimilatory selenate reduction; *SES-1 was lost from culture prior to further investigation; **_Pseudomonas_ sp. AX was reclassified as *Thauera selenatis*; ***Strain SES-3 was reclassified as *Sulfurospirillum barnesii* SES-3; [1]The chronology is given based on the date (month) of publication; n/a, not available

anaerobes. *T. selenatis* was shown to couple the oxidation of acetate with the reduction of selenate, while selenite was the main product of selenate reduction. Conversely, acetate was oxidized to $CO_2$ and around 32% was recovered intracellularly as polyhydroxybutyrate (PHB) granules. Growth is also supported by a number of carbon sources, including alanine, arginine, aspartate, benzoate, citrate, fructose, glutamate, 3-hydroxybutyrate, isobutyrate, lactate, lactose, proline, propionate, pyruvate, serine, succinate, and sucrose, but shows limited growth on glucose (Macy et al. 1993). *T. selenatis* grows autotrophically with hydrogen, carbon dioxide, and air. Optimal growth occurs at 25–30°C, pH 8 (on nitrate), and pH 7 (on selenate). This organism can also grow aerobically or anaerobically with nitrate (which is reduced to $N_2O$, therefore being not fully denitrified) as the final terminal electron acceptor. However, nitrate does not interfere with selenate reduction. This indicates the presence of an enzyme with high affinity for selenate. This set of results led the authors to the conclusion that *T. selenatis* possesses a unique type of anaerobic respiration, based on selenate. *T. selenatis* cannot respire selenite, but can respire nitrite, its nitrogen oxyanion counterpart. Because several nitrite-deficient mutants could not precipitate red $Se^0$ out of selenite, a reasonable explanation has pointed toward the involvement of a nitrite reductase or a component of the nitrite respiratory system in the reduction of $SeO_3^{2-}$ (Fig. 4a) (De-Moll Decker and Macy 1993). An alternative explanation, not fully supported experimentally, proposes GSH as the thiol that reduces selenite in the cytoplasm, indicative of a potential detoxification mechanism (Fig. 4b) (Debieux et al. 2011). Another challenge raised by the formation of $Se^0$ particles in the cytoplasmic compartment is the need for these particles to pass through two physical barriers (plasma membrane and cell wall) on their way to the extracellular environment during the transportation stage. Apart from its fundamental aspects, *T. selenatis* was also considered from a biotechnological perspective when employed for the biological treatment of selenate-laden drainage effluents in bioreactor systems (see chapter "Industrial Selenium Pollution: Sources and Biological Treatment Technologies" from this volume).

## 5 Growth Coupled to Selenate Reduction

In an anaerobic environment, the reduction of selenate to selenite coupled to the oxidation of formate, acetate (Eq. 1) or lactate (Eq. 2), provides sufficient energy to support bacterial growth (Macy et al. 1989; Oremland et al. 1994):

$$\begin{aligned} &4SeO_4^{2-} + \text{acetate} + H^+ \rightarrow 4SeO_3^{2-} + 2CO_2 + 2H_2O \\ &\Delta G_f^\circ = -556\ \text{kJ mol}^{-1}\ \text{acetate} \end{aligned} \tag{1}$$

$$\begin{aligned} &2SeO_4^{2-} + \text{lactate} \rightarrow 2SeO_3^{2-} + \text{acetate} + HCO_3^- + H^+ \\ &\Delta G_f^\circ = -343.1\ \text{kJ mol}^{-1}\ \text{lactate} \end{aligned} \tag{2}$$

With *T. selenatis,* acetate is the electron donor for selenate reduction (Macy et al. 1989) and a model of this activity is shown in Fig. 4. In a similar reaction, *S. barnesii* and *Bacillus arsenicoselenatis* reduce selenate to selenite; however, lactate is the preferred electron donor (Oremland et al. 1994; Switzer Blum et al. 1998). If the bacteria are capable of metabolizing both selenate and selenite, then elemental red selenium is the end product of selenate reduction according to Eq. 3 (Oremland et al. 1994):

$$\begin{aligned} &2\mathrm{SeO_4^{2-}} + 3\,\mathrm{Lactate} + H^+ \rightarrow 2\,\mathrm{Se^0} + 3\,\mathrm{acetate} + 3\,\mathrm{HCO_3} + 2\,\mathrm{H_2O} \\ &\Delta G_f^\circ = -467.4\ \mathrm{kJ\ mol^{-1}}\ \mathrm{lactate} \end{aligned} \tag{3}$$

Apart from bacteria growing by selenate respiration with $Se^0$ as the end product that are listed in Table 1, other isolates able to respire $SeO_4^{2-}$ were reported: *Salana multivorans* (von Wintzingerode et al. 2001); *Enterobacter taylorae*

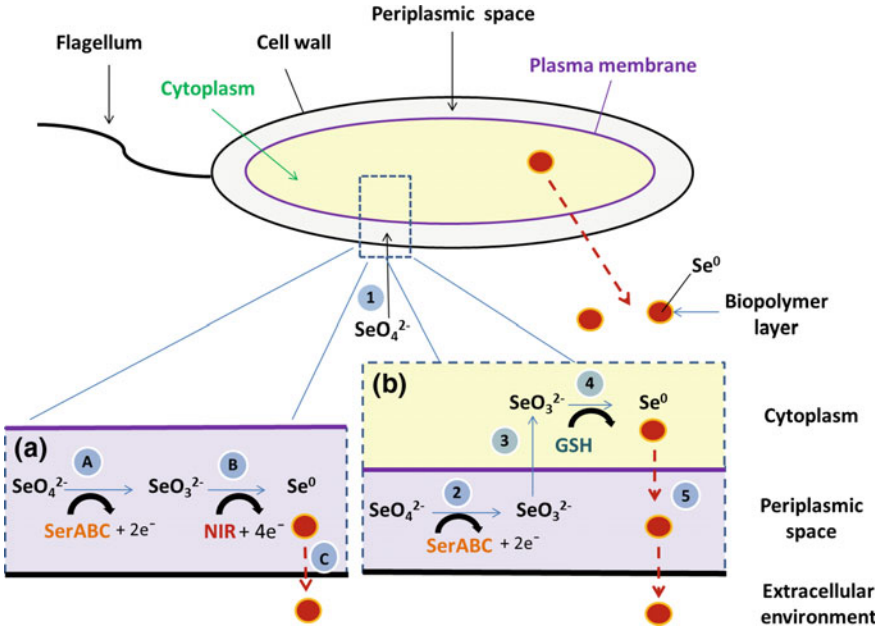


**Fig. 4** Selenium transformations in *Thauera selenatis.* **a** (*1*) Selenate enters the cell through the cell wall; (*A*) reduction of selenate to selenite in the periplasmic space, catalyzed by selenate reductase, SerABC; (*B*) reduction of selenite to elemental selenium in the periplasmic space, putatively catalyzed by nitrite reductase, NIR; (*C*) extrusion of elemental selenium coated with a biopolymer layer to the extracellular environment (Model according to Macy et al. 1993); **b** (*1*) selenate enters the cell through the cell wall; (*2*) reduction of selenate to selenite in the periplasmic space, catalyzed by selenate reductase, SerABC; (*3*) selenite transportation inside the cytoplasm; (*4*) reduction of selenite to elemental selenium mediated by thiols (Glutathione, GSH); (*5*) extrusion of elemental selenium coated with a biopolymer layer to the extracellular environment (Model according to Debieux et al. 2011). *Note* The electron donor was not represented

(Zahir et al. 2003); *Citrobacter freundii* (Zhang et al. 2004); *Ferrimonas futtsuensis* and *Ferrimonas kyonanensis* (Nakagawa et al. 2006); *Pelobacter seleniigenes* (Narasingarao and Haggblom 2007); *Pantoea* sp. SSS2, *Klebsiella* sp. WRS2, and *Shigella* sp. DW2 (Zhang et al. 2008); *Neisseria mucosa, Rahnella aquatilis,* and *Hafnia alvei* (Youssef et al. 2009); and *Desulfurispirillum indicum* (Rauschenbach et al. 2011).

Selenate respiration is not limited to members of the bacteria. Several species of hyperthermophilic archaea grow with selenate as the terminal electron acceptor and selenite accumulation (Huber et al. 2000). *Pyrobaculum arsenaticum* grows organotrophically with selenate respiration, and *Pyrobaculum aerophilum* grows lithoautotrophically with $CO_2$, $H_2$, and selenate. In order for selenate reduction to energize the cell, the electron flow would need to interface with the respiratory components in the cytoplasmic membrane and to generate a proton-motive force (PMF).

The reduction of selenate has been reported for other anaerobic bacteria; however, this reduction does not appear to energize cell growth. *Desulfovibrio desulfuricans*

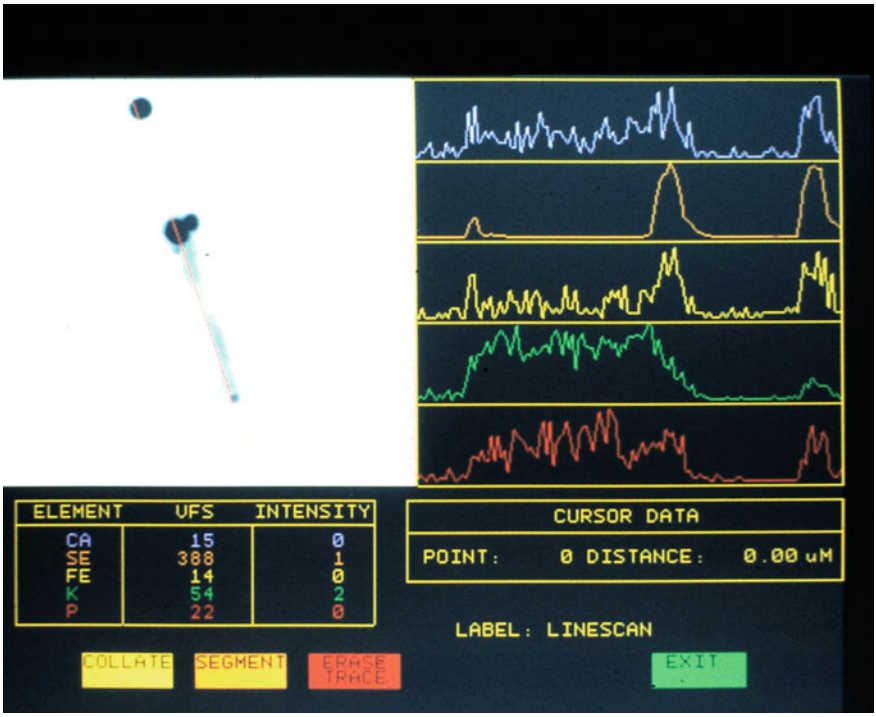


**Fig. 5** $Se^0$ produced by *Wolinella succinogenes* growing in media containing 0.1 mM selenite. The presence of Ca, Se, Fe, K, and P along the *red line* indicated in the figure is complemented with the analysis by energy-dispersive X-ray spectrometry provided in the right-hand panel (Barton et al. 2014, used with permission)

and *Wolinella succinogenes* reduce selenate to $Se^0$ without supporting growth on selenate reduction (Tomei et al. 1992, 1995). Chemical analysis of intracellular and extracellular $Se^0$ particles produced by *Wolinella succinogenes* is shown in Fig. 5. Suspended cells of *Desulfovibrio desulfuricans* subsp. *aestuarii* reduce selenate to selenide (Zehr and Oremland 1987). Under anaerobic conditions, *Azospira oryzae* reduces selenate by a protein with a molecular mass of about 500 kD, but this bacterium does not couple growth to the reduction of selenate (Hunter 2007).

## 6 Enzymatic Reduction of Selenate

The first selenate reductase purified and characterized, SerABC, was isolated from *T. selenatis* (Schroder et al. 1997). Later, the genes have been cloned and sequenced (Krafft et al. 2000). The enzyme was previously shown to be located in the periplasmic compartment of the cell, the place where selenate is reduced to selenite (DeMoll-Decker and Macy 1993). The selenate reductase is a soluble trimeric molybdo-enzyme comprising three heterologous subunits, αβγ, with an apparent molecular mass of ~180 kDa. The subunits have the following sizes: 96 kDa (α), 40 kDa (β), and 23 kDa (γ), as determined by SDS-PAGE. Molybdenum, iron, acid-labile sulfide, and heme *b* have been identified as cofactors. Ser A, which is the catalytic subunit, coordinates a molybdopterin cofactor. Ser B is an iron–sulfur protein rich in cysteine residues, comprising both [3Fe–4S] and [4Fe–4S] clusters (Dridge et al. 2007). SerC contains a *b*-type cytochrome with a standard reduction potential of +234 mV (Lowe et al. 2010). This means that the reduction of selenate could occur at an unusually high redox potential and one explanation for this could be the higher potential of the selenate/selenite couple, +475 mV. The enzyme displays high affinity ($K_m$) for selenate, 15.9 μM, as determined by using reduced benzyl viologen as the artificial electron donor. In contrast, other anions including nitrate, nitrite, chlorate, and sulfate did not serve as substrates, indicative of a separate reduction pathway characteristic to selenate. Even if *T. selenatis* can grow anaerobically using nitrate as the electron acceptor, selenate reductase was shown to be dissimilar to the periplasmic nitrate reductases purified from other bacteria. However, *T. selenatis* can reduce both selenate and nitrate concomitantly if co-present and, surprisingly, the reduction progresses to red $Se^0$ (Macy et al. 1989). The fact that nitrate and selenate were reduced at the same time led the authors to the conclusion that different terminal reductases were involved (Rech and Macy 1992). Since SerABC activity was not detected when *T. selenatis* was grown on nitrate, only on selenate, one plausible explanation could be that the enzyme is inducible. Furthermore, some periplasmic *c*-type cytochromes that have been identified as electron carriers to SerABC in vitro were shown to be upregulated in *T. selenatis* cultures exposed to selenate (Lowe et al. 2010). Interesting results came out when SerABC was subjected to increasing temperatures, showing activity and stability at temperatures up to 65 °C (Dridge and Butler 2010). Similar characteristics were found in thermostable reductases from hyperthermophilic bacteria,

suggesting that selenate respiration could be an ancient metabolic process that predates the advent of the oxygenic revolution. In addition, tungsten (W) was shown to substitute for molybdenum (Mo) at the active site of selenate reductase when *T. selenatis* was grown on tungstate-rich medium. The selenate binding affinity of W-Ser, 0.7 μM, was 23-fold higher than that of Mo-Ser.

*Enterobacter cloacae* SLD1a-1, a bacterium isolated from Se-contaminated drainage water in the San Joaquin Valley, California, expresses an insoluble membrane-bound selenate reductase only under aerobic conditions. When grown anaerobically on non-fermentable carbon sources, it cannot utilize selenate as the sole electron acceptor (Losi and Frankenberger 1997). The selenate reductase of *E. cloacae* SLD1a-1 is a heterotrimeric complex with a molecular mass of ~600 kDa (Watts et al. 2003). The enzyme was shown to be a molybdo-enzyme located in the plasma membrane facing the periplasmic compartment. The subunits are designated: α (~100 kDa), β (~55 kDa), and γ (~36 kDa). One major particularity of this enzyme is that it can convert selenate to $Se^0$, while selenite is a transitory intermediate. The reduction repertoire of this enzymes also includes chlorate [$Km(ClO_3^-)$ = 3.0 mM; $V_{max}$ = 0.035 μmol $ClO_3^-$ $min^{-1}$ $mg^{-1}$] and low levels of bromate. The enzyme does not display nitrate, nitrite, sulfate, perchlorate, DMSO, TMAO, and thiosulfate reductase activity (Ridley et al. 2006). Since selenate cannot be used for respiration, the selenate reductase of *E. cloacae* SLD1a-1 may function in the detoxification of Se oxyanions.

## 7 Structure of Selenate Reductases

Several selenate reductases have been described, but not all studied in detail (Table 2). The configuration of the selenate reductase subunits of *B. selenatarsenatis* and *T. selenatis* has been described by Kuroda et al. (2011). The subunit content and location of the selenate reductase of *B. selenatarsenatis* was deduced from *B. selenatarsenatis* molecular analysis. With both bacteria, the reduction of

**Table 2** Properties and kinetic parameters of bacterial selenate reductases

| Bacterial source | $K_m$ (mmol) | $V_{max}$ (μmol $min^{-1}$ $mg^{-1}$) | pH optimal | Molecular mass (kDa) | Cofactor | Localization |
|---|---|---|---|---|---|---|
| *Thauera selenatis*[a] | 0.016 | 3.84 | 6 | 180 | Mo | Periplasmic compartment |
| *Sulfurospirillum barnesii*[b] | 0.012 | 28.2 | n/a | n/a | n/a | Cytoplasmic membrane |
| *Escherichia coli*[c] | n/a | n/a | n/a | 115 | Mo | Cytoplasm |
| *Enterobacter cloacae*[d,*] | 2.1 | 0.5 | n/a | 600 | Mo | Cytoplasmic membrane |

[a]Schroder et al. 1997 (SerABC); [b]Oremland et al. 1999; [c]Bebien et al. 2002a; [d]Ridley et al. 2006; [*]Strain SLD1a-1; Mo, molybdenum; n/a, not available

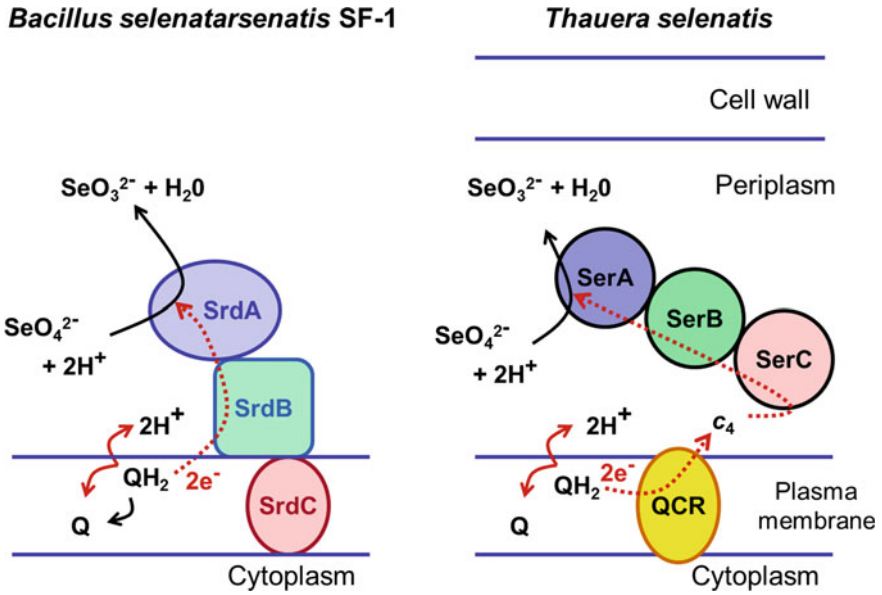


**Fig. 6** Models of dissimilatory selenate reduction in *Bacillus selenatarsenatis* SF-1 and *Thauera selenatis* (adapted from Kuroda et al. 2011). Designations are as follows: *c4* cytochrome c4; *Q* quinones; *QCR* quinol-cytochrome c oxidoreductase; *QH* reduced quinones; *SerABC* selenate reductase subunits A, B, and C

selenate is exterior to the plasma membrane and the electrons for the reduction of selenate are from quinol oxidation (Fig. 6). With *B. selenatarsenatis,* electrons from $QH_2$ are initially transferred to the 4[4Fe–4S] cluster in SrdB subunit, followed by electron transfer to the [4Fe–4S] cluster and Mo-cofactor in SrdA subunit. The active site for selenate reduction resides in SrdA and selenite release is exterior to the cytoplasmic membrane. With *T. selenatis,* electrons from quinol oxidation are transferred to the quinol-cytochrome c oxidoreductase (QCR) (Fig. 6). Electrons are acquired from QCR by a cytoplasmic cytochrome $c_4$ and transferred to heme *b* in SerC subunit. Electrons from the SerC subunit are transferred to the iron–sulfur clusters ([3Fe–4S] and 3[4Fe–4S]) in SerB and onto the [4Fe–4S] cluster and Mo-complex in SerA. $SeO_3^{2-}$ is produced as $SeO_4^{2-}$ acquires electrons from SerA subunit. While the selenate reductase enzyme is periplasmic in *T. selenatis,* the selenate reductase in *B. selenatarsenatis* is bound to the cytoplasmic membrane. In both selenate-reducing bacteria, the pair of protons ($2H^+$) released from quinol oxidation is used in the production of $H_2O$. Details of electron transport in selenate-respiring bacteria have not yet been developed to account for $H^+$ pumps to generate proton-motive force for ATP synthesis.

Selenate reductase from *Sulfurospirillum barnesii* showed greater activity for selenate, but also reduced nitrate, thiosulfate, and fumarate. When compared to the periplasmic selenate reductase of *T. selenatis*, the membrane-bound reductase from

*S. barnesii* has much broader substrate specificity (Stolz and Oremland 1999). The enzyme is a heterotetramer with the following subunits: 82, 53, 34, and 21 kDa, respectively. $K_m$ for selenate is 12 μM (see review by Stolz and Oremland 1999).

## 8 Additional Enzymes Reacting with Selenate

Several reports indicate that chemical mimicry enables selenate to substitute for the preferred substrate for different reductases. The periplasmic nitrate reductase of *Rhodobacter sphaeroides* and the membrane-bound nitrate reductase of *Escherichia coli* are able to reduce selenate. Additionally, the nitrate reductases of *Cupriavidus metallidurans* CH34 (previously classified as *Ralstonia eutropha*), *Paracoccus denitrificans*, and *Paracoccus pantotrophus* are also capable of using selenate as a final electron acceptor (Sabaty et al. 2001); however, these enzymes reduce selenate only in the absence of nitrate.

## 9 Bacterial Selenite Reduction

Selenite reduction to $Se^0$ has been used for decades in clinical laboratories to enrich for *Salmonella* and *Shigella* species in fecal samples. Selenite broth is commercially available, and it contains 0.4% sodium selenite along with peptone, lactose, and phosphate (Leifson 1939). When agar is added to selenite broth and plates are prepared, bright red colonies of selenium-resistant bacteria result. Selenite reduction in bacteria is more common than selenate reduction, and Doran (1982) indicates that 43% of bacteria isolated from soil reduce selenite, while only 17% of the isolates reduce selenate to $Se^0$. In addition to the bacterial strains discussed in this review, a list of 21 bacterial strains capable of reducing selenite to $Se^0$ is given in the article by Hunter and Manter (2009).

There are many reports of bacteria that are capable of reducing selenite to elemental selenium as a detoxifying process (Sura-de Jong et al. 2015). Bacteria growing on selenite produce bright red colonies containing $Se^0$ (Fig. 7) and potentially selenides. Thus far, it has not been conclusively established that energy from respiratory or dissimilatory reduction of selenite could provide energy for bacterial growth. Even though the reduction of selenite is a highly energetic reaction as seen below (Eq. 4), an appropriate enzymology is required for the energy to be conserved:

$$\begin{aligned} &SeO_3^{2-} + \text{lactate} + H^+ \rightarrow Se^0 + \text{acetate} + HCO_3^- + H_2O \\ &\Delta G_f^\circ = -529.5 \text{ kJ mol}^{-1} \text{ lactate} \end{aligned} \tag{4}$$

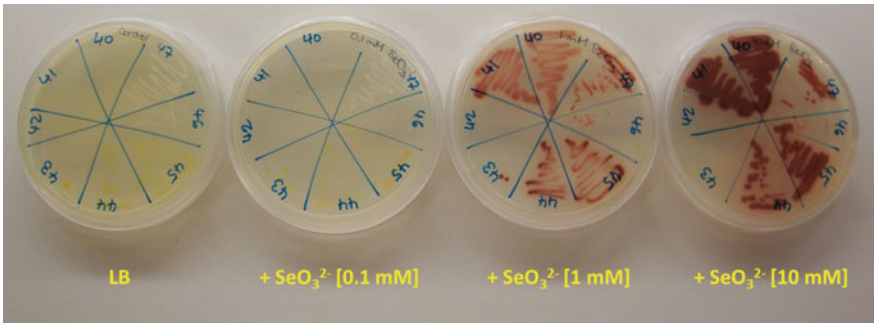


**Fig. 7** *Red* $Se^0$ produced by endophytic bacteria isolated from the root tissue of *Stanleya pinnata* and *Astragalus bisulcatus* and exposed to progressively higher concentrations of $SeO_3^{2-}$ (as $Na_2SeO_3$). LB represents control plates containing Luria Bertani growth medium without selenite (personal archive)

Several works have attempted to investigate the respiratory reduction of selenite by anaerobic bacteria. Taratus et al. (2000) used a strain of *Shewanella putrefaciens* to chemically produce mutants deficient in $SeO_3^{2-}$ reduction to red $Se^0$. The objective was to determine whether selected mutants were also deficient in other anaerobic respiratory capabilities. Several alternative electron acceptors including fumarate, nitrite, nitrate, sulfite, and thiosulfate could not support growth for various studied mutants, suggesting the presence of a complex network of shared electron transport chain components. In a recent study, Li et al. (2014) using a transposon mutant-screening approach proposed that periplasmic fumarate reductase is a potential enzyme involved in the respiration of selenite by *Shewanella oneidensis* MR-1. Interestingly, fumarate reductase-deficient mutants did not totally suppress selenite reduction, achieving a maximum of 60% decrease in $SeO_3^{2-}$ reduction compared with the wild type, suggestive of the involvement of other enzymes or reduction strategies. On the other hand, neither nitrate reductase nor nitrite reductase was involved in $SeO_3^{2-}$ reduction to red $Se^0$. Collectively, these results show the complexity of selenite reduction under anaerobic conditions. Additionally, a detoxification strategy could not totally be ruled out and it is worth exploring in future studies. It is interesting to note that while specialized selenate reductases have been described in detail in a number of publications, a typical selenite reductase awaits to be discovered.

Electron donors such as $H_2$, lactate, pyruvate, or acetate have been commonly employed for selenite reduction. As reflected in Eq. 4, the only end product of selenite reduction is $Se^0$ and it can accumulate in the culture media as a red precipitate, depending on the incubation temperature. One of the mechanisms accounting for selenite resistance in bacteria is that selenite is reduced to $Se^0$ by thiols (glutathione and thioredoxin) in the cytoplasm (see Fig. 3). The reduction sequence of selenite by reduced glutathione (GSH) is given in the following reactions (Eqs. 5–9) (Ganther 1968):

$$H_2SeO_3 + GSH \rightarrow GSSeO_2H + H_2O \quad (5)$$

$$GSSeO_2H + GSH \rightarrow GSSeOSG + H_2O \quad (6)$$

$$GSSeOSG + GSH \rightarrow GS{-}Se{-}SG + GSOH \quad (7)$$

$$GS{-}Se{-}SG + NADPH + H^+ \rightarrow GS{-}SeH + GSH + NADP^+ \quad (8)$$

$$GSSeH + GSH \rightarrow GSH + Se^0 \quad (9)$$

where $GSSeO_2H$, glutathione selenone; GSSeOSG, diglutathione selenone; GS-Se-SG, selenodiglutathione; GSSeH, L-gamma-glutamyl-S-selanyl-L-cysteinylglycine.

In *Pseudomonas seleniipraecipitans*, selenite reduction was suggested to be attributed to glutathione reductase and thioredoxin reductase (Hunter 2014). Selenite reduction in *Rhodospirillum rubrum, Rhodobacter capsulatus, Escherichia coli,* and *Bacillus subtilis* is in response to reduced thiols in the cytoplasm (Garbisu et al. 1999; Kessi and Hanselmann 2004; Kessi 2006). Reduction of selenite to $Se^0$ by *Pseudomonas* (now classified as *Stenotrophomonas*) *maltophilia* strain 0–2 was reported to be mediated by glutathione since butionine sulfoximine, an inhibitor of γ-glutamylcysteine synthetase, prevents the synthesis of glutathione and, thereby, increases bacterial sensitivity to selenite (Blake et al. 1993). Selenite induces the production of thioredoxin in *Bacillus subtilis* which is considered to facilitate the formation of $Se^0$ (Garbisu et al. 1996).

Several enzymes have been associated with the reduction of selenite to $Se^0$ in bacteria. An inducible and soluble protein of ~90 kD was isolated from *Tetrathiobacter kashmirensis,* and this protein of unknown function appears important in reduction of selenite and nitrate, but not selenate (Hunter and Manter 2008). Anaerobic selenite reduction by *Shewanella oneidensis* MR-1 is reported to be attributed to the periplasmic fumarate reductase which is energized by a *c*-type cytochrome (Li et al. 2014). Periplasmic nitrite reductase in *T. selenatis, Rhizobium sullae*, and *Rhizobium selenitireducens* are capable of catalyzing the formation of $Se^0$ from selenite (DeMoll-Decker and Macy 1993; Hunter 2007; Hunter and Kuyendall 2007). In *Bacillus selenitireducens*, selenite reduction has been reported by arsenate reductase (Afkar et al. 2003).

To further complicate the issue, selenite reduction to $Se^0$ may occur outside of the cell due to the chemicals released from bacteria or the presence of outer membrane cytochromes. Sulfate-respiring bacteria and heterotrophic bacteria with diverse metabolism release $H_2S$ which can chemically react with selenite to produce $Se^0$ (Barton and Fauque 2009). Glutathione and cysteine are secreted into the periplasm of *Escherichia coli* by CydDC, an ATP exit transporter (Pittman et al. 2005). It is significant to note that yeast defend against arsenite accumulation by exporting glutathione into the extracellular region (Thorsen et al. 2012). *Shewanella oneidensis* MR-1 secretes flavin mononucleotide (FMN) and riboflavin (Canstein et al. 2008; Marsili et al. 2008), and in an anaerobic environment, reduced FMN

and reduced riboflavin could produce $Se^0$ from selenite. Outer membrane cytochromes in *Shewanella* and *Geobacter* may mediate extracellular reduction of selenite to $Se^0$ (Shi et al. 2009; Li et al. 2014).

Sulfate-reducing bacteria (SRB) can obtain energy by the reduction of oxidized high-valence sulfur inorganic compounds (sulfate, $SO_4^{2-}$; sulfite, $SO_3^{2-}$; thiosulfate, $S_2O_3^{2-}$) with the formation of hydrogen sulfide ($H_2S$) (Muyzer and Stams 2008; Barton and Fauque 2009). Due to the structural similarity between sulfate and selenate, SRB have been reported to reduce $SeO_4^{2-}$ to intracellular red $Se^0$. *Desulfovibrio desulfuricans* could be adapted to increasing levels of Se oxyanions and developed tolerance through previous exposure to $SeO_x$ (Tomei et al. 1995). Since the *Desulfovibrio desulfuricans* cultures were more resistant to selenite when grown in cysteine-supplemented medium, the authors linked this response to the involvement of a non-enzymatic process by means of the reaction between $H_2S$ and $SeO_3^{2-}$. Overall, these results lead to the conclusion that $SeO_x$ reduction by SRB is a detoxification mechanism and not an energy conserving and growth sustaining one. Growth inhibition of SRB by selenate may be caused by the interference with sulfate activation, the critical step needed in the assimilatory or dissimilatory reduction of sulfate which results in reduction of ATP level produced (Wilson and Bandurski 1958).

## 10 Bacterial Response to Selenium Stress

The cultivation of bacteria in media containing selenite resulted in numerous phenotypic changes. Selenite was responsible for altered cell morphology which was reported for *Clostridium pasteurianum* (Laishley et al. 1980), *Wolinella succinogenes* (Tomei et al. 1992), *Desulfovibrio desulfuricans* DSM 1924 (Tomei et al. 1995), hydrothermal vent bacteria (Rathgeber et al. 2002), *Rhodobacter sphaeroides* (Bebien et al. 2001), and *Pseudomonas* strain CA5 (Hunter and Manter 2009). Cell lysis was observed when *Wolinella succinogenes* (Tomei et al. 1992) and *Stenotrophomonas* (formerly *Pseudomonas*) *maltophilia* (Blake et al. 1993) were grown in the presence of selenite. Cell lysis could be attributed to the production of highly destructive ROS such as superoxide anion ($O_2^-$) released following the reaction of reduced thiols with selenite (Kramer and Ames 1988; Zannoni et al. 2008).

The formation of superoxide anion occurs spontaneously in an aerobic environment according to the following reactions (Eqs. 10–13) (Mezes and Balogh 2009):

$$SeO_3^{2-} + 4GSH \rightarrow GSSeSG + GSSG \tag{10}$$

$$GSSeSG + GSH \rightarrow GSSeH + GSSG \tag{11}$$

$$GSSeH + GSH \rightarrow H_2Se + GSSG \quad (12)$$

$$H_2O + H_2Se + O_2 \rightarrow Se^0 + O_2^- \quad (13)$$

Reaction 6.4 is incomplete, and Zhao et al. (2006) propose that other activities may influence the production of $Se^0$ from selenite. Detoxification of the oxygen superoxide radical would be by the following reactions with the enzymes (Eqs. 14–16) associated with each reaction in parenthesis:

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \text{ (Super Oxide Dismutase Reaction)} \quad (14)$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 \text{ (Catalase Reaction)} \quad (15)$$

$$H_2O_2 + NADH + H^+ \rightarrow 2H_2O + NAD^+ \text{ (Peroxidase Reaction)} \quad (16)$$

Proteomic evaluations of aerobically growing *Escherichia coli* exposed to $SeO_x$ indicated the regulation of numerous proteins. There was an induction of 23 proteins that were present in cells exposed to either selenite or selenate. Selenite induced 8 antioxidant enzymes including a manganese-containing superoxide dismutase (Mn-SOD) and iron-containing superoxide dismutase (Fe-SOD) (Bebien et al. 2002b). In *Escherichia coli*, the induction of Mn-SOD is controlled by the SoxRS transcriptional regulator, while Fe-SOD is subject to the Fur transcriptional regulator. This induction of superoxide dismutases (SODs) following exposure to selenite in aerobic environments indicates the importance of SOD for cell resistance to selenium toxicity.

Selenium oxyanions have distinct regulatory effects on various strains of bacteria. Inoculation of bacteria into media containing selenite results in a prolonged lag phase. The reduction of selenite with formation of $Se^0$ has been reported to occur in the stationary phase with *Wolinella succinogenes* (Tomei et al. 1992), *Desulfovibrio desulfuricans* DSM 1924 (Tomei et al. 1995), and *Stenotrophomonas maltophilia* (Duran et al. 2003). *Pseudomonas moraviensis* stanleyae exposed to 10 mM selenite under aerobic conditions showed impaired growth (40% less bacterial cell density) and extended lag time (Staicu et al. 2015b). Red $Se^0$ enzyme assay of the same isolate identified glutathione, nitrite and sulfite reductases, suggestive of a detoxification mechanism of selenite reduction (Ni et al. 2015). With *Rhodobacter sphaeroides,* the reduction of selenite to $Se^0$ occurred during the exponential growth phase, while reduction of selenite to $Se^0$ by *Rhodobacter sphaeroides* occurs at the transition from exponential growth phase to stationary phase (Bebien et al. 2001). It is generally considered that bacteria in the stationary phase display resistance to toxic metals due to the induction of new sigma factors and this may explain selenite reduction by bacteria.

Global stress response may account for resistance and reduction of selenium oxyanions. Addition of selenite to a culture of *Rhodobacter sphaeroides* induces the production of 25 new proteins with the repression of 20 proteins. This reflects an induction of 16% of cell proteins with a repression of 21% of the proteins produced

(Bebien et al. 2001). Exposure of the same bacterial culture to selenate was less dramatic than selenite with 10% of the total number of proteins induced and 14% repressed. Proteomic evaluation of selenium proteins following of selenite exposure to *Rhodobacter sphaeroides* reveals the induction of enzymes associated with oxidative stress including glutathione reductase, thioredoxin, and an iron-containing SOD (Bebien et al. 2001). Resistance to and reduction of selenite in bacteria have been suggested to be a stress response activity as seen with *Bacillus subtilis* with elevated levels of thioredoxin and NADP-thioredoxin reductase in the presence of selenite (Garbisu et al. 1999).

An interesting article highlights the role of global transcription regulator known as fumarate nitrate reduction regulator (FNR) for selenate metabolism by *E. cloacae* SLD1a-1 (Yee et al. 2007). The FNR responds to oxygen levels in the environment and accounts for the induction of selenate reduction when oxygen is depleted in the cell. The FNR protein interacts with DNA only under anaerobic conditions and accounts for the regulation of over 100 operons including induction of selenate reductase. Numerous facultative anaerobes, including *Stenotrophomonas maltophilia* (Duran et al. 2003), reduce selenate to $Se^0$ only under suboxic conditions.

## 11 Elemental Selenium Oxidation/Reduction

Oxidation of $Se^0$ by bacteria was first inferred and later proven experimentally. The speculation was based upon the cycle of sulfur in which *Acidithiobacillus* (formally *Thiobacillus*) thiooxidans oxidizes elemental sulfur ($S^0$) to sulfuric acid. Additionally, the observation that reduced valence states of Se do not overaccumulate in soils suggested the presence of a cycle having also an oxidative component (Shrift 1964). A strain of *Bacillus megaterium* isolated from a selenium-rich environment was found to oxidize $Se^0$ mainly to selenite and to a lesser extent (less than 1%) to selenate. However, after 40 days of incubation only 1.5% of the initial $Se^0$ had been oxidized (Sarathchandra and Watkinson 1981). In a later study, mixed bacterial soil cultures, heterotrophic soil enrichment, and axenic cultures (*Leptothrix* strain MNB-1 and *Thiobacillus* ASN-1) were tested for elemental Se oxidation (Dowdle and Oremland 1998). In the case of soil slurries collected from a dried former freshwater lakebed pond from Kesterson National Wildlife Refuge, California, the inoculum (no electron donor added) oxidized 17% of added $Se^0$ after 25 days of incubation. In contrast, when amended with sodium acetate and $Na_2S$, higher oxidation yields of, respectively, 26.5 and 47% were determined. The main oxidation product was selenite. Enrichment culture established by growing soil slurry in Difco nutrient broth amended with 10 mM glucose for 2-month generated selenate and selenite ($\sim$40%), but the oxidation rates were inferior to those recorded for soil slurry. As for the pure cultures, *Leptothrix* oxidized only 1.6% of initial $Se^0$ and selenate was the major product. *Thiobacillus* oxidized 1.3% in the presence of 10 mM thiosulfate. Selenate was again the dominant Se oxyanion produced. Collectively, these results demonstrate the oxidation of $Se^0$ by bacterial

action, but the reaction rate is very slow, 3–4 orders of magnitude lower than the dissimilatory reduction of selenate. The benefit of $Se^0$ oxidation is unclear, and it is speculated to be more a co-metabolic process rather than an energy-yielding transformation. These studies contributed to the realization that a biogeochemical cycle of selenium exists and that bacteria play a vital role in closing the loop. It should not be overlooked the timescale characterizing the cycling of chemical elements in nature. What might appear to be a slow-paced process compared to the metabolic rates from bioreactor settings, the transformations at longer (geological) time frames are slower, but accumulative.

Bacterial reduction of $Se^0$ to Se(−II) was reported for axenic cultures (*B. selenitireducens*) and mixed microbial cultures (estuarine sediment slurries). In the case of the estuarine sediment, the majority of selenide produced was found in the sediments as a solid precipitate (FeSe), providing a possible biological explanation for the presence of the metal selenides in some sedimentary rocks (Herbel et al. 2003).

## 12 Transmembrane Movement of Selenium

Several different areas of the bacterial cell are associated with the metabolism of selenium. The plasma membrane is a major barrier to the uptake of selenate and selenite, as well for the potential export of $Se^0$ nanoparticles. In the case of Gram-negative bacteria, the outer membrane restricts release of $Se^0$ from the periplasm. It has been assumed that at high extracellular concentrations of selenate and selenite, the oxyanions would be transported across the plasma membrane by sulfate and sulfite transporters, respectively. The uptake of selenate in *Escherichia coli* is by the ABC sulfate transporter controlled by the *cysAWTP* operon (Turner et al. 1998). However, a separate uptake transporter for selenite has been reported for *Clostridium pasteurianum* (Bryant and Laishley 1988) and is implied for *Salmonella typhimurium* (Brown and Shrift 1980). In the phototrophic bacterium *Rhodobacter sphaeroides*, a putative ABC transporter involved in the uptake of sugars is proposed to be involved in the uptake of selenite (Bebien et al. 2001). The putative selenite transporter in *Escherichia coli* has a mass of ∼43 kDa with 12 transmembrane domains (Guzzo and Dubow 2000). In *Escherichia coli*, selenite may be acquired by the sulfate ATP transporter system, but when this sulfate transporter is repressed selenite continues to be transported inside the cell and this observation is used to indicate other uptake transport system(s) for selenite (Rosen and Liu 2009).

Selenate and selenite, like most salt compounds, traverse the outer membrane of Gram-negative bacteria through nonspecific porins. The export of $Se^0$ nanoparticles produced in the cytoplasm could be mediated by exporters in the plasma membrane and the outer membrane. Under aerobic conditions, selenate was shown to be relatively unreactive with certain bacterial species and it displayed no toxicity effect even at high concentrations (Sura-de Jong et al. 2015). An aerobic strain of

*P. moraviensis* isolated from the roots of Se hyperaccumulator *Stanleya pinnata* in Colorado could grow when supplemented with selenate concentration as high as 150 mM (Staicu et al. 2015b). However, no selenite or red $Se^0$ was measured during growth by high-resolution microchip capillary zone electrophoresis (Noblitt et al. 2014), suggesting that this high tolerance might be related to an efficient ion efflux pump. While bacterial efflux pumps function to export xenobiotics, the substrates for these efflux systems are soluble and not particulate as in the case of $Se^0$. The lysis of bacteria containing $Se^0$ has been often used to indicate the release of large cytoplasmic granules of $Se^0$. Homeostasis of selenium, particularly $Se^0$, in bacterial cells remains an important topic awaiting elucidation.

## 13 Biotechnological Applications of Microbial Selenium Metabolism

Microbial metabolism of selenium is a promising avenue for a handful of biotechnological applications. In the following section, we will briefly present the bioremediation of selenium-laden industrial effluents using selenium-respiring bacteria, as well as the production of functional materials ($Se^0$ and metal selenides nanoparticles) based on the microbial transformations of selenium oxyanions.

## 14 Bioremediation of Se-Laden Wastewaters

Various industrial activities, such as the combustion of fossil fuels for energy generation, mining, metal and oil refining, and the irrigation of seleniferous soils, result in the liberation of Se which ultimately accumulates in wastewater/products or in the environment. The biological treatment of Se-containing wastewaters using bacterial inocula (i.e., *bioremediation*) is an interesting alternative to the costly physical–chemical technologies. The bioremediation of Se-laden industrial effluents aims at converting soluble and toxic Se oxyanions into insoluble $Se^0$ particles, thus reducing their toxic potential (Lovley 1993; Barton et al. 1994; Tan et al. 2016). In such treatment systems, selenium oxyanions function as final electron acceptors, reducing their valence state to solid $Se^0$ particles. In general, selenium-containing industrial effluents are low in organic content, entailing the need to add exogenous carbon and electron sources. A broad spectrum of electron/carbon donors has been used including acetate, lactate, ethanol, or $H_2$. In a recent report, Lai et al. (2016) reported the use of methane gas ($CH_4$) as the electron donor for the bioconversion of $SeO_4^{2-}$ in a membrane biofilm reactor. Different bioreactor configurations exist, and all of these rely on the inoculation of a mixed microbial consortium having environmental or biotechnological (other bioreactors) origins. The reason for not using pure bacterial cultures is related to the non-sterile nature of industrial effluents and bioreactor settings, but also to the enhanced capacity of mixed cultures to

withstand the variability and toxicity of the feedstock. The inoculation of bioreactor systems with selenium-respiring bacteria is sometimes used for process optimization. A number of bioreactor types are commercially available and are currently in operation mostly in USA and Canada (see chapter "Industrial Selenium Pollution: Sources and Biological Treatment Technologies" from this volume). Because certain bacterial enzymes show high affinity for selenate, they will preferentially target this oxyanion even when sulfate and nitrate are present in excess within the wastewater matrix. This is a particular asset for the biological treatment, in comparison with various physical–chemical treatment systems that are non-selective for $SeO_x$ (Staicu et al. 2017). An important concern related to the biological treatment of Se oxyanions is the generation of colloidal $Se^0$ that should be collected before the treated effluent is discharged into the environment. Different post-treatment steps for the liquid–solid separation of $Se^0$ have been proposed including media filtration, chemical coagulation, and electrocoagulation (Staicu et al. 2015a, c). Chapter "Industrial Selenium Pollution: Sources and Biological Treatment Technologies" from this volume and Nancharaiah and Lens (2015a) provide an extensive presentation of the industrial selenium effluents and the biological treatment technologies founded on microbial metabolism. Physical–chemical treatment systems have also been tested for the removal of selenium at laboratory, pilot, and full scale, and they are presented in detail in chapter "Industrial Selenium Pollution: Wastewaters and Physical-Chemical Treatment Technologies."

## 15 Biogenic $Se^0$ NP Production by Microbial Factories

Biomineralization of $Se^0$ in bacteria is a poorly understood process (Butler et al. 2012; Nancharaiah and Lens 2015b). Unlike magnetic minerals that are biosynthesized to orient magnetotactic bacteria in the geomagnetic field in the search for preferred microaerophilic environments or the polyhydroxybutyrate (PHB) accumulation as a form of energy storage in certain bacterial groups, the formation of biogenic $Se^0$ does not seem to serve an ecological or nutritional function. The formation of $Se^0$ appears to be a by-product of bacterial Se metabolism. Various reports indicate the presence of $Se^0$ granules in the cytoplasm, in the periplasmic space or outside the cell (Fig. 8). What complicates the interpretation is the transport mechanism of elemental selenium particles. Since the size of the particles can sometimes go up to 300–400 nm, the extrusion process will inevitably result in cell lysis. However, many reports showed viable cells harboring $Se^0$ particles and higher amounts of particles in the extracellular environment. To date, no paper presented convincing evidence of the extrusion of Se particles. An interesting observation was made by Kessi et al. (1999) who correlated the decrease in buoyant density of *R. rubrum* cells to the extrusion of cytoplasmic $Se^0$ particles during stationary phase, suggestive of a two-phase process: (1) nucleation and growth of $Se^0$ particles inside the cell and (2) $Se^0$ particles extrusion to the extracellular environment at a certain growth stage.

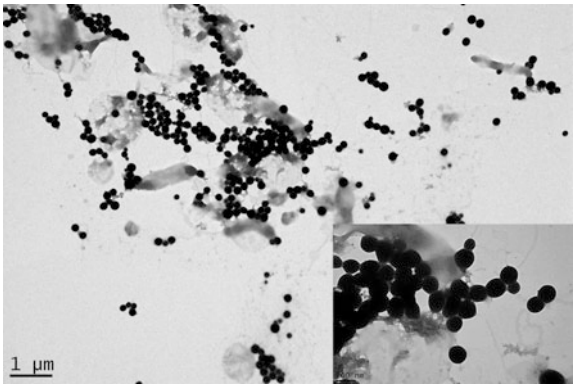


**Fig. 8** TEM micrographs of biogenic $Se^0$ nanoparticles produced by a mixed microbial culture (anaerobic granular sludge) by the reduction of 10 mM of sodium selenate under anaerobic conditions (personal archive). Inset: 200 nm scale

The process of Se nanoparticle assembly (Fig. 4) was investigated in *T. selenatis* identifying a 94.5 kDa protein (selenium factor A, SefA), putatively involved in $Se^0$ biomineralization (Debieux et al. 2011). It is not clear however to what extent SefA is involved in the production or in the stabilization of $Se^0$ particles, and the report does not elucidate the transport mechanism outside the cell. Other attempts have been made to identify proteins associated with $Se^0$ in search for a way to control their size distribution. Dobias et al. (2011) found four proteins (AdhP, Idh, OmpC, and AceA) specifically bound to $Se^0$. Of these, the purified AdhP (alcohol dehydrogenase propanol-preferring) was studied in more detail for the in vitro reduction–nucleation–growth process of elemental selenium resulting in smaller and less polydispersed $Se^0$ particles. A metalloid reductase (RarA) was identified associated with $Se^0$ particles produced by *S. barnesii*, but no enzymatic characterization of the protein was undertaken (Lenz et al. 2011). Interestingly, no observable membrane or structured protein shell associated with $Se^0$ particles was identified intracellularly in a novel aerobic strain of *P. moraviensis* using cryoelectron microscopy, thus indicating that the nanoparticles are freely exposed to cytosol (Ni et al. 2015). This finding might point to the subsequent acquisition of the protein shell during transportation.

The size of $Se^0$ particles varies widely especially when using mixed microbial cultures. Staicu et al. (2015a) reported polydisperse particles produced under anaerobic conditions by a granular sludge inoculum in the 50–300 nm range with an average size of 166 ± 29 nm and a polydispersity index of 0.18. Selenate- and selenite-respiring bacteria, namely *S. barnesii, Bacillus selenitireducens*, *and Selenihalanaerobacter shriftii*, produced $Se^0$ particles ranging from 200 to 400 nm, and the particles showed markedly different structural and spectral features (Oremland et al. 2004). The authors correlated these results with the potential diversity of enzymes involved in their production. Regarding the mineralogical nature of biogenic $Se^0$, many publications reported spherical amorphous particles, while some have found crystalline Se structures. Amorphous particles would have some advantages in that they are a fast way to get rid of toxic selenite and also because their globular shape is potentially less harmful to cell integrity. In the case

of magnetotactic bacteria, the magnetite crystals formed inside the cell are not exported to the intracellular milieu and are tightly fixed by invaginations of the inner membrane. It must be noted that for an accurate mineralogical determination of biogenic nanoparticles, their biopolymer coating should be totally removed in order to avoid artifacts. Proteins residues attached to $Se^0$ particles could bias the proper determination of the mineralogical state, and this aspect is often overlooked. Biogenic $Se^0$ nanoparticles are stabilized by a biopolymer coating composed of proteins (Dobias et al. 2011; Lenz et al. 2011) and extracellular polymeric substances (EPS) (Jain et al. 2015). The negatively charged biopolymers attach tightly onto $Se^0$ particles, preventing aggregation and thus imparting colloidal properties.

## *15.1 Metal Selenides*

Chalcogenide-based nanoparticles (e.g., CdS, CdSe, ZnSe, and CdTe) have multiple technological applications in electronics, nonlinear optics, luminescence, and catalysis. In order to avoid the physical–chemical preparation methods, the biological approach is proposed as a "greener" alternative. *Veillonella atypica* was shown to form selenides by the reduction of sodium selenite using hydrogen as an electron donor (Pearce et al. 2008). Transitory accumulation of biogenic $Se^0$ was also observed. The addition of an exogenous extracellular redox mediator, anthraquinone disulfonic acid (AQDS), increased sevenfold the reduction rate of selenite. For the formation of metal selenides, zinc chloride and cadmium perchlorate were added to a filtered biogenic selenide solution along with 2-mercatoethanol and glutathione, used as stabilizing agents. The ZnSe nanoparticles showed a size distribution of 3–6 nm, whereas the CdSe nanoparticles were in the 2–4 nm range. It should be noted that semiconductor quantum dots (QD) display tunable optical properties in the regime below 10 nm and this is due to the quantum confinement effect. However, several major drawbacks such as the financial aspect and the knowledge gap in our understanding of the bio-oriented approach currently tip the balance in favor of the traditional organochemical synthesis. Other concerns are raised by the presence of bound biopolymers onto biogenic QD, needing purification.

# 16 Summary

Bacteria are involved in the valence state transformations of selenium, covering both the oxidative and reductive side of its biogeochemical cycle. Se oxyanions, $SeO_4^{2-}$ and $SeO_3^{2-}$, are reduced by aerobic and anaerobic bacteria to elemental Se and selenides, thus alleviating their toxic potential exerted on aquatic ecosystems. A variety of enzymes is employed for these transformations, including several selenate reductases with high affinity for $SeO_4^{2-}$. Although it is considered a niche

metabolism compared with other anions such as nitrate or sulfate, selenium oxyanions reduction under anaerobic conditions still generate enough energy to sustain bacterial growth. It is yet to be elucidated the nature of the microbial aerobic reduction of $SeO_4^{2-}$ since only a limited number of species have been reported to achieve this. In the case of aerobic reduction of $SeO_3^{2-}$, it is a detoxification mechanism accompanied by the formation of particulate $Se^0$ and/or selenides, along with reactive oxygen species in need of further inactivation by the cell. Whether Se oxyanions are benefic or toxic to bacteria depends on a number of factors including: (1) bacterial species and enzymatic repertoire, (2) availability of nutritional resources, (3) aerobic, microaerophilic, and anaerobic conditions, and (4) the presence of other electron acceptors. $Se^0$ and selenides were shown to be oxidized by bacteria (pure or mixed cultures), but the reaction rates are several orders of magnitude lower than the opposite reactions. They appear, nevertheless, important in the global cycle of Se taking place at longer time frames. Bacterial transformations of Se are exploited for the biotreatment of Se-laden industrial effluents and could potentially be harnessed for the biofabrication of materials (e.g., $Se^0$ nanoparticles and selenide quantum dots).

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