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Chapter 5

Microbial-Based Bioremediation of Selenium and Tellurium Compounds

Elena Piacenza, Alessandro Presentato, Emanuele Zonaro, Silvia Lampis, Giovanni Vallini and Raymond J. Turner

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http://dx.doi.org/10.5772/intechopen.72096

Abstract

The chalcogens selenium (Se) and tellurium (Te) are rare earth elements, which are mainly present in the environment as toxic oxyanions, due to the anthropogenic activities. Thus, the increased presence of these chalcogen-species in the environment and the contamination of wastewaters nearby processing facilities led to the necessity in developing remediation strategies aimed to detoxify waters, soils and sediments. Among the different decontamination approaches, those based on the ability of microorganisms to bioaccumulate, biomethylate or bioconvert Se- and/or Te-oxyanions are considered the leading strategy for achieving a safe and eco-friendly bioremediation of polluted sites. Recently, several technologies based on the use of bacterial pure cultures, bacterial biofilms or microbial consortia grown in reactors with different configurations have been explored for Se- and Te-decontamination purposes. Further, the majority of microorganisms able to process chalcogen-oxyanions have been described to generate valuable Se- and/or Te-nanomaterials as end-products of their bioconversion, whose potential applications in biomedicine, optoelectronics and environmental engineering are still under investigation. Here, the occurrence, the use and the toxicity of Se- and Te-compounds will be briefly overviewed, while the microbial mechanisms of chalcogen-oxyanions bioprocessing, as well as the microbial-based strategies used for bioremediation approaches will be extensively described.

Keywords: selenium, tellurium, bioremediation, microbial consortia, biological reactors

1. Introduction

The chalcogens tellurium (Te) and selenium (Se) are naturally occurring rare elements of the Earth crust belonging to the group 16 of the periodic table that are defined as metalloids,

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due to their intermediate chemical–physical properties between metal and non-metals [1]. Te estimated average amount in the environment is around 0.027 ppm [2], while Se is unevenly distributed on the Earth's surface with a concentration ranging from 0.01 to 1200 ppm [3, 4]. These elements can be found in natural rocks and ores, soils, sediments or in association with rare minerals (e.g., calaverite $AuTe_2$, sylvanite $AgAuTe_4$, crooksite CuTlSe, calusthalite PbSe) [4–6]. Moreover, Se is an essential micronutrient for living systems, being part of the structure of several important enzymes, (i.e., glutathione peroxidases and thioredoxin reductases), as the 21st amino acid seleno-cysteine, in at least 25 human selenoproteins [7], while, to date, any biological function has been ascribed to Te [8]. Both these chalcogens exist in four different valence states in the environment (i.e., +VI, +IV, 0 and –II), and among them the oxyanion forms of Selenate (SeO_4^{2-}), Tellurate (TeO_4^{2-}), Selenite (SeO_3^{2-}) and Tellurite (TeO_3^{2-}) are the most abundant in soils and waters [9, 10].

The wide spread use of Se- and Te-compounds by anthropogenic activities related to oil refining, phosphate and metal ore mining, electronics and industrial glasses, have led to an increase in the presence of these chemicals in the environment [6, 11]. In this regard, although Se is an essential micronutrient, it is toxic at concentrations higher than the human dietary requirement (25–30 μg day^{-1}) [10], while the toxicity exerted by Te is even more dramatic, negatively affecting both prokaryotes and eukaryotes at concentration as low as 1 μg mL^{-1} [6]. Particularly, Se- and Te- oxyanions are recognized as harsh toxicants of public health and environmental concern due to their association with oxygen, which makes them highly bioavailable, enabling the mobilization of Se- and Te-compounds through water and soil [12, 13]. On the contrary, Se and Te organic forms (e.g., dimethyl selenide, trimethyl selenonium, selenomethionine, selenocysteine, Se-methilselenocysteine, dimethyl telluride), as well as their zero-valence states (Se^0 and Te^0) showed lower toxicity levels [2, 12, 14]. Considering the shared physical–chemical features of Se and Te, the suggested mechanism of toxicity exerted by the chalcogen-oxyanions is based on their interaction with glutathione molecules (GSHs) and related molecules, which are likely responsible for their reduction [8, 13, 15]. This bioconversion mechanism leads to the generation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) [16] or superoxide ions (O^{2-}) [17], therefore causing cell death [18–20]. An additional target of TeO_3^{2-} is the impairment of the heme metabolism in *E. coli* K-12 cells, by which this oxyanion is responsible for the accumulation of the heme precursor protoporphyrin IX, causing iron depletion and, subsequently, cell death [21].

Despite the toxic effects of Se- and Te-oxyanions, in the last 20 years several microorganisms able to sequester, bioconvert or biomethylate these chalcogen-ions have been isolated from extreme environments, such as ocean hydrothermal vents and the highly alkaline water Monolake (California), to name a few [22]. Mainly anaerobic or facultative-anaerobic bacteria capable of growing phototrophycally or chemotrophycally under oxic and anoxic conditions have been described for their metabolic potential in bioconverting these species, while much less is known about strictly aerobic microorganisms [23]. In this regard, anaerobic microorganisms have been described for their use of chalcogen-oxyanions as terminal electron acceptors to sustain their growth [19, 22, 24–29]. Although the exact biochemical mechanisms behind Se and Te metabolism and bioconversion in these microorganisms have not been fully elucidated,

there is a strong movement toward eco-friendly approaches for bioremediation of chalcogen-contaminated areas of interest. Moreover, among bacterial strains able to bioconvert Se- and Te-oxyanions in their less toxic and less bioavailable elemental form (i.e., Se^0 and Te^0), some of them were characterized for the generation of either intra- or extracellular precipitates and/or nanomaterials, for example, nanoparticles (NPs) and nanorods (NRs) [8, 19].

Here, we will overview the microbial-based strategies that, to date, are applied as tools for bioremediation purposes of chalcogens polluted environments, and briefly will be described the valuable role of bacteria for the recovery of metalloids in their zero-valence state in the form of nanomaterials.

1.1. Environmental toxicity of selenium and tellurium compounds

Annually, the total average amount of either Se or Te produced worldwide is 2500–2800 or 220 tons, respectively, with USA, Japan, Russia, Canada, Germany, Belgium and Sweden as main manufacturers [7, 30]. The accumulation of Se- or Te-compounds in the environment mainly relies on their anthropogenic use in several application fields, causing therefore their emission in the atmosphere [31, 32]. Se-accumulation derives from metallurgic industries, glass manufactures, pigments production, electronics and agriculture applications [33], while Te-containing compounds are used in copper refining [19], tarnishing metals [34], vulcanization of rubber [8], production of color glass or ceramics [19], photovoltaic cells and solar panels [8], as well as catalysis of several reactions [19]. Recently, the possibility to develop new Te-based nanomaterials such as fluorescent quantum dots (QDs) has been extensively investigated to create new high-tech probes in biological detection [8, 35], exasperating the already dramatic waste disposal circumstances.

Among the different Se-species present in the environment, the inorganic forms of Se^{2-}, SeO_4^{2-}, or SeO_3^{2-} are generally found in surface and ground waters as pollutants [36], while the organic and volatile ones (i.e., methylselenides, trimethylselenonium ions and selenoamino acids) occur in air and soils [37]. Similarly, Te-compounds result to be highly concentrated either in soils [38, 39] or waters [34] mainly in the form of TeO_4^{2-} and TeO_3^{2-}, being the latter highly soluble and toxic [35, 40, 41].

The presence of Se- and Te-compounds in water reservoirs has become a problem for both human health and ecological wildlife [42–45], which led to the development of several strategies aimed to protect aquatic and human life [46], as Se-poisoning events have occurred in the last few years worldwide, such as in the Kesterson Wildlife reservoir (California) [47], the uranium mine in Saskatchewan (Canada), and the Lake Sutton (USA) [48], causing physical deformities and mutations [46]. The major areas of the world affected by water contamination due to the presence of SeO_4^{2-} and SeO_3^{2-} are North America, Australia and New Zealand [23], while higher level of Te-oxyanions has been detected in the surface waters of Te-contaminated basins in Angola and Panama as compared to the deep ones, indicating a difference in behavior between Te and Se, which, as nutrient, is usually highly concentrated in the deep ocean [49]. Finally, Te-compounds emission in the atmosphere is now investigating, even if the implication related to the presence of Te-species in the air has not been established yet [19].

2. Bioremediation of chalcogen-contaminated environments

The exploitation of microorganisms for the decontamination of Se- and/or Te-polluted environments is based on the capability of several bacterial strains to sequester, bioconvert or biomethylate chalcogen-oxyanions [19]. Se- or Te-species sequestration is achieved by microorganisms through either their uptake in the bacterial cell or the interaction with charged surface biomolecules [19], while the bioconversion of these oxyanions in bacteria leads to their reduction to Se^0 and Te^0 in the form of metalloid precipitates [19]. Further, some microorganisms can biomethylate Se or Te-oxyanions, producing volatile methyl derivatives, which can react in the atmosphere with NO_3 radicals, ozone and atmospheric particles, increasing their residence times [19, 50].

2.1. Bioremediation of Se-polluted environments using bacterial pure cultures as planktonic cells

In the last 30 years, Se-oxyanions sequestration by microorganisms has been investigated as a potential strategy for the decontamination of Se-polluted environments. Indeed, several bacterial strains have been described for their ability to uptake SeO_4^{2-} and/or SeO_3^{2-} using several processes, such as the sulfate transporter in *E. coli* [51], the sulfate permease in *Salmonella typhimurium* [52], the sulfite uptake system in *Clostridium pasteurianum* [53], the polyol ABC transporter in *R. sphaeroides* [54]. Thus, once inside the bacterial cell, the sequestered Se-oxyanions are usually incorporated into Se-amino acids (i.e., seleno-cysteine and -methionine) to biosynthesize selenoproteins [55].

An alternative Se-bioremediation approach is based on the bacteria's ability to biomethylate Se-oxyanions, resulting in the production of Se-methyl derivates (i.e., dimethyl selenide, dimethyl selenyl sulfide, dimethyl diselenide), as in the case of *Aeromonas* sp. VS6, *Citrobacter freundii* KS8 and *P. fluorescens* K27 [56], *Clostridium collagenovorans*, *Desulfovibrio gigas* and *Desulfovibrio vulgaris* [57], *Enterobacter cloacae* SLS1a-1 [58], *R. sphaeroides* and *R. rubrum* S1 [59]. Se-oxyanions biomethylation is achieved in microorganisms through the Challenger mechanism [56], which consists of several reduction-methylation steps that change Se-redox state from either VI or IV to II [60].

Recently, the exploitation of microorganisms able to bioconvert Se-oxyanions to Se^0 has emerged as a cost-effective *green* alternative strategy for the decontamination of Se-polluted environments, with a particular focus on surface waters and wastewaters. To date, Se-bioremediation approaches exploit bacterial strains capable of reducing SeO_4^{2-} and SeO_3^{2-} [23] either to conserve energy [61–63] or to detoxify their environmental niches [23]. Since Se-oxyanions bio-reduction under anoxic conditions is more characterized as compared to the aerobic mode, mainly anaerobic bacterial strains have been used for Se-decontamination purposes [23]. However, studies evaluating either SeO_4^{2-} or SeO_3^{2-} bioconversion by aerobic or microaerophilic microorganisms have also been conducted [61, 64–67], highlighting some disadvantages of these experimental conditions: a competition between the dissolved oxygen and the Se-oxyanion as terminal electron acceptor [68, 69], and the additional energetic cost to aerate a bioreactor [23].

Regardless, aerobic bacterial strains have been explored as pure cultures at laboratory scale for Se-bioremediation purposes, yet little work about the use of these microorganisms for large-scale applications have been conducted [23].

Among the microorganisms described for their tolerance toward Se-oxyanions, bacterial strains belonging to *Pseudomonas, Desulfovibrio, Thauera, Enterobacter, Wolinella* and *Bacillus* genera have been characterized for their capability to bioconvert SeO_4^{2-} to SeO_3^{2-} mainly under anoxic growth conditions [61, 70, 71]. Moreover, several anaerobic microorganisms have been characterized for their use of SeO_4^{2-} as terminal electron acceptor to support their growth [26, 70–73], coupling the bioconversion of this Se-oxyanion to the oxidation of different carbon sources, such as aliphatic (pyruvate, lactate, acetate) as well as aromatic compounds (i.e., benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate) [61, 74, 75]. Nevertheless, facultative anaerobes, such as *Pseudomonas stutzeri*, showed their proficiency of bioreducing SeO_4^{2-} solely for detoxification purposes [70].

Unlike SeO_4^{2-}, both aerobic and anaerobic microorganisms can bioconvert the highly soluble and reactive SeO_3^{2-} [76] into Se^0 through either detoxification strategies or anaerobic respiration [77–79]. SeO_3^{2-} detoxification occurs through several mechanisms based on Painter-type reactions [17, 80–82], where glutaredoxin/thioredoxin reductase systems [19, 83] and siderophores mediate the oxyanion reduction [19, 65]. SeO_3^{2-} detoxification is mostly achieved by thiol molecules present in the cytoplasm of bacterial cells, such as GSHs, mycothiols (MSHs), and glutaredoxins [17, 84]. Moreover, GSHs can be exported into the periplasm of Gram-negative bacteria, leading to the bioreduction of SeO_3^{2-} in the periplasm or at their cell membrane [85]. Secondary SeO_3^{2-}-detoxification strategies exploited by microorganisms involved the interaction between SeO_3^{2-} and reactive biogenic sulfide, [86, 87], as well as the exploitation of iron siderophores [19, 88]. On the other hand, SeO_3^{2-} bioconversion during anaerobic respiration is mostly mediated by the presence of terminal nitrite, sulfite or fumarate reductases [19, 24, 61, 66, 67, 72, 89, 90], as described for *T. selenatis* AX, *Rhizobium sullae* HCNT1 and *C. pasteurianum*, to name a few [91–93]. Further, *Geobacter sulfurreducens* [94], *Shewanella oneidensis* MR-1 [90] and *Veillonella atypica* [94] showed high proficiency in bioreducing SeO_3^{2-} to Se^0 through dissimilatory reduction in anoxic conditions, while among the bacterial strains able to anaerobically bioconvert SeO_4^{2-} into SeO_3^{2-}, a high yield of Se^0 production by further reducing SeO_3^{2-} has been observed for *Bacillus beveridgei* [22], *D. indicum* [75], *Desulfovibrio desulfuricans* [95], *E. cloacae* SLD1a-1 [96] and *Sulfospirillum barnesii* SES-3 [25, 96]. Nevertheless, fewer bacterial species (i.e., *Bacillus selenitireducens* and *Aquificales* sp.) have been described for their ability to use SeO_3^{2-} as terminal electron acceptor as compared to those using SeO_4^{2-} [26, 27].

2.2. Bioremediation of Te-polluted environments using bacterial pure cultures as planktonic cells

Although Te does not have an essential biological role for living organisms [8], bacterial cells are able to uptake Te-oxyanions and to biomethylate and/or bioconvert them either as a decontamination strategy or during the anaerobic respiration [8, 19]. Particularly, TeO_3^{2-} uptake within bacterial cells has been ascribed to the phosphate transporter in *E. coli* [97],

Lactococcus lactis [98] and *R. capsulatus* [99, 100], considering that this Te-species is a strong competitive inhibitor of the phosphate group [19]. However, other carriers can be used to assist TeO_3^{2-} uptake in microorganisms, such as the ActP monocarboxylate transporter of *R. capsulatus* [101], as well as an ATP-dependent efflux pump responsible for the arsenite/arsenate/antimonite resistance in *E. coli* [102]. Since Te shares several chemical properties with Se, microorganisms tolerant and/or resistant toward Te-oxyanions process them exploiting similar mechanisms to those described above for Se-species. In this regard, the biomethylation of Te-oxyanions to produce dimethyl telluride and dimethyl ditelluride [56] has been observed in several bacteria able to biomethylate Se-oxyanions as well, such as *R. rubrum* G9, *R. capsulatus* [59], *P. fluorescens* K27 [103] and *D. gigas* [57]. Moreover, *P. aeruginosa* ML4262 [104], *G. stearothermophilus* V [105] and *Mycobacterium tuberculosis* [106] showed their capability of biomethylating only Te-oxyanions.

Despite of TeO_3^{2-} presence in lower amount in the environment compared to TeO_4^{2-} [39], tellurite showed toxicity 10 times higher than tellurate [40, 41], leading the experimental research to focus on the study of TeO_3^{2-}-tolerant/resistant microorganisms as ideal candidate for bioremediation purposes. Nevertheless, *B. beveridgei* [22], *B. selenitireducens*, *S. barnesii* [29] and *Shewanella frigidimarina* ER-Te-48 [28, 107] showed their ability under anaerobic growth conditions to use both TeO_4^{2-} and TeO_3^{2-} oxyanions as terminal electron acceptors in the respiratory chain to sustain their growth [8]. To date, the proposed mechanisms of Te-oxyanions bioconversion in microorganisms are similar to those described for Se-species [13, 56, 88, 104, 108]. Further, TeO_3^{2-} processing in microorganisms have been ascribed to enzymatic reductions by periplasmic or cytoplasmic oxidoreductases [107, 109], such as nitrate reductases [109, 110], catalases [111] and thiol:disulfide oxidoreductase [112]. However, the function of all these enzymes for bioconverting Te-oxyanions appears to be not specific, leading to a low resistance level toward Te-species in these microorganisms. To date, only one specific TeO_3^{2-} reductase has been identified as responsible for the anaerobic respiration of this Te-oxyanion in *Bacillus* sp. GT-83 [113].

2.3. Bioremediation of chalcogen-polluted environments based on bacterial biofilms

The majority of the investigations regarding the bioremediation of Se- and Te-contaminated environments have been focused on the exploitation of bacterial species grown as free planktonic cells [8]. However, in natural settings microorganisms are most often found in close association with surfaces and interfaces as complex communities, which are indicated as biofilms [114–116]. In bacterial biofilms, the cells are embedded and protected from the surrounding environments by the presence of a matrix defined as Extracellular Polymeric Substance, containing a high amount of water, polysaccharides, proteins, extracellular-DNA (e-DNA) and lipids [117, 118]. The communal life of bacterial cells in the form of biofilm offers them several advantages [114, 117, 119], resulting in their innate ability to populate a vast array of environments [119], including those contaminated by chalcogen-oxyanions. Thus, peculiar features of bacterial biofilms (i.e., quorum sensing signaling process, different cellular physiology, presence of the EPS and colony morphology variants) [120–124] confer them tolerance and/or resistance toward either Se- or Te-oxyanions without having specific Se- and Te- genetic resistant determinants [19]. In this regard, sulfate-reducing bacteria (SRB) within a biofilm produce sulfide (S_2), which

can abiotically bioconvert SeO_4^{2-} and/or SeO_3^{2-}, leading to the precipitation of Se^0 in the EPS [86]. Unlike SRB, *S. oneidensis* biofilms grown under anaerobic conditions can reduce TeO_3^{2-} and SeO_3^{2-}, accumulating Te^0 and Se^0 in both the cells and the EPS, respectively [125].

Since microorganisms grown as biofilms showed to play an important role in metal and chalcogen geochemistry [126], several biofilm-based reactors have been used to support the biosorption and the bioconversion of Se- and Te-oxyanions as detoxification strategy [8]. Indeed, *Burkholderia cepacia* biofilm grown on alumina surface [127], as well as a mixed species biofilm composed of *Dechloromonas* sp. and *Thauera* sp. [128] have been explored for Se-oxyanions bioremediation, resulting in the uptake and bioconversion of SeO_4^{2-} to Se^0 by the bacterial cells. Similarly, biofilms-containing denitrifying and sulfate-reducing microorganisms grown on a hallow-membrane biofilm reactor have been successfully used to remove SeO_4^{2-} from wastewater [129, 130], while the pre-grown biofilm of the SRB *Desulfomicrobium norvegicum* resulted able to abiotically reduce SeO_3^{2-} extracellularly through its production of S-Se granules within the EPS [86]. Further, biofilm formed by TeO_3^{2-}-resistant isolates of non-sulfur marine photosynthetic bacteria showed their proficiency in bioconverting this Te-oxyanion through intracellular reduction [131].

3. Microbial consortia for the treatment of selenium and tellurium contaminated wastewaters

3.1. Microbial consortia

In the environment, microorganisms usually thrive as communities composed by multiple species, generally referred as microbial consortia [132]. The employment of these microbial consortia in the treatment of environmental matrices contaminated with different inorganic or organic pollutants is currently a field of great interest for researchers [133]. There are significant advantages for the utilization of microbial consortia over pure cultures, such as the larger volumes of wastewaters treatable, the ability of microbial communities to adapt to diverse conditions, the presence of synergic interactions among members within the consortium and the possibility to work in non-aseptic conditions [23]. This last aspect is particularly significant, since it facilitates process control and it reduces both maintenance and operational costs [134].

In the following section, the different biological systems based on processes of biosorption and bioconversion of Se- and Te-oxyanions from contaminated matrices by using microbial consortia will be discussed.

3.2. Microbial consortia for Se-removal from contaminated environments

In recent years, the utilization of biological treatments based on the exploitation of microbial consortia has become the leading approach for the removal of toxic Se-species from environmental matrices, particularly from wastewaters (i.e., mine runoff, agricultural drainage, and flue gas desulfurization wastewater from plants) [23]. This decontamination strategy has

several advantages over chemical–physical remediation technologies, being: the cost-effectiveness of microbial-based remediation approach, the avoidance in employing hazardous chemicals, and the possibility to recover Se^0 in a recyclable form either as precipitates or as nanostructures, which are technologically and economically more valuable [23, 135]. Since using microbial consortia under aerobic conditions has a lower efficiency of the whole system compared to the anaerobic processes, microbial communities used in these systems are mostly capable of anaerobically bioconverting Se-oxyanions to their elemental state [136]. In this regard, the dissimilatory reduction of SeO_4^{2-} under anaerobic conditions by a microbial community was firstly reported for sediment slurries by Oremland and coworkers [89], while an anaerobic co-culture isolated from agricultural drainage water in the San Joaquin Valley in California of a not-identified Gram-positive rod-shaped bacterium and a *Pseudomonas* sp. was capable of bioconverting both SeO_4^{2-} and SeO_3^{2-} to Se^0 [72]. Further, several anaerobic microbial consortia able to process Se-species have been found in biological wastewaters, such as activated, denitrifying, sulfate-reducing and methanogenic sludges [135]. Among them, methanogenic anaerobic granular sludges were the most effective to remove high SeO_4^{2-} concentrations using different electron donors (e.g., methanol, ethanol, acetate, lactate, glucose) [137].

Considering the large amount of Se-oxyanions present in laden wastewaters, different technologies and reactor configurations have been developed in order to treat these environmental samples (**Figure 1**), such as the ABMet® biofilter system, the electro-biochemical reactors (EBR), the biofilm reactors (BSeR), the membrane biofilm reactors (MBfR), the upflow anaerobic sludge blanket reactors (UASB) and the sequencing batch reactors (SBR) [23]. In the following sub-sections, examples of bioreactor configurations used to bioremediate Se-contaminated waters and their operating procedures are briefly discussed.

3.2.1. The ABMet® reactor system

The ABMet® reactor is both a biological and a filtration system, in which microbial consortia are grown on porous granular activated carbon (GAC) beds, creating anoxic conditions for optimal SeO_4^{2-} and SeO_3^{2-} reduction [23]. The system consists of biofilter tanks where Se-oxyanions are bioconverted to their elemental state, followed by the removal of Se^0 from the biofilter through a backwash cycle [138, 139]. This reactor uses a nutrient dosage tank generally containing a molasses-based solution, which acts as an electron donor sink for the microbial consortia, allowing the bioconversion of Se-oxyanions [139]. Thus, in this reactor configuration, the microbial communities require only a small amount of supplemented nutrient, decreasing the maintenance costs of the entire system [23]. Further, the GAC beds are used as substratum to sustain the bacterial growth, allowing the formation of a biofilm, which is morphologically more robust as compared to planktonic cells, resisting to the washing steps of the reactor [23]. Recently, Se-oxyanions bioconversion using anaerobic microbial communities inoculated in a ABMet® biofilter system has been observed within 16 h of empty bed contact time (EBCT) (i.e., the residence time of the water in the reactor) with a removal efficiency of 99.3% at the Duke Energy and Progress Energy in North Carolina [138]. Moreover, co-contaminants present in these wastewaters, such as NO_3^- and heavy metals, along with Se-oxyanions resulted to be removed with a high efficacy by the microbial consortium grown on the ABMet® biofilter system [23].

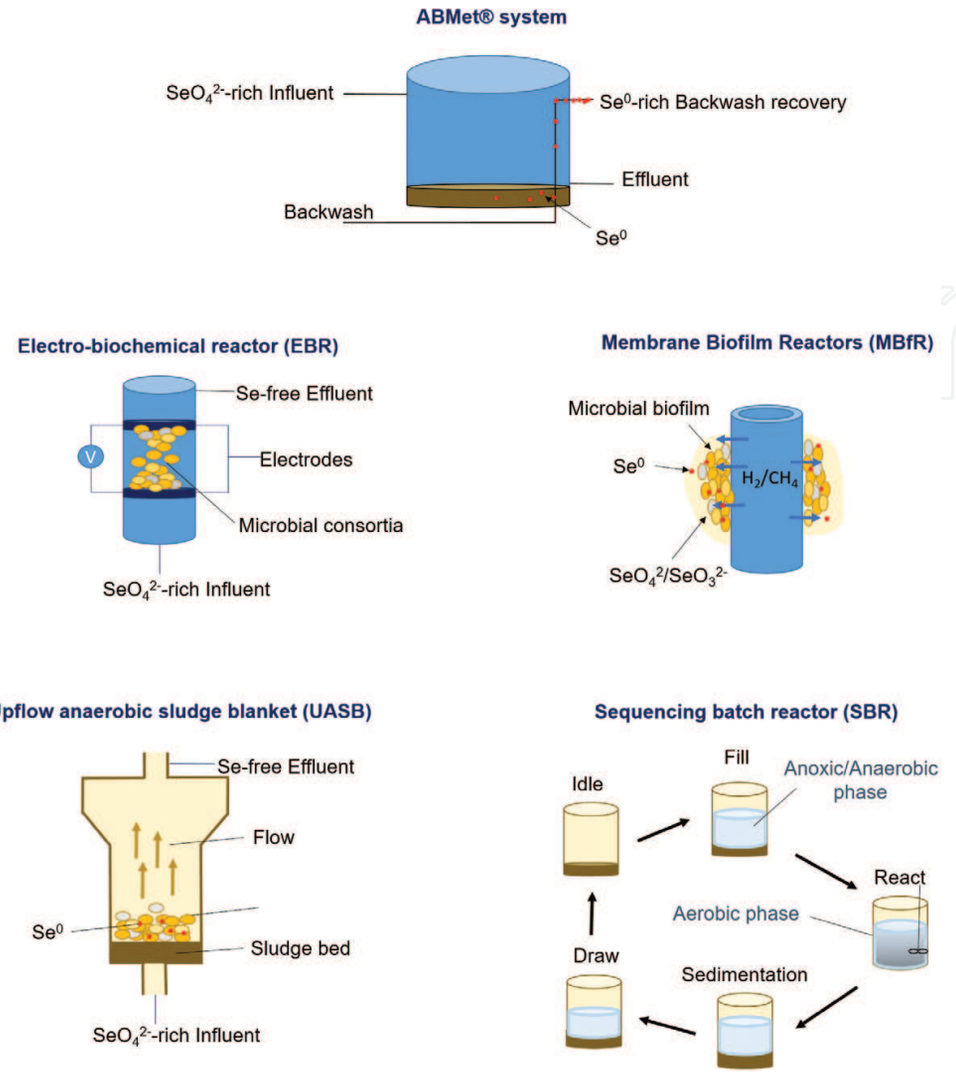


Figure 1. Schematic illustration of bioreactor configurations used for bioremediation of chalcogen-contaminated matrices.

3.2.2. The EBR system

Se-wastewater treatment is also achieved by using the electro-biochemical reactor (EBR), which utilizes the ability of certain microbial consortia to accept electrons from graphite electrodes reducing inorganic compounds (e.g., SeO_4^{2-} and SeO_3^{2-}) through direct interspecies electron transfer [140]. In this process, electrons obtained from the oxidation of electron donors (i.e., graphite electrodes) are transferred to the outer surface of a bacterial cell to reduce the extracellular terminal electron acceptor (i.e., Se-oxyanions) [140]. The efficiency of this system is strictly dependent on the retention times of the microbial consortia, with optimal performances between 6 to 18 h [141]. In this regard, on-site pilot scale study using an EBR system in British Columbia (Canada) for the decontamination of coal mine wastewaters from Se-oxyanions reported a decrease of their concentration from over 500–5 μg L^{-1} (below US discharge limits), showing its high effectiveness even with influent streams at temperature as low as 1°C [141].

3.2.3. The BSeR and MBfR systems

Reactors containing multispecies biofilms (BSeR) represent another promising approach for the treatment of Se-contaminated wastewaters. Indeed, microbial biofilms play a dominant role in the biogeochemical natural cycle of different inorganic compounds. In a recent study, a multispecies biofilm composed of strains (i.e., *Rhodococcus* sp., *Pseudomonas* sp., *Bacillus* sp. and *Arthrobacter* sp.) adapted to high concentration of SeO_3^{2-} has been investigated for its potential in converting these oxyanions to their elemental form (Se^0) [142]. Moreover, it has been highlighted the presence of specific biofilm regions where Se^0 was deposited as sub-micrometer-sized particles, associated with the microbial biomass [142]. In the BSeR methodology, bacterial biofilms are grown on granular activated carbon in anaerobic fixed-film reactors showing a high bioprocess proficiency toward both SeO_4^{2-} and SeO_3^{2-} [143], which resulted in the recovery of ca. 97% of Se^0 from agriculture drainage wastewater (Garfield Wetlands-Kessler Springs, Utah, USA) [144].

Another configuration of reactor based on microbial biofilms is the membrane biofilm reactor (MBfR) [129, 130, 145, 146]. MBfR in its standard configuration consists of a bundle of bubble-less gas transfer to a membrane delivering H_2 directly to the grown biofilm consisting of autohydrogenotrophic bacteria (e.g., *Cupriavidus metallidurans*) on the outer surface of the membrane [146], resulting in a higher efficiency of Se-oxyanions bioconversion as compared to other systems [143]. Although the membrane of the MBfR system can be made of either organic or inorganic materials, mostly hollow-fiber membranes are used at high gas pressures, providing a high surface-to-volume ratio [23]. Moreover, hydrophobic membranes are generally used in these systems, allowing to maintain the pores dry to achieve a fast diffusion of gas molecules [23]. In the MBfR system, the reduction of Se-oxyanions is coupled with the oxidation of H_2, acting as electron donor, which supports the growth of the autotrophic microbial consortia [129]. SeO_4^{2-} removal in this system has been improved to 94% by changing H_2 pressure, with Se^0 retained inside the microbial biofilm [129] in the form of crystalloid aggregates [147]. Similarly to the ABMet® system, the MBfR reactor resulted able to remove several oxidized toxic contaminants, such as chromium and arsenic, along with Se-oxyanions [23]. The microbial composition of a MBfR system exposed to different concentrations of SeO_4^{2-} was characterized by Ontiveros-Valencia and coworkers through 16S rRNA pyrosequencing [147]. Results showed that biofilms exposed to a high load of SeO_4^{2-} were composed principally by denitrifying bacteria belonging to the genera of *Denitratisoma* and *Dechloromonas*, which were previously reported as capable of reducing SeO_4^{2-} [147]. Recently, Lay and coworkers developed an MBfR system in which methane gas (CH_4) acted as electron donor instead of H_2, exploiting the microbial consortium capability to oxidize CH_4 coupled with SeO_4^{2-} reduction [148]. Particularly, the utilization of methane over H_2 has the advantages of lower cost and high availability from anaerobic digestion. Once again, the final product of the process are Se^0-nanospheres, accumulated in the microbial biomass [148]. A characterization of the microbial consortium by 16S rRNA sequencing revealed the presence of a specific methanotrophic genus (*Methylomonas*) that is able to simultaneously oxidize CH_4 and reduce SeO_4^{2-}, along with methanotrophic bacteria, which, upon methane utilization, are capable of generating organic metabolites suitable as electron donors for SeO_4^{2-}-reducing microorganisms present in the biofilm [148]. Although the MBfR system resulted to be a promising technology

to efficiently remove Se-oxyanions from contaminated environments, its implementation at industrial scale has not been investigated yet, likely due to the high cost of electron donors needed to the working-system, which is still prohibitive for large-scale applications [143].

3.2.4. The UASB system

Sludge-based reactors have also been employed for the treatment of Se-contaminated wastewaters [68]. Indeed, the most implemented process for anaerobic treatment of industrial effluents is the upflow anaerobic sludge blanket (UASB) reactor, because of the accumulation of microbial biomass and suspended solid, and a dense sludge bed at the bottom of the reactor, in which Se-oxyanions bioconversion occurs [68]. In this regard, the natural aggregation of some bacteria forming flocculates or granules leads to a high retention of active anaerobic sludge even at great organic load rates [149]. Additionally, the wastewater is kept in good contact with the bacterial biomass through both the turbulence of the upflow influent flow and the biogas produced by the anaerobic microorganisms [68]. UASB reactors have been pilot-tested for Se-removal at the Adams Avenue Agricultural Drainage Research Center in San Joaquin Valley (California) [150]. The influent had a total Se content of 500 μg L^{-1} and the removal efficiency ranged from 58 to 90% [150]. The efficiency of UASB reactors for the removal of Se-oxyanions was tested by Lenz and coworkers in a series of studies evaluating SeO_4^{2-} removal from synthetic wastewater by microbial consortia under methanogenic, sulfate-reducing and denitrifying conditions [151–153]. Using lactate as electron donor, a SeO_4^{2-} removal efficiency of 99% was obtained in both methanogenic and sulfate-reducing conditions, demonstrating that UASB reactors can be effectively applied to remove SeO_4^{2-} from contaminated wastewaters, with the involvement of sulfate-reducing bacteria (sulfate-reducing conditions) and a selenium-respiring sub-population (methanogenic conditions) [151]. Since the use of UASB reactors under methanogenic conditions leads to the recovery of decontaminated water, Se^0 and energy, methanogenic sludges are promising for Se-oxyanions bioconversion [143]. Further, Dessì and coworkers evaluate SeO_4^{2-} removal in UASB reactors as function of the temperature, observing that the maximum efficiency of removal was obtained at thermophilic conditions (55°C) [154]. Another advantage of working at this temperature is the better retention of reduced Se in the microbial biomass. Additionally, they performed a characterization of the microbial consortia through DGGE analysis, correlating the high SeO_4^{2-} removal efficiency to the presence of SeO_4^{2-}-respiring microorganisms, such as *Sulfurospirillum barnesii* and *D. indicum* [154]. UASB reactors are very promising for removing Se-oxyanions from contaminated wastewaters, however they require constant control, since any change in operation conditions may lead to an increase of the effluent Se-concentration through either biomethylation or bioconversion of Se-species [23].

3.2.5. The SBR system

Se-wastewater can be processed using a sequencing batch reactor (SBR), in which the biodegradation and solid separation take place in the same reactor [23]. In this configuration, the treatment is carried out in consecutive stages in the same tank: filling, reaction, sedimentation, draw, purging and inactivity [155]. The selection and enrichment of the desired microbial

consortia is achieved by the alternation of anaerobic and aerobic phases, which results in the complete integration of both oxic and anoxic conditions in the same reactor [69, 155]. The SRB systems have been mostly used in the treatment of textile wastewater, thanks to their efficiency in removing dyes [69]. Further, this system has been employed for Se-laden wastewater treatment by Rege and coworkers, which used a denitrifying bacterial consortium for the reduction of both SeO_3^{2-} and SeO_4^{2-} with acetate as electron donor, observing a lag phase of 150 h and a SeO_3^{2-} reduction rate higher than SeO_4^{2-} [156]. In other studies, SBR reactors have been used for the remediation of SeO_4^{2-} specifically inoculating the bacterial strains *Thauera selenatis* [157] and *Bacillus* sp. SF-1 [158]. However, SeO_3^{2-} accumulation in the reactor over the time exerted to a toxic effect toward the bacteria present in the system [158]. More recently, Mal and coworkers studied the potential of SeO_4^{2-} removal in the presence of NH_4^+ in an SBR inoculated with an activated sludge collected from a wastewater treatment plant [159]. In this study, the microbial consortium removed up to 100% of SeO_4^{2-} and 95% of ammonium through partial nitrification as well as nitrification/denitrification, with alternating between anaerobic and aerobic phases [159]. The efficiency of the system was improved by prolonging the anaerobic phase from 3 to 4.5 h. Interestingly, the effluent presented low concentrations of both volatile and elemental Se, suggesting that most part of biogenic Se^0 formed by the microbial consortium was retained in the activated sludge [159].

Even if the performances of the bioreactor configurations described above are promising, there are still challenges for the utilization of these approaches to remediate Se-laden wastewater, such as the presence of co-contaminations with different types of metals, the discharge limits for the effluent, and the disposal of the concentrated selenium solids [23, 143]. The bioremediation of Se-contaminated soils has been less explored than wastewater treatment. In this regard, a study by Prakash and coworkers, analyzing the capability of a microbial consortium, composed by aerobic rhizo-bacteria belonging to *Bacillus* genus, to remove SeO_4^{2-} and SeO_3^{2-} contamination from soils amended with different concentrations of these oxyanions [160]. The study revealed higher rate of removal for SeO_3^{2-} as compared to SeO_4^{2-}, due to the greater bioavailability in the soils of SeO_3^{2-} [160]. Moreover, microbial consortia can play a major role in assisting hyperaccumulator plants in phytoremediation approaches by enhancing both plant growth and Se-accumulation (**Figure 2**) [161, 162].

3.3. Microbial consortia for Te-removal from contaminated environments

Since Te-biogeochemistry is still poorly understood [34], to date few examples of microbial consortia employed for the bioconversion of Te-oxyanions into their elemental state (Te^0) are available in the literature [8]. Further, although Te-species are toxic for living organisms at very low concentrations [6], evaluating the actual amount of Te-contaminants present in environmental samples is challenging, due to their low general availability on Earth [34]. Indeed, even if TeO_4^{2-}- and/or TeO_3^{2-}- reducing bacteria are frequently isolated from natural microbial communities adapted to the stress exerted by Te-oxyanions [28, 107], the application of microbial consortia for their removal from contaminated matrices is still in its infancy.

One of the first studies regarding bioremediation of Te-contaminated environments was carried out by Baesman and coworkers, which isolated sediment slurries resistant to TeO_3^{2-} at Mono

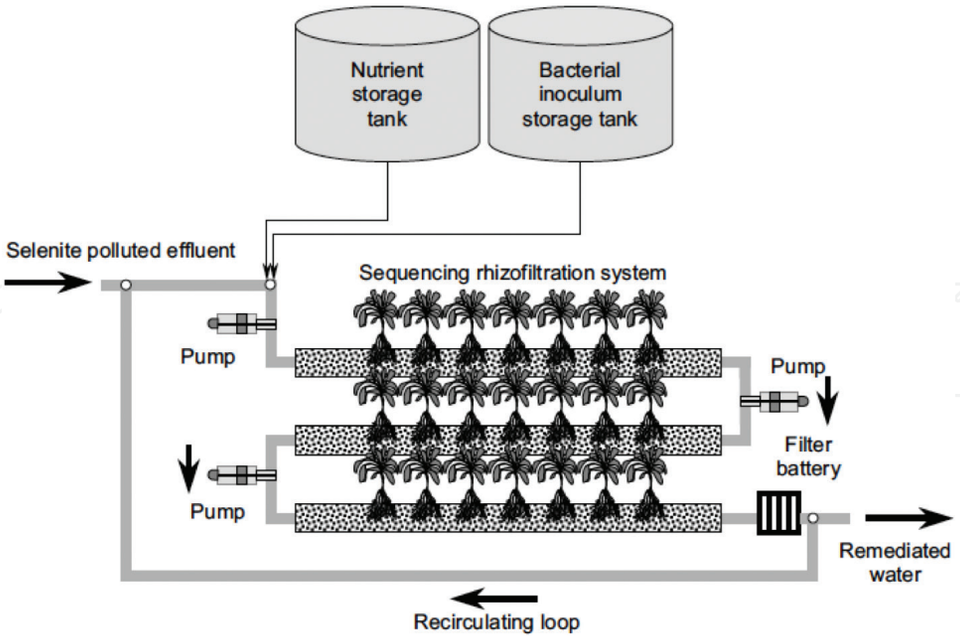


Figure 2. Schematic illustration of a phytoremediation system for the treatment of Se-wastewater through a synergistic cooperation of a Se-hyperaccumulator plant and selenite/selenate bioconverting bacteria of the rhizosphere [162].

Lake (California) [22]. Thus, the identified slurries were exposed under anaerobic conditions of growth to different concentrations of TeO_3^{2-} with either lactate or H_2 as electron donors, and they were incubated at 28°C for 30 days [22]. During the timeframe of microbial consortium's growth, a progressively blackening of the cultures has been observed, which indicated both Te-oxyanions bioreduction and the simultaneous accumulation of Te^0 precipitates, as proven by electron microscopy observations of the solid phase of the slurries [22].

More recently, Ramos-Ruiz and coworkers analyzed an anaerobic mixed microbial culture in a methanogenic granular sludge obtained from a wastewater treatment plant at Mahou's (beer brewery in Spain) [163]. In this regard, the granular sludge was chosen over planktonic cells considering that the latter one should be exposed more directly to the toxic Te-species [163]. As a result, the anaerobic sludge was able to catalyze the reduction of both TeO_4^{2-} and TeO_3^{2-} added to the system at a concentration of 20 mg L^{-1}, showing a rate of TeO_3^{2-} reduction seven-fold higher than TeO_4^{2-} one in all conditions tested [163]. As a consequence of Te-oxyanions bioconversion by the anaerobic sludge, the formation of intra and extracellular Te-nanoprecipitates has been detected through electron microscopy [163]. Interestingly, the microbial consortium did not show any lag phase when exposed to Te-oxyanions even in the case of a sludge originated from wastewater not contaminated with Te-species [163]. In order to avoid the possibility of an abiotic bioreduction of TeO_4^{2-} and/or TeO_3^{2-} by biogenic S^{2-} produced by SRB microorganisms generally present in microbial consortia, all the experiments have been performed in a (S)-free medium. Furthermore, the authors observed an increase of both TeO_4^{2-} and TeO_3^{2-} reduction rates after the amendment of different redox mediators, with riboflavin and lawsone causing the highest effect [163]. Finally, the addition of these redox mediators increased the percentage of extracellular Te-nanoprecipitates, determining a change in the shape of the nanomaterials produced [163].

A following study by the same research group evaluated the feasibility to use UASB reactors for the bioconversion of TeO_3^{2-} to Te-nanoprecipitates using a methanogenic microbial consortium in granular sludge, and the subsequent separation of the nanomaterials from the water effluent [164]. In this study, ethanol was added to the system as exogenous source of electron-donating substrate, while riboflavin was supplied as redox mediator during the biological process [164]. UASB reactors were operated with hydraulic retention time of 14 h at 28°C and supplemented with up to 20 mg L^{-1} of TeO_3^{2-} [164]. Similarly to the above-mentioned study [164], the presence of riboflavin as redox mediator enhanced the efficiency of TeO_3^{2-} bioconversion, lowering the toxicity of this oxyanion toward the microbial consortium. Moreover, a continuous removal of TeO_3^{2-} by the anaerobic microbial consortium was observed in the UASB reactor, showing a bioreduction efficiency ranging from 83%, when riboflavin was absent, to 99.5%, when riboflavin was added to the system [164].

TeO_3^{2-} removal from wastewater using a UASB bioreactor was also recently investigated by Mal and coworkers, which inoculated a UASB reactor with anaerobic granular sludge fed with lactate as carbon source, with a hydraulic retention time of 12 h at 30°C [165]. In the UASB reactor, firstly a concentration of 10 mg L^{-1} of TeO_3^{2-} was added, which was subsequently increased after 42 days to 20 mg L^{-1}. Te-oxyanion removal started immediately after the initial TeO_3^{2-} addition [165]. Particularly, after the first 3–4 weeks of sludge incubation in the reactor, a significant improvement of TeO_3^{2-} removal efficiency was observed, suggesting an adaptation of the microbial consortium to the presence of this oxyanion [165]. Furthermore, TeO_3^{2-} was almost completely bioconverted to its elemental state in the form of Te-nanostructures associated with the loosely bound EPS fraction surrounding the sludge, suggesting a pivotal role played by EPS and its functional groups in the biogenesis of Te-nanoprecipitates. In this regard, the possibility to easily recover Te-nanostructures associated with the EPS fraction opens new possibility to combine oxyanion removal with the recovery of Te^0 [165].

4. Microbial generation of Se- and Te-nanostructures

It is nowadays recognized the key role played by bacteria not only as tool for bioremediation purposes of highly contaminated Se- and Te-matrices, but also as a mean by which the less toxic and bioavailable elemental form of these chalcogens (i.e., Se^0 and Te^0) are generated and recovered. Indeed, yet Se and Te are elements featured by unique chemical-physical (i.e., semiconductive, photoconductive and catalytic) properties [166–169], which result to be emphasized in the nanosized material containing Se^0 and Te^0 as building blocks, forming nanoparticles (NPs) and/or nanorods (NRs). Se and Te as nanoscale structures are characterized by a large surface-to-volume ratio and a large surface energy as compared to their bulk counterparts [8], which make them suitable for biotechnological applications, such as: biomedicine, electronics, environmental engineering and agricultural industries [168, 170], to name a few. Since bacteria are considered inexpensive catalysts, their use for the production of Se- and Te-based nanostructures is an attractive choice over the chemical synthesis processes [79]. Thus, microorganisms capable of generating biogenic nanomaterials are seen as *green* and cost-effective exploitable methods to synthesize high-quality nanostructures [10],

whose process occurs at standard conditions (i.e., near neutral pH, controlled temperature and pressure), and, more importantly, avoiding the use of harsh reducing agents as well as the production of toxic wastes deriving from the chemical synthesis approaches [171].

Considering the peculiar photoconductive, semiconductive and optical properties of Se, the use of Se-based nanomaterials has been investigated in a wide range of applications, such as in the production of new optical devices, photovoltaic solar cells, photographic exposure meters and rectifiers and photo-assisted fuel cells [172–175]. Moreover, Se-nanostructures resulted to act as good catalyst for both the chelation of mercury ions (Hg^{2+}) present as contaminants in different polluted environments [176], and the degradation of several toxic chemical compounds (e.g., trypan blue dye) [177], as well as an efficient bio-sensor for H_2O_2 in different matrices [178]. Similarly, Te is a narrow band-gap *p*-type semiconductor, which is featured by high photoconductivity, piezoelectricity and thermoelectricity [168, 169]. These versatile properties led to the exploitation of Te-nanomaterials as optoelectronic, piezoelectric and thermoelectric devices, infrared detectors and gas sensors [179, 180], to name a few. Further, since these chalcogen-nanostructures showed great adsorptive ability, biological reactivity and antioxidant functions, their use in biomedicine have been recently explored [8, 170, 181]. Both Se- and Te-nanomaterials resulted efficient tools in protecting living organisms from DNA oxidation [181], as well as promising antimicrobial and anticancer agents [182–187]. In this regard, several Se-nanostructures produced by different microorganisms have been tested for their antimicrobial efficacy, highlighting their ability to prevent the growth of pathogenic bacteria (i.e., *E. coli*, *P. aeruginosa*, *S. aureus*) either in the form of planktonic cells or as biofilms [182, 183, 186, 187]. Particularly, biogenic Se-nanomaterials resulted to be more efficient as compared to those synthesized by mean of chemical processes, showing a strong inhibitory effect of pathogenic bacterial growth at lower concentrations [183]. Moreover, studies carried out to evaluate the cytotoxicity of biogenic Se-nanostructures toward human cell lines (i.e., fibroblasts and dendritic cells) revealed their high biocompatibility [187], which is a fundamental feature for their possible biomedical applications. Although Te-nanostructures produced by microorganisms are less studied for biomedical applications than those containing Se, recently the potential of such nanomaterials as antimicrobials has been assessed [186], showing their good efficacy in inhibiting pathogens growth. Further, a promising technological application of biogenic Te-based nanostructures regards the production of quantum dots (QDs), which are semiconductors nanocrystals featured by unique electronic and optical properties, due to quantum confinement effects [188].

5. Summary

Bioremediation strategies of Se- and Te-polluted environments based on the ability of microorganisms to bioprocess these toxic oxyanion species is an environmental-sustainable choice to reclaim contaminated soils, groundwater, surface water bodies and sediments. The primary microbial process after biosorption is the bioreduction of chalcogen-oxyanions into their less toxic and bioavailable elemental forms (i.e., Se^0 and Te^0) generating, as end-products nanoscale materials, which can be recovered from the biomasses and used for technological purposes.

Acknowledgements

We gratefully acknowledge Prof. Giovanni Vallini, Dr. Simona di Gregorio and Dr. Silvia Lampis for the concession of the use of **Figure 2** in the present manuscript. Funding for this work by RJT is through a discovery grant from NSERC.

Author details

Elena Piacenza[1], Alessandro Presentato[2], Emanuele Zonaro[2], Silvia Lampis[2], Giovanni Vallini[2] and Raymond J. Turner[1]*

*Address all correspondence to: turnerr@ucalgary.ca

1 Microbial Biochemistry Laboratory, Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

2 Environmental Microbiology Laboratory, Department of Biotechnology, University of Verona, Verona, Italy

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