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MINIREVIEW

Microbial Transformations of Selenium Species of Relevance to Bioremediation

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Selenium species, particularly the oxyanions selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}), are significant pollutants in the environment that leach from rocks and are released by anthropogenic activities. Selenium is also an essential micronutrient for organisms across the tree of life, including microorganisms and human beings, particularly because of its presence in the 21st genetically encoded amino acid, selenocysteine. Environmental microorganisms are known to be capable of a range of transformations of selenium species, including reduction, methylation, oxidation, and demethylation. Assimilatory reduction of selenium species is necessary for the synthesis of selenoproteins. Dissimilatory reduction of selenate is known to support the anaerobic respiration of a number of microorganisms, and the dissimilatory reduction of soluble selenate and selenite to nanoparticulate elemental selenium greatly reduces the toxicity and bioavailability of selenium and has a major role in bioremediation and potentially in the production of selenium nanospheres for technological applications. Also, microbial methylation after reduction of Se oxyanions is another potentially effective detoxification process if limitations with low reaction rates and capture of the volatile methylated selenium species can be overcome. This review discusses microbial transformations of different forms of Se in an environmental context, with special emphasis on bioremediation of Se pollution.

Since the discovery in 1954 by Pinsent that the oxidation of formate by cell suspensions of *Escherichia coli* requires growth medium containing molybdate and selenite, there has been a growing interest in the biochemical role of selenium in microorganisms (1). Se is an essential component of selenoamino acids, such as selenomethionine and selenocysteine (the 21st proteinogenic amino acid), that occur in certain types of prokaryotic enzymes. Indeed, the requirement for selenite in *E. coli* growing on formate is linked to the fact that formate dehydrogenase contains selenocysteine. Other prokaryotic enzymes that contain selenocysteine include glycine reductase in several clostridia, formate dehydrogenases in diverse prokaryotes, including *Salmonella*, *Clostridium*, and *Methanococcus*, as well as hydrogenases in *Methanococcus* and other anaerobes. In addition, other bacterial Se-dependent enzymes, in which the selenium is part of the active site molybdenum-containing cofactor, include nicotinic acid dehydrogenase and xanthine dehydrogenase, which is present in certain clostridial species (2–4).

Reactions that are involved in the cycling of Se in soil, including those influenced by microbes, are diagrammatically summarized in Fig. 1. Of the four transformation reactions, dissimilatory reduction and methylation are considered the most important in terms of bioremediation. For example, the microbial reduction of toxic Se oxyanions (SeO_4^{2-} and SeO_3^{2-}) to the insoluble and less biologically available elemental selenium (Se^0) results in its removal from solution. Microbial transformation of nonvolatile Se forms to volatile compounds is a significant pathway of Se transfer from aquatic and terrestrial environments to the atmosphere. Moreover, the reduction and methylation of SeO_4^{2-} and SeO_3^{2-} are effective detoxification processes because the product (dimethyl selenide [DMSe] or dimethyl diselenide [DMDSe]) is 500 to 700 times less toxic than SeO_4^{2-} or SeO_3^{2-} (5–8).

Zehr and Oremland (9) tested the assumption that microorganisms involved in the S cycle can also reduce Se oxyanions since Se is adjacent to S in group 16 of the periodic table and both commonly occur in the +6, +4, 0, and −2 oxidation states. Washed cell suspensions of *Desulfovibrio desulfuricans* (a sulfate-reducing bacterium) were found to be capable of reducing small (nanomolar) amounts of SeO_4^{2-} to Se^{2-} at the same time as reducing SO_4^{2-} to S^{2-}. The reduction was dependent on the relative concentrations of SeO_4^{2-} and SO_4^{2-}. Increasing concentrations of SO_4^{2-} inhibited rates of SeO_4^{2-} reduction but enhanced SO_4^{2-} reduction rates. Subsequently, however, Oremland et al. (10) reported a novel bacterial dissimilatory reduction of SeO_4^{2-}, which occurs by pathways different from those for SO_4^{2-} and was spatially separated from sulfate reduction in the environment despite the presence of substantial concentrations of sulfate where it occurred. Thus, it can be concluded that Se and S have different reductive biogeochemical cycles and appear to involve distinct populations of microorganisms.

With respect to the remediation of seleniferous environments, microbial oxidation and demethylation of Se compounds are not often considered because of the low rates at which these reactions proceed. Microbial demethylation of Se compounds occurs when some microorganisms utilize methylated Se forms as their sole source of carbon and energy (5, 11). The aim of this review is to discuss the reactions involved in the microbial transformation of different forms of selenium and to consider these in an environmental context, with reference to the bioremediation of the element in polluted environments.

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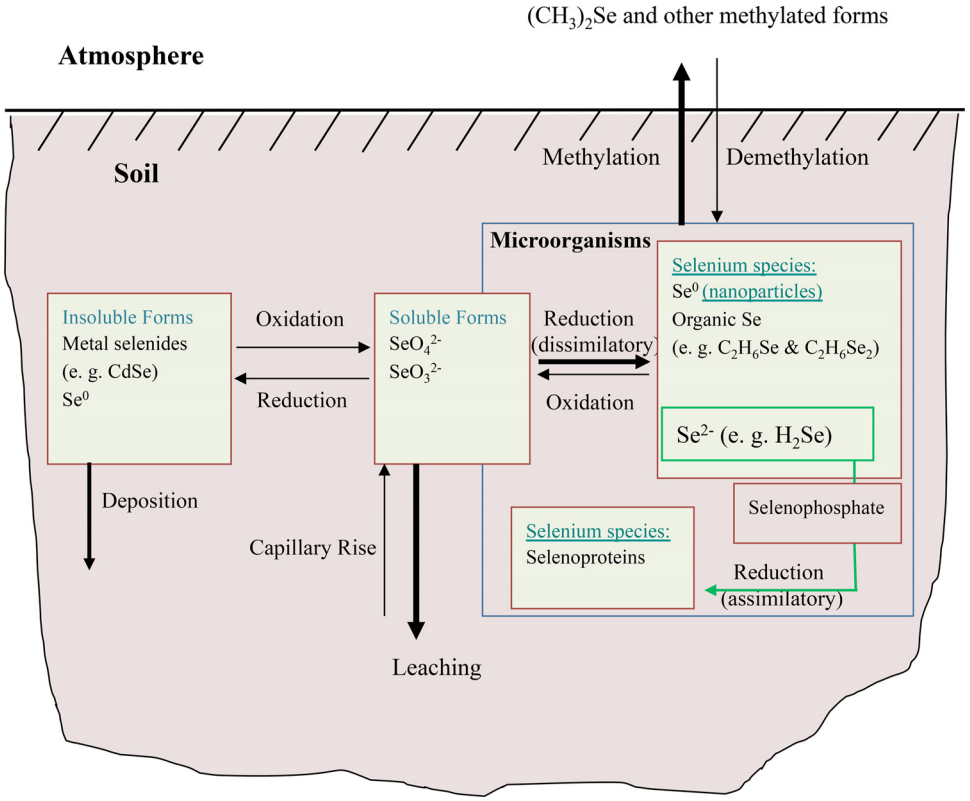

FIG 1 Schematic Se cycle in soil and the influence of microbial processes on the transformation of the element. The bold arrows indicate the dominant direction of the process. Modified from Flury et al. (110) with permission from Elsevier.

MICROBIAL REDUCTION OF SELENIUM SPECIES

During the microbial assimilation of Se oxyanions, selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are transported into the cells by different permeases. In the cell, the two oxyanions are reduced through assimilatory reduction to selenide (Se^{2-}) (12). In bacteria, selenophosphate is then produced by selenophosphate synthase. Selenocysteine is subsequently synthesized via the enzyme-catalyzed reaction of serine with selenophosphate, while the serine is attached to the $tRNA^{Sec}$ specific for insertion of selenocysteine into ribosomally synthesized proteins (13). Also, in the presence of excess available Se, cells begin to incorporate Se instead of S into cellular components that normally contain S (5).

In soil, sediment, and water, microbial reduction of SeO_3^{2-} and SeO_4^{2-} is known to be important process for removing toxic soluble Se oxyanions. In dissimilatory Se reactions, the reduction of Se oxyanions is a mechanism by which certain microorganisms can obtain metabolic energy (14). Dissimilatory Se-reducing microorganisms are known to use a number of different electron donors, such as alcohols, sugars, organic acids, humic substances, and hydrogen (15–19). In terms of the bioremediation of seleniferous environments, the assimilatory reduction of Se is expected to make only a minor contribution because of the small selenium fluxes involved. In contrast, the dissimilatory reduction of Se is considered to be the more important process for bioremediation. The reduction of selenium oxyanions, including reduction that is apparently not linked to respiration or assimilation, is a highly active reaction among many bacterial isolates and may play an important role in the environment (7). Research into dissimilatory reduction of Se is receiving increased attention, not least because results from these investigations offer a potentially cost-effective means of remediating selenium pollution. In contrast to insoluble Se^0, SeO_4^{2-} and SeO_3^{2-} are environmentally problematic in aqueous phases because of their high solubility. However, they become immobilized when the selenate and selenite are microbially reduced to Se^0 (20). Microbial reduction of Se^0 to selenide (Se^{2-}) has received limited attention, but it is noteworthy that insoluble Se^0 can be reduced microbiologically to soluble selenide (21, 22).

Certain bacteria are able to grow anaerobically through the dissimilatory reduction of selenium oxyanions. The product from dissimilatory reduction of selenite is generally Se^0, which appears in the form of Se nanoparticles. Microbial reduction occurs either in the periplasmic space (intracellularly) (23–25) or extracellularly (26–28). The reduction of Se oxyanions to Se^0 nanoparticles can also be mediated aerobically by diverse species of bacteria, namely, selenium-resistant bacteria (29–32). Several investigations have dealt with the mechanisms of microbial formation of Se nanoparticles (33–35). The Se nanoparticles are known to have microbial proteins associated with them, which play a role in the formation and growth of the Se nanoparticles (29) as well as in controlling their size distribution (33). Recently, Jain et al. (34) used biogenic elemental selenium nanoparticles (BioSeNPs), which were produced by anaerobic granular sludge in the treatment of pulp and paper wastewater, in an investigation of the presence of extracellular polymeric substances (EPSs) on the BioSeNPs. Functional group characteristics of proteins and car-

bohydrates were on the BioSeNPs, suggesting that EPSs form a coating that determines the surface charge on these BioSeNPs. EPSs also contribute to the colloidal properties of the BioSeNPs and thereby influence their fate in the environment and the efficiency of bioremediation technologies (34). Microbial reduction of Se may not only be exploited in Se bioremediation but also in the production of selenium nanoparticles for biotechnological applications (33, 36). However, the mechanisms involved in the formation of the nanoparticles and, more importantly, in their physical and chemical properties are yet to be fully elucidated.

Microorganisms that reduce the Se oxyanions SeO_3^{2-} and SeO_4^{2-} are not confined to any particular group of prokaryotes and are widely distributed throughout the bacterial and archaeal domains (37–49). However, compared to the SeO_3^{2-}-reducing microorganisms that have been isolated, the number of known SeO_4^{2-} reducers is relatively small. The reduction of SeO_4^{2-} to Se^0 is generally a two-step process in which SeO_3^{2-} is an intermediate product. Some bacteria are capable of reducing SeO_4^{2-} and SeO_3^{2-} to Se^0 (50–52), while other bacterial species can only reduce SeO_3^{2-} to Se^0 (53, 54). In some instances, dissimilatory reduction of SeO_4^{2-} supports growth via anaerobic respiration. In other cases, reduction of selenium oxyanions may serve a detoxifying function or be an adventitious reaction of enzymes with different functions. The reductions of SeO_4^{2-} and SeO_3^{2-} are considered in detail below. Major cultured selenium-reducing prokaryotes and their properties are summarized in Table 1.

Reduction of selenate. The mechanism of selenate reduction varies among the cultured microorganisms studied to date. Several selenate-respiring bacterial species (i.e., bacteria that can use selenate as the terminal electron acceptor to support growth), including *Thauera selenatis*, *Sulfurospirillum barnesii*, and *Bacillus arseniciselenatis*, have been well-characterized and shown to respire anaerobically by using SeO_4^{2-} as the terminal electron acceptor (55–57). Membrane-bound nitrate reductase (Nar), periplasmic nitrate reductase (Nap), and selenate reductase (Ser) have all been shown to be able to catalyze the reduction of SeO_4^{2-} to SeO_3^{2-}. Current evidence from *Enterobacter cloacae* (58) and other organisms indicates that selenate reductases have evolved specifically for the reduction of selenate and are more important in cultures of specific strains and, by implication, environmentally than the adventitious capacity of nitrate reductases to reduce selenate. Selenate reductase (Ser) has been purified and characterized from *T. selenatis* (20). It is a heterotrimer that is located in the periplasm, forming a complex of approximately 180 kDa containing the subunits SerA (96 kDa), SerB (40 kDa), and SerC (23 kDa). It contains molybdenum, iron, and acid-labile sulfur as prosthetic groups (20). Ser has been demonstrated to be specific for SeO_4^{2-} reduction to SeO_3^{2-} and does not use nitrate, nitrite, chlorate, or sulfate as electron acceptors. In contrast, the selenate reductase complex in *S. barnesii* is found in the membrane. It is a heterotetramer with subunits of 82, 53, 34, and 21 kDa and also contains molybdenum at the active site (59–61).

In the facultative anaerobe *Enterobacter cloacae* SLD1a-1, which can reduce selenate under aerobic conditions, the selenate reductase is located in the membrane fraction. It discriminates between SeO_4^{2-} and NO_3^- and is expressed under aerobic and anaerobic conditions. It is located in the cytoplasmic membrane, with its active site facing the periplasmic compartment (58). The enzyme is a heterotrimeric ($\alpha\beta\gamma$) complex with an apparent molecular mass of approximately 600 kDa. The individual subunit masses are 100 kDa (α), 55 kDa (β), and 36 kDa (γ). It contains molybdenum, heme, and nonheme iron in its prosthetic groups and displays activity on chlorate and bromate but none on nitrate (39, 62). It is noteworthy that the reductase of *E. cloacae* SLD1a-1 is similar to periplasmic Ser from *T. selenatis*. Both have active sites located in the periplasm, both are molybdoenzymes with catalytic α subunits of similar sizes (SerA is ~96 kDa), and both possess *b*-type cytochromes. Yee et al. (63) investigated the mechanisms of SeO_4^{2-} reduction using the Se-reducing bacterium *E. cloacae* SLD1a-1 in order to identify the gene(s) required for SeO_4^{2-} reduction. They demonstrated that the selenate reductase of the bacterium is controlled at the genetic level by the global anaerobic fumarate nitrate reduction (FNR) regulator and is induced under suboxic conditions.

Reduction of selenite. Microorganisms can carry out the conversion of SeO_3^{2-} to Se^0 via a number of different mechanisms (64–66). SeO_3^{2-} reduction can be catalyzed by reductases, including the periplasmic nitrite reductase, sulfite reductase, and dimethyl sulfoxide (DMSO) reductase (67–69). A number of thiol-mediated reactions have also been observed to reduce selenite to elemental selenium (14).

In *T. selenatis*, which is able to grow anaerobically with SeO_4^{2-} as the electron acceptor, little of the SeO_3^{2-} produced is reduced to Se^0 when SeO_4^{2-} is supplied as the sole electron acceptor. In contrast, SeO_3^{2-} formed during SeO_4^{2-} respiration is completely reduced to Se^0 by the same bacterium when NO_3^- and SeO_4^{2-} are available as electron acceptors. Mutants of *T. selenatis* that lack periplasmic NO_3^- reductase activity are unable to reduce either SeO_3^{2-} or NO_3^-, while mutants with increased nitrate reductase activity show rapid reduction of NO_3^- and SeO_3^{2-}. Together, these observations suggest that nitrate reductase is required for the reduction of SeO_3^{2-} to Se^0 by *T. selenatis* (67). *Pseudomonas seleniipraecipitans* strain CA-5 is capable of reducing SeO_3^{2-} and SeO_4^{2-} to Se^0. The strain is resistant to selenite at high concentrations (>150 mM). Two activities capable of reducing selenate were detected by zymography, one of which may correspond to nitrate reductase (70). Analyses of fractions from this strain indicate the presence of two reductases that can reduce SeO_3^{2-} to Se^0 in the presence of NADPH and that (based upon proteomics analysis of mixed protein samples) may correspond to glutathione reductase and thioredoxin reductase, both of which are able to reduce SeO_3^{2-} to Se^0 when derived from other sources (71). Similar zymography and proteomic analysis of fractions from *Rhizobium selenitireducens* suggest that a protein belonging to the old yellow enzyme (OYE) family of flavoproteins is capable of reducing SeO_3^{2-} to Se^0 using NADH as the electron donor (72). In a study by Li et al. (23), *Shewanella oneidensis* MR-1, an organism that shows substantial metabolic versatility and is known for its ability to perform biological electron transfer to solid minerals, is also able to reduce SeO_3^{2-} to Se^0. Specific mutants of *S. oneidensis* MR-1 have been used to investigate the contribution of the anaerobic respiration system to the microbial reduction of SeO_3^{2-}. Deletions of the genes that encode nitrate reductase (*napA*), nitrite reductase (*nrfA*), and two other periplasmic mediators of electron transfer for anaerobic respiration (*mtrA* and *dmsE*) were not impaired in their ability to reduce SeO_3^{2-}, which indicated that neither nitrate reductase nor nitrite reductase was essential for selenite reduction. In contrast, in the fumarate reductase (*fccA*) mutant of *S. oneidensis* MR-1, selenite reduction was decreased by 60% compared to that of the wild-type strain. This suggests that

TABLE 1 Cultured SeO_4^{2-}- and SeO_3^{2-}-reducing microorganisms and observed Se transformation reactions

Microorganism(s)	Se transformation	Reference
Bacteria with dissimilatory Se reduction supporting anaerobic respiration		
Thauera selenatis	Respiration via reduction of SeO_4^{2-} to SeO_3^{2-} in the absence of NO_3^-, minor reduction of SeO_3^{2-} to Se^0; in the presence of NO_3^-, SeO_4^{2-} is completely reduced to Se^0	40
Chrysiogenetes S5	Respiration via reduction of SeO_4^{2-} to Se^0	111
Deferribacteres S7		
Deltaproteobacteria KM		
Sulfurospirillum barnesii SES-3	Respiration via reduction of SeO_4^{2-} and SeO_3^{2-} to Se^0	37
Bacillus arseniciselenatis E-1H	Respiration via reduction of SeO_4^{2-} to SeO_3^{2-}	55
Bacillus selenitireducens MLS10	Respiration via reduction of SeO_3^{2-} to Se^0	55
Selenihalanaerobacter shriftii DSSe-1	Respiration via reduction of SeO_4^{2-} to Se^0	112
Archaea with dissimilatory Se reduction supporting anaerobic respiration		
Pyrobaculum arsenaticum and *Pyrobaculum aerophilum*	Anaerobic chemolithotrophs that also grow organotrophically with SeO_4^{2-} as electron acceptor; hyperthermophiles	113
Pyrobaculum ferrireducens	Anaerobic organotrophic growth on SeO_4^{2-} and SeO_3^{2-}; produces Se^0; hyperthermophile	49
Bacteria with dissimilatory Se reduction not clearly supporting respiration		
Rhodospirillum rubrum	Extracellular reduction of SeO_3^{2-} to Se^0; reduction under anoxic conditions is greater than that under oxic conditions	64
Rhodobacter sphaeroides	Reduction of SeO_3^{2-} to Se^0 with intracellular accumulation under aerobic and anaerobic conditions	53
Shewanella oneidensis MR-1	Extracellular reduction of SeO_3^{2-} to Se^0 under aerobic or anaerobic conditions	114
Clostridium pasteurianum	Enzymatic reduction of SeO_3^{2-} using hydrogenase I	42
Enterobacter cloacae SLD1a-1	Reduction of SeO_4^{2-} to Se^0 through SeO_3^{2-} as intermediate in the presence of NO_3^-	44
Azospira oryzae	Reduction of SeO_4^{2-} and SeO_3^{2-} to Se^0 under anaerobic and microaerobic conditions using O_2 or NO_3 as terminal electron acceptors for growth	45
Veillonella atypica	Reduction of SeO_3^{2-} to Se^0 and then to Se^{2-} under anaerobic conditions	22
Desulfovibrio desulfuricans	Reduction of SeO_4^{2-} and SeO_3^{2-} to Se^0 with formate as the electron donor and fumarate or sulfate as the electron acceptor	41
Enterobacter cloacae SLD1a-1	Reduction of SeO_4^{2-} to Se^0 through SeO_3^{2-} as an intermediate in the presence of NO_3^-	44
Pseudomonas stutzeri NT-1	Aerobic reduction of SeO_4^{2-} and SeO_3^{2-} to Se^0	46
Rhodopseudomonas palustris N	Aerobic reduction of SeO_4^{2-} and SeO_3^{2-} to Se^0	115
Wolinella succinogenes	Aerobic reduction of SeO_4^{2-} and SeO_3^{2-} to Se^0	116
Salmonella enterica serovar Heidleberg	Reduction of SeO_3^{2-} to intracellular granules Se^0	38
Ralstonia metallidurans CH34	Aerobic reduction of SeO_3^{2-} to Se^0	54
Salmonella Heidelberg	Aerobic reduction of SeO_3^{2-} to Se^0	38
Azospirillum brasilense	Reduction of SeO_3^{2-} to Se^0 nanoparticles	117
Pseudomonas sp. strain CA-5	Reduction of SeO_3^{2-} to Se^0 under aerobic conditions	70
Bacillus cereus CM100B	Reduction of SeO_3^{2-} to Se^0 under aerobic conditions	31
Bacillus megaterium BSB6 and BSB12	Aerobic reduction of SeO_3^{2-} to Se^0 at high salt concentrations	118
Duganella sp. strains C1 and C4	Reduction of SeO_3^{2-} to Se^0 nanoparticles	30
Agrobacterium sp. strains C 6 and C 7		
Pseudomonas sp. strain RB	Reduction of SeO_3^{2-} in the presence of cadmium producing CdSe nanoparticles	119
Archaea with dissimilatory Se reduction not clearly supporting respiration		
Halorubrum xinjiangense	Aerobic reduction of SeO_3^{2-} to Se^0; halophile	120
Well-studied example of assimilatory Se reduction		
Escherichia coli	Reduction of SeO_4^{2-} and SeO_3^{2-} to Se^0; incorporation of Se into proteins	51

FccA contributes substantially to selenite reduction in the organism. Deletion of *cymA*, which encodes a membrane-anchored *c*-type cytochrome that transfers electrons from the quinol pool in the cell membrane to various reductases (including fumarate reductase) that are involved in anaerobic respiration, resulted in a strain that exhibited only 9.6% of the selenite-reducing rate of the wild-type strain. While this indicates that a respiratory electron transport chain is involved in supplying electrons for the reduction of selenite, it is unclear whether this can support growth in *S. oneidensis* MR-1. In these experiments, the culture actually lost

biomass when it reduced selenite anaerobically, using lactate as an electron donor. Thus, the culture may have employed fumarate reductase to reduce the selenite and used it as a means of detoxifying selenite in the periplasm to prevent it from entering the cytoplasm, where it would be toxic (14, 23).

Reduction of selenite to elemental selenium has also been observed in living systems via a reaction that appears to be partly abiotic. Here, the selenite reacted chemically with biological thiol compounds, such as glutathione, via Painter-type reactions to produce molecules containing an S-Se-S bridge moiety known as a selenotrisulfide. It may break down spontaneously with the generation of reactive oxygen species, but it may also be reduced enzymatically by thioredoxin reductase or glutathione reductase, whose natural principal function is to regenerate the thiols in glutathione and thioredoxin through the oxidation of S-S bridges. When the substrate is a selenotrisulfide, the selenium is liberated as Se^0 (73). This may, of course, be the reaction via which glutathione and thioredoxin reductases are involved in the reduction of selenite to elemental selenium in *Pseudomonas seleniipraecipitans* (71) detailed above. Other reports of the reduction of SeO_3^{2-} to Se^0 by bacterial cultures include a detoxification mechanism in *Salmonella* (38).

The reduction of selenite to elemental selenium is clearly of pivotal importance to the bioremediation of selenium species, so further work is needed to provide information about the role and mechanisms of selenite reductases.

Reduction of selenium species to selenide. Dissimilatory reduction of selenium species to selenide (Se^{2-}) has been observed to at least a limited extent in environmental microorganisms. The obligate acidophile, *Thiobacillus ferrooxidans* can convert Se^0 to hydrogen selenide (H_2Se) under anaerobic conditions (74). The selenite-respiring bacterium *Bacillus selenitireducens* produces significant amounts of selenide when supplemented with Se^0. The strain is also able to reduce SeO_3^{2-} through Se^0 to Se^{2-} (21, 22).

OXIDATION OF SELENIUM COMPOUNDS

The oxidation of reduced selenium species may be relevant with respect to the availability of selenium as a trace nutrient for crop plants. It is not, however, considered to be of major relevance to the environmental toxicity of selenium species because of the low rates of transformation involved. Various studies indicate that microorganisms are capable of aerobic oxidation of Se^0 and SeO_3^{2-} in soil (75–77). A photosynthetic purple sulfur bacterium has been reported to use the oxidation of Se^0 to selenic acid (H_2SeO_4) as a sole source of energy (50), and *Acidithiobacillus ferrooxidans* has been shown to use copper selenide oxidation as a source of energy (76). Oxidation of Se^0 by an aerobic heterotrophic bacterium, a strain of *Bacillus megaterium*, that was isolated from soil via an enrichment procedure using elemental selenium has also been found to be capable of oxidizing Se^0 to SeO_3^{2-} and a trace of SeO_4^{2-} (<1% of SeO_3^{2-}) (77). The genes and enzymes and the pathways involved in the biological oxidation of selenium species have not yet been reported.

Studies with bulk soil have indicated that the oxidation of Se^0 in soils is largely biotic in nature, occurs at relatively low rates, and produces SeO_3^{2-} and SeO_4^{2-} (78). In a study of the oxidation of Se^0 in oxic soil slurries, SeO_3^{2-} was the predominant product, with small amounts of SeO_4^{2-} produced also. The oxidation rate constants were found to be between 0.0009 and 0.0117 day^{-1} in unamended soil slurries. Oxidation of Se^0 may have been carried out by heterotrophic bacteria, sulfur-oxidizing bacteria, and possibly fungi (79). These rates indicate that the removal of Se^0 from soil via biological oxidation would take hundreds of days. In contrast, field studies have shown that the SeO_4^{2-} pool of contaminated anoxic sediments can have turnover times of less than 1 h due to the reductive processes that are much more rapid (80). Oxidation, as well as reduction, of the selenium species also occurs during the methylation of the selenium species, which is considered in the next section.

METHYLATION OF SELENIUM SPECIES

Environmental microorganisms can use the Se methylation process as a mechanism to remove SeO_3^{2-} and SeO_4^{2-} by converting them to volatile compounds, such as dimethyl selenide (DMSe, CH_3SeCH_3) and dimethyl diselenide (DMDSe, $CH_3SeSeCH_3$). They may also be important in the natural cycling of Se to the atmosphere and may play a role as a detoxification mechanism, too (81).

A number of studies have shown microbial production of DMSe and DMDSe in various environmental samples, including soil, sewage sludge, and water, from selenium sources, including SeO_4^{2-}, SeO_3^{2-}, selenocysteine, and selenomethionine (82). A substantial number of cultured microorganisms, both bacteria and fungi, are now recognized as being able to produce methylated forms of selenium. Methylated forms of selenium produced by microorganisms also include dimethyl selenone [$(CH_3)_2SeO_2$, also known as methyl methylselenite] (83), dimethyl triselenide (DMTSe, $CH_3SeSeSeCH_3$), and mixed selenium/sulfur-methylated species, dimethyl selenyl sulfide (DMSeS, CH_3SeSCH_3,), dimethyl selenyl disulfide (DMSeDS, $CH_3SeSSCH_3$,), and dimethyl diselenenyl sulfide (DMDSeS, $CH_3SeSeSCH_3$) (84). Known cultured microorganisms that are capable of producing methylated selenium species are summarized in Table 2. The predominant groups of Se-methylating organisms that can be found in soils and sediments are bacteria and fungi, while bacteria are the active Se-methylating organisms in the aquatic environments (5, 50).

Selenium methylation pathways. If the initial form of selenium is one of the selenium oxyanions or elemental selenium, Se methylation must involve both reduction and methylation reactions. To date, a number of pathways have been suggested for the biomethylation of selenium, with evidence from proposed intermediates. Methyltransferases capable of methylating selenium species have been identified. The original pathway proposed by Challenger (85) suggested that methylation of SeO_3^{2-} by fungi involved the methylation and reduction of the Se atom in four steps to form DMSe as the final product (Fig. 2). Reamer and Zoller (83) subsequently reported that inorganic selenium compounds (SeO_3^{2-} or Se^0) are converted into DMDSe, DMSe, and dimethyl selenone (or possibly DMSeS [86]) by microorganisms in soil and sewage sludge. Challenger's proposed scheme was modified to introduce a branch that yielded DMDSe (Fig. 3). In this pathway, the methaneseleninic ion intermediate can form either methaneselenol or methaneseleninic acid, which would then be reduced to DMDSe. It was found that at low concentrations of SeO_3^{2-} (1 to 10 mg/liter Se), DMSe was the predominant product, while DMDSe or dimethyl selenone was produced at high concentrations of SeO_3^{2-} (10 to 1,000 mg/liter). In contrast, when Se^0 was added to sewage sludge, DMSe was the only product. There was a direct dependence of the production of DMDSe on the concentration of added SeO_3^{2-}, as at high concentrations of Se, DMSe

TABLE 2 Se methylating bacteria and fungi, with indications of selenium-containing substrate and methylated products

Organism(s)	Substrate(s)	Product(s)	Reference
Bacteria			
Corynebacterium spp.	SeO_4^{2-}, SeO_3^{2-}, Se^0	DMSe	11
Aeromonas spp.	SeO_4^{2-}	DMSe, DMDSe	121
Rhodocyclus tenuis	SeO_4^{2-}, SeO_3^{2-}	DMSe, DMDSe	122
Aeromonas veronii	SeO_4^{2-}, SeO_3^{2-}, Se^0, SeS_2, H_2SeO_3, NaSeH	DMSe, DMDSe, methylselenol, DMSeS	123
Bacillus spp.	SeO_3^{2-}, SeO_4^{2-}, selenocyanate	DMSe, DMSeS, DMDSe, DMSeDS, DMDSeS, DMTSe	84
Rhodospirillum rubrum S1	SeO_3^{2-}, Se^0	DMSe, DMDSe	124
Desulfovibrio gigas	SeO_3^{2-}	DMSe, DMDSe	125
Methanobacterium formicicum	SeO_3^{2-}	DMSe, DMDSe	125
Pseudomonas fluorescens K27	SeO_4^{2-}	DMSe, DMDSe, DMSeS	126
Citrobacter freundii KS8	SeO_4^{2-}	DMSe, DMDSe, DMSeS	126
Pseudomonas sp. strain Hsa.28	SeO_4^{2-}, SeO_3^{2-}	DMSe, DMDSe	126
Stenotrophomonas maltophilia	SeO_4^{2-}, SeO_3^{2-}	DMSe, DMDSe, DMSeS	127
Pseudomonas stutzeri NT-I	SeO_4^{2-}, SeO_3^{2-}, Bio-Se^0	DMSe, DMDSe	93
Fungi			
Scopulariopsis brevicaulis	SeO_4^{2-}, SeO_3^{2}	DMSe	128
Penicillium notatum/*Penicillium chrysogenum*	SeO_4^{2-}, SeO_3^{2-}	DMSe	129
Penicillium spp.	SeO_4^{2-}	DMSe	130
Cephalosporium spp.	SeO_4^{2-}, SeO_3^{2-}	DMSe	131
Fusarium spp.	SeO_4^{2-}, SeO_3^{2-}	DMSe	131
Candida humicola	SeO_4^{2-}, SeO_3^{2-}	DMSe	132
Alternaria alternata	SeO_4^{2-}, SeO_3^{2-}	DMSe	133
Penicillium citrinum	SeO_3^{2-}	DMSe, DMDSe	134
Acremonium falciforme	SeO_3^{2-}	DMSe, DMDSe	134

production was inhibited. During the 30-day period of the experiment, the maximum proportion of selenium across the tested concentration range that was volatilized was 7.9% (83).

Zhang and Chasteen (87) observed that the amounts of DMSe and DMDSe released from cultures of the Se-resistant bacterium *Pseudomonas fluorescens* K27 amended with dimethyl selenone were more than those formed from SeO_4^{2-}. This finding suggested that dimethyl selenone may be an intermediate in the reduction and methylation of selenium oxyanions (87), which is consistent with the proposed pathway for the production of DMSe (Fig. 2).

In the scheme proposed by Doran (50), the methylation of inorganic Se by soil *Corynebacterium* involved the reduction of SeO_3^{2-} to Se^0 and then a reduction to the selenide. The selenide was then methylated to form DMSe (Fig. 4). Although hydrogen selenide and methane selenol were not identified as intermediates, the roles of selenide and methane selenol as intermediates have been suggested in other investigations (88–90).

The bacterial thiopurine methyltransferase (bTPMT) from *Pseudomonas syringae*, which catalyzes methyl transfer reactions using *S*-adenosylmethionine (SAM) as the methyl donor, confers upon *Escherichia coli* the ability to transform selenite into DMSe and selenomethionine or (methyl)selenocysteine into DMSe and DMDSe (91). Production of methylated selenium species was also observed with an *E. coli* isolate that was transformed with a methyltransferase gene (*amtA*) from a freshwater isolate of *Hydrogenophaga* sp. that produced DMSe and DMDSe (92).

While rates for biological production of methylated selenium species are generally low, applications of selenium-methylating microorganisms in bioremediation and biotechnology have been suggested, such as for the recovery of selenium from seleniferous water via biovolatilization. A fermenter culture of *Pseudomonas stutzeri* NT-I under aerobic conditions was able to produce methylated selenium species at rate of 14 mol $liter^{-1}$ h^{-1}. The selenium could be recovered from the gas phase via a simple gas trap containing nitric acid (93).

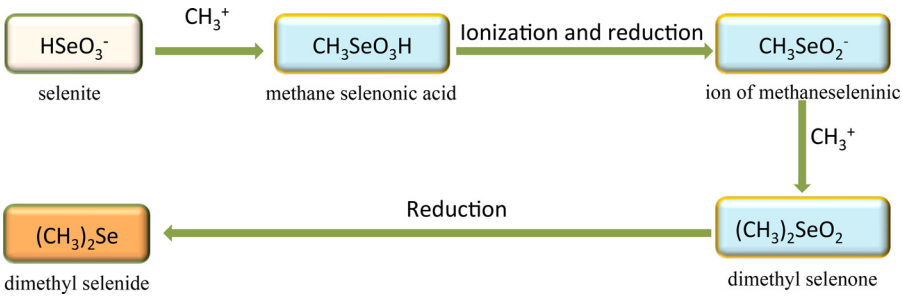

FIG 2 Challenger's pathway (85) for the microbial transformations of selenium.

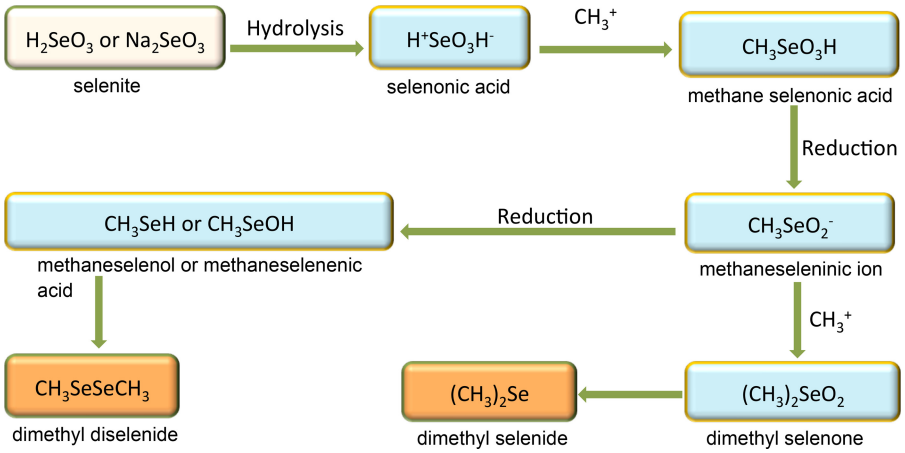

FIG 3 Reamer and Zoller's pathway (83) for the microbial transformations of selenium.

DEMETHYLATION OF SELENIUM COMPOUNDS

Doran and Alexander (11) isolated from seleniferous clay a pseudomonad able to grow on DMSe as well as strains of *Xanthomonas* and *Corynebacterium* that were able to grow on DMDSe as the sole carbon and energy sources. The pathways for breakdown of methylated selenium compounds, which presumably involve demethylation in such organisms, are currently unknown. In anoxic sediments, DMSe undergoes rapid demethylation. It has been suggested that DMSe could be anaerobically transformed to methane (CH_4), carbon dioxide (CO_2), and hydrogen selenide (H_2Se) by sediment organisms (methanogens and sulfate-reducing bacteria) in a pathway similar to dimethyl sulfide (DMS) degradation in freshwater and estuarine sediments (94).

SELENIUM BIOREMEDIATION

As its industrial and agricultural usage increases, increasing amounts of selenium (particularly in the forms of SeO_3^{2-} and SeO_4^{2-}) will be discharged into the environment, posing a threat to aquatic and terrestrial environments. Indeed, of the 2,700 tons of selenium that is produced annually, only about 15% is recycled (95). Therefore, there is a need to develop efficient, eco-friendly, and cost-effective methods for the remediation of Se pollution and also, where possible, for the recovery of this valuable element. As more stringent regulations come into force in order to limit the discharge of Se-containing waste, the use of bioremediation technologies are preferable because they will offer more cost-effective approaches for the removal of the pollutant. There has been a growing interest in the use of microorganisms in remediating Se-contaminated environments (96–99). In this context, a number of studies have been carried out in order to exploit the use of Se-oxyanion-reducing microorganisms in small/large-scale remediation schemes. These studies have demonstrated that many microorganisms may be used in remediation approaches designed for the treatment of Se-contaminated soil, sediments, and wastewater. Selenium is to a large extent immobilized and can be recovered in solid form after the biological reduction of selenium oxyanions to Se^0. Alternatively, if limitations due to low reaction rates can be overcome, the biological conversion of Se^0 to volatile methylated forms potentially permits remediation and subsequent removal and collection in a controlled manner.

A range of carbon and energy sources have been tested as electron donors for the microbial reduction of selenium species. These included inexpensive algal biomass, which has been explored as an electron donor and carbon source for bacterial reduction of SeO_4^{2-} to Se^0 as well as reduction of NO_3^- to N_2 in agricultural drainage (100). In another study, the SeO_4^{2-}-respiring bacterium *Thauera selenatis* was used to treat Se-oxyanion-containing oil refinery wastewater in a laboratory-scale bioreactor. A reduction of 95% of the soluble element was achieved from an initial concentration of 3.7 mg liter^{-1} (101). The SeO_4^{2-}-reducing bacterium, *Bacillus* sp. strain SF-1, has been tested in an anoxic continuous flow bioreactor under steady-state conditions for removing SeO_4^{2-} from a model wastewater containing 41.8 mg/liter SeO_4^{2-}, with lactate as the electron donor. The system effectively removed SeO_4^{2-} at short cell retention times (2.9 h), but there was accumulation of SeO_3^{2-} under these conditions. As the retention time was increased, more of the selenium was reduced to Se^0. Conversion of Se^0 was ≥99% at a cell retention time of 92.5 h and an Se^0 production rate of 0.45 mg liter^{-1} h^{-1} (102).

T. selenatis has been employed on a pilot scale for the remediation of Se-containing drainage water from the San Joaquin Valley, CA. The inflow to the reactor had a Se oxyanion (SeO_3^{2-} plus SeO_4^{2-}) concentration of 0.237 mg liter^{-1}. The reactor effected 97.9% conversion to recoverable insoluble Se^0 and left the treated water with only 5 μg liter^{-1} of selenium. This high removal of Se^0 was achieved via polymer coagulation with Nalmet 8072, which helped to overcome the general technical challenge of recovering Se^0 due to small particle size (103). The Se-reducing bacterium *Pseudomonas stutzeri* NT-I has also been effectively employed for the bioremediation of Se-containing refinery wastewater in 256-

FIG 4 Doran's pathway (50) for the microbial transformations of selenium.

liter pilot-scale bioreactors via reduction to elemental selenium (96). In a high-throughput sequencing study to investigate the effect of an electron acceptor on community structure during respiration of an activated-sludge-derived microbial population using hydrogen as the electron donor, principal-component analysis revealed a substantial shift in the composition of the microbial population upon the first addition of nitrate as an alternative to selenate as the electron acceptor (104). This gives additional evidence for the presence of environmental communities of microorganisms that utilize selenite as an electron acceptor and that these are, to a significant extent, distinct from nitrate-reducing microorganisms.

Since some algae can volatilize substantial quantities of inorganic Se compounds (105–107), algal methylation of selenium compounds offers a possible way to remove selenium from the aqueous phase. The inclusion of an algal pretreatment unit into a constructed wetland system was investigated in order to remove Se from river water entering the Salton Sea in California. The alga *Chlorella vulgaris* removed 96% of Se supplied as selenium oxyanions (1.58 mg liter^{-1}) from the microcosm water column within 72 h. With this arrangement, up to 61% of the selenium was removed by volatilization to the atmosphere, suggesting that an algal pretreatment stage can be included for selenium bioremediation into constructed wetland systems (108).

In addition to the problems that it causes as an environmental pollutant, selenium is an essential micronutrient and a valuable metalloid for which there are a dearth of high-yielding geological sources. Hence, the most advantageous systems for remediation of selenium pollution would put the recovered selenium to good nutritional or technological use. Elemental selenium is used in semiconductors. In this connection, it must be noted that a great diversity of prokaryotes are able to reduce selenium oxyanions to elemental selenium in the form of nanoparticles, which have properties that are difficult to mimic by chemical technologies. The microbially produced nanoparticles may have application in semiconductor and other technologies (14, 26). In effective selenium bioremediation, the selenium may have several acceptable fates. The likely fates of selenate in the presence of a variety of organisms have been demonstrated in an engineered aquatic ecosystem designed for brine shrimp production. In this investigation, selenate was taken up and metabolized differently by microalgae, bacteria, and diatoms to selenite, selenide, or elemental Se. Some of the biotransformed selenium species were incorporated and bioaccumulated as organic selenium compounds, as they were transferred between the different trophic levels. Organic selenium-enriched invertebrates suitable for human and animal consumption were produced as a result of these metabolic biotransformations (109).

Microbial methylation of inorganic Se oxyanions to volatile species offers a possible approach to bioremediation of selenium compounds in Se-polluted soils and aquatic environments. This has the attraction that the selenium may be completely removed in the vapor phase, although the limitation of low reaction rates would have to be overcome. In principle, organisms that demethylate selenium species may be used to recover vapor-phase selenium, provided that the reaction rate limitations and the possible production of toxic and volatile H_2Se can be overcome. Genetic characterizations of the pathways of selenium methylation and demethylation may enable their modification by overexpressing the necessary enzymes, resulting in acceleration of these processes.

CONCLUSIONS

Selenium species may be transformed in a diversity of metabolic reactions. Interest in the microorganisms capable of transforming selenium compounds involved in environmental pollution and in making selenium nutritionally available will increase as the activities of these organisms become better understood. Further characterizations of the mechanisms of selenite reduction to elemental selenium and of selenium methylation and demethylation are needed. Culture-independent analysis will be useful in studying the diversity and distribution of selenium-transforming organisms in a range of environments using a combination of functional gene analysis and metagenomics. Sequencing with 16S rRNA gene analysis should be fruitful in unraveling the role of microorganisms in the global selenium cycle. Their ability to produce selenium nanoparticles will be industrially exploited. Their ability to transform different selenium species by reduction, methylation, and demethylation will be harnessed further in the remediation of selenium-containing wastewater.

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