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REVIEW

Biological transformations of selenium by microorganisms

Lieke Riadi[1*] and John P. Barford[2]

[1]*Department of Chemical Engineering, The University of Surabaya, Surabaya 60292, Indonesia;*
[2]*Department of Chemical Engineering, The University of Sydney, NSW 2006, Australia*
Current Address: Department of Chemical Engineering, The Hongkong University of Science and Technology, Clear Water Bay, Kowloon, Hongkong.

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Abstract Microorganisms can enzymatically reduce a variety of metals in metabolic processes. Selenate [Se(VI)] and selenite [Se(IV)] which are known toxicants serve as terminal electron acceptors to support the growth of some microorganisms. Reduction of Se(VI) or Se(IV) to Se(0) is an important mechanism for the precipitation of selenium from contaminated waters. In addition, biomethylation of Se(VI) or Se(IV) is also known as one of the detoxification processes of these selenium oxyanions. A problem still remains in the characterisation of the carbon metabolism in selenium oxidising-reducing bacteria. Some researchers have investigated the utilisation of some carbon compounds for selenium reducing bacteria. However, a detailed study of carbon metabolism has not been reported. Furthermore, the effect of other ions (except nitrate) as competitive electron acceptor for selenium oxyanions has not been studied in detail. Therefore, it is of interest to fill the existing gap in this research area. In this paper, we review the biological transformation of selenium by microorganisms and describe initial experiments in our laboratories on the carbon metabolism of a selenium reducing microorganism and the effect of nitrate on electron acceptance by selenium oxyanions.

Key Words: Biological transformation, selenium, microorganism

INTRODUCTION

Among the elements, selenium (Se) ranks seventieth in order of abundance and it is widely dispersed in the earth's crust at low concentrations. It has been considered as a toxicant in the environment. Toxic effects in wildlife caused by excessive levels of selenium have been widely reported in the San Fransisco Bay Estuary (WSPA, 1993) which were due to oil refinery discharges, the liquid waste from a nickel-copper smelter in Ontario, Canada (Nriagu and Wong, 1983) the water of a coal fired power station in North Carolina (Merrill *et al.*, 1987) and the Kesterson wildlife refuge, San Joaquin Valley, California due to agricultural drainage water (Tanji *et al.*, 1986).

PROPERTIES OF SELENIUM

Selenium consists of 4 oxidation states: Se(VI), Se(IV), Se(0), Se(-II). Selenate [Se(VI)] the form most readily taken up by plants, is stable and very soluble in alkaline and oxidising conditions. Selenite [Se(IV)] tends to occur in mildly oxidising condtions at neutral pH environments. It is stable, soluble and can also be oxidised slowly to Se(VI) in alkaline environment. Elemental selenium is highly insoluble and very stable in water. It is not readily oxidised nor reduced chemically. It can appear as red crystalline, red or black amorphous or metallic hexagonal crystalline form. Selenide [Se (-II)] exists as hydrogen selenide, metal selenide, Se-sulfide salts, or in an organic combination which can either be in a methylated (volatile) form or a selenoaminoacid (non volatile) form. The stability, solubility and toxicity differ in each of the oxidation states of Se, as shown in Table 1 (Brooks,1984).

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*Author for correspondence. Mailing address: Mailing Address: Department of Chemical Engineering, The University of Surabaya, Surabaya 60292, Indonesia, Tel: 62-31-8491915 Extn. 1338, Fax : 62-31-8439167, E-mail: lieke@indo.net.id

Table 1. Some properties of selenium in different oxidation states (Brooks, 1984)

	Oxidation state	Solubility Stability	Relative	Toxicity
selenate	+6	highly soluble	stable	moderate
selenite	+4	moderately soluble	stable	moderate
selenide (M)$_2$Se M= heavy metal	-2	highly insoluble	stable	-
selenide, in methylated form, as organic compound,	-2	-	stable	low
selenide, in amino acid form asa organic compound,	-2	-	least stable	low
selenide, H$_2$Se	-2	-	least stable	high
elemental Se	0	highly insoluble	most stable	-

SELENIUM IN ENVIRONMENT

Selenium in aquatic systems. Under most pH and redox conditions, Se (IV) and Se(VI) are the dominant forms of selenium in water (Cutter and Bruland,1984). These forms also predominate in saline and alkaline evaporation pond water. Marine studies have shown that both Se (IV) and Se (VI) are present at higher concentrations in deep water than at the surface of sea water (Sugimura *et al.*, 1977). Inorganic selenium can dissolve in natural waters as Se(-II), primarily as biselenide (HSe^-), Se(0) as colloidal elemental selenium, Se(IV) as the selenite oxyanions ($HSeO_3^-$ and SeO_3^{2-}) and Se(VI) as the selenate oxyanion (SeO_4^{2-}).

Selenium in terrestrial systems. As in aquatic systems, the dominant forms of selenium found in soils under most pH and redox conditions are Se (IV) and Se(VI). The concentration and speciation of selenium in soil depends on the following conditions: pH, redox potential, solubility, complexing ability of soluble and soil ligands, biological interactions and reaction kinetics. Under the most oxidising conditions (>400 mV), Se (VI) is the dominant species in solution. In moderately oxidising groundwater (0 mV-400 mV), Se (IV) is the dominant species, whereas under strongly reducing and neutral pH conditions (< 0 mV), Se (-II) stability is dominated by HSe^-. In Hawaii and Puerto Rico, some soils with high concentrations of Se (6-15 mg/kg) have been found. However, they are non-hazardous because of the soils had a low pH (4.5-6.5), a high iron content and there being in a humid climate (Thompson-Eagle and Frankenberger, 1992).

Selenium in atmosphere. Atmospheric selenium is usually described in terms of its total concentration. A potentially important source of atmospheric selenium is natural biomethylation. Three different organoselenium species (DMSe, DMDSe and dimethylselenone) were identified in the atmospheric vapour phase originating from a coal fired plant, a smelter and some lakes (Jiang and Adams, 1983). In contrast, it has been shown that the form of the vapour phase selenium produced from a coal-fired plant was the elemental form (Andren *et al.*, 1975).

SOURCES OF SELENIUM

Selenium commonly exists in igneous rock and fossil fuels. The concentrations of selenium vary considerably depending on the location of the source. Coals and fuel oils contain up to 10 mg Se/kg and 7.5 mg Se/kg respectively (McNeal and Balistrieri, 1990). It enters the atmosphere through low temperature processes such as aerosol generation at the sea surface and biomethylation by soils and plants and high temperature processes such as industrial activities (smelting), the burning of fossil fuels (coal, oil) and volcanic activities (Frankenberg and Karlson, 1992). Some environmental parameters such as pH and redox conditions strongly influence the concentration, mobility and distribution of selenium in the environment.

The major objectives of this article are to review: (1) the biotransformation of selenium by a wide range of microorganisms; (2) the mechanism of carbon metabolism in selenium oxidising and reducing bacteria; (3) the biochemical pathway of selenium methylation and (4) the factors influencing the reduction and methylation of selenium by microorganisms.

BIOLOGICAL SELENIUM TRANSFORMATION

Reduction and Oxidation. Higher states of Se [Se(IV), Se(VI)] can be used by bacteria as electron acceptors in their energy-converting metabolisms resulting in lower oxidation states [Se(0), Se(-II)]. However, some microorganisms have been reported capable of oxidising selenium from lower ones as shown in equation (1) to higher oxidation states (Torma and Habashi 1972; Saratchandra and Watkinson, 1981)

$$Se(-II) \rightleftarrows Se(0) \rightleftarrows Se(IV) \rightleftarrows Se(VI) \quad (1)$$

Immobilization and mineralization. These biological processes reduce the accessibility of an essential element or a toxic element to microorganisms as summarised in equation (2); immobilisation is a process in which inorganic forms of Se are transformed to organic forms by microorganisms, animals or plants in terrestrial or aquatic environment, whereas mineralisation is the reverse process of that (Doran, 1982).

$$\text{inorganic Se} \;\underset{\text{mineralisation}}{\overset{\text{immobilization}}{\rightleftarrows}}\; \text{organic Se} \qquad (2)$$

One example of the immobilization of selenium is the synthesis of selenomethionine from Se(IV) by *Escherichia coli* (Tuve and Williams, 1961) and *Candida albicans* (Falcone and Giambaco, 1967).

Methylation. Methylation of most elements or compounds involves the enzymatic transfer of a methyl group from a donor compound. There are three cofactors known to be involved in the transfer of methyl groups in biological systems: (1) N^5-methyltetrahydrofolate derivatives, (2) cobalamin derivatives, and (3) S-adenosyl methionine. Both of the cobalamin derivatives and S-adenosyl-methionine are methyl donors in the microbial methylation of inorganic selenium (Doran, 1982).

Methyl-cobalamine (CH_3B_{12}), the methylated form of vitamin B_{12}, is able to transfer a methyl group to either cations, anions or nonionized species yielding reduced vitamin B_{12}. S-adenosyl-selenomethionine is also known as a biological methyl donor and usually involved in many biosynthetic methylations.

GROUPS OF ORGANISMS

Table 2 lists the groups of microorganisms which transform selenium from one state to another. The aerobic or anaerobic conditions present during these process are also noted. It is possible under this condition that the culture varied from aerobic at the top to microaerobic or anaerobic at the bottom. The term anaerobic sometimes refers to culture in vessels filled to the top and capped, as well as referring to cultures grown under strictly anaerobic conditions in reduced media. In other cases, it refers to anoxic conditions using a nitrogen atmosphere. The overall scheme of biological Se transformation which relates to the various groups of microorganisms is presented in Fig.1.

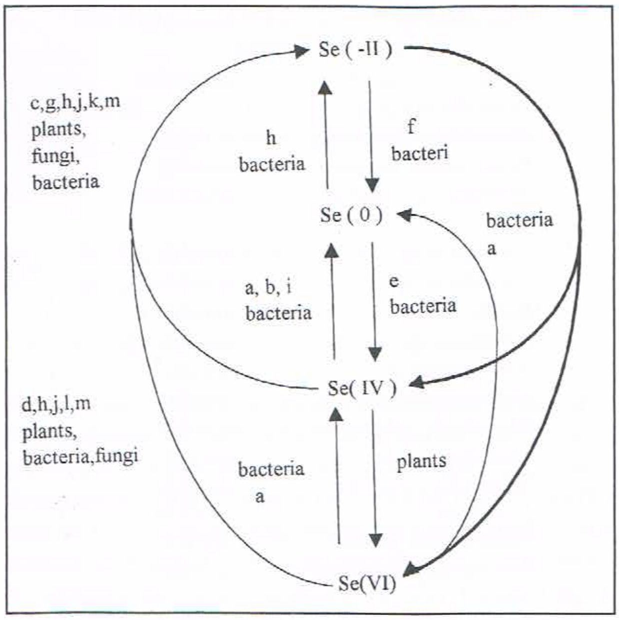

Figure 1. Biological selenium transformation, adapted from WHO (1987), slightly modified according to the report of Doran and Alexander (1977), Macy *et al.* (1989), Oremland *et al.* (1989), Steinberg *et al.* (1992) and Torma and Habashi (1972). Bold lines indicate processes requiring further investigation. The alphabets showed the types of microorganisms involved in transformation as can be seen in Table 2.

Bacteria. The major transformations of Se by bacteria are reduction, oxidation and methylation. In living systems, the tendency of Se to be reduced is greater than the tendency to be oxidised (Sugimura *et al.*, 1977). The reduction of a Se compound (Se (IV) to elemental Se by *Enterobacteriaceae* was reported to occur both extracellularly and intracellularly (Smith, 1959). It has been shown that the intensity of reduction was related to the density of the microorganisms. McCready *et al.* (1966) showed that *Salmonella heidelberg* reduced selenite to amorphous red Se as red intracellular granules when cultured in trypticase soy broth media. An anaerobic cell extract of *Micrococcus lactilyticus* has also been shown to use H_2 to reduce

Table 2. List of organisms and selenium transformation

Organism(s)	Condition	Selenium transformation	References
I. Bacteria :		reduction :	
a. mixed culture	anaerobic	Se(VI) → Se(IV)	Maiers *et al.* (1988)
		→ Se(0)	Oremland *et al.* (1989)
pure culture, gram negative, vibrio shaped	anaerobic		Steinberg *et al.* (1992)
Pseudomonas sp.	aerobic		Burton *et al.* (1987) Gersberg *et al.* (1986) Larsen *et al.* (1989)
Flavobacterium sp.	aerobic		Burton *et al.* (1987)
Citrobacter sp.	aerobic		Burton *et al.* (1987)
Salmonella typhimurium	aerobic		Gersberg et al. (1986)
Clostridium sp.	anaerobic		Kauffman *et al.* (1986) Baldwin *et al.* (1985)
*Pseudomonas sp.*AX + anaerobe strain E	anaerobic		Macy *et al.* (1989) Macy *et al.* (1993)
Rhodobacter sphaeroides	aerobic		Moore and Kaplan (1992)
Pseudomonas stutzeri	aerobic		Lortie *et al.* (1992)
Wolinella succinogenes	anaerobic		Tomei *et al.* (1992)
b. *Coryneform sp.*	aerobic	reduction:	Kauffman *et al.* (1986)
Arthrobacter sp.	aerobic	Se(IV) → Se(0)	Burton *et al.* (1987)
Bacillus sp.	aerobic		Burton *et al.* (1987)
Aeromonas sp.	aerobic		Burton *et al.* (1987)
Acinetobacter sp.	aerobic		Burton *et al.* (1987)
Streptococcus faecalis	aerobic		Burton *et al.* (1987)
Rhodobacter sphaeroides	aerobic		Moore and Kaplan (1992)
Enterobactericeae	aerobic		Smith (1959)
Salmonella heidelberg	aerobic		McCready *et al.* (1966)
S. faecium	aerobic		Tilton *et al.* (1967)
Pseudomonas pickettii	aerobic		Rialdi *et al.* (1994a)
Shewanella putrefaciens	aerobic		Rialdi *et al.* (1994a)
c. *Micrococcus lactilyticus*	anaerobic	reduction : Se(IV) → Se(0) → Se(-II)	Woolfolk and Whiteley (1962)
d. *Desulfovibrio desulfuricans subsp.estuarii*	anaerobic	reduction : Se(VI) → Se(-II)	Zehr and Oremland (1987)
e. *Bacillus megaterium*	aerobic	oxidation : Se(0) → Se(IV)	Sarathchandra and Watkinson (1981)
f. *Thiobacillus ferroxidans*	aerobic	oxidation : Se(-II) → Se(0)	Torma and Habashi (1972)
g. *Aeromonas sp.*	aerobic	methylation :	Chau *et al.* (1976)
Falvobacterium sp.	aerobic	Se(IV) → Se(-II)	Chau *et al.* (1976)
Rhodocyclus tenuis	aerobic		McCarty *et al.* (1993)
h. *Corynebacterium sp.*	aerobic	methylation : Se(0) → Se(-II)	Doran (1982)
Pseudomonas fluorescens	aerobic	Se(IV) → Se(-II)	Chasteen *et al.* (1990)
Rhodocyclus tenuis	aerobic	Se(VI) → Se(-II)	McCarty *et al.* (1993)
Rhodospirillum rubrum S1	aerobic	Se(VI) → Se(-II)	McCarty *et al.* (1993)

Cont. Table 2

II. Fungi		reduction :		
i. *Neurospora crassa*	aerobic	Se(IV) →	Se(0)	Zalokar (1953)
		methylation :		
j. *Acremonum falciforme*	aerobic	{Se(IV) →	Se(-II),	Karlson and Frankenberger (1989)
Penicillium citrinum	aerobic	Se(IV) →	Se (-II)}	Karlson and Frankenberger (1989)
Alternaria alternata	aerobic			Thompson-Eagle *et al.* (1989)
Fusarium sp.	aerobic			Barkes and Fleming (1974)
Cepahlosporum sp.	aerobic			Barkes and Fleming (1974)
Scopulariopsis sp.	aerobic			Barkes and Fleming (1974)
Candida humicola	aerobic			Cox and Alexander (1974)
Scopulariopsis brevicaulis	aerobic			Challenger and North (1934)
k. *Penicillium sp.*	aerobic	Se(IV) →	Se(-II)	Fleming and Alexander (1972)
Ulocladum tuberculatum	aerobic			Karlson and Frankenberger (1989)
l. *Aspergilus niger*	aerobic	Se(VI) →	Se(-II)	Challenger *et al.* (1954)
Schizopyllum commune	aerobic			Challenger and Charlton (1947)
III. Algae :		methylation :		
m. *Ankistrodesmus sp.*	aerobic	{Se(VI) →	Se(-II),	Oyamada *et al.* (1991)
Chlorella sp.	aerobic	Se(IV) →	Se(-II)}	
Selenastrum sp.	aerobic			

Se(IV) to Se (-II) (Woolfolk and Whiteley, 1962). Burton *et al.* (1987) reported that some heterotrophic bacteria (*Corynebacterium sp., Arthrobacter sp., Bacillus sp., Pseudomonas sp., Aeromonas sp., Flavobacterium sp., acinetobacter sp., Citrobacter sp.*) were capable of reducing Se(IV) or Se(VI) to Se metal but their study was inconclusive.

The Coryneform group has been demonstrated to reduce Se (IV) by observation of a red colour (indicating the presence of elemental selenium) when cultured on tryptone-glucose yeast agar with Se(IV) and the non appearance of a red colour when the microorganisms were grown on agar without Se (IV) (Kaufmann *et al.*, 1986). These bacteria have been demonstrated to have the ability to reduce Se (IV) to elemental selenum. *Clostridium sp.* are also reported to be able to reduce both Se (IV) and Se (VI) to selenium metal by making up to 90-95 % of colonies with an associated red colour on the anaerobic agar supplemented with Se (IV) and Se (VI).

Sulfate-respiring bacteria, *Desulfovibrio desulfuricans* subsp. *estuarii* reduce nanomolar Se (VI) to Se (-II). Based on the pathway of Se (VI) reduction, trace concentrations of Se (VI) could enter the pathway of dissimilatory sulfate reduction. It would then be converted to Se(-II) due to the presence adenosine-5-phosphoselenate (APSeO$_4^{2-}$ formed from ATP-sulfurylase) had greater stability than adenosine-5'-phosposulffate, APSO$_4^{2-}$ (Zehr and Oremland, 1987).

Macy *et al.* (1993) isolated a facultative anaerobe, *Pseudomonas sp.* AX which was named as *Thauera selenatis* and a strictly anaerobic rod shaped bacterium designated strain'E' which reduced Se (VI) to Se(IV) and Se (IV) to elemental selenium respectively. Only a small amount of elemental selenium was formed by *T. selenatis.* In their coculture, Se(IV) formed by *T. selenatis* was reduced by the anaerobe to elemental selenium.

A detailed study on a population of selenate reducing bacteria (mixed culture) and a pure culture of gram negative bacterium (vibrio shaped) known as strain SES-3 (Orremland *et al.*, 1994) showed the ability of these bacteria to reduce Se (VI) to elemental selenium under anaerobic conditions (Steinberg *et al.*, 1992). The presence of nitrate inhibited the reduction of Se (VI) to elemental selenium by mixed culture and totally inhibited the reduction by strain SES-3 (Orremland *et al.*, 1994). Hence, nitrate was the preferable electron acceptor to Se (VI) and was most likely reduced to NO$_2^-$. Oremland *et al.* (1990) also demonstrated in situ bacterial selenate reduction to elemental selenium in anoxic sediments.

A detail study of the fundamental kinetic of Se(IV) reduction to elemental selenium has been caried out in *Shewanella putrefaciens* under microaerobic conditions (Riadi *et al.*, 1994). The elemental selenium was formed outside the cells as examined using a transmission electron microscope equipped with an energy dispersive spectrometer. This should enable the recovery of selenium without breaking the cells. Furthermore, a phototrophic bacterium, *Rhodobacter sphaeroides* has high capacity to transform and reduce oxyanions, SeO_4^{2-} and SeO_3^{2-} to elemental selenium (Moore and Kaplan, 1992). With regards to strict anaerobe, *Wolinella succinogens* can precipitate elemental selenium from Se (VI) or Se (IV) after adaptation to selenium oxyanions. However, this bacterium cannot use selenium oxyanions for their respiratory growth (Tomei *et al.*,1992).

Although several studies have been conducted on selenium reduction, there are few studies done on selenium oxidation and methylation by bacteria. *Bacillus megaterium* has been reported to oxidize elemental selenium to Se (IV) (Sarathchandra and Watkinson, 1981). It utilized glucose as a carbon source, elemental selenium as an energy source, and oxygen as an electron acceptor. By deriving its energy from copper selenide, *Thiobacillus ferroxidans* (Torma and Habashi, 1972) oxidised Se(-II) to elemental selenium. It used Se(-II) as an energy source, CO_2 as a carbon source and oxygen as an electron acceptor.

Studies on methylating bacteria, *Aeromonas sp.* and *Flavobacterium sp.* showed that they were able to methylate sodium selenite to DMSe and DMDSe when grown in nutrient broth (Chau *et al.*, 1976). The production of these volatile selenium compounds depends on temperature. The lower the temperature, the less volatile selenium compounds are formed. Doran and Alexander (1977) isolated *Corynebacterium sp.* from soil and showed this bacteria can methylate elemental selenium, Se(IV) and Se(VI) to DMSe with a methyl donor S-adenosylmethionine. In addition, McCarty *et al.* (1993) reported some phototrophic bacteria, *Rhodocyclus tenuis* produced DMDSe from selenate and DMSe either from Se (IV) or Se (VI) whereas *Rhodospirillum rubrum S1* produce DMSe and DMDSe from selenate.

Fungi. Fungi have some potential value in selenium transformation, due to the ability to reduce and methylate selenium. *Scopulariopsis brevicaulis* was first confirmed by Challenger and North (1934) to be capable of producing DMSe from Se(IV) and Se(VI) when growing on bread crumbs. *Neurospora crassa* was able to reduce Se(IV) and precipitate selenium inside its mycelium as red droplets which are known to be amorphous selenium (Zalokar, 1953). There was no evidence of a detectable amount of DMSe. However, it was assumed that this fungus went through a methylation step followed by a reduction step to produce elemental selenium. Some soil fungi have been identified as active isolates in biomethylation of selenium, such as *Acremonum falciforme, Penicillium citrinum* and *Ulocladium tuberculacum* (Karlson and Frankenberg, 1989), which methylated either Se(IV) or Se(VI) to DMSe from soils spiked with radioactive labeled selenium. The methyl donor in this methylation was not reported. It could be already present in the soil.

Methyl cobalamin and L-methionine were used as a methyl donor in the methylation of Se(VI) and Se(IV) to DMSe by *Alternaria alternata* at pH 6.5 and T = 30°C (Thompson-Eagle *et al.*, 1989). *Fusarium sp., Cepahlosporum sp., Scopulariopsis sp.,* and *Penicillium sp.* have also been reported to methylate Se(IV) or Se(VI) to DMSe (Fleming and Alexander, 1972; Barkes and Fleming, 1974). Hydroxymethyl-aminomethane was used in the media and could be the methyl donor in this methylation.

Algae. Some green algae such as *Ankistrodesmus sp., Chlorella vulgaris* and *Selenastrum sp.* also play role in the methylation of Se (VI) and Se (IV) (Oyamada *et al.*, 1991). The volatile methylated selenium formed from these green algae are DMSe and TMSe (trimethylselenide). However, the mechanism of methylation in these algae has not been reported.

MECHANISM

Carbon catabolism and its interaction with oxidation and reduction of selenium oxyanions by microorganisms. The most common carbon and energy sources used by Se reducing bacteria are lactate and acetate. However, some Se reducing bacteria also use lactose and amino acids as their carbon and energy sources. Smith (1959) used semi-defined media containing lactose and peptone as their carbon and energy sources for the reduction of Se(IV) to elemental selenium by *Enterobacteriaceae.* Peptone contains amino acids with the major components being glutamate, aspartate and glycine. Aspartate and glutamate are degraded to oxaloacetate and α ketoglutarate prior to entry into the TCA cycle (Gottschalk, 1986). The predicted pathway of utilization lactose and major amino acids in this catabolism is shown in Fig.2. Tables 3 and 4 illustrate the mechanisms involved in the process of selenium transformation by some bacteria listed in Table 2. Some of these bacteria use a carbon source which differs from the energy sources, while others use a carbon source as their energy sources.

In our work with *S. putrefaciens,* glutamate, aspartate, alanine, serine and arginine are the preferred amino acids used by this bacterium. The fraction of

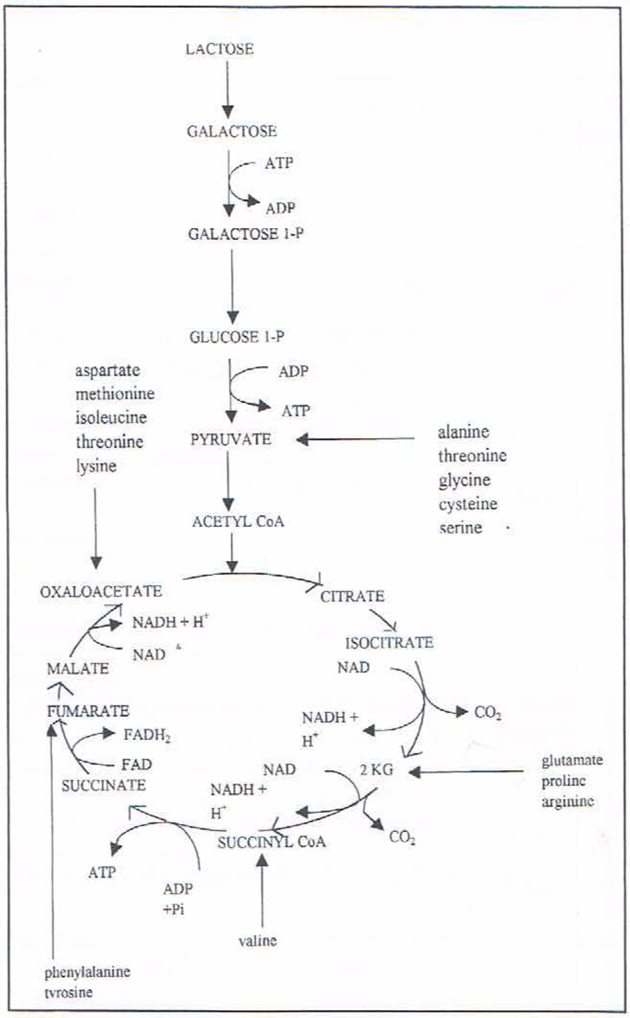

Figure 2. The predicted pathway for degradation of lactose and amino acids.

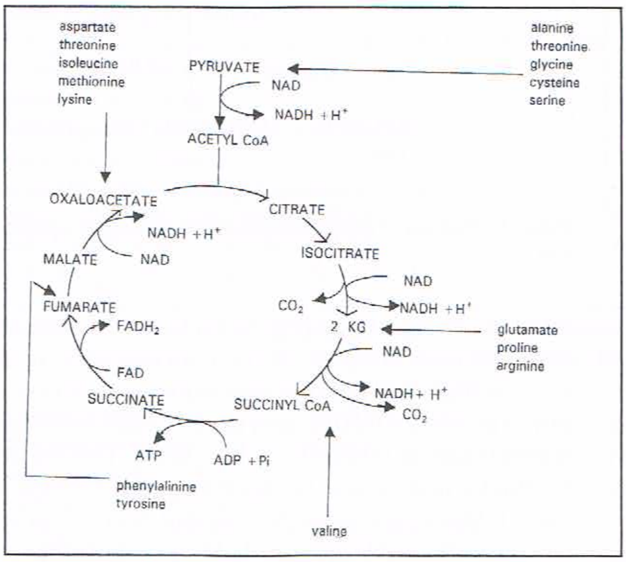

Figure 3. Biochemical pathway for degradation of amino acids in S. putrefaciens.

Table 3. Mechanism of selenium transformation by some selected bacteria which use different compounds for their carbon and energy source

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Bacteria : *Bacillus megaterium* (Sarathchandra and Watkinson, 1981)
Carbon source : glucose
Energy source : elemental selenium
Electron acceptor : O_2
Mechanism :
indirect energy formation:
$Se + 3H_2O \rightarrow SeO_3^{2-} + 6H^+ + 4e^-$
$2NAD^+ + 4H^+ + 4e^- \rightarrow 2NADH + 2H^+$
release of electrons :
$2NADH + 2H^+ \rightarrow 2NAD^+ + 4H^+ + 4e^-$
electrons acceptance :
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

Bacteria : *Thiobacillus ferroxidans* (Torma and Habashi, 1972)
Carbon source : Carbon dioxide
Energy source : Copper selenide
Electron acceptor : O_2
Mechanism :
indirect energy formation:
$Cu\,Se + 2H^+ \rightarrow Cu^{2+} + Se + 2e^- + 2H^+$
$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+$
release of electrons :
$NADH + H^+ \rightarrow NAD^+ + 2H^+ + 2e^-$
electrons acceptance :
$1/2O_2 + 2H^+ + 2e^- \rightarrow H_2O$

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amino acids used for anabolism is lower than that for catabolism. The fraction of glutamate, aspartate, alanine, serine and arginine for anabolism and catabolism are as follows: 0.0373, (0.9627); 0.04824, (0.9518); 0.0966, (0.9034); 0.0722, (0.9278); and 0.0845, (0.9155) respectively. The biochemical pathway of this bacterium grown in complex medium is presented in Fig.5 (Riadi and Barford, 1994).

Biochemical pathway of methylation of selenium oxyanions. As previously discussed , the major groups of selenium methylating organisms isolated from soil and sediment are bacteria and fungi. However, in water, bacteria play a more dominant role (Thompson-Eagle and Frankenberger, 1991). DMSe is the major metabolite of selenium volatilization, although DMDSe and dimethylselenone have also been detected.

The selenium compounds involved in methylation by fungi were considered as negative ions (Bird and Challenger, 1942). The biochemical pathway of methylation of Se(IV) with methionine as methyl donor, was proposed by Challenger (1951). It involves a series of steps in which the selenium atom is methy-

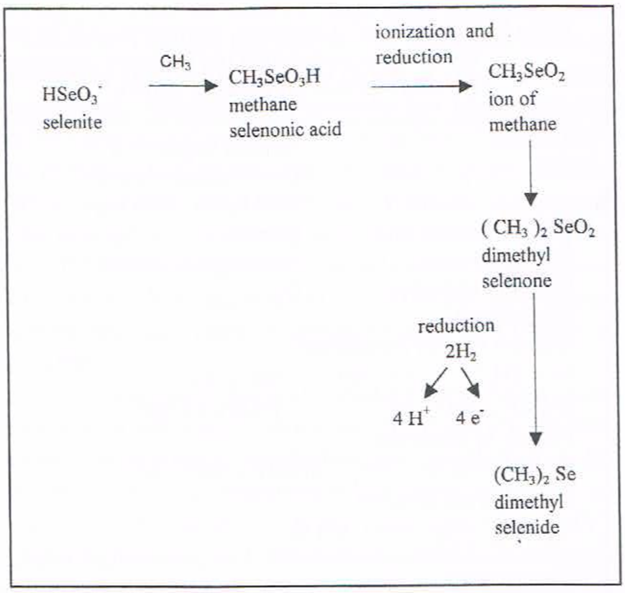

Figure 4. Biochemical pathway of methylation of selenite proposed by Challenger (1951).

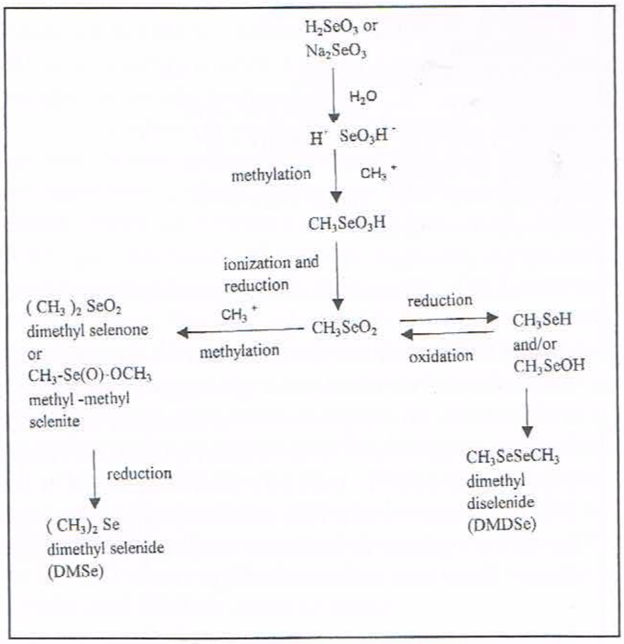

Figure 5. Biochemical pathway of selenium methylation proposed by Reamer and Zoller (1980).

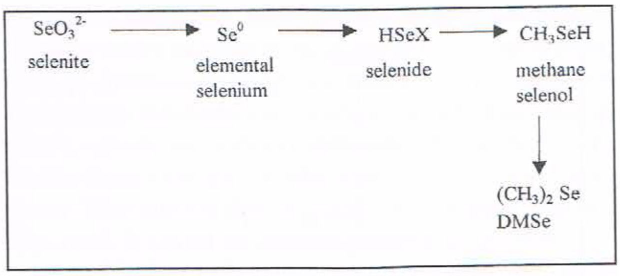

Figure 6. Selenium methylation pathway proposed by Doran (1982).

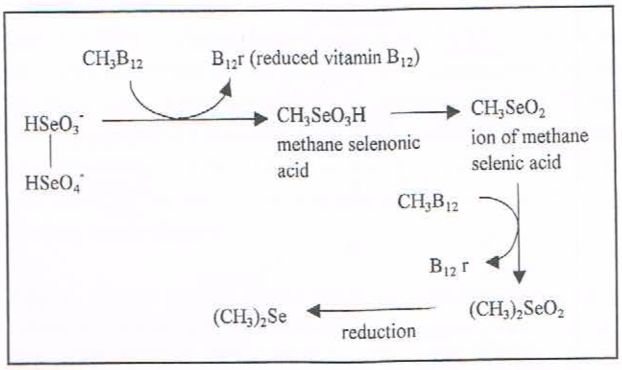

Figure 7. Propopsed pathway of selenium methylation in A. alternata.

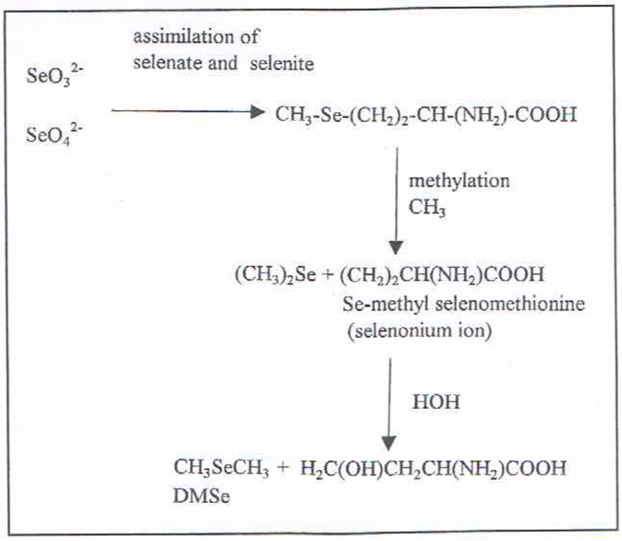

Figure 8. Pathway of selenium methylation in natural aqueous system.

lated and reduced to give DMSe as the final product (Fig. 4).

Reamer and Zoller (1980) suggested that the methylation pathway proposed by Challenger (1951) could be modified to include a concentration-dependent branch at the dimethylselenone [$(CH_3)_2SeO_2$] intermediate, where reduction could form either methyl-selenium-hydroxide (CH_3SeOH) or methane selenol (CH_3SeH), which would rapidly produce DMDSe. This pathway (Fig. 5) includes the formation of DMSe and DMDSe. If the concentration of selenium is high enough, the mechanism continues rapidly to produce DMDSe, and the left hand mechanism is shortened at $DMSeO_2$, $(CH_3)_2SeO_2$. The evidence for this formation are: (1) microorganisms which produce DMDSe are more Se tolerant and (2) at high concentration of Se, it is probably energetically easier for organisms to produce $DMSeO_2$ than to reduce it to DMSe (Reamer and Zoller, 1980). Hence, less DMSe

Table 4. Possible mechanisms involved in biological selenium transformation.

Bacteria : Enterobactericeae : *Salmonella typhimurium*, *E.coli* I, *Citrobacter freundii* I, *Klebsiella aerogenes* I, *Proteus vulgaris* (Smith, 1959)
Carbon and energy source : lactose , peptone
Electron acceptors : SeO_3^{2-}, O_2
Mechanism :
indirect energy formation*:
$4/23C_{12}H_{22}O_{12} + 48/23H_2O \rightarrow 48/23CO_2 + 8H^+ + 8e^-$
$4NAD^+ + 8H^+ + 8e^- \rightarrow 4NADH + 4H^+$
release of electrons :
$4NADH + 4H^+ \rightarrow 4NAD^+ + 4H^+ + 8e^-$
electrons acceptance:
$48\ (SeO_3^{2-} + O_2 + 10H^+ + 8e^- \rightarrow Se + 5H_2O)$

Bacteria : *D.desulfuricans* (Zehr and Oremland, 1987)

Carbon and energy source : sodium lactate
Electron acceptors : SO_4^{2-},SeO_4^{2-}
Mechanism :
indirect energy formation :
$4C_3H_6O_3 + 4H_2O \rightarrow 4C_2H_4O_2 + 4CO_2 + 16\ H^+ + 16e^-$
$8NAD^+ + 16H^+ + 16e^- \rightarrow 8NADH + 8H^+$
release of electrons :
$8NADH + 8H^+ \rightarrow 8NAD^+ + 16H^+ + 16e^-$
electrons acceptance :
$SeO_4^{2-} + SO_4^{2-} + 16H^+ + 16e^- \rightarrow Se^{2-} + S^{2-} + 8H_2O$

Bacteria : Mixed culture (Maiers *et al.*, 1988)
Carbon source : sodium lactate
Electron acceptors : O_2, SeO_4^{2-}
Mechanism :

indirect energy formation * :
$2.5C_3H_6O_3 + 7.5H_2O \rightarrow 7.5CO_2 + 2.5C_2H_4O_2 + 10H^+ + 10e^-$
$5NAD^+ + 10H^+ + 10e^- \rightarrow 5NADH + 5H^+$
release of electrons :
$5NADH + 5H^+ \rightarrow 5NAD^+ + 10H^+ + 10e^-$
electrons acceptance :
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$
$SeO_4^{2-} + 2H^+ + 2e^- \rightarrow SeO_3^{2-} + H_2O$
$SeO_3^{2-} + 6H^+ + 4e^- \rightarrow Se + 3H_2O$
$(O_2 + SeO_4^{2-} + 12H^+ + 10e^- \rightarrow Se + 6H_2O)$

Bacteria : Mixed culture : *Pseudomonas sp.*AX, anaerobe strain 'E' (Macy *et al.*, 1989)
Carbon source : sodium acetate
Electron acceptor : SeO_4^{2-}
Mechanism :
indirect energy formation :
$0.75C_2H_4O_2 + 1.5\ H_2O \rightarrow 1.5CO_2 + 6H^+ + 6\ e^-$
$3NAD^+ + 6H^+ + 6e^- \rightarrow 3NADH + 3H^+$

release of electrons :
$3NADH + 3H^+ \rightarrow 3NAD^+ + 6H^+ + 6e^-$
electrons acceptance :
$SeO_4^{2-} + 2H^+ + 2e^- \rightarrow SeO_3^{2-} + H_2O$
$SeO3^{2-} + 6H^+ + 4e^- \rightarrow Se + 3H_2O$
$SeO_4^{2-} + 8H^+ + 6e^- \rightarrow Se + 4H_2O$

Bacteria : Mixed culture (Oremland *et al.*, 1989)
Carbon and energy source : acetate or lactate
Electron acceptor : SeO_4^{2-}
Mechanism :
indirect energy formation :
(acetate as carbon and energy source)
$0.75C_2H_4O_2 + 1.5H_2O \rightarrow 1.5CO_2 + 6H^+ + 6e^-$
$3NAD^+ + 6H^+ + 6e^- \rightarrow 3NADH + 3H^+$
release of electrons :
$3NADH + 3H^+ \rightarrow 3NAD^+ + 6H^+ + 6e^-$
electrons acceptance :
$SeO_4^{2-} + 8H^+ + 6e^- \rightarrow Se + 4H_2O$
indirect energy formation * :
(lactate as carbon and energy source)
$1.5C_3H_6O_3 + 4.5H_2O \rightarrow 4.5CO_2 + 1.5C_2H_4O_2 + 6H^+ + 6e^-$
$3NAD^+ + 6H^+ + 6e^- \rightarrow 3NADH + 3H^+$
release of electrons :
$3NADH + 3H^+ \rightarrow 3NAD^+ + 6H^+ + 6e^-$
electrons acceptance :
$SeO_4^{2-} + 8H^+ + 6e^- \rightarrow Se + 4H_2O$

Bacteria : mixed culture (Steinberg *et al.*, 1992)
Carbon and energy source : acetate
Electron acceptors : SeO_4^{2-}& NO_3^-
Mechanism :
indirect energy formation :
$11/8C_2H_4O_2 + 11/8H_2O \rightarrow 11/4CO_2 + 11H^+ + 11e^-$
$5.5\ NAD^+ + 11H^+ + 11e \rightarrow 5.5NADH + 5.5H^+$
release of electrons :
$5.5NADH + 5.5H^+ \rightarrow 5.5NAD^+ + 11H^+ + 11e^-$
electrons acceptance :
$SeO_4^{2-} + 8H^+ + 6e^- \rightarrow Se + 4H_2O$
$NO_3 + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$
$NO_2^- + 4H^+ + 3e^- \rightarrow 1/2N_2 + 2H_2O$
$SeO_4^{2-} + NO_3^- + 14H^+ + 11e^- \rightarrow Se + 1/2N_2 + 7H_2O$

Bacteria : Mixed culture (Steinberg *et al.*, 1992)
Carbon and energy source : acetate
Electron acceptor : SeO_4^{2-}
Mechanism :
indirect energy formation :
$0.75C_2H_4O_2 + 1.5H_2O \rightarrow 1.5CO_2 + 6H^+ + 6e^-$
$3NAD^+ + 6H^+ + 6e^- \rightarrow 3NADH + 3H^+$
release of electrons :
$3NADH + 3H^+ \rightarrow 3NAD^+ + 6H^+ + 6e^-$
electrons acceptance :
$SeO_4^{2-} + 8H^+ + 6e^- \rightarrow Se + 4H_2O$

Table 4: Cont.

Bacteria : pure culture, gram negative,vibrio (Steinberg *et al.*, 1992)
Carbon and energy source : sodium lactate
Electron acceptors : SeO_4^{2-} &NO_3^-,
Mechanism :
indirect energy formation :
$C_3H_6O_3 + H_2O \rightarrow C_2H_4O_2 + CO_2 + 4H^+ + 4e^-$
$2NAD^+ + 4H^+ + 4e^- \rightarrow 2NADH + 2H^+$
release of electrons :
$2NADH + 2H^+ \rightarrow 2NAD^+ + 4H^+ + 4e^-$
electrons acceptance :
$2(NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O)$
Se (VI) is not used as electron acceptor in presence of NO_3^-

Bacteria : pure culture (gram negative vibrio) (Steinberg *et al.*, 1992)
Carbon and energy source : sodium lactate
Electron acceptor : SeO_4^{2-}
Mechanism :
indirect energy formation :
$1.5C_3H_6O_3 + 1.5H_2O \rightarrow 1.5C_2H_4O_2 + 1.5CO_2 + 6H^+ + 6e^-$
$3NAD^+ + 6H^+ + 6e^- \rightarrow 3NADH + 3H^+$
release of electrons :
$3NADH + 3H^+ \rightarrow 3NAD^+ + 6H^+ + 6e^-$
electrons acceptance :
$SeO_4^{2-} + 8H^+ + 6e^- \rightarrow Se + 4H_2O$

Bacteria : Mixed culture : *Pseudomonas sp.* (Larsen *et al.*, 1
Carbon and energy source : peptone, lactose
Electron acceptors : SeO_4^{2-}, O2
Mechanism :
indirect energy formation *:
$5/23C_{12}H_{22}O_{12} + 60/23H_2O \rightarrow 60/23CO_2 + 10H^+ + 10e^-$
$5NAD^+ + 10H^+ + 10e^- \rightarrow 5NADH + 5H^+$
release of electrons:
$5NADH + 5H^+ \rightarrow 5NAD^+ + 10H^+ + 10e^-$
electrons acceptance:
$SeO_4^{2-} + 8H^+ + 6e^- \rightarrow Se + 4H_2O$
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$
$SeO_4^{2-} + O_2 + 12H^+ + 10e^- \rightarrow Se + 6H_2O$

Bacteria : *Clostridium sp.* (Baldwin *et al.*, 1985)
Carbon and energy source : sucrose
Electron acceptor : SeO_4^{2-}
Mechanism :
indirect energy formation * :
$3/23C_{12}H_{22}O_{12} + 36/23H_2O \rightarrow 36/23CO_2 + 6H^+ + 6e^-$
$3NAD^+ + 6H^+ + 6e^- \rightarrow 3NADH + 3H^+$
release of electrons:
$3NADH + 3H^+ \rightarrow 3NAD^+ + 6H^+ + 6e^-$
electrons acceptance:
$SeO_4^{2-} + 8H^+ + 6e^- \rightarrow Se + 4H_2O$

Bacteria : Mixed culture : *Pseudomonas sp.* (Gersberg *et al.*, 1986)
Carbon and energy source : acetate & glucose
Electron acceptors : SeO_4^{2-}, O_2
Mechanism :
inderect energy formation *:
$5/16C_6H_{12}O_6 + 5/16C_2H_4O_2 + 10H_2O \rightarrow 10CO_2 + 10H^+ + 10e^-$
$5NAD^+ + 10H^+ + 10e^- \rightarrow 5NADH + 5H^+$
release of electrons :
$5NADH + 5H^+ \rightarrow 5NAD^+ + 10H^+ + 10e^-$
electrons acceptance :
$SeO_4^{2-} + O_2 + 12H^+ + 10e^- \rightarrow Se + 6H_2O$

* end product predicted theoretically

and more DMDSe is produced.

Doran (1982) disagreed with the pathway proposed by Challenger and suggested that the reduction of Se(IV) to Se(-II) occurs before methylation (Fig. 6). A study on the methylation of Se(IV) and Se(VI) by *Alternaria alternata* has been conducted, but the methylation pathway of this fungus was not reported. Based on the pathway proposed by Challenger (1951); Atlas and Bartha (1987) suggested that a predictive methylation pathway by the microorganism used methyl-cobalamin and L-methionine as a methyl donor (Fig. 7).

Cooke and Bruland (1987) gave evidence for a selenium biomethylation pathway in a natural aqueous system (Fig. 8) by detecting DMSe which is similar to sulfur. They suggested that the occurrence of DMSe is due to transformation of biogenically derived dimethylselenonium ions (DMSe+ -R) which is released from organically bound Se(-II) to DMSe intra- and/or extracellularly. The most readily utilized organo-Se compounds for the production of DMSe was selenomethione (CH_3-Se-$(CH_2)_2$-CH-(NH_2)-COOH) (Frankenberger and Karlson, 1989).

ECOLOGY OF SELENIUM REDUCING BACTERIA

In the natural environment, the role of selenium reducing bacteria is highly/moderately influenced by a range of physical, chemical and biological factors. For example, the depth of sediment influences the reduction rate Se(VI). The deeper the sediment the lower is the reduction rate (Oremland *et al.*, 1990).

Effect of pH. Since most bacteria grow well at neutral pH, the optimum pH for selenium reduction is around 7.0-7.5. For example, *Pseudomonas sp.* that reduces Se(VI) to Se(0), grows well at pH 7.2-7.5 (Macy *et al.*, 1989; Steinberg *et al.*, 1992). This pH range is also favoured by bacterial cultures from anaerobic sediment slurries (Oremland *et al.*,1989). Some bacteria such as *S. heidelberg* and *M. lactilyticus*, that reduce Se(IV) to elemental selenium and Se(-II) respectively, exhibit optimum growth at pH 8.5 and 6.5-7.0 respectively. In contrast, *Thiobacillus ferroxidans*, which oxidises Se(-II) to elemental selenium, grew under extremely low pH (pH 2.3). Some reduction processes of selenium also need critical value of pH. For instance, the preferable pH of treatment mine discharge water by *Clostridium* bacteria is 7.0 to 10.0 whereas the general pH of this effluent is 6.0-10.0 (Kauffman *et al.*, 1986). Hence at pH 6.0, *Clostridium* is not able to reduce selenium to any extent.

Salt tolerance. Dissimilatory reduction of selenate to elemental selenium by anaerobic sediment could occur in a mild-saline environment and does not correlate with salinity. There was a reduction of Se(VI) in sediments containing 60-250 g/l salinity, however there was no reduction of Se(VI) in hypersaline sediment containing 320 g/l salinity (Steinberg and Oremland, 1990).

Effect of temperature. The optimal temperature required for most Se(VI) and Se(IV) reducing bacteria is about 25-30°C (Maiers *et al.*,1988; Macy *et al.*, 1989). *Pseudomonas sp.* grow well at 26°C-28°C undertaking Se(VI) reduction (Steinberg *et al.*, 1992). *Desulfovibrio desulfuricans* and *Desulfotomaculum sp.* reduces Se(VI) to Se(-II) at T = 30 °C and some *Enterobacteriaceae* grow well at 37°C with Se(IV) reduction. Laboratory soil column tests, using *Clostridium sp.* (Kauffman *et al.*, 1986) showed that the impact on selenium removal of temperature variation. Colder temperature inhibits microbiological activity and hence retards the removal of selenium (Oremland *et al.*, 1989).

Inhibitors. The inhibitors of Se(VI) reduction are NO$_3$, NO$_2^-$, CrO$_4^{2-}$, tungstate, MnO$_2$, MoO$_4^{2-}$, WO$_4^{2-}$ (Oremland *et al.*, 1989; Steinberg *et al.*, 1992). The most common mechanism for this inhibition is illustrated by competition for electrons as alternative electron acceptors. NO$_3^-$ was reported as a preferable electron acceptor for selenate respiring bacteria (Steinberg *et al.*, 1992). It fully inhibits Se(VI) reduction whereas MnO$_2$ only partially inhibits Se(VI) reduction. In the presence of nitrate, Se(VI) respiring bacteria (Steinberg *et al.*, 1992) do not reduce Se(VI) to elemental selenium but convert NO$_3^-$ to NO$_2^-$. These bacteria use these nitrogen compounds as alternative electron acceptors. However, *Thauera selenatis* (Macy *et al.*, 1993) utilised Se(VI) as an electron acceptor without interference by NO$_3^-$. Our study also showed that *S. putrefaciens* transformed Se(IV) to elemental selenium without any interference of NO$_2^-$ (Riadi and Barford, 1995).

Other inhibitors for Se(VI) reduction could be sulfate (SO$_4^{2-}$) , high level of Se(VI) (1 mM) (Zehr and Oremland, 1987) or its end products which are below an oxidation state Se(IV) (Maiers *et al.*, 1988). The rate of Se(VI) reduction is inhibited by increasing concentrations of sulfate. Maiers *et al.* (1988) reported that the remaining Se(IV) reduction from Se(VI) appeared to be inhibited if more than 50 mg/l of Se(VI) has been reduced to lower than the oxidation state Se (IV) in an isolated mixed culture. This effect was considered as an end product inhibition. The excess of oxygen can also decrease the reduction of Se (VI) (Larsen *et al.*, 1989).

FACTORS AFFECTING SELENIUM METHYLATION ORGANISMS

Selenium species and concentration. Selenium in certain organic forms (Secysteine, Semethionine, TMSe) is readily transformed into volatile derivatives other than inorganic selenium (Doran and Alexander, 1977). Frankenberger and Karlson (1989) found that seleniferous soils methylated organic selenium compounds in the following orders: selenomethionine> selenocysteine = selenoquanosine = n selenoinosine > selenoethionine = selenopurine > selenourea. Not all microorganisms are able to transform organoselenium compounds as easily as they can transform some inorganic compounds. An *A. alternata* species was found to methylate various selenium species in pure culture in the following orders : SeO$_4^{-2}$ > SeO$_3^{-2}$ >> selenoinosine > selenomethionine > selenopurine > selenium sulfide (Thompson- Eagle *et al.*, 1989). Inorganic selenium are more effective subtrates for selenium methylation than that the organic selenium compounds as shown by symbol >>.

Elemental selenium is poorly methylated compared to other inorganic selenium species. It is probably due to its low solubility (Reamer and Zooler, 1980). However, Weres *et al.* (1989) showed that elemental selenium was readily available for microbial selenium methylation. Selenium concentration reduces the capacity of selenium biomethylation of a soil. The major volatile selenium species which evolved with an initial Se(IV) concentration between 1 and

10 mg/kg was DMSe, while at concentrations of ≥ 100 mg/kg Se(IV), DMSe clearly decreased, but the relative concentration of DMDSe and dimethylselenone increased (Thompson-Eagle and Frankenberger, 1992).

Temperature. Selenium volatilization is temperature dependent. However, maximum DMSe emission occurred at the maximum temperature tested in each case. Therefore the optimum temperature for biomethylation may not have been reached. In winter, selenium emission is relatively low, but it increases during spring and summer (Zieve and Peterson, 1981). The maximum release of DMSe from lake sediments occurs at 20 °C (Chau *et al.*, 1976). Thompson-Eagle *et al.* (1989) showed that the optimum temperature for methylation of selenium by *A. alternata* was 30 °C. In addition, the rate of selenium methylation from seleniferous soil (Kesterson reservoir) increased with increasing temperature (5 - 35 °C) with the optimum temperature for optimum production of DMSe being 35 °C (Frankenberger and Karlson, 1989).

Moisture. Selenium biomethylation requires the presence of water. Air drying the soil severely inhibits methylation (Zieve and Petrson, 1981). Field studies have also shown that the emission rates of selenium are much lower in dry sites than in corresponding damp or wet conditions (Weres *et al.*, 1989; Thompson and Frankenberger, 1992). The changes in the soil water content seem to promote selenium volatilization (Handy and Gissel-Nielsen, 1976). This, explained by the fact that the decomposition of organic matter in soil, is also directly related to repeated drying-rewetting cycle and hence nutrients may become more available for the soil microflora and increase the metabolic activity under these conditions (Sorensen, 1974).

pH and salinity. The optimum pH for selenium transformation varies. For example, *A. alternata* isolated from evaporated pond water was reported to have an optimum pH of 6.5 (Thompson-Eagle *et al.*, 1989). The optimum pH for biomethylation in seleniferous Kesterson sediments was 8.0 (Karlson and Frankenberger, 1989). Hamdy and Gissel-Nielson (1976) showed that selenium volatilization in soil was increased by increasing pH 6.0 to 7.0. Hence, each methylating microorganism has a different optimal pH for growth. Some selenium methylating microorganisms have been reported to be extremely tolerant in saline conditions (Cox and Alexander, 1974). It has been demonstrated that selenium volatilisation in soils and water occur with (ECe) values as high as 22dSm^{-1} and 10-30 dSm^{-1} respectively (Karlson and Frankenberger, 1990a,b; Thompson-Eagle and Frankenberger, 1990a). Therefore, it seems that some methylating organisms can adapt to these conditions and tolerate extreme fluctuations in the environment.

Aeration. Most Studies showed that, under aerobic conditions, greater quantities of volatile selenium are transformed than under an anaerobic condition. Glucose- and sodium selenite-amended seleniferous clay soil produced trace quantities of DMSe under argon where as more DMSe (about > 38 and > 64 fold) were produced under air (Francis *et al.*, 1974). The sample from soil, sewage sludge, and seleniferous pond water exposed to air have been reported to produce larger amounts of volatile selenium than these samples exposed to nitrogen (Abu-Erreish *et al.*, 1968; Reamer and Zoller, 1980; Thompson-Eagle and Frankenberger, 1991).

Cofactors. Selenium volatilisation consists of reduction and methylation reactions. Therefore, reducing agents, methyl donors and prosthetic groups are involved in the volatilisation. Methyl donors include methyl-cobalamine, S-adenosylmethionine and its precursors or derivatives such as homocysteine and methionine are involved in selenium volatilization.

Methionine is a stimulator to selenium volatilisation in soil, dewatered sediments, and water. The methyl donors were found to stimulate selenium volatilization (1 mg/kg) in dewatered sediments include S-adenosyl-L-methionine chloride, S-adenosyl-L-homocysteine, methionine sulfone and methionine sulfoxide (Thompson-Eagle and Frankenberger, 1992). However, the biological methyl donors, SAM (S-adenosylmethionine) and methylcobalamine had little effect on the methylation of selenium in pond water (Thompson-Eagle and Frankenberger, 1991).

Nutrients. The rate of selenium evolution from soils, sediments and water increases with the addition of certain organic materials (Thompson-Eagle and Frankenberger, 1992). Such as glycerol, glucose, wheat grain, cellulose, pectin, orange peel, galacturonic acid (Francis *et al.*, 1974; Karlson and Frankenberger,1988). However, the addition of mono-, poly- and acidic saccharides, alcohol, amino acids, fats and oils had little effect on biomethylation in water than soils (Thompson-Eagle and Frankenberger, 1990a; 1991). Proteins such as caseins, albumen strongly stimulated biomethylation either in water or soils (Thompson-Eagle and Frankenberger, 1992).

INHIBITORS

Heavy metals. There are few studies on the effect of heavy metals on the biomethylation of selenium. Addition of Mo, Hg, Cr, Pb 5 mmol/Kg to seleniferous soils greatly inhibited selenium volatilization (Karlson and Frankenberger, 1988). However, As, B, Mn had little effect on selenium volatilization. It has also been reported that addition of Co, Zn, Ni to seleniferous sediments stimulated volatilization of selenium (Karlson and Frankenberger, 1988). They postulated that Zn, Ni may inhibit the utilization of a readily available organic source by the non-methylating microbial population, thus more carbon will be more available to the selenium methylating microorganisms.

Other ions. The addition of Na_2SO_4 and $CaCl_2$ increases methylation whereas addition of NaH_2PO_4 had no effect on selenium-biomethylation in water (Karlson and Frankenberger, 1990). Microbial production of volatile selenium in soil was slightly more sensitive to chloride than to sulfate ions and more sensitive to Na^+ than to Ca^{2+} ions. Fleming and Alexander (1972) found that selenite alkylation increased by increasing the concentration of sulfate.

The presence of high levels of NO_3^- and NO_2^- in evaporating pond water inhibits selenium biomethylation at 0.1 M and above (Thompson-Eagle and Frankenberger,1990a;b).

Antibiotics. Cycloheximide, a fungicide (200 mg/L) has been reported to slightly stimulate biomethylation of selenium when it is added to protein-amended evaporation pond water or the addition did not give effect on volatilisation. A number of bactericides tested such as penicillin G 100 mg/L, polymixyn B sulfate 100 mg/L, crystal violet 10 mg/L were strongly inhibitory to biomethylation (Thompson-Eagle and Frankenberger, 1991). In addition some dewatered sediments microflora showed resistance to some bactericides such as 40 mg/kg penicillin G and streptomycin concentration (Weres *et al.*, 1989).

CONCLUSION

Numerous environmental assessments of selenium methylation and demethylation have been conducted, also the discovery of dissimilatory Se(VI) and Se(IV) reduction, however more work needs to be done on the oxidative part of cycle in order to eliminate significant gaps in research area about the reductive side.

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