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Research Paper

Biotransformation and detoxification of selenite by microbial biogenesis of selenium-sulfur nanoparticles

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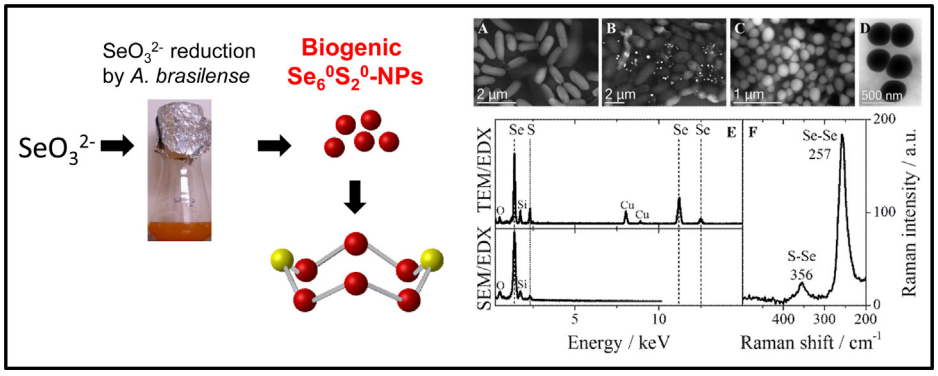
HIGHLIGHTS

- *A. brasilense* is able to efficiently reduce toxic selenite to Se^0S^0-nanoparticles.
- Reduction was also possible in environmental waters supplemented with selenite.
- Biogenic nanoparticles are $Se_{8-n}S_n$ structured spheres, most likely Se_6S_2.
- Se^0S^0- nanoparticles occur extracellularly with an average size of 400 nm.
- Se^0S^0-nanoparticles form a (destabilized) colloidal suspension (ζ-potential −18 mV).

GRAPHICAL ABSTRACT



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ABSTRACT

This study combines the interaction between the toxic oxyanions selenite and selenate and the plant growth promoting bacterium *Azospirillum brasilense* with a comprehensive characterization of the formed selenium particles. As selenium is an essential trace element, but also toxic in high concentrations, its state of occurrence in nature is of major concern. Growth of the bacterium was affected by selenite (1–5 mM) only, observable as a prolonged growth lag-phase of 3 days. Subsequently, selenite reduction occurred under aerobic conditions resulting in extracellularly formed insoluble Se^0 particles. Complementary studies by microscopic and spectroscopic techniques revealed the particles to be homogeneous and stable $Se_{8-n}S_n$ structured spheres with an average size of 400 nm and highly negative surface charge of −18 mV in the neutral pH range. As this is the first study showing *Azospirillum brasilense* being able to biotransform selenite to selenium particles containing a certain amount of sulfur, even if environmental waters supplemented with selenite were used, they may significantly contribute to the biogeochemical cycling of both elements in soil as well as to their soil-plant transfer. Therefore, microbial biotransformation of selenite under certain circumstances may be used for various bio-remediation and bio-technological applications.

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1. Introduction

In the present time, water contamination by substances such as heavy metals, toxic organics, radionuclides or nanoparticles poses an increasing problem of global concern, especially in drinking water treatment and in the purification of process water from many industrial applications [1]. For the improvement of water quality in the contaminated streams, detailed knowledge on chemical, biological and physical interaction of these noxious chemicals are mandatory. With respect to synthetic submicron- or nanoparticles in water matrices, respective knowledge is still limited. In this context, selenium with its ambivalent biologic characteristics is an especially challenging case. For living organisms, selenium is a key trace element, but the healthy level between selenium deficiency (<40 μg/day) and acute selenium poisoning (>400 μg/day) is rather narrow [2].

Selenium can exist in different oxidation states. The oxyanions selenate [SeO_4^{2-}] and selenite [SeO_3^{2-}] represent soluble species in aqueous media, whereas the reduced species Se^0 and Se^{2-} form mainly colloidal particles or hardly soluble precipitates [3]. Chemical equilibrium speciation information on selenium oxidation state are challenging, discussed and summarized in "Chemical Thermodynamics of Selenium." by OECD Nuclear Energy Agency [4]. The standard electrode potential of the redox couples are given in the supplementary information. Selenium is ubiquitous in natural environments (e.g. associated with various sulfide ores of copper, silver, lead, mercury and uranium) and has also anthropogenic origins, e.g. coal burning for power generation, agricultural irrigation of seleniferous soils [1,5–8]. Besides the potential chemotoxicity, the isotope ^{79}Se as a fission product with a long half-life ($\sim 3.27 \times 10^5$ years [9]) is present in spent nuclear fuel. Several national reports for the long-term safety assessment of high-level nuclear waste disposals show that ^{79}Se is one of the radionuclides that dominate the long-term dose rate [10,11].

These days, selenium becomes more and more important for technological applications, in consequence of its photoelectric and semiconducting properties [5]. Respective releases from industrial process waters and wastes have to be considered, too. Eventually, to understand the cycling of selenium in the environment is of great importance for the well-being of humans as well as for saving resources.

One possible way to address these hazards is to transform toxic soluble selenium species into insoluble selenium species like nanoparticles promoting their technological separation by sedimentation, coagulation and filtration.

Reactions between selenium and microorganisms can significantly influence the selenium oxidation state and therefore the transport through geological environment. Recently, many investigations have shown that bacteria are able to form Se^0 particles under anaerobic as well as aerobic conditions (e.g. *Geobacter sulfurreducens*, *Veillonella atypical*, *Bacillus subtilis, Bacillus cereus*, *Shewanella putrefaciens*, *Agrobacterium* sp., *Pseudomonas aeruginosa, Stenotrophomonas maltophilia*) [12–19].

In addition to the better separation of particulate selenium from water, nanoparticles attract special interest since their properties usually differ significantly from those of the bulk material. Especially Se^0 nanoparticles have various attractive features, like higher biological activity [20], lower toxicity [21,22] and larger surface area [23]. They have novel *in vitro* and *in vivo* antioxidant activities and provide new pathways for medical application like cancer treatment as well as anti-bacterial coating material [24–28]. In the photovoltaic and semiconductor industry Se^0 nanoparticles are used because of their high particle dispersion and unique electrical and optical properties. Other practical applications in the field of nanotechnology are under development [23,27,29] Finally, recent studies have shown Se^0 nanoparticles being good adsorbents for heavy metals such as Zn, Hg or Cu [29–31].

In the present study, the interaction of SeO_3^{2-} and SeO_4^{2-} with the plant growth promoting rhizobacterium *Azospirillum brasilense* was investigated, which were reported to have the ability to form Se^0 nanoparticles [32,33] earlier. As this bacterium might be used for biological fertilization also in regions with heavily selenite-loaded soils, its influence on the transfer of the toxic selenium oxyanion as well as the reduction potential needs to be further elucidated. As it was reported that *Azospirillum* forms the Se^0 particles mainly inside the cells [32,33], this bacteria might be helpful to prevent migration of (radio)toxic selenium through soil and water, as the selenium remains entrapped inside the biomass [34,35]. Kamnev et al. [36] used *A. brasilense* to obtain extracellular Se nanoparticles, which were than further characterized by infrared spectroscopy and electron microscopy. In this study, after comparable growth experiments to Tugarova et al. [32,33] special focus was set on the physico-chemical and structural characterization of the formed Se^0 particles, confirming results already reported, but revealing also some more profound and interesting new structural aspects on the Se particles. The formation of hardly soluble Se(0) particles during reduction of selenium oxyanions might be of interest for an industrial application. Moreover, if the Se^0 particles will be released from the biomass (e.g. cell death), the mobility of the selenium particles in the environment will be governed by their physico-chemical properties.

So in this study, the elemental selenium particles for further investigation were produced by *Azospirillum brasilense.* The process of microbial selenium reduction was tracked by inductively coupled plasma mass spectrometry (ICP-MS), hydride generation atomic absorption spectrometry (HG-AAS) and light microscopy. Scanning and transmission electron microscopy (SEM and TEM) with energy dispersive X-ray (EDX) microanalysis, Raman spectroscopy, X-ray absorption spectroscopy (XAS) and UV/Vis spectroscopy (UV/Vis) as well as zeta potential measurements and photon correlation spectroscopy (PCS) were used to characterize the formed selenium particles.

2. Material and methods

2.1. Medium and growth conditions

Growth medium for *Azospirillum brasilense* (DSMZ 1843) was a malate-containing Azo-medium (DSMZ 2007). Medium components (yeast extract 0.05 g, K_2HPO_4 0.25 g, $FeSO_4 \cdot 7\,H_2O$ 0.01 g, $Na_2MoO_4 \cdot 2\,H_2O$ 1.00 mg, $MnSO_4 \cdot H_2O$ 2.00 mg, $MgSO_4 \cdot 7\,H_2O$ 0.20 g NaCl 0.10 g, $CaCl_2 \cdot 2\,H_2O$ 0.02 g, $(NH_4)_2SO_4$ 1.00 g, Biotin 0.10 mg) were solved in 950 mL distilled water and pH was adjusted to 7.1 before autoclaving. After sterilization 25 mL each of filter-sterilized 20% glucose and 20% Na-malate were added.

Cells were grown under aerobic conditions in liquid medium in flasks on a rotary shaker at 100 rpm at 30 °C.

2.2. Incubation with selenate and selenite

For growth experiments in the presence of selenate and selenite, pre-cultured cells were inoculated into 250 mL flasks containing 150 mL of Azo-medium reaching a starting optical density at 600 nm (OD_{600}) of 0.25. The different concentrations of selenite (1 mM to 5 mM) or selenate (1 mM) in the assay were obtained by addition of the required volume of stock solutions of 0.1 M $Na_2SeO_3 \cdot 5H_2O$ or 0.1 M Na_2SeO_4. Two replicates of each concentration were incubated as described above and sampled at intervals. Samples were assayed for bacterial density, whole cell protein concentration, selenite and selenate.