



The microbial impact on the sorption behaviour of selenite in an acidic, nutrient-poor boreal bog



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ABSTRACT

⁷⁹Se is among the most important long lived radionuclides in spent nuclear fuel and selenite, SeO_3^{2-} , is its typical form in intermediate redox potential. The sorption behaviour of selenite and the bacterial impact on the selenite sorption in a 7-m-deep profile of a nutrient-poor boreal bog was studied using batch sorption experiments. The batch distribution coefficient (K_d) values of selenite decreased as a function of sampling depth and highest K_d values, 6600 L/kg dry weight (DW), were observed in the surface moss and the lowest in the bottom clay at 1700 L/kg DW. The overall maximum sorption was observed at pH between 3 and 4 and the K_d values were significantly higher in unsterilized compared to sterilized samples. The removal of selenite from solution by *Pseudomonas* sp., *Burkholderia* sp., *Rhodococcus* sp. and *Paenibacillus* sp. strains isolated from the bog was affected by incubation temperature and time. In addition, the incubation of sterilized surface moss, subsurface peat and gyttja samples with added bacteria effectively removed selenite from the solution and on average 65% of selenite was removed when *Pseudomonas* sp. or *Burkholderia* sp. strains were used. Our results demonstrate the important role of bacteria for the removal of selenite from the solution phase in the bog environment, having a high organic matter content and a low pH.

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1. Introduction

Mires are important biotopes in the Finnish environment. Peatlands cover up to a third of Finland's land area and in the northern regions peatlands are important carbon and fresh water reservoirs. Olkiluoto Island, located on the Finnish west coast, has been chosen as the repository site for the final disposal of spent nuclear fuel in Finland. The long term safety of the repository is based on several barriers, such as the copper sheeted waste canister, the bentonite clay protecting the canister and the bedrock of the repository. However, if the waste canisters were to lose their integrity, radionuclides could escape from the repository. In the Olkiluoto area, the post-glacial land uplift continues and as a result Olkiluoto Island will be joined into the Finnish mainland after the next 6000 years (Haapanen et al., 2013). During this period new bogs will be formed in the area and according to the biosphere assessment, these will coincide with the first potential radionuclide

emissions from the repository to the surface ecosystem if the canisters would leak (Posiva, 2012). Lastensuo bog is considered representing the mire type found in the Olkiluoto area in the future, and has therefore been chosen as an analogue biotope in biosphere safety assessment for the long-lived radionuclides present in spent nuclear fuel (Haapanen et al., 2011). ⁷⁹Se is classified as a high priority radionuclide in the long-term safety assessments when the possible radiation dose for humans in the future is considered (Helin et al., 2010). ⁷⁹Se is a fission product and in addition it is formed by neutron activation from stable selenium by reaction $^{78}\text{Se}(n, \gamma)^{79}\text{Se}$. Besides radioactive selenium, considerable amounts of stable selenium enter the environment via anthropogenic activities including coal combustion, mining, refining of sour crude oils and agricultural irrigation of seleniferous soils (Coppin et al., 2009; Sharmasarkar and Vance, 2002; Manceau and Gallup, 1997; Yasin et al., 2014; de Souza et al., 1999). Although selenium is an essential micronutrient for animals and humans, it is toxic in high concentrations characterized by a narrow range between toxic and deficient doses (Terry et al., 2000; Barceloux, 1999).

The behaviour of selenium in the environment is influenced by several factors such as pH, chemical form, soil mineral composition,

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redox conditions, as well as micro-organisms (Nakamaru and Altansuvd, 2014; Sarret et al., 2005; Nelson et al., 1996; Oremland et al., 2004; de Souza et al., 1999). In the environment selenium occurs with different oxidation states forming selenide (Se^{2-}), elemental Se (Se^0), selenite (SeO_3^{2-}), selenate (SeO_4^{2-}), and organic Se (Kausch et al., 2012; Pezzarossa et al., 1999). At high redox potential selenate dominates and at intermediate redox conditions selenite becomes more prevailing (Nakamaru and Altansuvd, 2014; Pezzarossa et al., 1999). Se^{2-} and Se^0 are found typically in most reducing environments with low pH (Nakamaru and Altansuvd, 2014; Pezzarossa et al., 1999). Under non-flooded conditions (mineral soils) the solubility of selenium is affected by adsorption on oxy-hydroxides of aluminium (Al), iron (Fe) and manganese (Mn), but in wetland environments, such as acidic bogs, reduced selenium (Se^0) and organic matter bound Se (Se-org) are the most important forms (Nakamaru and Altansuvd, 2014). Selenite forms inner-sphere bidentate surface complexes with haematite (Catalano et al., 2006; Balistieri and Chao, 1990) and bridging bidentate complexes on adjacent singly coordinated oxygen groups with amorphous $\text{Fe}(\text{OH})_3$ (Balistieri and Chao, 1990; Su and Suarez, 2000) and goethite ($\alpha\text{-FeOOH}$) (Su and Suarez, 2000). When comparing the sorption of the two oxyanions, selenite is known to adsorb more strongly on amorphous iron oxyhydroxide and manganese dioxide compared to selenate (Balistieri and Chao, 1990). Selenite adsorption on amorphous iron oxyhydroxides and manganese dioxide has been reported to increase with decreasing pH (Balistieri and Chao, 1990).

In organic wetland soils the reduction of selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) into insoluble elemental selenium (Se^0) by microorganisms is an important process which greatly affects the environmental distribution and biological effects of selenium (Li et al., 2014). Several bacterial genera are known to reduce both selenate and selenite into Se^0 (e.g. Fujita et al., 1997; Sarret et al., 2005; Li et al., 2014) primarily via microbial dissimilatory reduction involving enzymes with molybdenum co-factors (Stolz and Oremland, 1999). Slow abiotic reduction (>1 month) of selenite into Se^0 has also been observed in the presence of Fe(II), but only under reducing conditions at pH below 7 (Charlet et al., 2007). Selenium respiring bacteria are found in a wide range of environments and they are dispersed throughout the bacterial domain (Stolz and Oremland, 1999; Oremland et al., 2004; Li et al., 2014). A number of organic substrates (i.e. acetate, lactate, pyruvate, glycerol, and ethanol) or hydrogen can be coupled to the reduction of selenium (Stolz and Oremland, 1999). Both intracellular and extracellular selenium granules have been found in phylogenetically and physiologically distinct bacteria like *Chromatium vinosum*, *Desulfosporospora desulfuricans*, *Sulfospirillum barnesii*, *Bacillus selenitireducens*, *Selenihalanaerobacter shriftii*, *Shewanella oneidensis* MR-1, *Paenibacillus selenitireducens* sp. nov., and *Ralstonia metallidurans* CH34 (e.g. Nelson et al., 1996; Oremland et al., 2004; Li et al., 2014). For example in *B. selenitireducens*, *S. shriftii*, *S. barnesii* and *R. metallidurans* CH34 Se^0 nanospheres on the exterior of the cell envelope as well as intracellular Se^0 granules have been reported (Oremland et al., 2004; Sarret et al., 2005). In *R. metallidurans* CH34 organoselenium compounds of form R–Se–R are also formed (Sarret et al., 2005). Therefore the selenate/selenite reduction appears to be widely spread among bacteria and reduction has been reported both in anoxic (e.g. Oremland et al., 1989, 2004; Nelson et al., 1996) and oxic (e.g. Fujita et al., 1997; Sarret et al., 2005; Lortie et al., 1992) conditions.

Although only a few studies exist on the specific interactions of selenium with organic matter (e.g. Bruggerman et al., 2007; Kamei-Ishikawa et al., 2008), organic bound selenium is expected to have an important role in the biogeochemistry of wetland soils (Nakamaru and Altansuvd, 2014). The actual mechanism of

selenium association with organic matter is still not explained, but organic fractions have been shown to be the major carriers of selenium (Coppin et al., 2009). It has been suggested that selenium sorption on organic particles could only be indirect, mainly resulting from association with Fe oxides or clays residing either on the organic matter surface or fixed within its matrix (Coppin et al., 2009) and is linked to the microbial reduction, which in turn is affected by the local chemical conditions of the soil (Kausch et al., 2012).

In our study, the sorption of selenite on bog samples (surface *Sphagnum* moss, peat, gyttja and clay) from an acidic, nutrient-poor boreal bog was examined using both unsterilized and sterilized samples under oxic and anoxic conditions. The effect of temperature and sterilization on the sorption kinetics of selenite on bog samples was examined using Elovich kinetics and pseudo-second-order (PSO) models. In addition the removal of selenite from the solution by bacteria isolated from the surface moss, peat, gyttja and clay samples was studied using four different growth media and sterilized bog samples in model bog water solution as nutrient. The primary motivation of our study was to assess the sorption characteristics and the microbial impact on the sorption characteristics of selenite in an acidic bog for the long-term safety assessment of the final disposal of spent nuclear fuel.

2. Materials and methods

2.1. Sampling site, sample pretreatment, and peat and bog water analysis

Our study site, Lastensuo bog, is a raised, ombrotrophic boreal bog located on the western coast of Finland. This bog has a surface area of 440 ha and the surface level of the bog is 44–48 m above the sea level (Mäkilä and Grundström, 2008; Haapanen et al., 2013). The maximum thickness of the peat layer is approximately 6 m and below the peat layers a clayish bottom soil layer derived from a former seabed is found. In the middle parts of the bog, gyttja is found on top of the clay layer (Mäkilä and Grundström, 2008). Different mire types are found on the Lastensuo area and the centre parts of the bog consist of treeless or near-treeless *Sphagnum fuscum* bog, *S. fuscum* pine bog, ridge hollow pine bog and hollow bog. At the edges of the bog, low sedge bog, cotton grass pine bog, tall sedge pine fen and forested peatland are found (Mäkilä and Grundström, 2008). The main peat types include *Sphagnum* peat (58%), sedge peat (19%) and few-flowered sedge (15%) (Mäkilä and Grundström, 2008). The average peat accumulation rate is 1.1 mm/year and has started 5300 years ago (Mäkilä and Grundström, 2008). The bacterial community of the Lastensuo bog profile mainly consists of Acidobacteria and Proteobacteria in the surface moss and Acidobacteria in the peat layers, with increasing abundances of Chloroflexi, Verrucomicrobia, Bacteroidetes, Spirochaeta and OP8 groups at greater depths (Tsitko et al., 2014).

Our sampling point is located in the middle part of the Lastensuo bog (61°17'31", 21°50'22", WGS84 coordinate system) and consists of almost treeless hummock-hollow pine bog (Haapanen et al., 2013). Samples from seven bog layers, 0.5–1.0 m, 1.5–2.0 m, 2.5–3.0 m, 3.5–4.0 m, 4.5–5.0 m, 5.5–6.0 m and 6.5–7.0 m were retrieved using a peat corer made of stainless steel with a nest length of 50 cm and a diameter of 15 cm. Surface moss (mainly *Sphagnum* spp.) was also collected. The layers from 0.5 to 5.0 m consisted of peat with variable degrees of humification and the deeper layers from 5.5 to 6.0 m and 6.5–7.0 m of gyttja and light grey clay, respectively. From each depth a total of six full 50-mL sterile centrifuge tubes of sampling material were aseptically taken and Parafilm was wrapped around the caps. Closed tubes were sealed in plastic, brought to the laboratory in cool bags and

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