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Responses of the bentonite microbial community to Se(IV)exposure: insights into selenium biogeochemical cycle.

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Deep geological repository (DGR) is the internationally accepted option for the storage of highly radioactive wastes in the near future. In this multi-barrier system bentonite clay is considered as the best backfilling and sealing material. The ^{79}Se is one of the most critical radionuclides in DGRs as it was predicted by several safety assessment studies. Furthermore, autochthonous microorganisms inhabiting this clay formation could affect the mobility of the stored radionuclides (e,g, Se) if a waste leak occurs. Therefore, the aim of the present work is to investigate the impact of bentonite microbial populations on the biotransformation of Se(IV) and formation of Se(0) nanoparticles.

Water-saturated bentonite microcosms were treated with Se(IV), and amended with acetate and glycerol-2-phosphate (G2P) as electron donors. In addition, the microcosms were spiked with a bacterial consortium (*Pseudomonas*, *Stenotrophomonas*, *Shewanella*, *Bacillus* and *Amycolatopsis*) isolated from bentonite. After 3 and 6 months of anaerobic incubation, DNA extractions and 16S rRNA gene Next Generation Sequencing were performed to study the microbial diversity of the different microcosms obtaining bacteria such as *Pseudomonas, Stenotrophomonas, Symbiobacterium,* and *Desulfosporosinus*, which were highly enriched in the Se(IV)-treated microcosms. These bacteria have the potential to produce Se(0) nanoparticles through Se(IV) reduction. In contrast, bacterial genera such as *Pelosinus, Desulfovibrio,* and *Lentimicrobium,* as well as the archaea *Methanosarcina* decreased their relative abundance in presence of selenite.

Along the anaerobic incubation, orange and black precipitates were observed in all the Se-treated microcosms. Microscopic and spectroscopic techniques (VP-FESEM, STEM, EDX, Raman spectroscopy, HRTEM) of the orange precipitates corresponded to the selenium amorphous or monoclinic crystalline phases, while the black precipitates showed the typical structure of the trigonal selenium (*t*-Se).

These results revealed a key role of the native bentonite microbial community in the reduction of toxic and soluble Se(IV) to non-toxic, insoluble and stable *t*-Se(0) through a biotransformation process, which could contribute to the immobilization of Se, increasing the safety of the future DGRs.