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A novel and efficient DNA extraction method focus to the bacterial diversity in compacted bentonites: insights into biogeochemical processes within the Deep Geological Disposal concept

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Compacted bentonite-based materials are often considered as suitable sealing and backfilling materials for Deep Geological Repository (DGR) of radioactive wastes. Studies covering the presence of indigenous microorganisms and their survival and mobility in compacted clay-based buffer materials under relevant conditions are gaining importance since indigenous microbes could contribute to the metal corrosion, structural Fe(III) bentonite transformations and/or to affect the mobility of radionuclides. However, bentonites comprise highly challenging systems to nucleic acid extractions due to their cation exchange and swelling capacities. Here we provide an optimized phenol:chloroform based protocol that facilitates higher DNA-yields when other traditional methods or commercial kits failed. To test this novel method, untreated and acetate-treated bentonites at 1.5 and 1.7 g/cm^3 dry densities were incubated for 24 months under anaerobic conditions. DNA was extracted and sequenced to reveal an Actinobacteria and Proteobacteria dominated community. 16S rRNA gene sequences most similar to taxa described for their role in biogeochemical sulfur cycling included sulfur oxidizing (e.g., *Thiobacillus*, *Sulfurifustis*, and *Sulfurimonas*) and sulfate reducing (e.g., *Desulfuromonas* and *Desulfosporosinus*) bacteria. In addition, iron-cycling populations included iron oxidizing such as *Thiobacillus* and *Rhodobacter* plus reducing *Geobacillus* and *Stenotrophomonas* taxa. Some genera described for their capacity to utilize acetate as a carbon source such as *Delftia, Paracoccus, Stenotrophomonas, and Thermicanus* were also detected. In addition, the data obtained from mineralogical (X-Ray Diffraction) analyses showed up the bentonite stability and microscopic (Scanning Electron Microscopy) studies revealed pores and cracks that could host nanobacteria or spores.

This study highlights the effect of microbial driven biogeochemical processes in compacted bentonites, the potential role of compaction densities on the bacterial diversity, and the importance of these populations for the safety of DGRs.

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