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Detection of living bacterial cells in clay - bentonite

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Bentonite is a swelling clay, consisting mainly of montmorillonire, being planned to be used as a backfill material in the nuclear waste repository. It contains indigenous microbial populations that can negatively influence the long-term safety of the geological repository due to their metabolic activity (canister corrosion, illitization of bentonite, gas production, degradation of cementitious materials). However, reliable detection of microorganisms in clayish material is generally very difficult. Although the compactness of bentonite will undoubtedly limit the microbial activity, in the extremely long-time frame of repository lifetime this condition can fail. It is thus crucial to understand the potential of the naturally present microbial community in bentonite to compromise the safety of repository, if not limited by the compactness. Higher metabolic activity can be mainly expected at the interfaces or in the places with a lower density of bentonite.

Here we present an optimized cell extraction method enabling direct estimation of bacterial density and viability in bentonite. Indigenous bacterial cells were extracted from bentonite suspensions by an improved step-wise protocol and their viability was detected using live/dead staining and epifluorescence microscopy. We used dispersant (2.5 mM natrium pyrophosphate-based solution or 1% methanol) to partially disintegrate the bentonite and detach the vital and dead microbial cells from its surface. The dispersed material was subsequently stepwise centrifuged over two high-density media (sucrose and Histodenz) to remove most of the heavy bentonite particles while keeping the light bentonite particles and cells in the final extract. We were able to detect and enumerate the cells concentrated at the surface of the light bentonite particles, which served as a sieve to retain all free cells during centrifugation.

Different extraction procedures were tested and their efficiency was estimated by comparing live/dead ratios of resulting extracts and was also proved by implementing both NGS and quantitative PCR. The results show that most of the microbial genera present in the original suspension are also present in extracts but as proved by Deseq2 analysis some genera tend to settle down with heavier bentonite particles during the first centrifugation step.

To conclude, we present a protocol for extraction and detection of metabolically active cells in clayish material – bentonite. The quality of the extraction procedure was estimated both by a combination of fluorescent microscopy and genetic methods. The protocol was successfully tested on different bentonite types showing general applicability of this approach for clay materials.