## Constraining microbial life in future radioactive waste repositories: growth inhibition by bentonite

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Deep Geological Repositories (DGRs) are engineered to isolate radioactive waste within a stable host rock. This process entails sealing the waste within steel canisters and placing the canisters in tunnels backfilled with bentonite clay. The latter serves as a barrier material to prevent the migration of radionuclides out of the repository. An additional safety component of the bentonite barrier is preventing microbially induced corrosion that could alter the longevity of waste canisters. However, the inhibitory clay density threshold for microbial growth, growth dynamics in clay, and the underlying mechanism of growth limitation by bentonite remain poorly defined. To address these knowledge gaps, we built 12 diffusion reactors and investigated microbial growth as a function of time and bentonite dry density. The reactors were filled with MX80 Wyoming bentonite compacted to 1.25 g/cm<sup>3</sup>, supplied continuously with artificial porewater for 1.5 or 4 months under anoxic conditions and sampled to monitor bacterial growth over time. In addition, 12 other vessels compacted to varying densities, i.e., 1.25, 1.45, 1.55 and 1.75 g/cm<sup>3</sup>, were run in parallel to pinpoint the inhibitory density threshold. Firstly, 16S rRNA gene quantification and sequencing revealed that, at a low bentonite density (1.25 g/cm<sup>3</sup>), microbial growth started after approximately 30 days and ceased within 3 months. Secondly, despite anoxic conditions, aerobes dominated the microbial community, while sulfate-reducing bacteria (SRB) were present at low abundance. Altogether, these findings show that in a swelling bentonite barrier, microbial growth occurs within a short window, primarily supporting aerobes. In addition, this research contributes to identifying the minimal dry density threshold of compacted bentonite for optimization of DGR design. Overall, this study provides new insights into microbial communities under conditions relevant to anoxic DGRs environments and key information for modelling their long-term evolution.

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