

# **PLFA assessment of bentonite-associated microbial communities: Influence of water activity and comparison to a deep geological repository analogue**

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Isolation in a deep geological repository (DGR) is proposed as the most effective way to manage used nuclear fuel. Bentonite clay is a key component of the engineered barrier system for Canada's DGR designed by the Nuclear Waste Management Organization. Assessment of the potential for microbial growth within bentonite is being undertaken as part of the engineered barrier design process. One parameter that may influence microbial growth within bentonite is water activity ( $a_w$ ). This study explored the impact of  $a_w$  on microbial growth in as-received MX-80 Wyoming bentonite. Additionally, it evaluated microbial abundances in Miocene age bentonite from Tsukinuno Mine (Japan), which is an analogue for the bentonites proposed for use in a DGR. The MX-80 bentonite was mixed with water to achieve a low  $a_w$  of 0.93 and a high  $a_w$  of 0.99. Phospholipid fatty acids (PLFA) were extracted to quantify and characterize viable microbial communities. Duplicate microcosms were established for each  $a_w$  and extracted at three different timepoints: day 0, representing initial community, 1 week, and 1 month. Microbial abundances, as indicated by PLFA, peaked after one week, then decreased slightly by one month, indicating early growth of the microbial community. No resolvable difference in PLFA abundance between the two water activities suggests that  $a_w$  alone did not control changes in PLFA abundances, even though the low  $a_w$  samples were below the minimum target value of 0.96 previously identified for bacterial suppression when in conjunction with high compaction of >2 MPa [1]. Concurrent 16S rRNA gene analysis found the community to be dominated by sequences associated with *Actinobacteria* and 18S rRNA gene analysis confirmed the presence of fungi, which can grow at lower  $a_w$  values. *In situ* bentonite clay cores from Tsukinuno Mine consisted of three horizontal and one vertical core from two bentonite clay beds with average PLFA abundances in these samples were comparable to or below those observed in the as-received bentonite used in the  $a_w$  experiment, indicating a common level of abundance in saturated bentonite.

[1] Stroes-Gascoyne, Hamon, Maak, and Russell (2010), Applied Clay Science 47, 155-162.