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Trapped Air, Trapped Assumptions: Rethinking Microbial Life in Radioactive Waste Repositories

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The safety of future deep geological radioactive waste repositories will depend on a multibarrier system, with the disposal canister providing an absolute containment of radionuclides for a certain period. The lifetime of the canisters will depend on *in-situ* degradation processes, including microbially-driven corrosion (MIC). Indeed, after repository closure, partial saturation of the backfill (bentonite clay) around waste canisters is expected, which may favor the growth of sulfate-reducing bacteria (SRB). However, a long-term (8.5 years) experiment conducted within the rock formation that will host Swiss radioactive waste, Opalinus Clay, shows that despite anoxic conditions, SRB remain in the minority (<0.2%), and instead, aerobes dominate. This surprising observation may result from oxygen (O_2) trapped within bentonite particles surrounding the canister, which can persist for an extended period, shaping the microbial communities. To test this hypothesis, we varied the O_2 amount in bentonite by equilibrating it with atmospheres containing 0%, 21%, or 100% O_2 and placed it, along with metal coupons, into stainless steel modules. These were deployed into a borehole within the Opalinus Clay formation for 1.5 or 3 years. We also varied the bentonite water saturation to study the colonization of the bentonite clay by porewater bacteria in this ecosystem. The full-length 16S rRNA gene analysis revealed that bentonite-associated O_2 indeed increased the number of gene copies, stimulating the growth of aerobes, but also boosted specific SRB species (e.g., *Desulfatitalea* sp.). XRD, µXRF and 6M HCl and HF digestions demonstrated that the initial O_2 level significantly influenced the precipitation of reduced sulfur and iron within bentonite and on carbon steel surfaces. Additionally, bacteria from the borehole colonized the bentonite, but their mobility within bentonite was limited to a 1-cm range. These findings suggest a more complex microbial succession than conventionally viewed, with an important role for aerobes, and highlight the role of the often-overlooked repository oxic-to-anoxic transition in microbially mediated processes within the clay backfill.