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Impact of bentonite-associated oxygen on microbial activity and growth

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The effectiveness of future nuclear waste repositories is partly dependent on canister integrity for the first 100 years after closure. The waste canisters will be placed in the deep subsurface in galleries that will be backfilled with bentonite clay. Canister corrosion could be induced by sulfate-reducing bacteria (SRB) under the reducing conditions that will develop. However, recent long-term incubation studies demonstrated the inhibition of SRB despite anoxic conditions, most likely due to the long-term persistence of residual oxygen entrapped within bentonite clay. Thus, a question about the influence of residual oxygen on the delay in the onset of SRB growth in bentonite and its impact on the corrosion rates remains. Here, we performed a 1.5-year-long borehole incubation experiment using stainless steel modules filled with Wyoming bentonite compacted to 1.25 g/cm^3 and equilibrated at varying initial oxygen saturation: 0%, 21%, and 100% before the deployment. As a sterile control, gamma-irradiated bentonite, exposed to 21% O_2 was used. Additionally, all modules contained carbon steel and copper coupons embedded within the clay. First, 16S rRNA gene quantification showed that microbial growth occurred at all conditions, with higher cell numbers within the first 13 mm from the bentonite surface in direct contact with anoxic porewater. Second, we found that with decreasing oxygen levels, fewer bacterial cells were present, while concentrations of S, Fe(II) and Fe(total) increased, with magnetite and siderite as the main reduction products identified by a combination of XRD, μXRF mapping and the 6M-HCl extractions. Carbon steel corrosion occurred at all conditions with the thickest layer of corrosion products observed for steel in contact with bentonite pre-treated with 21% of O_2. Altogether, this demonstrates that both porewater- and bentonite-derived microbial communities contribute to the alteration of bentonite composition, precipitating sulfide phases and affecting the stability of Fe(III)-bearing minerals, most likely including reduction of clays when O_2 is absent or consumed. Oxygen shapes microbial communities, and its decrease causes a shift towards less abundant but more specialized corrosion-inducing microorganisms, with corrosion being the most prominent in bentonite equilibrated at atmospheric O_2, due to a combination of simultaneous abiotic and biotic processes.