MICROBIAL ANALYSES OF CLAY AND WATER FROM DIFFERENT SAMPLES FROM THE MONT TERRI ROCK LABORATORY (RL), SWITZERLAND

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Exploration of deep subsurface microbial life has increased for very diverse motives. One of them is that these environments are potential host rocks for radioactive waste repositories and that microorganisms may influence geochemical conditions around such sites and migration properties of radionuclides.

The Porewater Chemistry experiment (PC) was conducted at the Mont Terri-RL to measure in situ the pH, Eh, and other geochemical parameters within the porewater of the Opalinus Clay formation. The borehole for PC was drilled with N_2 under clean but not aseptic conditions, filled immediately with synthetic porewater, which was circulated and monitored for five years. Soon after initiation of PC it was evident that microbial activity affected the borehole water geochemistry. Microbial analyses, including molecular biology and culturing methods, were performed repeatedly during PC (2003-2006), with detailed analysis of water and overcore clay upon termination in 2007.

Results indicated the presence of heterotrophic aerobes and anaerobes, nitrate-reducers, iron-reducers, sulphate-reducers and Archaea, which together with geochemical data suggested a reducing environment with sulphate reduction in the water and adjacent clay. A black precipitate containing pyrite and a strong H_2S smell confirmed the occurrence of sulphate reduction. Specific species identified (> 98% similarity) in PC water included *Pseudomonas stutzeri, Bacillus licheniformis*, and *Desulfosporosinus sp.*, with similar and additional species (e.g., *Trichococcus sp.*; *Koccuria sp.*) in the clay. The origin of these (mostly anaerobic) species cannot be determined with certainty. Some species likely resulted from contamination, but others could be revived species indigenous in the Opalinus Clay. The microbial processes that occurred in PC are not representative of the processes in the undisturbed formation but illustrate the potential for microbial processes that may develop locally in Opalinus Clay upon disturbance by drilling and excavation (i.e., introduction of space, water, microbes and nutrients).

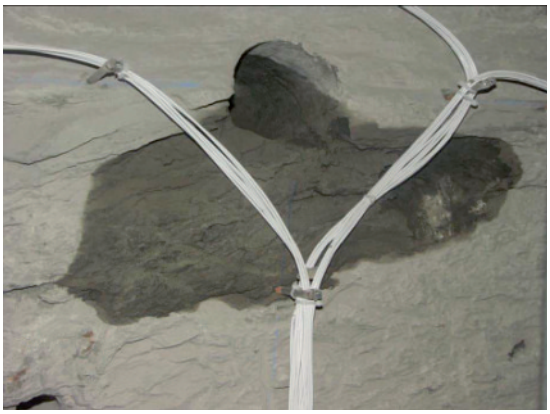

Figure 1: BEZ-G5 after water accumulation.

In a second part of this work, results are presented here from detection and identification of microorganisms in water which accumulated in an open borehole drilled in the Opalinus Clay (BEZ-G5).

Besides introduced (exogenous) microorganisms, such water may contain autochthonous species. The latter may be easier to detect in water samples than by direct analysis of the clay rock. Both culturing (aerobic and anaerobic media) and direct (DNA extraction and PCR-DGGE) methods were used. Pure cultures of bacteria isolated in several enrichment media and identified by DNA extraction, PCR and sequencing indicated that most isolated bacteria are heterotrophic aerobes or facultative anaerobes commonly isolated from soil and water (such as *Dietzia sp.*, *Pseudomonas sp.*) and therefore probably contaminants. In parallel, direct DNA extraction from water and PCR-DGGE revealed other contaminant bacteria (such as *Staphylococcus sp.*, *Rhizobium sp.*). Nevertheless, some species may be indigenous in the Opalinus Clay such as *Desulfosporosinus sp.* isolated on sulfate-reducing media or *Speleomyces sp.*, identified by PCR-DGGE and previously isolated from a medieval mine. Complementary characterizations of these bacteria are required to confirm these first results. The diversity of microorganisms detected shows that both culturing and molecular approaches are essential to study this type of environment.

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