Bentonite – Geotechnical barrier and source for microbial life

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Due to their properties, namely a high swelling capacity and a low hydraulic conductivity, Bentonites fulfil as geotechnical barrier a sealing and buffering function in the nuclear waste repository. Depending on the mineral composition Bentonites contain many suitable electron-donors and -acceptors, enabling potential microbial life. For the potential repository of highly radioactive waste the microbial mediated transformation of Bentonite could influence its properties as a barrier material. Microcosms were set up containing Bentonite and anaerobic synthetic Opalinus-clay-pore water solution under an N_2/CO_2-atmosphere to elucidate the microbial potential within selected Bentonites. Substrates like acetate and lactate were supplemented to stimulate potential microbial activity. First results show that bentonites represent a source for microbial life, demonstrated by the consumption of lactate and the formation of pyruvate. Furthermore, microbial iron-reduction was determined, which plays a crucial role in Betonite-transformation.

EXPERIMENTAL. Microcosm-experiments were set up by applying 20 g Bentonite B36 (processed Bentonite) with 40 mL sterile synthetic Opalinus-clay-pore water solution (212 mM NaCl, 26 mM $CaCl_2$, 14 mM Na_2SO_4, 1.6 mM KCl, 17 mM $MgCl_2$, 0.51 mM $SrCl_2$ and 0.47 mM $NaHCO_3$, degassed for 1.5 h with a N_2/CO_2 gas mixture (80/20) while stirring). Selected microcosms were supplemented with organic substrates (50 mM acetate/lactate, 3 mM methanol, 0.1 mM AQDS). The microcosms were incubated at 30 °C in the dark without shaking and during a period of 98 days samples were analyzed. All procedures were carried out under anaerobic conditions. Sensory measurement of O_2-concentration, redox potential and pH-value were performed within portions of the respective, well-mixed suspensions. For iron-determination the Ferrozine-method based on a modified protocol by Viollier was applied to the cleared supernatant after digestion of the suspension with 0.5 M HCl for 30 min.[1] The centrifuged and filtrated supernatant of the suspension was used for determining sulfate-concentration *via* IC and lactate- and acetate-concentration *via* HPLC. For analyzing the microbial diversity within the respective sample, DNA was extracted using a protocol from Selenska-Pobell.[2] For sequencing, the 16S rDNA was amplified by using oligonucleotides 28f/519r.[3]

RESULTS. In order to analyze microbial effects on Bentonite-transformation, we analyzed different geochemical and biological parameters over a period of 98 days. No significant changes in O_2-concentration, pH-value or sulphate concentration could have been detected (data not shown).
Only substrate-containing microcosms showed significant changes in geochemical and biological parameters. Here, a significant decrease in redox potential from 505 mV to 270 mV was demonstrated (data not shown). Furthermore, HPLC-analysis of the supernatant showed a decrease in lactate- and an increase in acetate-concentration. Surprisingly, an additional metabolite, namely pyruvate, was formed and secreted. Thus, we were able to show that indigenous microbes are able to metabolize the provided carbon sources. Strikingly, a reduction of Fe(III) to Fe(II) has been observed (Fig. 1), demonstrating that in Bentonite contained iron serves as a potential electron-acceptor for microbial anaerobic respiration and, thus, showing that microbial metabolism could affect the structure of Bentonite directly, which has to be elucidated in structural analysis.

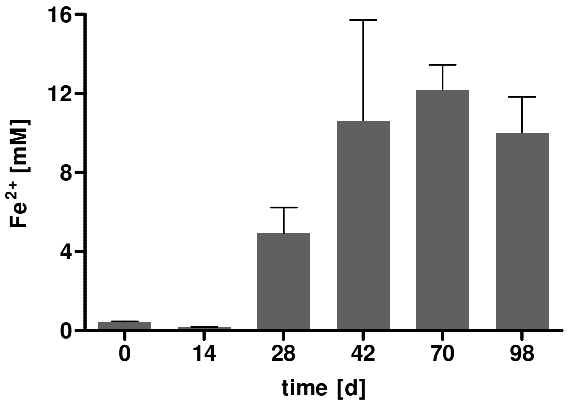

Fig. 1: Microbial influence on Fe^{2+}-concentration within the Bentonite B36. Iron determination was carried out according to a modified Ferrozine-method based on Viollier *et al.*[1] Shown are mean values with standard deviations from at least two independent experiments conducted in duplicates.

First analysis of the microbial composition within Bentonite B36 showed that bacteria from the genus of *Bacillus* and *Saccharopolyspora* were and/or became very dominant in microcosms containing substrates. Both genera are known for spore-formation, which enable the respective organisms to outlast during environmental and nutritional stresses. Mature endospores of *Bacillus* and other related genera are resistant to heat, UV and γ-radiation treatments even if the respective exposure lasts years.[4] Colonization starts again when environmental conditions are favorable. It has already been shown that *Bacilli* are able to reduce iron(III).[5]
Our results evidence the occurrence of metabolic active microbes in Bentonites under ideal conditions. Organic acids are metabolized and furthermore converted to other organic acids serving as potential substrates for other microbes. Thus, our results reveal the importance of the selection of the best suitable Bentonite in order to avoid transformation by indigenous microbes. By using a Bentonite containing only low organic carbon and low iron, microbial activity during deposition of highly radioactive waste can be avoided or retarded.

ACKNOWLEDGEMENTS. We thank Falk Lehmann, Jana Seibt and Susanne Sachs for HPLC-analysis and Carola Eckardt for IC-measurements. Furthermore, we thank Stephan Kaufhold (BGR, Hannover, Germany) for providing the Bentonites. This work was supported by the Federal Ministry for Economic Affairs (No. 02E11344B).

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