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Research Paper

Microbial sulphide-producing activity in water saturated Wyoming MX-80, Asha and Calcigel bentonites at wet densities from 1500 to 2000 kg m^{-3}

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A B S T R A C T

Highly compacted bentonite is projected to function as a buffer against outward transport of radionuclides and inward transport of corrosive groundwater components for copper canisters with spent nuclear fuel in future Scandinavian geological repositories. The dominant long-term copper corrosive species will be sulphide from dissimilatory reduction of sulphate to hydrogen sulphide by sulphate-reducing bacteria (SRB). The effects from varying wet densities of MX-80, Asha and Calcigel bentonites, doped with SRB, on cultivability and sulphide-production of SRB were investigated. The studied commercial bentonites were all infested with cultivable SRB. While cultivability of SRB clearly decreased with increasing wet density of MX-80 and Calcigel, it remained relatively constant for most tested wet densities applied to Asha. The sulphide-production results for the three clays indicated intervals between 1740 and 1880 kg m^{-3} in wet densities within which sulphide-producing activity dropped from high to very low or below detection. This work demonstrated that a high density of bentonite buffers in future spent nuclear fuel repositories will significantly reduce the risk for sulphide production in the buffer and concomitant corrosion of copper canisters.

1. Introduction

High-level radioactive wastes from energy production, most spent nuclear fuel (SNF) and waste from re-processed nuclear fuel, will be encapsulated in iron canisters in the present French and Swiss concepts and in copper canisters in the Swedish and Finnish concept (see http://www.igdtp.eu for details). These canisters will be disposed in deep underground hard host rock formations and they will generally be surrounded by an engineered barrier consisting of a swelling clay. In the present Belgian concept, so-called super-containers with a concrete barrier encapsulating iron shells with waste will be emplaced directly in the Boom Clay formation. The man-maid metal and clay barriers are commonly denoted engineered barrier systems (EBS) and are susceptible to deterioration processes. A possible deterioration process is metal corrosion by sulphide that eventually may cause the canister to prematurely breach, leading to radionuclide release. Microbial sulphide-producing activity could consequently impact the safety case by compromising the canisters isolation and containment functions.

In the Finnish and Swedish concepts for geodisposal of SNF the bentonite barrier has an important function in maintaining the integrity of the copper canisters isolating the spent fuel (SKB, 2010). In the repository a highly compacted bentonite with a bulk wet density between 1950 and 2050 kg m^{-3} is projected. The bentonite is intended to hinder outward transport of radionuclides and inward transport of corrosive groundwater components, and to act as a buffer against rock movements. The presence and activity of sulphide-producing bacteria have been detected in groundwater at repository depth (Hallbeck and Pedersen, 2012; Pedersen et al., 2014a) as well as in various types of commercially available bentonites including Asha, Calcigel and Wyoming MX-80 (Svensson et al., 2011). Sulphide-producing bacteria have been found in a full scale demonstration repository (Arlinger et al., 2013), in various pilot and full scale tests of bentonite performance (Karnland et al., 2009; Lydmark and Pedersen, 2011) and in the Boom Clay formation (Bengtsson and Pedersen, 2016). In a future SNF repository, the dominant long-term copper corrosive species may, therefore, be sulphide. The anaerobic microbial process of concern is consequently the dissimilatory reduction of sulphate to hydrogen sulphide by sulphate-reducing bacteria (SRB).

Microbial sulphide-producing activity has previously been found to decrease with increasing density of MX-80 bentonite (Masurat et al., 2010b; Pedersen, 2010). Variables of importance for such activity, in addition to bentonite density can be pore space, swelling pressure and pore water composition, transport conditions to and from the bentonite boundaries, usability of the naturally occurring organic matter present in the bentonite and molecular hydrogen from corroding metals,

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hydrogen and methane from geological sources and temperature. Until now, our laboratory research on survival and activity of SRB in bentonite as functions of wet density has only been performed with MX-80 bentonite and a precise cut-off density for sulphide-production could not be established (Bengtsson et al., 2015). Further, it is not known if different bentonites have diverse influence on SRB activity and survival. This work was aimed to identify if there are threshold bentonite densities with corresponding swelling pressures for MX-80, Asha and Calcigel above which microbial sulphide-producing activity is practically inhibited with all others conditions being favourable for growth of SRB. This work also aimed to evaluate if cultivability of SRB decreased with increasing wet density.

Cylindrical test cells made of titanium were used in the experiments. The cells were filled with the respective bentonite clay powder with addition of a bacterial cocktail consisting of three different species of SRB, except control cells without addition of SRB. By adjustment of the amount of bentonite in a confined space, the wet density and the swelling pressure generated by the bentonite could be regulated and also monitored by force transducers connected to a data collection system. The bentonite clay powders were compacted to a specific volume and then water saturated with a salt solution. A copper disc that simulated a copper canister was installed in the clay core bottom when the clay cores had reached the planned wet densities and were fully water saturated. On the opposite clay core side to the copper disc, $^{35}SO_4^{2-}$ together with lactate (carbon and energy source for SRB) were added. The test cells were then closed again and the force transducer, piston and top lid were refitted. The test cells were sampled in series after up to 4 months from the addition of $^{35}SO_4^{2-}$ and lactate. The radioactivity of $Cu_x^{35}S$ that had formed on the copper discs was located and quantified using electronic autoradiography. Samples were taken from different layers of the bentonite core and analysed for distribution of ^{35}S, of sulphate and most probable number of cultivable SRB. Sulphide production rates were calculated.

2. Material and methods

2.1. Experiments

This paper describes five different, consecutive experimental series with three different bentonite types and three different species of added SRB exposed to varying levels of wet density. To more easily refer to each experiment, a numbered list with information about bentonite types, planned densities and the time frame of the respective experiment 1 to 5 is given in Table 1. Experiment 1 with two densities of MX-80 was performed differently compared to experiments 2–5 in several aspects and these procedures and the obtained results have been reported (Bengtsson et al., 2015). The results from that report are included in this paper for comparison with one additional MX-80 density in experiment 2 and with Asha and Calcigel. In experiment 1, negative controls were heat treated in an oven for one week at 110 °C in a partly unsuccessful attempt to sterilize the clay without altering the clay minerals. The copper discs were not bevelled which resulted in a skewed distribution of results as previously described for Boom Clay experiments (Bengtsson and Pedersen, 2016, see Section 2.7) and the number of clay sampling positions were different. Else, all procedures in experiment 1 were similar compared to the procedures in experiment 2–5.

2.2. Test cells

The experiments utilized test cells consisting of a titanium cylinder with a piston, a top and bottom lid attached by six Allen screws for each lid. The test cells and their functions have been recently been described in detail by Bengtsson and Pedersen (2016). Briefly, a 35 × 20 mm cavity inside the cylinders was filled with MX-80, Asha or Calcigel bentonite powder and water saturated. The pressures created by the swelling bentonites were recorded by force transducers.

2.3. Chemical and mineralogical characters of the bentonites

The main mineralogical and chemical compositions of the clays are given in Table 2. All clays have a high content of montmorillonite and minor amounts of various clay minerals. MX-80 and Asha are sodium bentonites while Calcigel is a calcium bentonite as reflected by the respective amounts of CaO and Na_2O (Table 2). The MX-80 contains more silica than Asha and Calcigel. Asha distinguishes from the other clays by a three times larger amount of iron and Calcigel distinguishes form the other clays by a smaller amount of montmorillonite and absence of sulphate. All clays contain carbon, possibly in carbonates, and organic carbon is present in MX-80 and Asha. Sulphur is found in all clays.

2.4. Bentonite slurries

Three different species of SRB were used in the experiment. *Desulfovibrio aespoeensis* (DSM 10631), *Desulfotomaculum nigrificans* (DSM 574) and *Desulfosporosinus orientis* (DSM 765). *D. aespoeensis* was isolated from deep groundwater (Motamedi and Pedersen, 1998), *D. nigrificans* is a thermophilic, spore-forming sulphide-producing bacterium and *D. orientis* is spore-forming sulphide-producing bacterium with the ability to grow with molecular hydrogen as source of energy. The bacteria were grown in appropriate media and temperature as specified by the German collection of microorganisms and cell cultures (https://www.dsmz.de). At the start day of the experiments bacterial numbers for each of the three bacterial cultures were determined in 1 mL samples using the acridine orange direct count method as devised by Hobbie et al. (1977) and modified by Pedersen and Ekendahl (1990).

The three different bacterial cultures were mixed into one cocktail and poured or sprayed carefully out on a bed of the respective bentonite powder in a large glass Petri dish. This created small lumps of bentonite and cocktail. The whole content of the Petri dish was then passed through a mesh where the lumps were pulverised with sterile spoons. This created batches of SRB doped bentonite with a content of approximately 1×10^7 SRB g^{-1}. Additional batches with the respective clays without the addition of SRB were produced similarly. Experiments with clays without addition of viable SRB were denoted as negative controls. However, as the results will show, SRB were already present in the studied clays. All clay preparations were performed inside of an anaerobic box with an atmosphere consisting of 97% N_2 and 3% H_2, $O_2 < 1$ ppm (COY Laboratory Products, Grass Lake, MI, USA).

Table 1
List of performed experiments with MX-80, Asha and Calcigel bentonites with the planned wet and dry densities.

Experiment number	Year	Bentonite(s) tested	Planned wet densities (kg m^{-3})	Planned dry densities (kg m^{-3})
1	2012–2013	MX-80	1750, 2000	1171, 1562
2	2013–2014	Asha, MX-80	1850, 1900, 1950, 2000 1900	1300, 1406, 1453, 1529 1406
3	2014–2015	Calcigel	1850, 1900, 1950	1333, 1411, 1490
4	2015–2016	Asha	1500, 1750, 1850	765, 1147, 1300
5	2016	Asha	1600, 1700	917, 1070