The effect of compaction and microbial activity on the quantity and release rate of water-soluble organic matter from bentonites

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Abstract

Swelling bentonites, planned to be installed around spent nuclear fuel canisters made of copper/cast iron in geological repositories, contain organic matter. Organic matter can act as substrates for microorganisms, such as sulfate-reducing microorganisms (SRM), which produce sulfide, a copper corrosion agent. Thus, it is important to study the quantity and release rate of organic matter from bentonites. In this study, the soluble organic matter (sOM) quantity of three bentonites (Wyoming, Indian, and Bulgarian) determined by dynamic leaching assays was originally 85, 16 and >163 mg kg^{-1}, respectively. To assess the effect of simulated repository conditions on the quantity and release rate of bentonite sOM, the original bentonites were compacted to an average dry density of 1314–1368 kg m^{-3} and connected to sand layers in anaerobic cell systems. The sand layers were either inoculated or uninoculated with microorganisms. Afterwards, the cells were operated on for 12–15 months. As shown by postmortem dynamic leaching assays, the bentonite sOM quantities either increased (Indian and Bulgarian) or decreased (Wyoming) relative to the initial sOM quantities in original bentonites. The release rate of sOM increased in all bentonites in comparison with the original bentonites, and the increase was higher for the bentonites of the inoculated cells than for the uninoculated cells. The findings suggest that the interaction of the microorganisms with the bentonites increased the quantity and/or release rate of the bentonite sOM. Compaction of the bentonites, leading to changes in the mineral size and microstructure of bentonites, was also hypothesized to affect the amount of sOM released. Additional processes (e.g., diffusive transport) might also be relevant for the release of sOM from bentonites, but they were beyond the scope of this study. The present study represents an advance in the understanding of the effect of different possible repository conditions on the quantity and role of bentonite sOM as a source of substrates for the microbial community, especially considering the sulfide-producing SRM.

Keywords: Engineered barrier system, sulfate-reducing microorganism, spent nuclear fuel, deep geological repository, smectite

1. Introduction

Many countries plan to dispose of spent nuclear fuel (SNF) in a repository consisting of tunnels and deposition holes excavated 250–1000 m below ground (Birkholzer et al., 2012). The long-term safety of the geological disposal of SNF is based on the use of multiple release barriers, which include—in the context of the Finnish spent fuel geologic disposal program—the crystalline bedrock of Olkiluoto, copper/cast iron canisters sealing the SNF, bentonite encasing the canisters in the deposition holes, backfilling the tunnels at the depth of 400–450 m, and closure of the underground openings (Posiva, 2012a). The plan to use copper/cast iron canister is specific to the Finnish and Swedish disposal concepts (Padovani et al., 2017). The long-term safety functions of the bentonite surrounding the canister (also called the "buffer") are to protect the canister from corroding substances, such as sulfide, and from other chemical, mechanical, and hydraulic disturbances and to retard the release of radionuclides from the canisters to the environment (Juvankoski, 2013). The long-term safety functions of the buffer are achieved by installing the bentonite at a high dry density (1595 kg m^{-3}; Posiva, 2012a). Upon saturation with groundwater, the bentonite swells, creating diffusion-dominated and pressurized conditions (hydraulic conductivity 10^{-13}–10^{-14} m s^{-1}, swelling pressure 6–8 MPa; Karnland et al., 2006), which restrict biological sulfide production inside the buffer (inhibition at ≥ 2 MPa created with a dry density of ≥1400 kg m^{-3}) (Stroes-Gascoyne et al., 2010; Dixon, 2019).

The main source of sulfide in the groundwater system of the Olkiluoto repository is the activity of sulfate-reducing microorganisms (SRM), a microbial group including both bacteria and archaea, which reduce sulfate in the groundwater to sulfide using low-molar-mass organic compounds or H_2 as electron donors (Muyzer & Stams, 2008; Pedersen et al.,

2008). The availability of organic matter is naturally low in deep groundwater, but a significant amount of organic matter can be introduced to the repository environment within the bentonites (up to 10,000 mg kg^{-1} according to the Finnish buffer design specification) (Hallbeck, 2010; Juvankoski, 2013), and SRM and other microorganisms have been shown to be able to grow on bentonite organic matter in laboratory-scale experiments (Maanoja et al., 2020). However, one factor expected to restrict the availability of bentonite organic matter for microbial consumption in the repository is the adsorption of organic matter to bentonite (Hallbeck, 2010). Organic matter molecules are mostly bound to the surfaces and edge sites of the smectite by forces of different strengths, such as ligand exchange, cation bridging, hydrogen bonding, and van der Waals forces (Arnarson & Keil, 2000; Wattel-Koekkoek et al., 2003; Kleber et al., 2007). Due to strong mineral surface binding or inclusion in bentonite aggregates (Broz, 2020), the majority of the organic matter is expected to be immobile (e.g., 92–94% for the Wyoming type bentonite), and the small mobile fraction consists of soluble and reversibly adsorbed (i.e., leachable) organic matter (Marshall et al., 2015). In addition, the transport of organic matter from the dense bentonite to the groundwater can be expected to be retarded by the low interconnectivity of the pores in the bentonite and the large size and complex structure (e.g., aromaticity, branched chains) of the organic compounds, which leads to a high affinity toward the mineral surfaces (Vilks et al., 1998; Arnarson & Keil, 2000; Durce et al., 2018).

Even though the high density and the resulting low permeability and high swelling pressure of the saturated bentonite can be met in the bulk buffer, it may still contain areas of somewhat lower density, for example, at the interface with the bedrock (Villar et al., 2020). At the interfaces and lower-density areas, groundwater can interact with the bentonite, leading to the release of organic matter from the bentonite and increased microbial activity (Stroes-Gascoyne et al., 2011; Durce et al., 2018). Consequently, resolving the effect of

microbial activity on bentonite characteristics and performance has been a target of intensive research (for a review, see Mueller, 2015). For example, microorganisms have been shown to decrease the swelling capacity of bentonites by reducing structural Fe(III) (Kim et al., 2019) and to increase the interlayer space of smectites by intermediating the exchange of monovalent cations to divalent cations (at least in favorable laboratory conditions; Dai et al., 2014; Gregory et al., 2019). It is likely that the activity of the SRM and other microorganisms may also affect the quantity and release rate of organic matter adsorbed to the bentonites, but to the authors' knowledge, the interaction has not been studied until now.

Thus, this study aimed to resolve the effect of simulated repository conditions, including microbial activity, compaction of the bentonite, and groundwater salinity, on the quantity and release rate of the soluble organic matter (sOM) of three bentonites (Wyoming, Indian, and Bulgarian), selected as an example of materials that could be used in the final disposal of SNF. The study was carried out using six laboratory-scale cell systems, in which the interface of the bentonite and bedrock was simulated by separating the compacted bentonites from layers of saturated sand with a porous sinter enabling the migration of dissolved compounds and microorganisms. The sand layer represents an interface with a higher hydraulic conductivity than in the bulk of the bentonite. Two possibilities for microbial origin in the interface were mimicked: intentionally added cultured microorganisms and microorganisms indigenous to the bentonites. The sOM contents of both the bentonites exposed to simulated repository conditions and the original bentonites (as received) were analyzed by leaching assays. Understanding the effect of different possible repository conditions on the fate of bentonite sOM is crucial for assessing the role of the introduced bentonite material as a source of substrates for the microbial community of the repository environment, especially considering the sulfide-producing SRM.

2. Materials and Methods

The effect of the simulated repository conditions on the quantity and release rate of bentonite sOM was studied using six experimental cells (Fig. 1a). Setting up the cell systems included grinding, saturation, and compaction of the bentonites (before and at later stages of the operation) and installation of the sand layers on top of the bentonites (described in Section 2.2; Fig. 1a). In total, two cells were prepared for each bentonite: one cell of each bentonite received sand that was inoculated with SRM and groundwater microorganisms (called 'inoculated cells'), while the second cell of each bentonite received clean sand (called 'uninoculated cells'). The cells were operated on for 12–15 months. During this time, the growth of SRM and other microorganisms on bentonite organic matter was studied by monitoring the evolution of the concentrations of different analytes in the sand layers of the cells. The results are reported in detail by Maanoja et al. (2020). After operation, the cells were dismantled, and the sOM quantity and release rate were determined from both the bentonites exposed to the simulated repository conditions and from the original bentonites (as received) by dynamic leaching assays (see Section 2.3.2; Fig. 1d). The leachant used in the dynamic leaching assays was selected based on the results of a separate batch equilibrium assay (see Section 2.3.1; Fig. 1c).

2.1 Bentonites, quartz sand, and artificial groundwater

Two of the bentonites studied were Na-bentonites originating from Wyoming and India, and one was a Ca-bentonite from Bulgaria (Table 1). For the batch equilibrium assay, the bentonites (referred to as 'original bentonites') were used in the form as received (Indian and Bulgarian particle size of 0.5–3 mm, Wyoming 0.25–1 mm, 68% ≤0.5 mm; Kumpulainen et al., 2016; Kiviranta et al., 2018). For the dynamic leaching assays and exposing the bentonites to the simulated repository conditions in the cells, the original Indian and Bulgarian bentonites were ground to ensure homogeneity and reduce their particle size (100% <0.2 mm) approximately to the same level with the Wyoming bentonite to harmonize the

level of physical changes the bentonites had experienced before arriving to the laboratory, while the Wyoming bentonite was used as received due to its finer homogenous state. The quartz sand (NFQ Nilsiä QUARTZ, Sibelco Nordic Ab, particle size of $0.63 \leq x < 1$ mm) was combusted (4 h at 450°C) to remove organic residues.

Two types of artificial groundwaters (AGWs) were used as saturation solutions for the sand and bentonites in the cells and as leachants in the leaching assays: saline and dilute AGW with 11 and 1 g total dissolved solids (TDS) L^{-1}, respectively (Table 2). The saline AGW mimicked the current composition of Olkiluoto groundwater at the repository depth (Hellä et al., 2014). The dilute AGW was formulated to mimic the composition of the water at the repository depth after mixing with infiltrated fresh water of melting glaciers and traces of sulfate-rich marine water, which is predicted to occur in the far future (>10,000 years after closure) according to a potential scenario of climatic evolution (Posiva, 2012b; Hellä et al., 2014). Both AGW solutions were prepared in ultrapure water (Milli-Q®, MilliporeSigma), using carbon-free glassware (combusted at 450°C for 2 h) to minimize the amount of organic matter in the solutions (Table 2).

2.2 Compaction of bentonite blocks and cell setup

Preparation of the cells with the three bentonites is described in detail by Maanoja et al. (2020) and summarized here. For the preparation of the bentonite blocks, the original ground bentonites were deoxygenated with N_2 (99.5% v/v, Aga Ltd.), mixed with anaerobic saline AGW (target moisture content 25–27% of wet mass depending on the bentonite to saturate the pores at the target density), and then compacted inside the cells to produce saturated bentonite blocks (Table 3). A dry density of 1400 kg m^{-3} was selected as the target density with the objective of creating a swelling pressure to inhibit the growth of microorganisms in the bentonite blocks (approximately 2 MPa; Dixon, 2019; Taborowski et al., 2019). Porous titanium sinters (pore size 1–2 µm) were pressed on top of the bentonite blocks, and layers

consisting of quartz sand saturated with saline AGW were installed on top of the sinters (Fig. 1a). Microorganisms pre-enriched from Olkiluoto groundwater and known SRM (*Desulfobacula phenolica, Desulfobulbus mediterraneus, Desulfobulbus rhabdoformis*, *Pseudodesulfovibrio aespoeensis*, and *Desulfotomaculum acetoxidans* from DSMZ GmbH) were added to the sand layers of the inoculated cells after washing with saline AGW to remove residues of their original media (for further details, see Maanoja et al., 2020). In the uninoculated cells, the unsterile bentonites acted as a potential source of SRM and other microorganisms. Inoculated and uninoculated cells represented scenarios of high and low microbial activity, respectively, at the bentonite-bedrock interface. Next, the cells were sealed with airtight plungers and operated on for 12–15 months. During the operation, samples were withdrawn every three weeks from the sand layer solution, replaced with an equal volume of saline AGW through the sampling valves of the plungers (Fig. 1a) and analyzed for different parameters, such as dissolved organic carbon (DOC) as a measure of sOM.

The theoretical dry densities of the compacted bentonite blocks were eventually lower (1314–1368 kg m^{-3}; Table 3) than the target density (1400 kg m^{-3}) due to the unexpected swelling of the bentonite blocks before and during the operation of the cells. The swelling was caused by the unconstrained blocks adsorbing saline AGW from the sand layers before the operation was started and after breakage of cell parts controlling the volume of the bentonite blocks during operation. The swollen bentonite blocks were recompacted before the operation was started, as well as on operational days 146 and 176, but the initial densities could not be restored, and they decreased by 6% in the uninoculated cell of the Bulgarian bentonite and by 3% on average in the other cells relative to the initial densities (Table 3). The mass balance calculations conducted after the swelling and re-compaction occasions (described in Maanoja et al., 2020) suggested that only the sOM quantity of the Bulgarian bentonite in the uninoculated cell was decreased by the re-compaction occasions (by 14

weight-% [w-%] relative to the theoretical sOM quantity). However, an error was discovered from the calculations (theoretical sOM contents assumed for the original bentonites were too low), and the calculation was re-done using the sOM quantity determined for the particular batch of Bulgarian bentonite used in this study (>163 mg kg^{-1}; Section 3.3.1). As a result, the decrease in the sOM quantity of the Bulgarian bentonite block in the uninoculated cell resulting from swelling and re-compaction occasions was found to be only 0.1 w-%.

After 12–15 months, the cells were opened one after another, and the sand layers were emptied. After that, the middle pieces of the bentonite blocks (5 × 5 cm; Fig. 1a) were detached by sawing and cut horizontally into layers (1–4 cm, narrower layering next to the sand layer interface than on the bottom of the block; Fig. 1b). The pieces of Wyoming and Bulgarian bentonites were divided into layers in a slightly different way than the pieces of Indian bentonite (Fig. 1b), because the sampling approach was improved after dismantling the cells of Indian bentonite. The edges of the bentonite layers that had been in contact with the saw blade were removed by cutting with surgical blades to exclude possible contamination or drying of the bentonite caused by sawing. Then, the layers, referred to as 'exposed bentonites', were sampled for determination of dry density by moisture content (unhomogenized pieces to avoid drying of the bentonite) and sOM quantity and release rate (bentonite homogenized in size by cutting to a particle size of 5 ± 2 mm).

2.3 Leaching assays

2.3.1 Batch equilibrium assay

The composition of the leachant in terms of salinity was selected for the dynamic leaching assays with a preliminary batch equilibrium assay (Fig. 1c; CEN ISO/TS 21268-2) aimed at achieving a maximal release of organic matter from the bentonites. The assay was conducted at a liquid-to-solid (L/S) ratio of 40 L kg^{-1} by mixing 7.5 ± 0.02 g dry mass of all three unground original bentonites with 0.3 L of saline or dilute AGW (Table 2) in carbon-free

aerobic bottles. The preliminary tests showed no difference between aerobic and anaerobic conditions in terms of the amount of sOM released from the bentonites (data not shown). For each bentonite, in total, eight parallel samples were prepared, four of which were leached with saline AGW and four with dilute AGW. In addition, two parallel samples without bentonite were prepared from both leachants to serve as analytical blank samples. Leaching was conducted at 21 ± 2ºC and 150 revolutions per minute (rpm) for seven days to allow time for the liquid-solid system to reach equilibrium (based on preliminary testing, data not shown), after which the eluate was separated from the bentonites by centrifugation (30 min at 10,000 × *g*) and analyzed for DOC and cation concentrations.

2.3.2 Dynamic leaching assay

The sOM quantity and release rate of the original bentonites and the bentonites exposed to the simulated repository conditions were determined by dynamic leaching assays under aerobic conditions (Fig. 1d; Marshall et al., 2015). Dilute AGW was used as a leachant to maximize the quantity of sOM released from the bentonites based on the results obtained from the batch equilibrium assay (see the results in Section 3.1). The samples were prepared for leaching by mixing 0.75 ± 0.03 g dry mass of each bentonite collected from six (Indian) or seven (Wyoming and Bulgarian) different depth layers of the bentonite blocks (Fig. 1b; n = 3) and 0.5 g dry mass of each three original bentonites (n = 4) with 0.04 L of dilute AGW in acid-washed polypropylene tubes (soaked 24 h in 1 M HCl, rinsed with ultrapure water and dried). Thus, the initial L/S ratio was 54 L kg^{-1} on average for the bentonites exposed to the simulated repository conditions and 80 L kg^{-1} for the original bentonites. The L/S ratio was increased to accelerate the release of sOM from the original bentonites based on the results obtained from leaching of the exposed bentonites. In addition to samples containing bentonites, sets of analytical blank samples were prepared from dilute AGW without bentonites (n = 3–4).

Leaching was conducted at 21 ± 2°C and 170 rpm, and samples were initially collected five times per week for two weeks and then approximately once in three weeks. At sampling, the eluate was separated from the bentonite by centrifugation (30 min at 10,000 × *g*) and removed from the tubes to determine DOC concentration as a measure of sOM (Fig. 1d). The mass of the bentonite residue in the tube was weighed to account for the mass of leachant adsorbed by the bentonite, and a new batch of leachant was added to achieve the original L/S ratio (54 or 80 L kg^{-1}). Next, the leaching was conducted, and at the next sampling point, eluate was separated from the bentonite and replaced with fresh leachant, as described above; these leaching steps were repeated in total 4–24 times depending on how many steps it required to reach a state where no more sOM was released by the bentonites. To determine the cumulative L/S ratio required to exhaust the release of sOM, the cumulative volume of the leachant used in the progressing leaching steps was calculated. For the following samples, the leaching was stopped before exhaustion of sOM release was reached due to time constraints: Wyoming bentonite from inoculated (depths 4–16 cm) and uninoculated cells (1–6 and 12–16 cm), Bulgarian bentonite from uninoculated cells (depths 1–16 cm), and original Bulgarian bentonite.

2.4 Analytical methods and calculations

The moisture content of the bentonite samples (*n* = 2–3) was measured by drying the samples at 105°C to a constant mass, and the moisture content and solid mass were calculated from the mass loss of the samples during drying (APHA, 1995). The concentration of DOC was measured from filtered (0.45 μm) eluates with a total organic carbon analyzer (*n* = 1; Shimadzu TOC-V_{CPH}; SFS-EN 1484). The concentration of cations was determined from filtered and preserved eluates (0.45 μm, 1 volume-% 1 M HNO_3; *n* = 1) by an ion chromatograph (Dionex DX-120; SFS-EN ISO 14911) with an IonPac CS12 A 4 × 250 mm

column, CSRS 300 4 mm suppressor at 100 mA, and 20 mM methanesulfonic acid as an eluent at 1.0 mL min^{-1}.

The moisture contents (Fig. S1 in the supplementary material, only available online) and solid masses were used for calculating the dry densities of the bentonites exposed to the simulated repository conditions with the following assumptions: bentonites at full water saturation, 1016 kg m^{-3} as an average density for pore waters in the bentonites (calculated using salinity 23 g TDS L^{-1}; UNESCO, 1981; Wersin et al., 2016) and 2780, 2910, and 2670 kg m^{-3} as density of solid particles for Wyoming, Indian, and Bulgarian bentonites, respectively (Kiviranta & Kumpulainen, 2011; Kumpulainen & Kiviranta, 2015; Kumpulainen et al., 2016).

As the leachants originally contained 1 or 11 g TDS L^{-1} and 0.04 or 0.4 mg DOC L^{-1} (Table 2), the analytical blank samples without bentonites were used to calculate the amount of DOC adsorbed or desorbed by the bentonites during the leaching steps (Eq. 1):

$$m(DOC)_{Bentonite} = [c(DOC)_{Eluate-S} \cdot V_{Tot}] - [c(DOC)_{Eluate-B} \cdot V_{Leachant}] \quad (1)$$

where $m(DOC)_{Bentonite}$ is the mass of DOC released by the bentonite sample (mg), $c(DOC)_{Eluate-S}$ is the DOC concentration in the eluate of the sample containing bentonite (mg L^{-1}), V_{Tot} is the total volume of liquid in a sample containing bentonite (i.e., the sum of the volume of added leachant and moisture content of the bentonite (L)), $c(DOC)_{Eluate-B}$ is the DOC concentration in the eluate of a blank sample (mg L^{-1}) and $V_{Leachant}$ is the volume of leachant added in the samples containing bentonites (L). To convert the volumes of leachants to masses and vice versa, theoretical densities of 998.8 kg m^{-3} and 1006.0 kg m^{-3} were used for dilute and saline AGWs, respectively (UNESCO, 1981). The sOM quantities of the bentonites were calculated as cumulative values from the obtained DOC concentrations of the

eluates. In some samples, the bentonites started adsorbing DOC from the leachants toward the end of the dynamic leaching assay, which was observed as a decrease in the cumulative sOM quantity (e.g., see Fig. 5), but the maximum value obtained in the assay was considered the result for the sOM quantity. All the sOM and cation quantities were calculated for the dry mass of the bentonite samples (mg kg^{-1}). The sOM quantities were considered in three ways: 1) as weighted average values for the entire bentonite blocks, taking into account the mass factor of each depth layer (dry masses ranging from 47–66 g in the 1-cm layers to 222–236 g in the 4-cm layers); 2) as proportional values relative to the total organic carbon (TOC) quantities of the bentonites (680–2023 mg kg^{-1}; Table 1); and 3) as individual results for each original bentonite and depth layer (0–16 cm) of the six bentonite blocks. The sOM release rate was defined as the quantity of sOM released relative to the cumulative L/S ratio, not the leaching time, because the time did not affect the release of sOM from the bentonites as shown by preliminary testing (data not shown).

3. Results and Discussion

3.1 Release of organic matter from bentonites at different salinities

To study the effect of leachant salinity on the release of bentonite sOM, a batch equilibrium assay was carried out with the three studied bentonites and two leachants (saline and dilute AGWs). It was demonstrated that the sOM quantities of the Wyoming, Indian, and Bulgarian bentonites determined with the dilute AGW were higher than those determined with the saline AGW (Fig. 2). For the Wyoming and Indian bentonites, the difference between the sOM contents released into saline and dilute AGW was greater than for the Bulgarian bentonite (Fig. 2). The amount of cations exchanged by the three bentonites was lower in the dilute AGW than in the saline AGW (Fig. 3). For the Wyoming and Indian bentonites, having sodium as the main cation at the exchange sites, the main cation released was Na^+ and the main cation adsorbed was Ca^{2+} in both leachants (Fig. 3). In the case of the Bulgarian Ca-

bentonite, a lower amount of cations with a less distinct pattern were released and adsorbed from the leachant than for the other two bentonites (Fig. 3). It should be noted that dissolution of accessory minerals, such as calcite and gypsum (Table 1), and precipitation of different minerals also contribute to the total pool of cations (e.g., Ca^{2+}), but these processes and the mechanisms of the cation exchange are not considered further here.

The higher release of bentonite sOM to dilute (1 g TDS L^{-1}) than to saline (11 g TDS L^{-1}; Table 2) solution could be explained by the charge effects on the smectite surfaces. The majority of the sOM in the bentonites studied can be assumed to be negatively charged because the release of sOM from the bentonite did not increase with an increased release of cations in the saline solution (Fig. 3; Arnarson & Keil, 2000). In dilute AGW with low salinity, the negative charge of the smectite is not as screened by the cations at the wider diffuse double layers of the mineral surfaces, thus leading to a higher anion exclusion effect (Van Loon et al., 2007). Preferably, the negatively charged bentonite sOM would then be driven from the bentonite to the solution. In saline AGW, the smectite surfaces are closely shielded by cations in the narrower diffuse double layer, which decreases the repulsive forces of the smectite surfaces and leads to lower exclusion/higher adsorption of the anionic organic compounds in the bentonite than in the low saline solution (Van Loon et al., 2007; Rao & Thyagaraj, 2007; Xiang et al., 2019). The effect of leachant salinity on the release of sOM from the Bulgarian bentonite was lower than for the other bentonites (Fig. 2), possibly because the divalent calcium at the Bulgarian bentonite's exchange sites has greater attraction to the smectites than monovalent cations and, thus, the anion exclusion effect is weaker (Likos & Wayllance, 2010; Muurinen & Carlsson, 2013) or the cation bridges between organic compounds and calcium are stronger than in Na-bentonites (Arnarson & Keil, 2000).

Based on the observed differences in the release of sOM with the dilute and saline AGWs, dilute AGW was used as a leachant in the dynamic leaching assays to induce a

maximal release of bentonite sOM from the original and exposed bentonites. Saline AGW was used instead as a saturating solution in the cells, as it is considered representative of the current repository conditions.

3.2 Density of the bentonite blocks and indications of microbial activity

The effects of the simulated repository conditions on the quantity and release rate of bentonite sOM from the three bentonites were studied with the cell systems. To check whether the swelling and re-compaction occasions (described in Section 2.2) had affected the final densities of the bentonite blocks in comparison to the theoretical dry densities (1314–1368 kg m^{-3}; Table 3) and possibly enabled microbial activity inside the bentonite blocks, the dry densities at different depths of the blocks (Fig. 1b) were determined after the cells were dismantled. In all six blocks, the dry density was 5–15% lower near the sinter interface (0–4 cm; 1140–1310 kg m^{-3}) than at the bottom of the blocks (4–16 cm; 1260–1380 kg m^{-3}), where it was close to the theoretical average dry density (Fig. 4). In the cells containing Bulgarian bentonite, the dry densities were lower (by 1–6%) at all depths of the bentonite block of the uninoculated cell than in the inoculated cell (Fig. 4c) likely because the block in the uninoculated cell of the Bulgarian bentonite swelled the most before and during operation of the cells (Section 2.2; Maanoja et al., 2020).

During the operation of the cells, microorganisms were shown to be active and, thus, to potentially have interacted with bentonite organic matter in all six cells, based on the observations summarized below (reported in detail in Maanoja et al., 2020, and for the cells of Wyoming and Bulgarian bentonites in Kiczka et al., 2021). In all three inoculated cells, the activity of SRM and iron-reducing bacteria (IRB) was concluded from sulfate concentrations being lower and iron concentrations higher in the sand layer solutions of the inoculated cells than in the sand layer solutions of the uninoculated cells. In addition, the iron sulfide precipitates (FeS, Fe_3S_4) identified from the sand layers of the inoculated cells of the

Wyoming and Bulgarian bentonites indicated activity of SRM. The SRM indigenous to the bentonites were also believed to have become active in the uninoculated cells of Wyoming and Bulgarian bentonites because SRM were detected from the sand layers of these cells after operation (measured as gene copies of dissimilatory sulfite reductase subunit B enzyme). The activity of the methanogens in all six cells was determined based on the detection of CH_4 in the sand layer solutions. The overall microbial activity was higher in the inoculated cells than in the uninoculated cells, and the uninoculated cells of the Indian bentonite exhibited the lowest microbial activity of all the cells based on the concentration of adenosine triphosphate in the sand layers. The exact locations of the active microorganisms inside each of the six cells could not be confirmed, but the results suggested that microorganisms had been active in all six sand layers, and at least in the bentonite blocks of the inoculated cells of Wyoming and Indian bentonites. The activity in the bentonite blocks could be hypothesized based on the most probable number of SRM being higher in the surface layers (0–1 cm) of Wyoming and Indian bentonites of the inoculated cells after exposure to the simulated repository conditions in comparison to the original bentonites (Maanoja et al., 2020). The reactive transport modeling of the evolution of dissolved inorganic carbon and sulfate concentrations in the sand layers of the cells supported this hypothesis and further indicated that SRM were also active in the top layer of the bentonite in the Wyoming and Bulgarian bentonite blocks of both cells (Kiczka et al., 2021).

The lower dry densities in the top depths of the bentonite blocks (0–4 cm), particularly in the uppermost 1 cm, where dry density was the lowest, might have enabled microbial activity and, therefore, a direct interaction of microorganisms with bentonite organic matter. This is hypothesized because the dry densities of the Wyoming and Indian bentonite blocks (at 0–4 cm; 1270–1330 kg m^{-3} and 1140–1300 kg m^{-3}, respectively; Fig. 4a, b) were lower than the dry densities reported in the literature needed to restrict the activity of SRM in the

Wyoming and Indian bentonites (approximately 1350 and 1280 kg m^{-3}, respectively, based on the saturated densities of 1830–1870 kg m^{-3} and other information given by the authors; Bengtsson & Pedersen, 2017). For the Bulgarian bentonite, a similar density threshold inhibiting microbial activity has not been reported in the literature. The reactive transport calculations of Kiczka et al. (2021) indicated that SRM were active at the top layers of the Bulgarian bentonite in both cells and, thus, presumably dry densities of <1250 kg m^{-3} do not inhibit microbial activity in the Bulgarian bentonite. It is possible that the swelling pressures in the lower depths of the Bulgarian blocks were inhibitive for microbial activity (≥ 2 MPa; Stroes-Gascoyne et al., 2010). Swelling pressures of the exposed bentonites were not measured in this study, but in comparable conditions, the Bulgarian bentonite has been reported to have an exceptionally high swelling pressure (11 MPa at dry density of 1460 kg m^{-3}, saturated with 10 g TDS L^{-1} Na:Ca 2:1 w/w) for an unknown reason (Svensson et al., 2019), in comparison to the Wyoming and Indian bentonites (2 and 4 MPa at 1460 kg m^{-3}, saturated with 6 g NaCl L^{-1} and 10 g TDS L^{-1} Na:Ca 2:1 w/w, respectively) (Karnland et al., 2006; Kumpulainen et al., 2016).

3.3 Bentonite sOM before and after exposure to simulated repository conditions

To assess the effect of the simulated repository conditions on the quantity and release rate of bentonite sOM, the sOM was quantified from the three original and six exposed bentonites (each block divided vertically into 7–8 layers) by dynamic leaching with dilute AGW as a leachant. During the assays, the bentonites were sequentially leached until exhaustion of sOM release, which took 4–24 leaching steps over 14–344 days, resulting in a cumulative L/S ratio of 195–1819 L kg^{-1} (Fig. 5). For some samples, the leaching was stopped before exhaustion of sOM release (Fig. 5). The cumulative bentonite sOM quantities determined after exposure to the simulated repository conditions were compared with the sOM quantities of the original bentonites as follows: 1) as average values for the entire bentonite blocks to identify the

overall effect of the simulated repository conditions (inoculated vs. uninoculated cells) on the quantity of sOM in the bentonites; 2) as separate values for each cumulative L/S ratio to show the changes in the sOM release rate; and 3) as a function of depth in the bentonite block to identify changes in the vertical distribution of the bentonite sOM quantity in the blocks, potentially induced by the simulated repository conditions.

3.3.1 Average sOM quantities of the bentonites

The maximum sOM quantities of the original bentonites were 85, 16, and >163 mg kg^{-1} for the Wyoming, Indian, and Bulgarian bentonites, respectively (Table 4), which was approximately in the same range as the sOM quantities reported in the literature for Wyoming bentonite (8–90 mg kg^{-1}; Marshall et al., 2015; Usman & Simpson, 2020). For the Bulgarian and Indian bentonites, to the authors' knowledge, no such data have been published before. After exposure to the simulated repository conditions, the average sOM quantity in the Wyoming and Bulgarian bentonite cells was higher in the bentonite blocks of the inoculated cells than in the blocks of the uninoculated cells (Table 4). In the cells with Indian bentonite, the average sOM quantity was lower in the bentonite block of the inoculated cells than in the block of the uninoculated cells (Table 4).

Exposing the bentonites to simulated repository conditions increased the sOM quantities of the Indian and Bulgarian bentonites compared to the original bentonites. The highest increase in the average sOM quantity was in the inoculated cell of Bulgarian bentonite, followed by the uninoculated and inoculated cell of Indian bentonite (Table 4). In the Wyoming bentonite, the average sOM quantity (Table 4) was lower in both cells compared to the original bentonite, probably because of the incomplete dynamic leaching assays. Indeed, the end point for the sOM release was not achieved during the dynamic leaching assay for the Wyoming bentonite in most samples of the inoculated (depths 4–16 cm) and uninoculated cells (depths 1–6 cm and 12–16 cm; Fig. 5a, b; Table 4), the Bulgarian

bentonite in the uninoculated cell (all depths; Fig. 5f; Table 4), and the original Bulgarian bentonite (Fig. 5e, f; Table 4). Thus, the resulting sOM quantities obtained for these bentonites were probably lower than they would have been if the leaching continued further. More research is needed to confirm the observed response of the sOM quantity of the Wyoming bentonite to the simulated repository conditions because it seemingly differed from the response of the other studied bentonites.

3.3.2 Quantity of sOM released by bentonites relative to cumulative L/S ratio

The amount of sOM released by the bentonites with the increasing L/S ratio (Fig. 5) was assessed relative to the total cumulative sOM quantity to identify the possible effects of the simulated repository conditions on the release rate of sOM from the bentonites. Release rate of bentonite sOM after the exposure was higher than from the original bentonites, except for the uninoculated cell of the Bulgarian bentonite, for which the sOM release rate was like that of the original Bulgarian bentonite (Fig. 5). With the inoculated cells of the Wyoming and Bulgarian bentonites, the majority of the sOM was dissolved during the first leaching cycle (80% and >90% of the total sOM on average, respectively), whereas it took 3–6 cycles to obtain a similar level of release (approximately 90% of the total sOM) from the bentonites of the corresponding uninoculated cells (Figs. 5, S2a–b, g–h). For the cells of Indian bentonite, the difference between the inoculated and uninoculated cells was not as obvious, but a lower number of leaching cycles was required to release the same quantity of sOM from the bentonite of the inoculated cells than of the uninoculated cells (e.g., 80% of total sOM in 3–4 and 4–6 cycles, respectively; Figs. 5, S2d–e).

3.3.3 Vertical distribution of sOM in exposed bentonite blocks

The sOM quantities of the bentonite blocks were examined relative to the sampling depths of the bentonite blocks (Table 4). In the inoculated cells of the Wyoming and Bulgarian bentonites, the sOM quantity was lower in the surface layers (0–4 cm and 0–1 cm,

respectively) than in the deeper layers of the bentonite blocks (4–16 cm and 1–16 cm; Table 4). Similarly, in the uninoculated cells, the sOM quantity of the surface layers of the Wyoming and Bulgarian bentonites was lower (0–1 cm) than that of the deeper layers (1–16 cm; Table 4). Even though the leaching of some of the layers from the Wyoming and Bulgarian blocks was finished before the exhaustion of organic matter release, it did not affect the observed patterns in the vertical distribution of the sOM quantity in the Wyoming and Bulgarian blocks; the sOM quantity of these bentonites was lower in the top than in the bottom layers of the blocks (Table 4). In the blocks of the Indian bentonite, the vertical distribution of the sOM quantity differed from the pattern observed in the blocks of Wyoming and Bulgarian bentonites. In the inoculated cell of Indian bentonite, the sOM quantity was slightly higher at the top layers (0–2 cm) than at the deeper layers (2–16 cm), while in the uninoculated cell, the sOM quantity did not differ between the layers (Table 4).

3.3.4 Discussion of the observed changes in sOM quantity and release rate

Exposing the bentonites to the simulated repository conditions increased the quantity and/or the release rate of the sOM from the bentonites in both inoculated and uninoculated cells relative to the original bentonites, so the increase could have been attributed to both the compaction of the bentonites and the microbial activity inside the cells. The individual effects of compaction or microbial activity, however, could not be separated. The suggested abiotic mechanism, potentially explaining the increased sOM quantity or release rate after exposure, is based on changes in the smectite microstructure and particle size as a result of compaction. In compaction, the microstructure of the smectite particles becomes altered due to a decrease in the inter-aggregate porosity (Delage et al., 2006; Likos & Wayllace, 2010), which presumably induces the breaking of microaggregates and, thus, results in the release of organic matter. The relationship between the density and the sOM quantities of the bentonites (Fig. S3), however, did not confirm the hypothesis that a higher compaction degree (i.e.,

density of the bentonite in the cell) would have induced a higher quantity of sOM in the bentonite after dismantling.

The decrease of sOM quantity from the top layers of the bentonite blocks could be attributed to microbial consumption. In the surface layers of the Wyoming bentonite blocks of the inoculated and uninoculated cells, the sOM quantity was in total 145 mg kg^{-1} (0–4 cm) and 17 mg kg^{-1} (0–1 cm), respectively, lower than in the bottom layers of the corresponding blocks, while in the Bulgarian bentonite of the inoculated cell, the difference between the surface (0–1 cm) and bottom layers was 224 mg kg^{-1} as calculated from the sOM contents presented in Table 4 (see Table S1 for details). Part of the quantity of sOM missing from the bentonite blocks could have diffused to the sand layers during the operation of the cells, but the amount of DOC measured from the sand layer solutions accounted only for 1–8 mg kg^{-1} and 10 mg kg^{-1} of relative to the mass of Wyoming and Bulgarian bentonites at depths having the decreased sOM quantities, respectively (Table S1). As the sOM quantity measured from the sand layers did not correspond to the sOM quantity missing from the blocks, this is a clear indication of the microbial consumption of sOM in these cells. For the cells with Indian bentonite, no decrease of sOM quantity from the surface layers of the blocks could be seen, but the average sOM quantity being lower in the bentonite block of the inoculated cell than in the uninoculated cell suggests that the sOM in the bentonite of the inoculated cell had been consumed by microorganisms (Table 4). Similarly, microbial activity in the cells of the Wyoming bentonite could also explain why the sOM quantity of the exposed bentonite blocks in both cells (on average 52 and 68 mg kg^{-1} in inoculated and uninoculated) was lower than in the original Wyoming bentonite (85 mg kg^{-1}). If the sOM was consumed microbially during the operation of the cells, it could have masked the possible increasing effect of exposing the bentonites to the simulated repository conditions on the sOM content of the Wyoming bentonite.

The other mechanism proposed to explain the increased quantity and faster release of the bentonite sOM was the activity of microorganisms inside the cells during the operation. The microorganisms that were shown to be active in the cells, SRM, IRB, and methanogens (Section 3.2), could have induced the release of bentonite sOM by reducing the structural iron(III) directly (IRB, methanogens) or indirectly (S^{2-} produced by SRM), leading to the release of organic matter adsorbed to smectite surfaces (Zhang et al., 2014; Cuadros, 2017; Broz, 2020). Another possibility is that the microorganisms decreased the complexity of the bentonite organic matter by decomposing it into smaller and simpler compounds, which could have decreased their adsorption and facilitated their release from the bentonite relative to the unreacted compounds (Arnarson & Keil, 2000; Condron et al., 2010; Durce et al., 2018), as suggested by the faster release of sOM from the exposed bentonites during the first dynamic leaching steps relative to the original bentonites (Fig. S2), in particular in the top layers of the bentonite blocks.

Overall, the bentonite organic matter was shown to be poorly soluble, as the majority of the TOC in the original bentonites (>88 wt-% of TOC) and in the bentonites after exposure to the simulated repository conditions (≥80 wt-%) remained immobile even when the release was maximized by dynamic leaching (Table 4). However, the conditions created in the dynamic leaching assays, L/S ratios of 195–1819 L kg^{-1} (Fig. 5) and loosely arranged, free-swelling bentonite differed considerably from the diffusion-controlled conditions prevailing inside the compacted bentonite blocks of the cells (L/S ratio 0.4 L kg^{-1}, dry density 1140–1380 kg m^{-3}; Table 3, Fig. 4) and in the engineered barrier system of the SNF repository (theoretical L/S 0.27 L kg^{-1}, based on the expected average dry density of the buffer bentonite, 1595 kg m^{-3}; Posiva, 2012a). In compacted bentonites, the release and transport of organic matter, like other compounds, is based on diffusion through the inter-particle voids and is affected by the charges of the different internal and external surfaces, the size of the

organic molecules, the size and connectivity of the pores, the density of the bentonite, and the ionic strength of the pore water, among other factors (Alt-Epping et al., 2015; Durce et al., 2018).

The diffusion coefficients (D_e) for the anion accessible porosity of Wyoming and Bulgarian bentonites in the cells described here (2.3 and $4.1 \cdot 10^{-11}$ m^2 s^{-1}, respectively) were determined by Kiczka et al. (2021) by using average dry densities for the blocks (1355 and 1364 kg m^{-3}). Even though the range of densities in the bentonite blocks (Fig. 4) was neglected, the determined coefficients were shown to predict the evolution of different ion concentrations in the sand layers accurately (Kiczka et al., 2021). The relatively constant DOC concentrations observed in the sand layers of the experimental cells (2–23 mg L^{-1}; Maanoja et al., 2020) were interpreted to reflect an attained diffusive equilibrium with the DOC concentrations in the bentonite porewater, while the overall flux of DOC from the bentonite to the sand layer was also affected by the microbial consumption and bioavailability of the sOM (Kiczka et al., 2021). Thus, even though the microbial activity and compaction of the bentonites have the potential to increase the quantity of sOM in the bentonites, there are other processes that control the release of organic compounds to the body of water interacting with the bentonite.

4. Conclusions

Bentonites planned to be used in a geological repository for spent nuclear fuel (SNF) contain soluble organic matter (sOM) that can be utilized by sulfate-reducing microorganisms in production of sulfide, an agent corroding SNF canisters. In this study, the effect of the simulated repository conditions, compaction to the target dry density of 1400 kg m^{-3} and microbial activity (introduced and/or indigenous), on the quantity of bentonite sOM and its release rate was assessed for three different bentonites. The two Na-bentonites from Wyoming and India and one Ca-bentonite from Bulgaria were shown to differ in their sOM

quantities originally (84.5, 16.4 and > 163 mg kg^{-1}, respectively). Exposing the bentonites to the simulated repository conditions in the laboratory cell systems for 12–15 months was shown to increase the sOM quantity from two of the bentonites (up to 61 and 270 mg kg^{-1} in Indian and Bulgarian bentonites) and the sOM release rate from all exposed bentonites relative to the original bentonites (>60 wt-% vs. 30 wt-% of sOM released in the first leaching step of a dynamic leaching assay, respectively). Both the compaction and interaction of the bentonite with the microorganisms, directly or indirectly, were possible mechanisms increasing the quantity and release rate of the bentonite sOM because the increase was higher in the bentonites of the cells inoculated with external microorganisms than in the uninoculated cells containing only microorganisms indigenous to bentonites. For Wyoming bentonite, the simulated repository conditions seemingly decreased the bentonite sOM quantity relative to the original bentonite, but the decreased sOM quantities particularly in the top layers of the exposed bentonite blocks suggested that the sOM quantities had been affected by microbial consumption in the cells of Wyoming bentonite. The effect of solution salinity on the release of sOM from the original bentonites was studied in a separate batch equilibrium assay, and the results showed that the release of sOM was lower in salinity corresponding to the salinity of the current brackish groundwater in Olkiluoto (the SNF repository site in Finland), rather than to fresh water (11 and 1 g total dissolved solids L^{-1}, respectively). This is attributed to the charge effects on smectite surfaces. The results of this study demonstrated that the different conditions and factors occurring in the SNF repository could increase the sOM quantity of the bentonites available for microbial consumption, for example, up to 20 wt-% of total organic carbon, as shown with Bulgarian bentonite. However, additional processes occurring in actual repository conditions (e.g., slow diffusion) might also influence the quantity and release of sOM from bentonite, but they were beyond the scope of this study.

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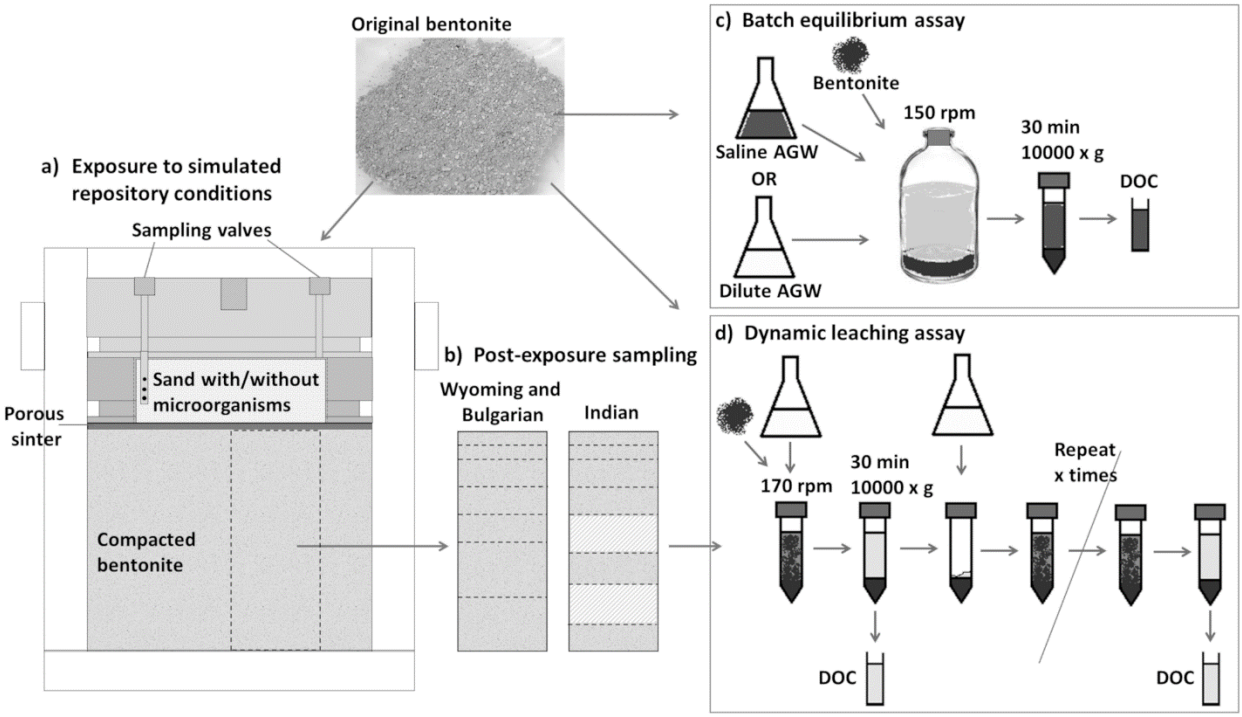

Figure 1. Schematic of the experimental setup used to study the effect of the simulated repository conditions (compaction of bentonite and microbial activity) on the quantity and release rate of soluble bentonite organic matter (sOM) in four working stages (a–d) (AGW, artificial groundwater; rpm, rounds per minute; DOC, dissolved organic carbon, used as a measure of sOM).

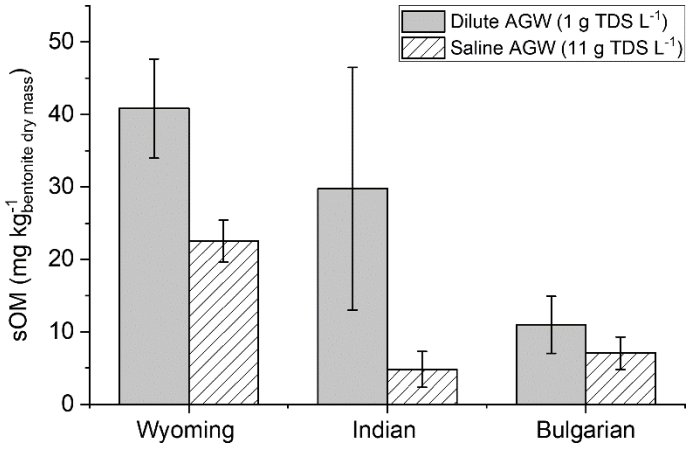

Figure 2. Amount of sOM released from the bentonites to leachants with different salinities in a batch equilibrium assay (liquid-to-solid ratio 40 L kg^{-1}) (mean ± standard deviation, n = 3–4) (sOM, soluble organic matter; AGW, artificial groundwater; TDS, total dissolved solids).

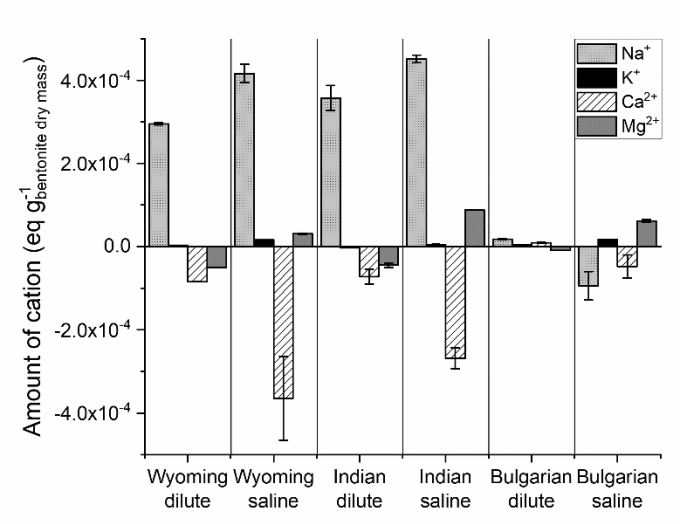

Figure 3. Amount of cations released from the bentonites using leachants with different salinities in a batch equilibrium assay (liquid-to-solid ratio 40 L kg^{-1}) relative to the amount of cations in the leachant (mean ± standard deviation, n = 3–4) (dilute and saline 1 and 11 g total dissolved solids L^{-1}, respectively).

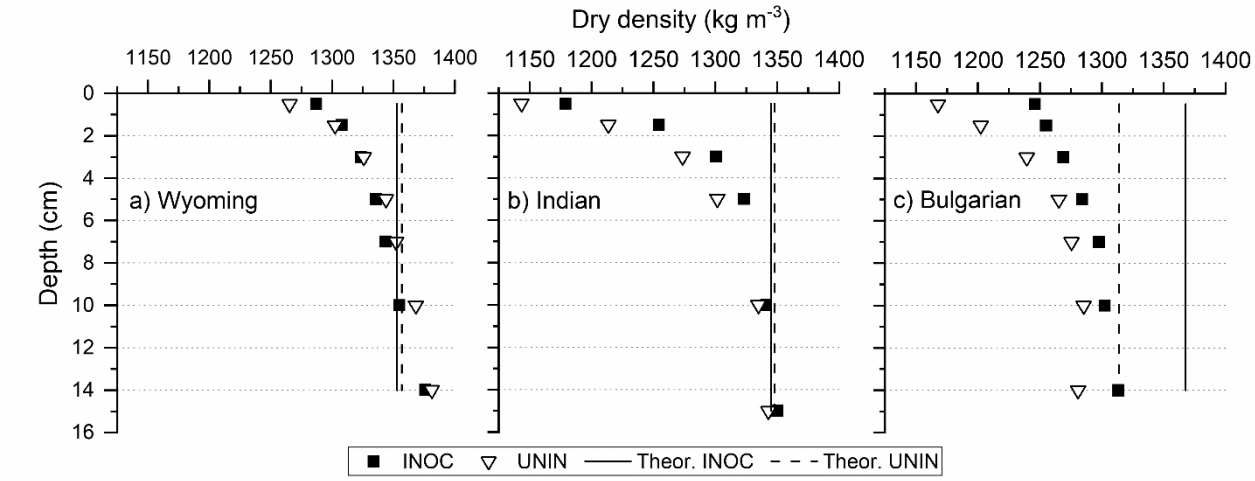

Figure 4. Theoretical average and measured dry densities at different depths of the bentonite blocks (a–c) in the cells (inoculated [INOC] or uninoculated [UNIN] with microorganisms) after operation of 12–15 months (mean, n = 2–3).

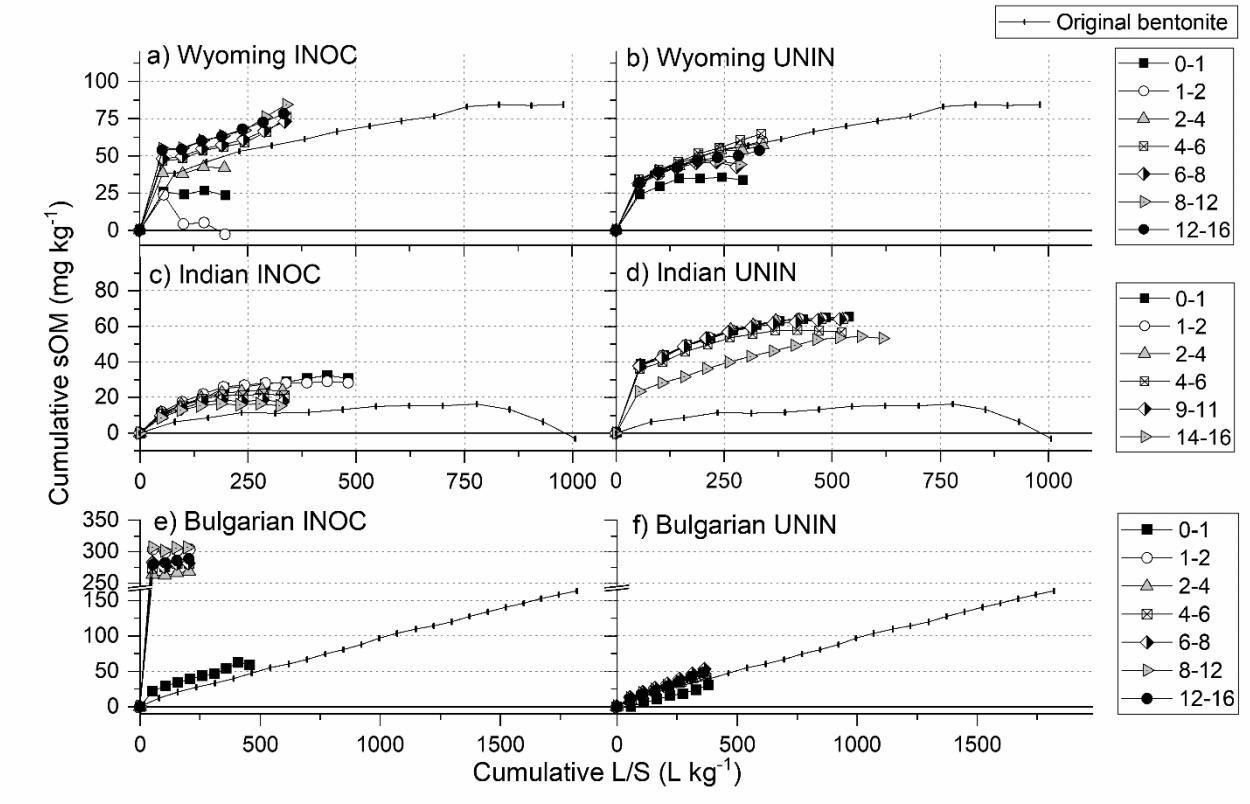

Figure 5. Cumulative amount of soluble organic matter (sOM) released by the bentonites before (Original i.e., as received) and after exposing the bentonites to the simulated repository conditions in cells uninoculated (UNIN) or inoculated (INOC) with microorganisms (bentonite sampled from different depths of the blocks e.g., 0–1 cm) as a function of cumulative liquid-to-solid (L/S) ratio (mean, n = 2–4). Note different scales on the axes.

Table 1. Mineral and major element compositions of the bentonites relative to dry mass.

Bentonite		Wyoming[a]	Indian[b]	Bulgarian[c]
Minerals				
Smectite	w-%	88	74	68
Illite	w-%	<1	1	7
Calcite	w-%	<1	9	11
Gypsum	w-%	<1	1	<1
Plagioclase	w-%	3	tr.	<1
Pyrite	w-%	1	0	0
Other	w-%	8	15	14
Elements				
Fe^{3+}	w-%	2.1	10.6	3.2
Fe^{2+}	w-%	0.57	0.05	0.26
CEC	eq. g^{-1}	930	880	630
Na	eq. g^{-1}	580	450	60
K	eq. g^{-1}	20	0	20
Ca	eq. g^{-1}	240	270	450
Mg	eq. g^{-1}	90	160	100
LOI	g kg^{-1}	63	121	167
TOC[d]	mg kg^{-1}	2032	680	<1400
Moisture[d]	% of wet mass	9.2	11.3	11.0

w-%, weight-%; tr., trace amount; CEC, cation exchange capacity (NH_4Cl method); eq., equivalent; LOI, loss on ignition; TOC, total organic carbon.

[a]Kiviranta & Kumpulainen, 2011; Kiviranta et al., 2018

[b]Kumpulainen & Kiviranta, 2015; Kumpulainen et al., 2016

[c]Kumpulainen et al., 2016

[d]Determined in this study (CSN ISO 10694, CSN EN 13137).

Table 2. Composition of the saline and dilute AGWs used in the study (modified from Hellä et al., 2014[a]).

Analyte (mg L^{-1})	Saline AGW	Dilute AGW
TDS	10636	1035
SO_4^{2-}	20	92
Cl^-	6536	555
Na^+	2640	302
K^+	11	10
Ca^{2+}	1300	54
Mg^{2+}	62	18
Sr^{2+}	14	0.5
F^-	1.0	0.6
Br^-	44	1.4
NH_4^+	0.02	0.8
B^{3+}	1.3	0.3
pH[b]	7.4 ±0.2	6.2 ±0.5
DOC[b,c]	0.14 ±0.15	0.04 ±0.05

AGW, artificial groundwater; TDS, total dissolved solids; DOC, dissolved organic carbon.

[a]HCO_3^- was excluded from the composition, as $NaHCO_3$ reagent was found to contain a substantial amount of DOC (1.8–2.7 mg g^{-1} depending on the product, several were tested), which increased the background concentration of DOC in the leachates up to 0.30 and 2.04 mg L^{-1} for saline and dilute AGW, respectively.

[b]Mean ± standard deviation, n = 28 and 107 for DOC, n = 14 and 40 for pH, respectively.

[c]The traces of organic carbon in AGWs originated from the solid reagents used for preparing the solutions.

Table 3. Characteristics of the saturated bentonite blocks in the cells at different stages of the operation (modified from Maanoja et al., 2020).

Bentonite /	Wyoming		Indian		Bulgarian	
Cell	UNIN	INOC	UNIN	INOC	UNIN	INOC
In the beginning of operation						
Bentonite (kg_{dry})	6.95	6.98	6.99	7.02	6.86	7.05
Liquid (kg)[a]	2.51	2.59	2.60	2.60	2.33	2.43
Moisture content (% of wet mass)[b]	27.3	27.2	28.2	28.1	26.4	26.3
Volume of the block (L)	4.95	5.02	5.02	5.06	4.94	5.04
Dry density (kg m^{-3})[d]	1405	1390	1393	1386	1388	1398
As compacted after 167 days of operation						
Bentonite (kg_{dry})	6.95	6.97	6.99	7.01	6.85	7.05
Liquid (kg)[a]	2.69	2.63	2.83	2.83	2.89	2.80
L/S (L kg^{-1})[c]	0.4	0.4	0.4	0.4	0.4	0.4
Volume of the block (L)	5.12	5.15	5.18	5.22	5.22	5.15
Dry density (kg m^{-3})[d]	1357	1353	1348	1345	1314	1368

UNIN and INOC, cells in which the sand layers of the cells were uninoculated or inoculated with microorganisms; L/S, liquid-to-solid ratio.

[a]Sum of the ambient water content and the volume of saline artificial groundwater (AGW) used for saturating the bentonites (11 g total dissolved solids L^{-1}, theoretical density 1006.3 kg m^{-3} at 21°C).

[b]At 100% saturation.

[c]Theoretical density of 1006.3 kg m^{-3} (21°C) assumed for the liquid within the bentonites.

[d]Theoretical values calculated from the masses and volumes are presented in the table.

Table 4. Soluble organic matter (sOM) contents (mg kg^{-1})[a] of the original bentonites and bentonites exposed to simulated repository conditions determined by dynamic leaching assays (mean ± standard deviation, n = 3–4).

Bentonite	Wyoming		Indian		Bulgarian	
Original (as received)	85 ±9.0		16 ± 6.4		163† ± 15.5	
Relative to TOC (%)[b]	4.2		2.4		11.6	
Cell ► Depth from the sinter ▼	UNIN	INOC	UNIN	INOC	UNIN	INOC
0–1 cm	36 ±2.6	26 ±5.1	65 ±2.2	32 ±4.7	31† ±12.0	62 ±9.9
1–2 cm	60† ±12.9	24 ±0.3	64 ±7.6	29 ±4.9	46† ±9.4	303 ±21
2–4 cm	57† ±6.8	42 ±4.8	64 ±2.2	24 ±5.0	46† ±16.5	264 ±6.1
4–6 cm	65† ±6.9	76† ±8.0	58 ±2.9	22 ±4.4	47† ±3.1	274 ±1.4
6–8 cm	46 ±9.3	73† ±5.4	n.a.	n.a.	53† ±8.6	283 ±32
8–12 cm	47 ±4.7	84† ±11.3	n.a.	n.a.	46† ±13.8	307 ±12
9–11 cm	n.a.	n.a.	64 ±19.5	19 ±1.4	n.a.	n.a.
12–16 cm	54† ±11.6	78† ±8.0	n.a.	n.a.	48† ±8.9	279 ±8.6
14–16 cm	n.a.	n.a.	54 ±18.4	16 ±2.1	n.a.	n.a.
Weighted average all depths[c]	52	68	61	22	47	270
Relative to TOC (%)[b]	2.6	3.4	9.0	3.3	3.3	19.3

UNIN and INOC, cells in which the sand layers were uninoculated or inoculated with microorganisms; n.a., not applicable; TOC, total organic carbon.

[a]sOM contents normalized to the dry mass of the sample.

[b]sOM contents given relative to the TOC content of the original bentonites (2023, 680, and 1400 mg kg^{-1} for Wyoming, Indian, and Bulgarian bentonites, respectively; Table 1).

[c]The mass factor of each depth layer was taken into account in the calculation of the average values for the entire bentonite blocks.

†The exhaustion of sOM release from the sample was not reached during the assay, so the actual sOM quantity was likely to be higher than reported.

Supplementary material for:

The effect of compaction and microbial activity on the quantity and release rate of water-soluble organic matter from bentonites

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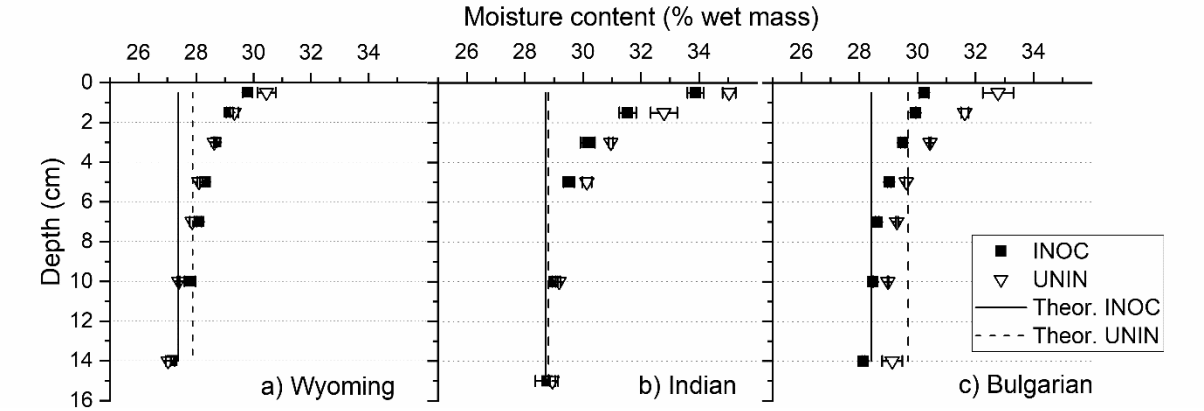

Figure S1. *Theoretical and measured moisture contents of the compacted bentonites at different depths of the experimental cells (inoculated [INOC] or uninoculated [UNIN] with microorganisms) at the end of the operation of the cells (mean ± standard deviation, n = 2–3).*

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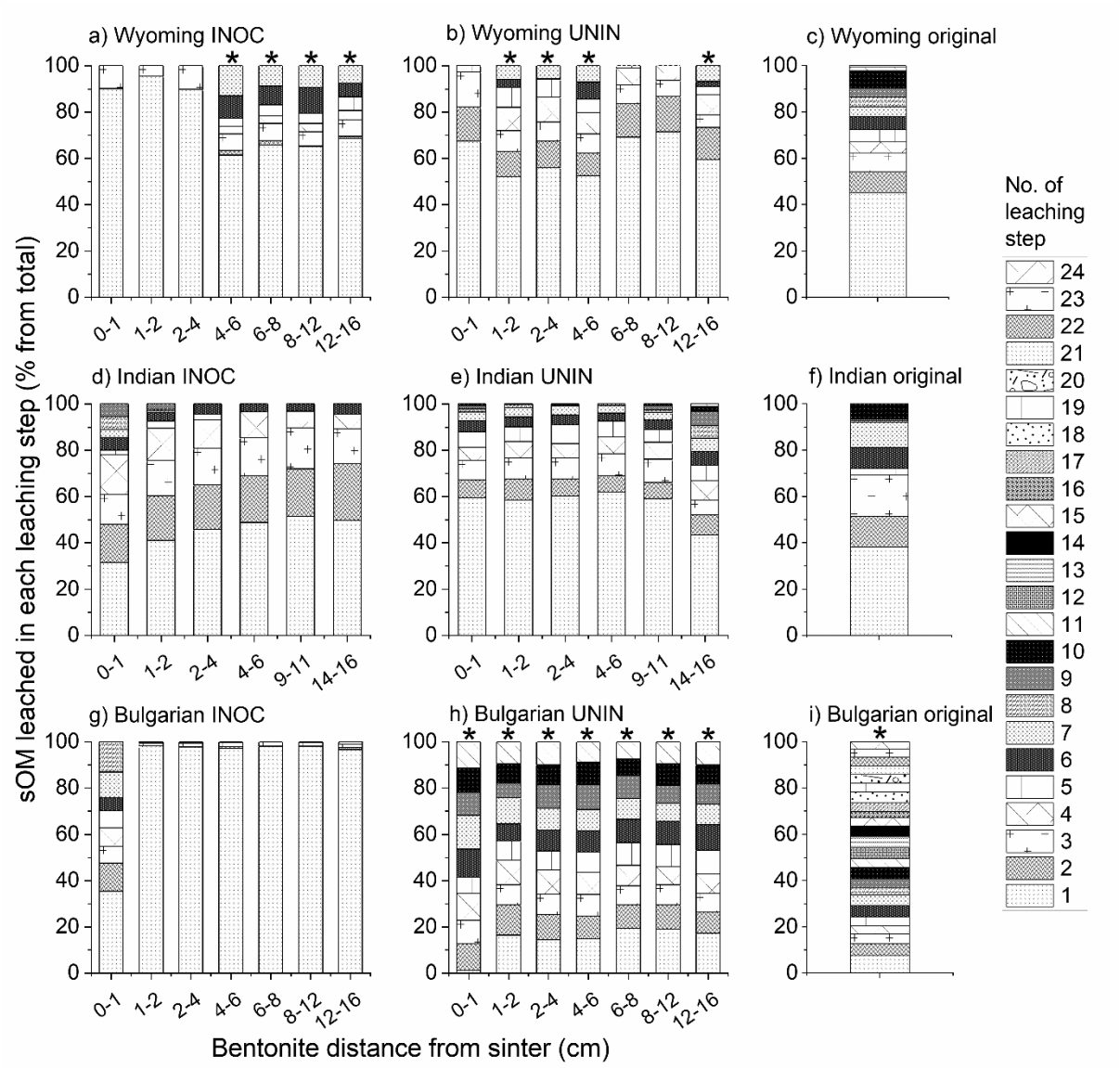

Figure S2. *Relative amount of soluble organic matter (sOM) released by the original bentonites and bentonites exposed to simulated repository conditions (cells inoculated [INOC] or uninoculated [UNIN] with microorganisms, bentonite sampled from different depths of the blocks e.g. 0–1 cm) in the individual steps of the dynamic leaching assay (1–24) versus the maximum cumulative sOM quantity (mean, n = 2–4; *the actual sOM quantity is higher than obtained as a result from the dynamic leaching test; see the main text for details). Note: the L/S increment at each leaching step was 54 L kg^{-1} for the exposed bentonites and 80 L kg^{-1} for the original bentonites.*

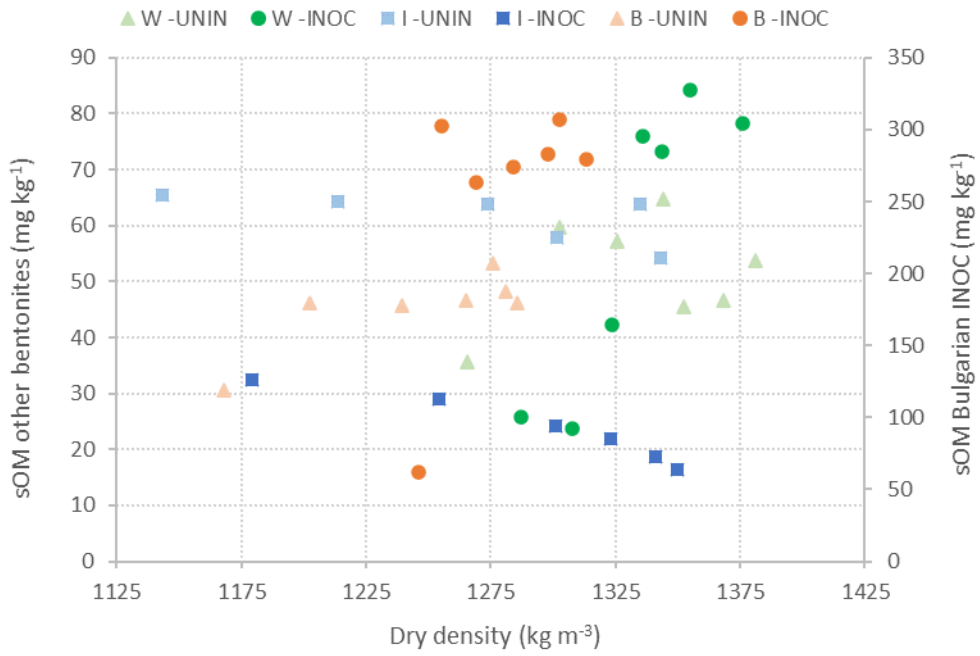

Figure S3. *Relationships of the soluble organic matter (sOM) quantities and the dry densities of the bentonite blocks in the experimental cells inoculated (INOC) or uninoculated (UNIN) with microorganisms after operation of 12–15 months. The markers represent different depths of the bentonite blocks (0–16 cm) (W, Wyoming bentonite; I, Indian bentonite; B, Bulgarian bentonite).*

Table S1. *Comparison of the reduction of sOM contents from the bentonites exposed to simulated repository conditions and the quantity of sOM measured as DOC from the sand layers of the cells during the operation.*

Cell	Depth from the sinter (cm)	sOM (mg kg^{-1})	Dry mass of the layer (kg)	Weighted average sOM (mg kg^{-1})	Reduction of sOM from top relative to bottom layers (mg kg^{-1})	Reduction of sOM from top relative to bottom layers in total (mg kg^{-1})	DOC in the sand layer of the cell during operation[a] (mg L^{-1})	DOC in the sand layer of the cell during operation[b] (mg)	DOC in the sand layer of the cell during operation normalized for the dry mass of the top layers with reduced sOM contents (mg kg^{-1})
Wyoming UNIN	0–1	36	0.42	36	17	17	11.0	3.2	7.6 (layer 0–1 cm)
	1–2	60[†]	0.41	53 (layers 1–16 cm)	n.a.	n.a.			
	2–4	57[†]	0.91						
	4–6	65[†]	0.85						
	6–8	46	0.86						
	8–12	47	1.8						
	12–16	54[†]	1.7						
Wyoming INOC	0–1	26	0.55	26	53	145	7.2	2.0	1.1 (layers 0–4 cm)
	1–2	24	0.41	24	55				
	2–4	42	0.91	42	37				
	4–6	76[†]	0.79	79 (layers 4–16 cm)	n.a.	n.a.			
	6–8	73[†]	0.86						
	8–12	84[†]	1.8						
	12–16	78[†]	1.7						
Bulgarian INOC	0–1	62	0.48	62	224	224	15.8	4.6	9.6 (layer 0–1 cm)
	1–2	303	0.47	286 (layers 1–16 cm)	n.a.	n.a.			
	2–4	264	0.86						
	4–6	274	0.84						
	6–8	283	0.91						
	8–12	307	1.7						
	12–16	279	1.7						

DOC, dissolved organic carbon; UNIN and INOC, cells in which the sand layers were uninoculated or inoculated with microorganisms; n.a., not applicable.

[a]DOC on average. For the full data set, see Maanoja et al. 2020. [b]Volume of solution in the sand layers: 0.281–0.296 L

[†]The exhaustion of sOM release from the sample was not reached during the assay, so the actual sOM quantity was likely to be higher than reported.