Compacted bentonite as a source of substrates for sulfate-reducing microorganisms in a simulated excavation-damaged zone of a spent nuclear fuel repository

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HIGHLIGHTS

- The interface of compacted bentonite and host rock was simulated experimentally.
- Organics and inorganics dissolved at varying quantities in Na- and Ca-bentonites.
- Bentonite organic matter sustained the growth of different microorganisms.
- Microbially produced sulfide was immobilized via precipitation by bentonite iron.

Abstract

Sulfide formed by sulfate-reducing microorganisms (SRM) is a potential safety risk in the geological disposal of spent nuclear fuel (SNF) enclosed in copper canisters because it can corrode copper. The canisters will be isolated from the environment by surrounding them with compacted bentonite. This study shows experimentally that the organic matter naturally present in compacted bentonites can become dissolved and sustain biological sulfate reduction. The experiment was conducted in cell systems consisting of an interface of compacted bentonite (at dry density of 1314–1368 kg m^{-3}) and a low-porosity sand layer representing an excavation-damaged zone of the host rock. Some cells were inoculated with SRM and groundwater microorganisms and some were not. Varying concentrations of organic matter and sulfate in the sand layer solution resulted from partial dissolution of the studied bentonites (Wyoming, Indian, and Bulgarian). The dissolved organic matter promoted biological sulfate reduction, as demonstrated by the decrease in sulfate concentration in the sand layer solution and the formation of sulfide iron precipitates in the inoculated cells relative to the uninoculated cells. Other anaerobic microorganisms (e.g., methanogens) also became active in the cells and they along with the SRM were found to grow within the sand and/or bentonite layers of the cells. The findings of this study show that bentonites can sustain biological sulfate reduction in areas with lower density and immobilize possibly formed sulfides. However, the extent of these capabilities seems to be affected by the mineralogy of bentonites in the studied density range.

Keywords: Sulfide formation, microbial activity, organic matter, engineered barrier system, buffer-host rock interface, geologic disposal

1. Introduction

One of the management options for spent nuclear fuel (SNF) generated by the nuclear power industry is direct disposal in geologic repositories. For example in Finland, the disposal of SNF to the Olkiluoto bedrock is planned to be initiated in the 2020s (Posiva, 2018). According to the plan, the SNF will be disposed of in deposition holes drilled along the tunnels of the underground repository and isolated from the surrounding environment by an engineered barrier system. The

barrier system will consist of a copper/iron canister designed to contain the SNF, buffer bentonite to protect the canister in the deposition hole, and backfill bentonite to seal the tunnels leading to the holes (Posiva, 2012a). Based on the expected decay of the radioactivity of the SNF, the repository should provide passive safety for hundreds of thousands of years (Posiva, 2012b). One factor that can possibly affect canister integrity is biologically produced sulfide as sulfide is a well-known corroding substance for copper (King et al., 2012).

Ubiquitous in nature, anaerobic sulfate-reducing microorganisms (SRM) including both bacterial and archaeal species have been found in the bedrock and groundwater of Olkiluoto (Muyzer & Stams, 2008; Pedersen et al., 2014). While reducing sulfate to sulfide, SRM use organic compounds, CO_2 and/or H_2 to obtain energy and carbon for growth (Liamleam & Annachhatre, 2007; Muyzer & Stams, 2008). The growth of SRM in bedrock and groundwater is limited by the low availability of organic matter (Wolfaardt & Korber, 2012; Rajala et al., 2015). However, as a result of the final disposal of SNF, the buffer and backfill bentonites will introduce organic matter (Hallbeck, 2010), which could serve as a substrate for microorganisms in the repository. The organic matter in bentonite is chemically recalcitrant and contains long-chain and aromatic compounds (Durce et al., 2015; Marshall et al., 2015), which are assumed to be thermodynamically unfavorable substrates for SRM (Cassidy et al., 2015). However, when SRM live in a diverse microbial community, such as that typically present in groundwater (e.g., Bomberg et al., 2015) or bentonites (Masurat et al., 2010; Stone et al., 2016), hydrolytic and fermentative microorganisms can degrade the complex organic compounds into a more utilizable form for the SRM (Zavarzin et al., 2008). The ability of SRM to grow on the organic matter of bentonite has been hypothesized (Bengtsson et al., 2017) but never demonstrated experimentally.

One function of the bentonite buffer is to limit microbial activity in the near field of the SNF canisters and retard the migration of possibly formed sulfides to the canisters (Posiva, 2012a). Earlier studies suggest that this function can be achieved by compacting the buffer bentonite to a high dry density ($\geq$1370 kg m^{-3}, corresponding to a wet density of 1880 kg m^{-3}; Bengtsson & Pedersen, 2017). In a compacted buffer, low porosity hinders the dissolution of the

complex organic matter from bentonite to water (Durce et al., 2015), and low water activity (a_w <0.96) suppresses the activity of microorganisms (Stroes-Gascoyne et al., 2010). The repository, however, will feature lower density areas where the microbial activity and sulfide formation can occur (Stroes-Gascoyne et al., 2011). These areas include interfaces of the buffer and backfill with the host rock, particularly in the microfractures in excavation-damaged zones (EDZs) of the tunnels, where the groundwater can more readily interact with the bentonite and cause the dissolution of ions and organic matter from the bentonite (Stroes-Gascoyne et al., 2011; Wolfaardt & Korber, 2012).

This work aimed to study experimentally whether organic matter can dissolve from compacted bentonite and sustain biological sulfate reduction in cell systems that mimic the interface of the host rock and the compacted bentonite in an EDZ of a SNF repository. Quartz sand was used as a material conservatively mimicking the conditions in a lower density area (such as the EDZ), where the hydraulic conductivity and porosity are considerably higher than in intact rock (Posiva, 2013). In the experiment, two possible sources for microbial growth into the EDZ were studied: external, a groundwater-originated community enriched with known SRM, and indigenous, a bentonite-originated community. Three bentonites from different origins (Wyoming, India, and Bulgaria) were tested as an example of materials that could be used as part of the engineered barrier system for a geologic repository for SNF. The characteristics of Wyoming and Indian bentonites, including their physical, chemical, and mineralogical properties, hydromechanical behavior in repository-related conditions, and threshold density for suppressing microbial activity, have been studied extensively (e.g., Rautioaho & Korkiala-Tanttu, 2009; Kiviranta & Kumpulainen, 2011; Bengtsson & Pedersen, 2017; Cui, 2017), whereas the corresponding characteristics of Bulgarian bentonite have been studied less. To the authors' knowledge, the possible effect of the three bentonites on microbial activity by acting as a potential source of organic matter has never been studied.

2. Materials and methods

The experiment was carried out in six uniquely designed experimental cells having two sections, which consisted of a layer of saturated compacted bentonite and a layer of loosely packed quartz sand (Fig. 1). Cells were prepared by using three different bentonites and the preparation steps included saturation and compaction of the bentonite blocks (described in Chapters 2.1 and 2.2). Furthermore, the blocks were re-compacted twice at the later stages of the experiment (Chapter 2.2). The sand layers were assembled on top of the compacted bentonite blocks, and the sand layer of one cell of each bentonite was inoculated with a mixture of known SRM and microorganisms enriched from Olkiluoto groundwater (Chapter 2.3). These cells were referred to as "inoculated cells." The sand layer of the other cell of each bentonite was not inoculated with external microorganisms, but as the used bentonites were not sterilized, the presence of microorganisms indigenous to bentonites was anticipated. These cells were referred to as "uninoculated cells."

2.1 Bentonites, quartz sand, and artificial groundwater

The bentonites used in the experiment included two sodium bentonites from Wyoming and India and one calcium bentonite from Bulgaria (Table 1). The Indian and Bulgarian bentonites (grain size 0.5–3 mm) were ground to ensure homogeneity (100% <0.2 mm), while the Wyoming bentonite was used as is because it was originally finer material than the other bentonites (68% ≤0.5 mm; Kiviranta & Kumpulainen, 2011). After this, the bentonites were stored in sealed plastic bags in aerobic conditions at 6°C. The quartz sand (NFQ Nilsiä QUARTZ, Sibelco Nordic Oy Ab, grain size of $0.63 \leq x < 1$ mm) was combusted (4 h at 450°C) to remove organic residues. Prior to the experiment, O_2 was removed from the bentonites and sand by purging them with N_2 in a desiccator as follows; the desiccator holding the bentonite or sand was first evacuated (10 min, <10 mbar) and then filled with N_2 (99.5% v/v, Aga Ltd.). These steps were repeated four more times after minimum of 20 min of equilibration time as determined in preliminary testing. After deoxygenation, all the following preparation steps (i.e. saturation and wetting) took place in an anaerobic hood (Whitley A35 Anaerobic Workstation, 100% v/v N_2).

Artificial groundwater (AGW) with controlled concentration of dissolved organic carbon (DOC; <0.2 mg L^{-1}) was used to saturate the bentonites and sand in the cells. Composition

of the AGW corresponded to a defined reference water expected to occur at the Olkiluoto repository: 6.65 g NaCl, 4.77 g $CaCl_2 \cdot 2H_2O$, 519 mg $MgCl_2 \cdot 6H_2O$, 56.7 mg NaBr, 42.6 mg $SrCl_2 \cdot 6H_2O$, 29.6 mg Na_2SO_4, 21.0 mg KCl, 7.4 mg H_3BO_3, 2.2 mg NaF, and 0.03 mg NH_4Cl in 1 L ultrapure water (Milli-Q®, MilliporeSigma; pH 6.5; modified from Hellä et al., 2014).

2.2 Cell design and preparation of the bentonite blocks

The cells (Fig. 1) were made of polyether ether ketone with high chemical resistance and low reactivity and stainless steel (316-L) with high mechanical strength. In the cells, the bentonite layer (5.0 L) located at the bottom, and the sand layer was assembled on top of the bentonite inside a separate sleeve (662 mL). A porous titanium sinter (pore size 1–2 µm; GKN Sinter Metals GmbH; purified with 8% w/v HNO_3 and ultrapure water) separating the bentonite and sand allowed the migration of ions, molecules and microbial cells between the layers. The O-rings used for sealing the cells were inert and had low gas permeability (Viton®). Microvalves (VICI AG) were attached to the plunger for sampling and balancing the pressure during compaction. Organic residues were removed from the parts by washing with propanol, 1 M HCl or AGW, and ultrapure water.

Preparation of the bentonite blocks was started by saturating and compacting the bentonites by using the method described by Herbert et al. (2008) as follows. First, batches of deoxygenated bentonite and degassed AGW (≥30 min with N_2) required for preparing saturated bentonite blocks (Eqs. S1–S5; Chapter S1; Table 2) were mixed. Then, the mixture was allowed to wet for 1–4 days before compaction, except for some of the mixtures of Indian and Wyoming bentonites for uninoculated cells, which wetted for 20 days due to the unexpected additional adjustments required for these cells. If any organic matter dissolved in AGW from the bentonite during the wetting period, it became compacted inside the bentonite block. After wetting, the bentonites were compacted to the target dry density of 1400 kg m^{-3}, which results in a target swelling pressure currently assumed to suppress activity of microorganisms in Wyoming type bentonite (approximately 2 MPa; Kaufhold et al., 2015; Taborowski et al., 2019). The bentonite blocks were compacted in four layers (4 cm each) to obtain as homogeneous density as possible throughout the blocks. Compaction (with hydraulic pressure at 20 MPa) took 2–192 h for each

layer to reach the target height and, thus, density calculated according to Eq. S1 (Table 2). A sinter saturated with AGW was pressed on top of the compacted bentonite block, and AGW was added on the sinter to prevent it from desaturation (50–65 mL, until the sinter was soaked).

After completing the initial compaction of all six bentonite blocks one after another (hence stored for 10–108 days), re-compaction of the blocks was needed as it was noticed that the unconstrained bentonite blocks had swelled during storage (by 61–326 mL, to a dry density of 1302–1380 kg m^{-3}; details given in Chapter S2.1, Table S1). Thus, all the bentonite blocks were re-compacted to the original target density (Table 2). The changes in sulfate and DOC contents of all bentonite blocks caused by swelling and re-compaction were <0.2% (w/w) of the initial contents except for Bulgarian bentonite in the uninoculated cell, where the decrease in DOC content was 14% (w/w) (Chapter S2.2, Tables S2, S3). The change in that block was greater than in the others because the assemblance of the sand layers was started with the uninoculated cell of the Bulgarian bentonite (as described in Chapter 2.3), and the bentonite adsorbed the AGW added both on the sinter and in the sand sleeve before it was noticed that re-compaction was required (Chapter S2.1).

Another re-compaction was required during the experiment (at operational days 146 and 167) due to swelling of bentonites inside the cells (by 184–328 mL, to a dry density of 1309–1337 kg m^{-3}; Table S1), which resulted from breakage of the plunger height adapters (Fig. 1). The bentonite blocks were re-compacted to dry densities of 1314–1369 kg m^{-3} (Table 2) and the cells were equipped with new plunger height adapters. The swelling and re-compaction of bentonite did not cause the release of sulfate or other inorganic compounds from the bentonites (Chapter 3.2; no anomalies in data at days 146 and 167). The implications of swelling and re-compaction on the bentonite densities (decrease of 6% in the uninoculated cell of the Bulgarian bentonite, 3% on average in the other cells in comparison to the density in the beginning of the experiment) and increased release of DOC are further discussed in Chapters 3.1 and 3.4.

2.3 Preparation of the sand layers and start-up of the experiment

To start the experiment, the sand layers were assembled for the uninoculated and inoculated cells separately. For the uninoculated cells, combusted, uninoculated sand, and unsterilized, uninoculated AGW deoxygenated with sterile-filtered N_2 (0.2 µm) were used (Table 2).

For the inoculated cells, the sand and AGW were both amended with microorganisms. The sand was mixed with groundwater from Olkiluoto (drill hole ONK-PVA06), and the groundwater microorganisms were fed sequentially with leachates of bentonites in a pre-enrichment step described in Chapter S3.1. The AGW was amended with microorganisms from three sources: 1) pure cultures of SRM (*Desulfobacula phenolica*, *Desulfobulbus mediterraneus*, *Desulfobulbus rhabdoformis*, *Pseudodesulfovibrio aespoeensis*, and *Desulfotomaculum acetoxidans* from DSMZ GmbH) previously identified from Olkiluoto groundwater (Pedersen et al., 2014; Bomberg et al., 2015; Rajala et al., 2015), 2) microorganisms present in fresh Olkiluoto groundwater (ONK-PVA06), and 3) pre-enriched pelagic microorganisms not attached to the sand during the pre-enrichment step (details given in Chapter S3.2). The AGW and sand amended with microorganisms were inserted to the inoculated cells (Table 2).

Samples were taken from the uninoculated and inoculated sand and AGW to determine the initial number of microorganisms (SRM and total bacteria) and overall microbial activity in the sand.

2.4 Cell operation, monitoring and sampling

After the sand layers were assembled, the cells were closed with plungers and a sealant (Sikaflex®-11FC, Oy Sika Finland Ab). The following day, AGW (16–33 mL) was added in the sand layer through the sampling valve to ensure saturation of the sand. Shortly after, the first sample (11 mL; denoted as day 0) was collected from the sand layer solution by a gas-tight glass syringe (VICI AG). The sampled volume was optimized with respect to the volume of the sand layer solution (3% v/v) to minimize perturbation of the sand layer solution during sampling. A corresponding volume of fresh AGW was inserted to the sand layer while the sample was extracted. All the following samplings were carried out in a similar manner unless stated otherwise.

The sand layer solution was sampled at days 0, 14, and 21 and then every three weeks until days 370–454, when the cells were terminated and processed for sampling one after another. The dissolution of bentonite constituents to the sand layer solution was monitored by measuring the concentrations of DOC and dissolved inorganic carbon (DIC), sulfide, sulfate, and total iron and the redox potential (E_h) and pH of the sand layer solution. Only E_h was measured from the samples taken from the cells of the Indian bentonite between days 43 and 85 because before analyzing the other parameters, the E_h was anticipated to decrease < –50 mV (vs. standard hydrogen electrode, SHE) for enabling the activity of SRM similarly as occurred in the other inoculated cells. As the E_h did not drop below –50 mV in the cells of the Indian bentonite, measurement of other parameters was continued from day 106 onwards. For monitoring possible methanogenic activity in the cells, the CH_4 concentration was measured from the sand layer solutions from day 127 onward.

At day 21, it was noticed that some of the solution in the sand layers had drained out. Subsequently, the lost solution volume was replenished with AGW 24 h before each sampling for the next six sampling points (until day 125). Draining of the sand layer solutions (in total 168–226 mL; Table S1) presumably resulted from the swelling of bentonites. At days 146 and 167, sampling of the sand layer solution was performed simultaneously with re-compaction of the bentonite blocks (described in Chapter 2.2) by using the solution exiting the cells (Table S1) as a sample. At the end of the experiment (days 370–454), the cells were opened in the anaerobic hood. Samples were collected from the sand to determine the number of microorganisms, the total microbial activity and the elemental composition of precipitates. After that, samples were collected from the bentonite blocks. The surface layer (0–1 cm) was cut off from the block and the bentonite was homogenized, after which a sample was collected for enumerating the viable SRM.

2.5 Analytical methods and calculations

All the sand layer solution samples were filtered (0.45 µm) and analyzed immediately after sampling, except for the redox and pH sample (not filtered) and the sulfate and total iron samples (stored at –20°C and 4°C after filtration, respectively). One parallel sample of the sand layer solution was used for each analysis. The E_h (BlueLine 31 Rx; Ag/AgCl reference system) and pH

(Hamilton Slim Trode; SFS 3021) were measured from open vials in the anaerobic hood (in the absence of O_2) at 21°C. The measurement took 10–30 min due to the redox electrode slowly reaching a thermal equilibrium with the sample (Nordstrom & Wilde, 2005). Redox potentials were corrected for temperature with respect to SHE (25°C; Sawyer et al., 1995). The concentrations of DIC (as CO_2) and dissolved CH_4 were determined by a headspace technique from an acidified sample (Trimmer et al., 2009) via gas chromatography (Kinnunen et al., 2015; Maanoja & Rintala, 2015). The concentration of DOC was measured with a total organic carbon analyzer (Shimadzu TOC-V_{CPH}) by manual injections (SFS-EN 1484; Stubbins & Dittmar, 2012). The concentration of dissolved sulfide was determined from an alkalified sample with an ultraviolet-visible spectrophotometer (Shimadzu UV-1700; Cord-Ruwisch, 1985). The concentration of total iron was analyzed from preserved samples (1% v/v 67%–69% HNO_3) with inductively coupled plasma spectrometry by ALS Finland Oy. The concentration of sulfate was measured via ion chromatography (Dionex ICS-1600; SFS-EN ISO 10304). The column (IonPac AS22 4 × 250 mm), suppressor (ASRS 300, 4 mm), and detector were at 30°C, 31 mA, and 30°C, respectively, and the eluent was 4.5 mM Na_2CO_3/1.4 mM $NaHCO_3$ at 1.2 mL min^{-1}.

The moisture and total solids content of bentonite and sand were determined by gravimetry (n = 2–3; APHA, 1995). The morphology of precipitates in sand grains (n = 23–33) was determined by a scanning electron microscope (Jeol JSM-IT-500) at 15 or 20kV accelerating voltages. Quantitative elemental analysis of the precipitates was determined by energy dispersive spectrometry (EDS) from sand grains mounted on carbon adhesive tape after carbon evaporation (Agar Turbo Carbon Coater). Iron and sulfur were targeted in the EDS analysis because it was assumed that the sulfide formed in the sand layers would precipitate out as mackinawite (FeS) or greigite (Fe_3S_4) having a Fe/S molar ratio of 1.0 or 0.75, respectively (Gramp et al., 2010).

The activity of the overall microorganisms in the sand and AGW samples (400 mg wet mass or 120 µL, both n = 3) was estimated by measuring the adenosine triphosphate (ATP) concentration with a luminometer (Plate Chameleon Multilabel Detection Platform, Hidex; Velten et al., 2007). To estimate the potential activity of the SRM in the sand, sulfate reduction rates

(SRRs) were determined with a post-experiment batch assay. Subsamples of sand (10 g wet mass, *n* = 4) were incubated in 140 mL of medium described in DSMZ (2017) with the following modifications (in 1 L of AGW): 500 mg Na-lactate, 50 mg yeast extract, 50 mg K_2HPO_4, 100 mg NH_4Cl, 680 mg SO_4^{2-} from Na_2SO_4 and 55 mg $NaHCO_3$. Three parallel samples were used to monitor sulfate consumption by the SRM, and one sample was sterilized (60 min at 121°C) to serve as an abiotic control sample. The samples were incubated (200 rpm) at 30°C for 8–75 days and sampled for sulfate analysis. The SRRs were calculated from the linear part of the slope of sulfate concentration decreasing over time.

To determine the number of microorganisms, DNA was extracted from the sand and from microbial samples collected from AGW on filters (0.2 µm, Supor-200) in the beginning and at the end of the experiment. Samples (400 mg–10 g wet mass, *n* = 3–5) were stored at –20°C before extraction (QIAGEN DNeasy PowerSoil® Kit and PowerMax Soil® Kit). The DNA extracts were analyzed for the number of SRM and total bacteria by using quantitative polymerase chain reaction (qPCR; StepOne Plus Real-Time PCR System, Applied Biosystems) to measure the number of *dsrB* (dissimilatory sulfite reductase subunit B) and bacterial 16S rRNA gene copies according to a method described in Supplementary material (Table S4). The obtained copy number of the *dsrB* gene corresponded directly to the number of SRM (Klein et al., 2001), but the number of 16S rRNA gene only gave an approximate of the number of total bacteria possessing a varying number of 16S rRNA gene copies in their genome (Stoddard et al., 2015; Větrovský & Baldrian, 2013). Thus, the number of 16S rRNA and *dsrB* gene copies measured here enabled the comparison of each microbial group size between samples but did not enable comparison of the different microbial group sizes within a sample.

The number of cultivable SRM in the compacted bentonites collected from the cells and in the uncompacted original bentonites was determined post-experiment by most probable number (MPN) technique. Serial ten-fold dilutions of an initial sample (10 g bentonite per 90 mL medium) were incubated in the medium used for the SRR batch assay at 27°C for 28 days (Stroes-Gascoyne et al., 2010; Bengtsson & Pedersen, 2017). The bentonite samples collected from the

cells were stored anaerobically in vacuum bags at 4°C for 42–127 days (in a descending order from Indian to Bulgarian and then to Wyoming bentonites) prior to the assay. The number of cultivable SRM in each bentonite was calculated with a statistical method based on the number of tubes showing detectable growth (n = 4; Koch, 1981). The molecular biological methods were not applied for the bentonite samples as they are cumbersome for the purpose and often the yield of the extracted DNA or ATP is low (Contin et al., 1995; Taborowski et al., 2019).

3. Results and discussion

3.1 Dissolution of organic matter from the bentonites

In all the cells, the concentration of DOC was 2–23 mg L^{-1} on average throughout the experiment apart from the initial peak (16–215 mg L^{-1}) observed between days 0 and 22 (Fig. 2). The initial high concentration of DOC likely resulted from mobilization of organic matter from the repeatedly compacted bentonite, and the following decrease from re-adsorption of organic matter to bentonite. Presumably, the observed DOC consisted mostly of the organic matter dissolved from the bentonites and to smaller extent organic products (e.g., volatile fatty acids) and dissolved extracellular polymeric substances released from the biofilm and microorganisms attached to the pre-enriched sand (Muyzer & Stams, 2008; Decho & Gutierrez, 2017). However, the possible amount of microbiologically produced organic matter was assumed to be insignificant relative to the amount of organic matter dissolving from the bentonites as the DOC concentrations remained generally lower in the inoculated than in the uninoculated cells during the experiment.

In the inoculated cells of Wyoming and Indian bentonites, the concentrations of DOC were lower (10 and 2 mg L^{-1} on average, respectively) than in their corresponding uninoculated cells (11 mg L^{-1} on average, and in the range of 9–53 mg L^{-1}, respectively; Figs. 2a, b). This difference was likely resulting from microbial consumption of DOC in the inoculated cells. By contrast, in the inoculated cell of the Bulgarian bentonite, the concentration of DOC was higher than in the corresponding uninoculated cell (16 and 3.6 mg L^{-1} on average, respectively; Fig. 2c). The opposite trend compared to the cells of the other two bentonites possibly resulted from re-compaction of the swelled Bulgarian bentonite of the uninoculated cell before the experiment,

when more DOC desorbed from that bentonite block (14% of the initial DOC) than from the other blocks (<0.2%; Chapter S3, Table S3). The second re-compaction of the bentonites at days 146 and 167 induced an increase in the DOC concentrations of the uninoculated and inoculated cells of Indian and Bulgarian bentonites (by 25 and 18 mg L^{-1}, respectively; Fig. 2b, c). As a result, the microbial activity could have increased temporarily in these cells, but that was not indicated by the other measured parameters (for example no increase in concentration of DIC due to increased respiration at days 146 and 167; Fig. 2; Chapter 3.2).

When the average DOC concentrations in the cells (2–23 mg L^{-1}) were compared to the TOC "reservoir" in the bentonite blocks (1100–1500 mg TOC kg^{-1}; Table 1), it could be concluded that in case of all studied bentonites only a small part of the bentonite organic matter (0.01%–0.06% TOC w/w) was readily soluble to the water phase of the sand layer. This outcome agreed with the earlier findings of, for example, Marshall et al. (2015) who reported that only <0.1% of TOC was water-soluble from uncompacted Wyoming bentonite. Although the fraction of water-soluble organic matter in the bentonites was low, the results indicated that organic matter became dissolved from bentonites with dry densities of 1314–1368 kg m^{-3}, which are similar to the densities potentially occurring at the interfaces of bentonite and host rock and in the backfill of a SNF repository (Autio et al., 2013). The use of higher dry density (e.g. 1780 kg m^{-3}) could result in lower dissolution of organic matter (Hallbeck, 2010).

3.2 Concentration of inorganic compounds in the sand layer solution

The concentration of DIC increased in all cells with time (until days 106–440) partly because of dissolution of calcite from the bentonites (Melamed & Pitkänen, 1996). The DIC concentrations in the inoculated cells (48, 25, and 57 mg L^{-1} on average at the highest in the cells with Wyoming, Indian, and Bulgarian bentonites, respectively) were higher than the ones observed in their corresponding uninoculated cells (18, 22, and 26 mg L^{-1}, respectively; Fig. 2) likely because of greater microbial respiration in the inoculated cells. In both cells of Wyoming and Bulgarian bentonites, the concentration of DIC remained at the highest reached concentration (Figs. 2a, c), while in both cells of the Indian bentonite, the concentration of DIC decreased from 23 mg L^{-1} to

14 mg L^{-1} on average by the end of the experiment (Fig. 2b). One possible explanation for the decrease could be precipitation of calcium carbonates, which was induced either by SRM activity (Braissant et al., 2007) or by abiotic processes occurring at high calcium concentration (1300 mg L^{-1} in AGW alone) and alkaline pH (Morse et al., 2007). The pH value of the sand layer solution was initially 6.0–6.5 in all cells, and it increased in both cells of Wyoming and Bulgarian bentonites to 8.0 and 7.5, respectively (Figs. 3a, c). In the cells of Indian bentonite, the pH increased to a slightly lower value in the inoculated cell (7.7 on average) than in the uninoculated cell (8.0; Fig. 3b).

The concentration of sulfate increased with time in all cells due to dissolution of gypsum (Melamed & Pitkänen, 1996), and the highest concentrations were observed in the uninoculated cells (3200, 2100, and 245 mg L^{-1} with Wyoming, Indian, and Bulgarian bentonites, respectively; Fig. 4). After approximately 100 days of operation, the concentrations of sulfate started becoming increasingly lower in the inoculated than in the uninoculated cells of all bentonites (Fig. 4) indicating activation of SRM in the inoculated cells. The results suggested that the SRM could have been active also in the uninoculated cells of Wyoming and Bulgarian bentonites because the highest concentrations of sulfate observed in these cells were not as high as could have been expected based on the sulfate contents of the bentonites (0.12, 0.06 and 0.05 wt-% SO_4-S in Wyoming, Indian and Bulgarian bentonites; Table 1).

The differences in the sulfate concentration between the inoculated and uninoculated cells in Wyoming, Indian, and Bulgarian bentonites (at highest 263, 683, and 247 mg L^{-1}) could have theoretically resulted in the production of 88, 228, and 83 mg L^{-1} of sulfide, respectively. However, the measured concentration of sulfide was low in all cells during the experiment (<4 mg L^{-1}). The evolution of total iron concentration in the cells (Fig. 4) suggested that the formed sulfide had precipitated as iron sulfide. In the inoculated cells, the total iron concentration decreased from 1.9, 0.13, and 7.8 mg L^{-1} in Wyoming, Indian, and Bulgarian bentonites, respectively to <0.01–0.2 mg L^{-1} around the same time when the difference in the sulfate concentration between inoculated and uninoculated cells developed (Fig. 4). The concentration of total iron was lower in the uninoculated cells than in the corresponding inoculated cells (Fig. 4), which could have resulted

from the activity of microorganisms that promoted dissolution of iron from the bentonites in the inoculated cells (Colombo et al., 2013). In the uninoculated cells of Wyoming and Indian bentonites, the concentration of iron did not vary to a great extent (Figs. 4a, b). However, in the uninoculated cell of the Bulgarian bentonite, there was a period (days 127–288) when the concentration increased from 0.2 to 2.1 mg Fe L^{-1} on average (Fig. 4c). This increase could also have attributed to the activity of microorganisms (Colombo et al., 2013) as the presence of ATP in the sand layer of the uninoculated cell of the Bulgarian bentonite in the end of the experiment indicated activation of indigenous microorganisms (discussed in Chapter 3.2).

The possible precipitation of sulfide as iron sulfides was confirmed by determining the elemental composition of the precipitates in the sand after the experiment. In the inoculated cells of Wyoming and Bulgarian bentonites, the molar ratio of Fe/S in the precipitates was 0.97 and 6.5 on average, respectively (ranges of 0.1–3.4 and 0.5–22.9; Table 3), and these precipitates were identified as iron sulfides (FeS, Fe_3S_4). In the other cells, the molar ratio of Fe/S was considerably higher, 23.3 on average in the inoculated cell of the Indian bentonite and 38.8–46.4 in the uninoculated cells of all bentonites (Table 3). Thus, these precipitates were likely not FeS or Fe_3S_4, but possibly different iron oxides or hydroxides such as hematite (Fe_2O_3), magnetite (Fe_3O_4), goethite (FeOOH) or ferrous hydroxide ($Fe(OH)_2$) (Anthony et al., 2001). These findings highlight the role of bentonites as a sink of the formed sulfide, at least via dissolving iron, which immobilizes soluble sulfide as solid iron sulfides. Another possible route of immobilization of sulfide would be via diffusion of sulfide in the bentonite, where it reacts with the ferric iron of the bentonite minerals forming FeS or S^0 (Pedersen et al., 2017).

In the sand layer solution of the inoculated cells of Wyoming and Bulgarian bentonites, the E_h decreased from 200–325 mV to <0 mV (vs. SHE) by days 150 and 110, respectively (Figs. 3a, c) and continued to decrease throughout the experiment (to –150 mV on average; Figs. 3a, c). The E_h likewise decreased in the sand layer solution of the uninoculated cells of Wyoming and Bulgarian bentonites (to 10 and 100 mV, respectively) but did not reach values as low as those in the corresponding inoculated cells. Similar evolution of the redox conditions, from oxidizing to

anaerobic initially and then to increasingly reducing, is expected to occur in the repository following chemical oxidation reactions (e.g., those of Cu^0 and minerals of bentonite and host rock), microbial respiration, and reduction activity (Wolfaardt & Korber, 2012; King et al., 2017). The increasingly negative E_h values observed in the inoculated cells of the Wyoming and Bulgarian bentonites (Fig. 3a, c) were reducing enough to enable activity of SRM, which has been reported to occur below –50 mV (vs. SHE; Frindte et al., 2015). The cells with Indian bentonite deviated from the other cells in terms of evolution of E_h as in both cells of Indian bentonite, the E_h of the sand layer solution remained >200 mV throughout the experiment (Fig. 3b). Consequently, the growth of SRM requiring reduced conditions was highly unlikely in the sand layers of the cells with Indian bentonite. The redox potential of bentonites is mostly governed by their content of redox active iron species, which include both electron donating Fe^{3+} (e.g. hematite) and electron accepting Fe^{2+} minerals (e.g. siderite, pyrite). The lower the ratio of Fe^{2+} to Fe^{3+} in the bentonite, the higher the redox potential can be (Hofstetter et al., 2014). Consequently, the high redox potentials observed in the cells with Indian bentonite were most likely resulting from the low Fe^{2+}/Fe^{3+} ratio of the Indian bentonite (0.005, calculated from the iron contents shown in Table 1). In Wyoming and Bulgarian bentonites, the Fe^{2+}/ Fe^{3+} ratios were higher (0.27 and 0.08) and, thus, resulted in lower E_h values than in the Indian bentonite.

3.3 Activity and number of SRM and other microorganisms in the sand layers

Methane was detected from the sand layer solutions of all the six cells throughout the experiment, but the CH_4 concentration did not have a clear increasing or decreasing trend with time (Fig. S1). The observed CH_4 could be attributed to active methanogens, which were originating from the bentonites, and possibly from the groundwater of Olkiluoto in case of the inoculated cells. As CH_4 produced by methanogens was detected also from the cells of the Indian bentonite, it indicated that the conditions in the cells were anoxic even though they were not reducing (based on the measured E_h). Some methanogens have reported to be less sensitive to redox conditions than SRM, and they only require anaerobic, but not necessarily reducing, conditions for active growth (Frindte et al., 2015). In the inoculated cells of Wyoming and Indian bentonites, the concentration of CH_4 was

lower (<50 µg L^{-1}) than in their corresponding uninoculated cells (<100 and <650 µg L^{-1}, respectively; Fig. S1a, b), which could have resulted from the added SRM outcompeting the methanogens in the competition for the same organic compounds in the inoculated cells (Muyzer & Stams, 2008). In the cells of Bulgarian bentonite, however, the CH_4 concentration was higher in the inoculated than in the uninoculated cell (<100 and <10 µg L^{-1}; Fig. S1c), which could have been resulting from the lower availability of DOC in the uninoculated cell of Bulgarian bentonite due to re-compaction of the bentonite block before the experiment.

At the start and at the end of the experiment, the total microbial activity was measured as a concentration of ATP. At the end of the experiment, for all bentonites, the concentration of ATP was higher in the inoculated than in the uninoculated cells (Table 3). The highest ATP concentration was observed in the inoculated cell of the Bulgarian bentonite (Table 3), which suggested that the organic matter or other growth conditions in that cell were more favorable for the mixed microbial community than in the cells of the other two bentonites. In the uninoculated cells of the Wyoming and Indian bentonites, the concentration of ATP was lower at the end than at the start of the experiment (≤0.13 vs. 0.20 nmol ATP kg^{-1}). In the uninoculated cell of the Bulgarian bentonite instead, the concentration of ATP was higher at the end of the experiment than in the sand initially (Table 3), which was likely resulting from propagation and activation of the indigenous bentonite microorganisms in the sand layer of this cell.

For determining the activity of SRM in the sand, the SRRs were measured in a post-experiment batch assay after the cells were opened. The SRM were active in the sands collected from all inoculated cells and from the uninoculated cell of the Bulgarian bentonite (Fig. 5; data for abiotic control samples in Fig. S2). The highest SRRs were observed in samples taken from the inoculated cells of Wyoming and Indian bentonites (Table 3). With the Bulgarian bentonite, the observed SRR was slightly higher in the uninoculated than in the corresponding inoculated cell even though the uninoculated cell showed considerably lower total microbial activity than the inoculated cell based on the ATP concentration (Table 3). This discrepancy could be explained by the fact that ATP was produced by other microorganisms than SRM in the sand of the inoculated

cell of the Bulgarian bentonite as the ATP concentration represent a summed activity of the whole microbial population (Velten et al., 2007), while SRRs only the activity of SRM.

To further explore the number of different microbial groups in the sand and sand layer solutions, the number of *dsrB* gene copies for SRM and 16S rRNA gene copies for total bacterial community were quantified by qPCR. At the end of the experiment, more SRM and bacterial gene copies were found from the sand layers of the inoculated cells than of the uninoculated cells (Table 3). Some SRM and bacterial gene copies were also found from the uninoculated cells, and they were most likely originating from the microorganisms indigenous to the bentonites (Masurat et al., 2010; Stone et al., 2016). In the uninoculated cell of the Indian bentonite, the number of SRM gene copies remained below the limit of detection (Table 3). The number of SRM gene copies in the sand of the inoculated cells of the Indian and Bulgarian bentonites were higher and lower, respectively, than could have been expected based on the high E_h value (inhibiting the growth of SRM) and low SRR determined for these cells (Fig. 3b; Table 3). A possible reason for the observed inconsistency is that most of the SRM gene copies in the sand layers of these cells were originating from dormant cells (Burkert et al., 2019), which, in case of the inoculated cell of the Bulgarian bentonite, might not have regained their activity in the SRR batch assay.

3.4 Indications of microbial activity inside the bentonite blocks

After the experiment, the original uncompacted bentonite, which had not been in the cells, and the surface layer of the bentonite blocks (0–1 cm) were studied for the number of viable SRM using an MPN technique. The results showed the presence of viable SRM in all original uncompacted bentonites, Indian bentonite having the highest number and Wyoming bentonite the lowest number of indigenous SRM (Table 4). In the bentonite blocks of the inoculated cells of Wyoming and Indian bentonites, the number of viable SRM was higher, while in the bentonite block of the inoculated cell of the Bulgarian bentonite it was lower than the number of SRM in the original uncompacted bentonites (Table 4). In the bentonite blocks of all uninoculated cells, the number of SRM was lower than in the original uncompacted bentonites. It should be noted, however, that the samples were stored for 42–172 days, which could have decreased the number of viable SRM as

the viability of anaerobic microorganisms has been shown to decrease with increasing storing time at 4°C (Haavisto et al., 2019).

The MPN results suggested that some of the microbial activity, which was observed in the cells, but could not be shown to have occurred in the sand layers, had occurred inside the bentonite blocks. For example, the largest difference in the sulfate concentration was observed between the inoculated and uninoculated cells of the Indian bentonite (683 mg L^{-1}; Fig. 4b), although the E_h was not optimal for the SRM activity. Therefore, the increase in the number of SRM in the bentonite block of the inoculated cell of the Indian bentonite indicated that the active SRM were likely located within the bentonite. Given that a similar increase in the number of viable SRM was likewise observed in bentonite of the inoculated cell of the Wyoming bentonite, where the sulfate concentration of the sand layer solution remained unexpectedly low (compared to SO_4-S content; Chapter 3.2), the microbial activity in the Wyoming bentonite cannot be excluded either. While demonstrating the threshold densities for microbial activities in the bentonites was not the purpose of this study, the results indicate that the dry densities of the bentonites (1314–1368 kg m^{-3}) in the experiment were not high enough to fully inhibit the microbial activity. These findings agree with the threshold dry densities of sulfate-reducing activity reported for Wyoming and Indian bentonites (<1374 kg m^{-3}; Bengtsson & Pedersen, 2017). For the Bulgarian bentonite, no threshold density for sulfate-reducing activity has been reported. Nonetheless, the present results suggested that the cultivability of the bentonite SRM (external and indigenous) slightly decreased at the dry density created in the experiment (1341 kg m^{-3} on average) compared to the number of SRM in the uncompacted bentonite. Even less microbial activity could have occurred within the compacted bentonite blocks if the dry densities had not decreased during the experiment following from the swelling occasions.

3.5 Implications

Several results evidenced that the SRM had been active in the cells during the experiment; the difference in the sulfate concentrations of the sand layer solution between the inoculated and uninoculated cells (Fig. 4), formation of sulfide-containing precipitates in the sand (Table 3), and

491 increased SRRs and numbers of SRM gene copies in the sand of the inoculated versus uninoculated
492 cells. Thus, the results indicate that the organic matter dissolving from all the studied bentonites
493 can sustain microbial sulfate reduction. The SRM were either utilizing the organic matter as such
494 or it was first degraded by other microorganisms to simpler compounds and then used by the SRM
495 (Zavarzin et al., 2008). This experimental study therefore confirms the earlier hypothesis that SRM
496 can use bentonite-bound organic matter as a substrate for growth, either directly or indirectly
497 (Stone et al., 2016; Bengtsson et al., 2017). The activity of autotrophic SRM was not measured in
498 the present experiment, but it is possible that the bentonite organic matter was first consumed by
499 fermentative bacteria and the resulting CO_2 and H_2 by the SRM (Cassidy et al., 2015).

500 Several factors must be considered when evaluating the performance of the engineered
501 barrier system. From a microbiological point of view, the Indian bentonite would be an appealing
502 material to use in the repository because it seems to result in redox conditions that suppress SRM
503 activity in the EDZ. The oxidizing conditions, however, could expose the copper of the SNF
504 canisters to corrosion and increase radionuclide mobility in case of canister failure (Posiva, 2012a,
505 b). In case sulfide was formed in the EDZ, the Indian bentonite, which contains the highest level of
506 ferric iron, would immobilize the sulfide by precipitating it as FeS. Out of the three studied
507 bentonites, the Bulgarian bentonite, due to its low gypsum content, released the lowest sulfate
508 concentration, which could result in a lower amount of sulfide produced by the SRM. However, the
509 organic matter dissolving from the Bulgarian bentonite was found to sustain the highest overall
510 microbial activity as measured as the highest concentration of ATP and copy number of SRM gene
511 copies in the sand layer of the inoculated cell (Table 3). The organic matter dissolving from the
512 Bulgarian bentonite would be beneficial for the microorganisms inhabiting the areas with lower
513 density at the EDZ, but not necessarily for the ones inside the Bulgarian bentonite (decrease in
514 number of SRM in the compacted bentonite blocks relative to the uncompacted bentonite; Table 4).
515 Meanwhile, out of all the uncompacted bentonites, the Wyoming bentonite contained the lowest
516 number of indigenous cultivable SRM, which could be considered a beneficial characteristic in
517 terms of introducing less SRM to the repository with the bentonite.

4. Conclusions

In this study, microbial activity was studied in a unique experimental setup with microorganisms growing on a porous sand layer interconnected with compacted bentonite. Results showed that organic matter, sulfate, and iron, among other compounds, dissolved from the compacted bentonites into the sand layer solution. The microorganisms, including sulfate-reducing microorganisms (SRM), present in the sand layers grew on the organic matter and other compounds that dissolved from compacted bentonites. The SRM indigenous at least to the Bulgarian bentonite became active in the uninoculated cells during the experiment. Organic matter dissolving from all the studied bentonites (Wyoming, Indian, and Bulgarian) sustained equally the growth of microorganisms. The bentonites differed in the ions leached to the sand layer solution, which, in turn, created conditions where the SRM activity was suppressed in the cells with the Indian bentonite. These findings showed that the bentonites used to seal spent nuclear fuel repositories were able to sustain sulfate reduction in areas with lower density and immobilize possibly formed sulfides through precipitation with iron; clay mineralogy seemed to play a role in the extent of sulfate reduction and immobilization in the studied density range.

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FIGURE CAPTIONS

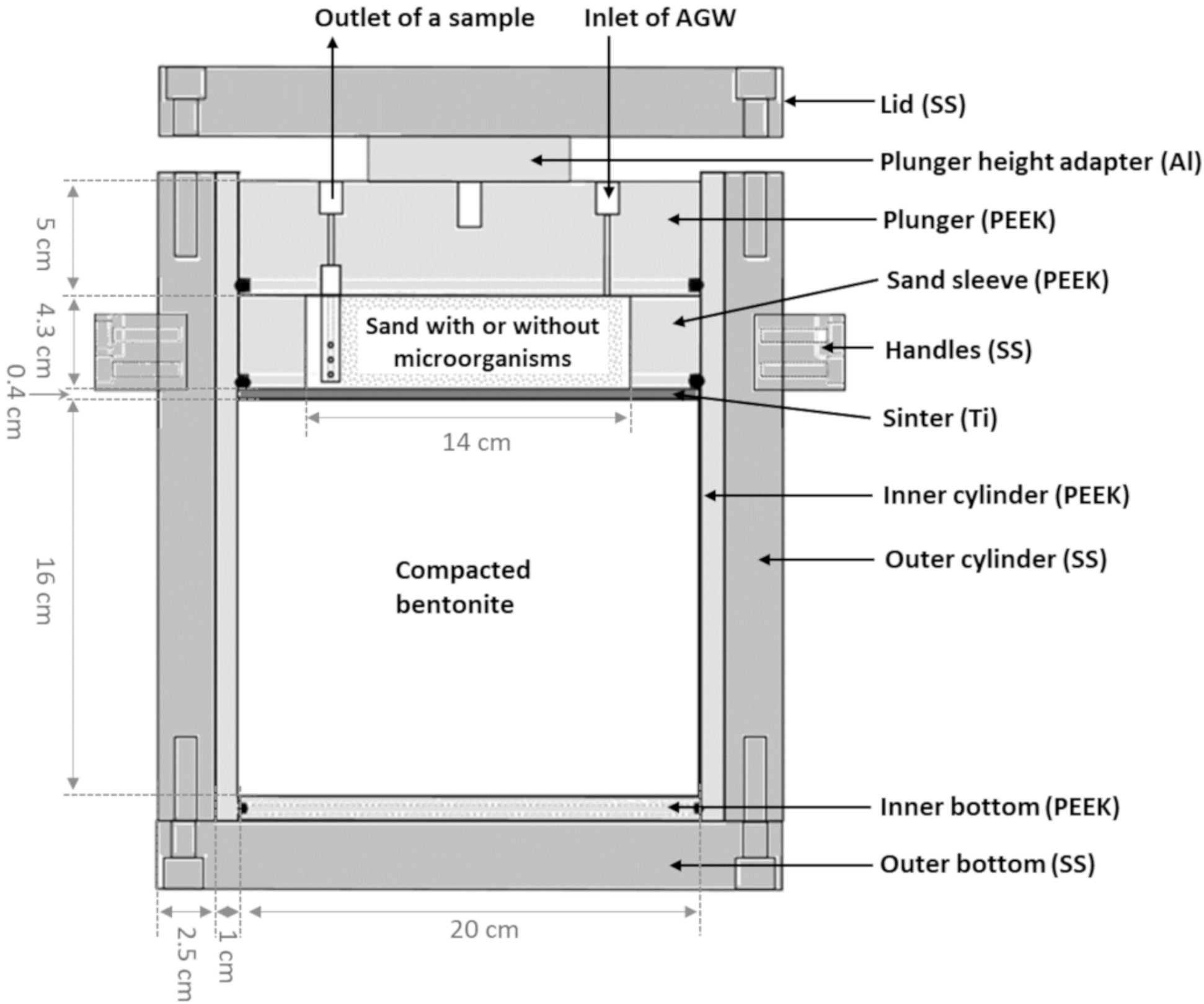
Figure 1. Schematic of the experimental cell (dimensions are in scale; AGW, artificial groundwater; SS, stainless steel; PEEK, polyether ether ketone).

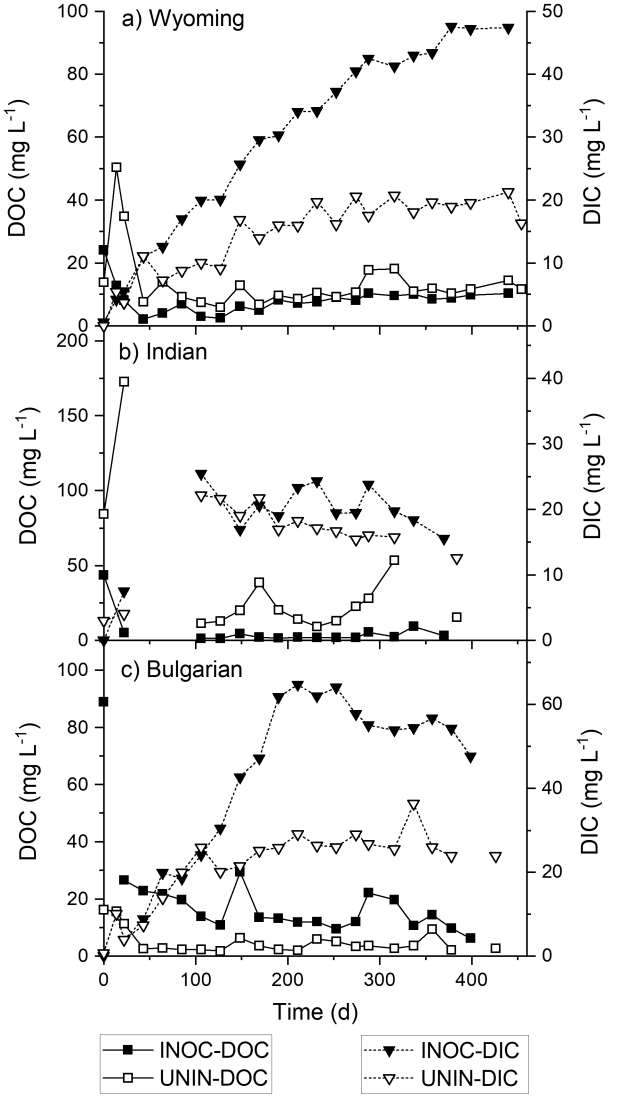
Figure 2. Dissolved organic and inorganic carbon (DOC, DIC) in the solution of sand layers (inoculated [INOC] or uninoculated [UNIN] with microorganisms) of the experimental cells with different bentonites (a–c). Note the different scales on the y-axes.

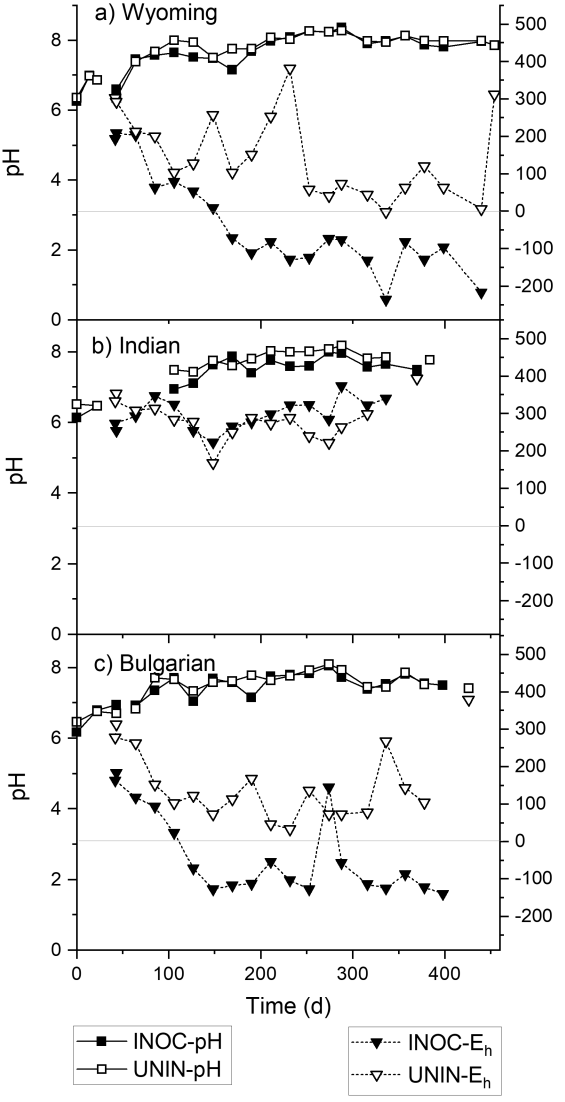
Figure 3. Measured pH and redox potential (E_h vs. standard hydrogen electrode) of the solution in sand layers (inoculated [INOC] or uninoculated [UNIN] with microorganisms) of the experimental cells with different bentonites (a–c).

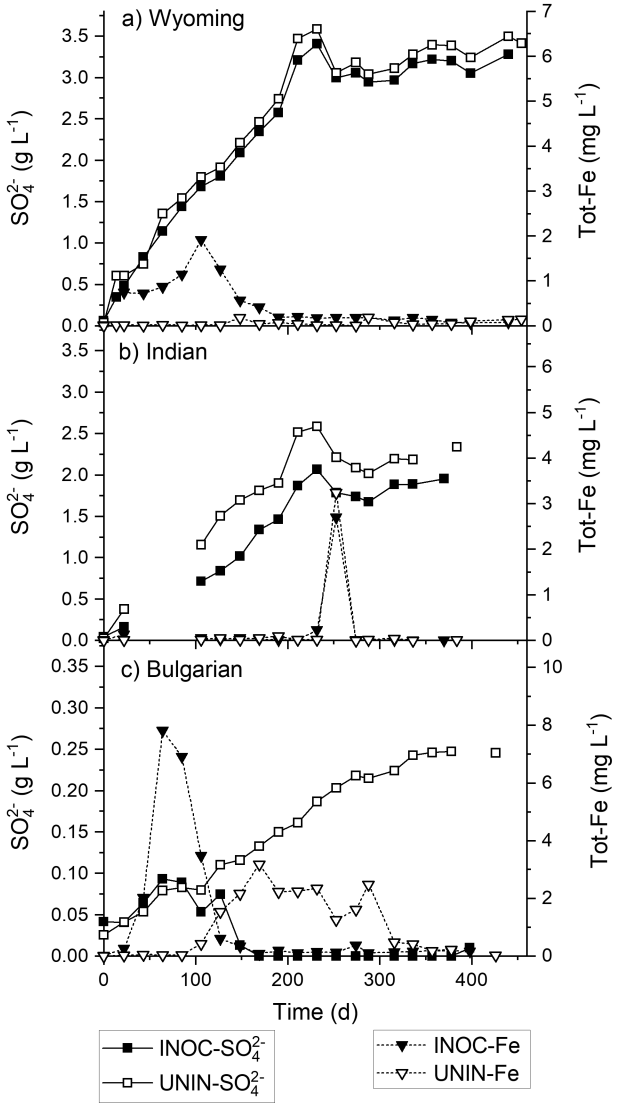
Figure 4. Dissolved sulfate and total iron in the solution of sand layers (inoculated [INOC] or uninoculated [UNIN] with microorganisms) of the experimental cells with different bentonites (a–c). Note the different scales on the y-axes.

Figure 5. Consumption of sulfate in sand inoculated (INOC) or uninoculated (UNIN) with microorganisms of the experimental cells with Wyoming (W), Indian (I) and Bulgarian (B) bentonites in a post-experiment batch assay (mean ± standard deviation, n = 2–9).


Outlet of a sample
Inlet of AGW
Lid (SS)
Plunger height adapter (Al)
Plunger (PEEK)
Sand sleeve (PEEK)
Sand with or without microorganisms
Handles (SS)
Sinter (Ti)
14 cm
Inner cylinder (PEEK)
Outer cylinder (SS)
Compacted bentonite
Inner bottom (PEEK)
Outer bottom (SS)
5 cm
4.3 cm
0.4 cm
16 cm
20 cm
2.5 cm
1 cm


a) Wyoming
b) Indian
c) Bulgarian
DOC (mg L-1)
DIC (mg L-1)
Time (d)
INOC-DOC
UNIN-DOC
INOC-DIC
UNIN-DIC


a) Wyoming
b) Indian
c) Bulgarian
pH
E_h (mV)
Time (d)
INOC-pH
UNIN-pH
INOC-E_h
UNIN-E_h


a) Wyoming
b) Indian
c) Bulgarian
SO_4^{2-} (g L^{-1})
Tot-Fe (mg L^{-1})
Time (d)
INOC-SO_4^{2-}
UNIN-SO_4^{2-}
INOC-Fe
UNIN-Fe

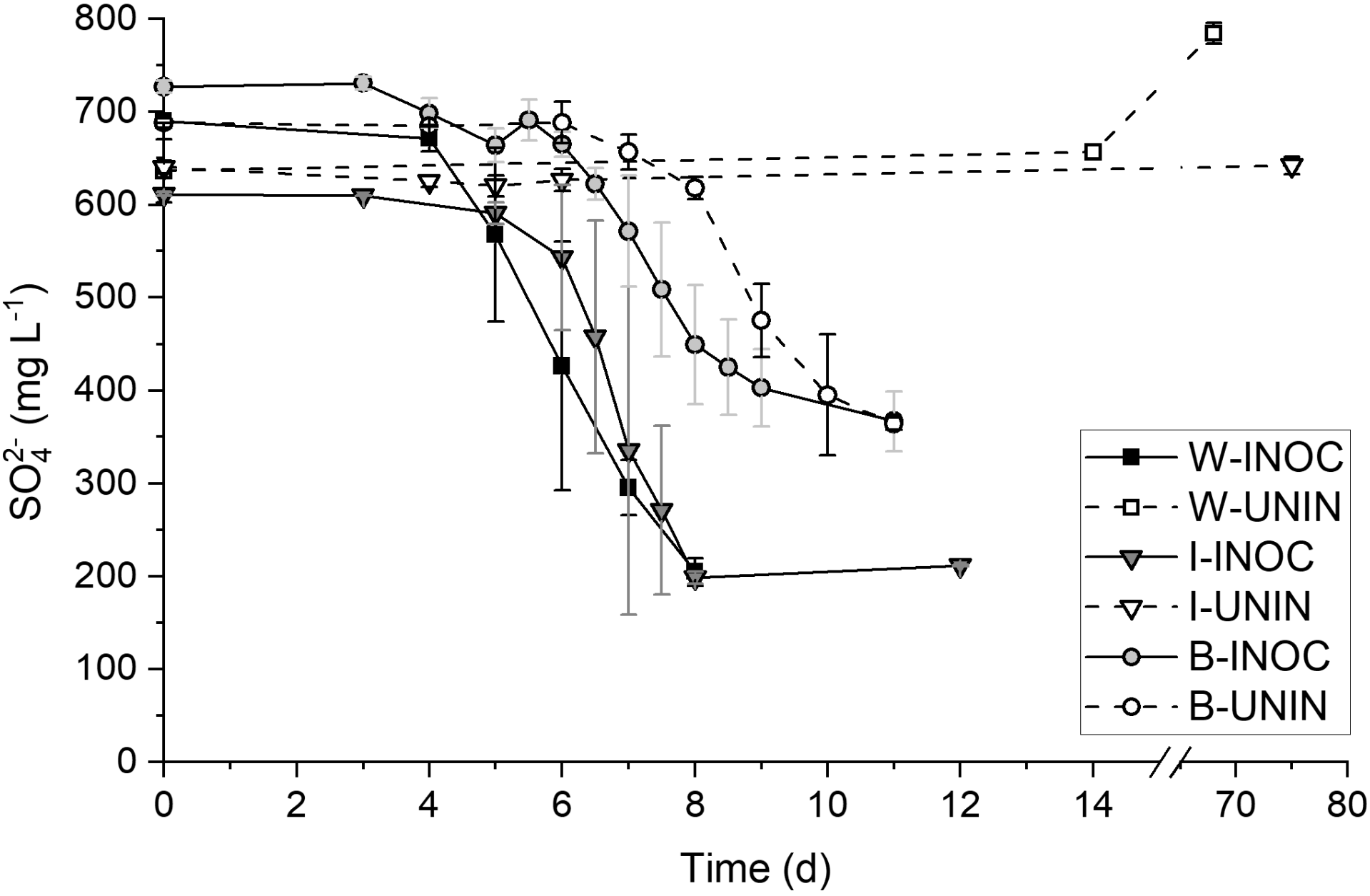

800
700
600
500
400
300
200
100
0
SO_4^{2-} (mg L^{-1})
0
2
4
6
8
10
12
14
70
80
Time (d)
W-INOC
W-UNIN
I-INOC
I-UNIN
B-INOC
B-UNIN

Table 1. Mineral composition and major elements as oxides of Wyoming, Indian, and Bulgarian bentonites expressed as mass-% of the dry material.

Bentonite	**Wyoming[a]**	**Indian[b]**	**Bulgarian[c]**
Minerals			
Smectite	88	74	68
Illite	<1	1	7
Calcite	<1	9	11
Gypsum	<1	1	<1
Plagioclase	3	tr.	<1
Pyrite	1	0	0
Other	8	15	14
Elements			
Sulfate-S	0.12	0.06	0.05
Sulfide-S	0.15	<0.02	<0.02
Fe^{3+}	2.1	10.6	3.2
Fe^{2+}	0.57	0.05	0.26
Inorganic C	0.15	0.36	n.r.
Organic C	0.15	0.15	0.11
Total C	n.r.	n.r.	0.87
LOI	6.3	12	17
CEC	863	850	700
Exchangeable cations			
Na	580	450	60
K	20	0	20
Ca	240	270	450
Mg	90	160	100
SiO_2	62	45	53
Al_2O_3	21	19	14
Fe_2O_3	3.9	15	5.0
FeO	0.53	n.r.	n.r.
TiO_2	0.17	1.0	0.82
MgO	2.5	2.5	2.8
CaO	1.3	3.0	5.7
Na_2O	2.4	1.5	0.34
K_2O	0.78	0.10	1.2
P_2O_5	0	0.10	0.36
Cr_2O_3	0	0.036	<0.002
MnO	0	0.13	0.10

n.r., not reported; tr., trace amount; CEC, cation exchange capacity and exchangeable cations (equivalent g^{-1}); LOI, loss on ignition.

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

[b]Kumpulainen & Kiviranta, 2015

[c]Kumpulainen et al., 2016

Table 2. Grain density and TS content (mean ± SD, *n* = 2–3) of the bentonites, and the characteristics of the bentonite blocks and sand layers in the experimental cells with different bentonites at different stages of the experiment.

Bentonite	**Wyoming**		**Indian**		**Bulgarian**	
Grain density (kg m^{-3})	2780[a]		2910[b]		2670[c]	
TS (ground, deoxygenated) ($\%_{wm}$)	90.8 ± 0.093		88.6 ± 0.239		89.0 ± 0.010	
Object / Cell	**UNIN**	**INOC**	**UNIN**	**INOC**	**UNIN**	**INOC**
Bentonite as compacted initially						
Bentonite (kg_{dm})	6.95	6.98	6.99	7.02	6.86	7.05
Liquid (kg)[d]	2.51	2.59	2.60	2.60	2.33	2.43
Volume of the block (L)	4.95	5.02	5.02	5.06	4.94	5.04
Dry density (kg m^{-3})	1405	1390	1393	1386	1388	1398
Bentonite as compacted in the beginning of the experiment (after storage of 10–108 days)						
Bentonite (kg_{dm})[e]	6.95	6.97	6.99	7.01	6.85	7.05
Liquid (kg)[d]	2.57	2.65	2.66	2.65	2.55	2.49
Volume of the block (L)	5.01	5.05	5.05	5.09	4.99	5.03
Dry density (kg m^{-3})	1387	1380	1384	1379	1374	1401
Bentonite as compacted after 167 days of experiment						
Liquid (kg)[d]	2.69	2.63	2.83	2.83	2.89	2.80
Volume of the block (L)	5.12	5.15	5.18	5.22	5.22	5.15
Dry density (kg m^{-3})	1357	1353	1348	1345	1314	1368
Contents of the sand layers						
Quartz sand (kg_{dm})	0.908	0.864	0.911	0.848	0.871	0.886
Liquid (L)[d,f]	0.296	0.281	0.295	0.303	0.327	0.289
Porosity (mL mL^{-1})[g]	0.47	0.47	0.47	0.49	0.50	0.47

UNIN and INOC, sand layers of the cells uninoculated or inoculated with microorganisms; TS, total solids; SD, standard deviation; SRM, sulfate-reducing microorganisms; wm, wet mass; dm, dry mass.

[a]Kiviranta & Kumpulainen, 2011

[b]Kumpulainen & Kiviranta, 2015

[c]Kumpulainen et al., 2016

[d]The liquid includes the volume of artificial groundwater (11 g total dissolved solids L^{-1}, theoretical density 1006.3 kg m^{-3} at 21°C) added to the bentonite and the ambient pore water of the bentonite.

[e]Some bentonites exited the cells during this compaction.

[f]Volume of the liquid remaining in the sand layers after installation of the plungers.

[g]Porosity was calculated using 2703 kg m^{-3} as the density of quartz sand (determined separately, data not shown).

Table 3. Molar ratio of Fe/S in the precipitates of the sand grains (mean [min–max], *n* = 24–43) and ATP concentration, SRRs, and copy numbers of *dsrB* and 16S rRNA genes in the sand and solution of the experimental cells with different bentonites (mean ± SD, *n* = 2–9).

Cell		**Fe/S (mol mol^{-1})**	**ATP (nmol L^{-1})**	**SRR[a] (mg SO_4^{2-} L^{-1} d^{-1})**	***dsrB* of SRM (·10^5 copies L^{-1})**	**16S rRNA of bacteria (·10^5 copies L^{-1})**
Artificial groundwater-based solution added in the cells in the beginning						
UNIN	Initial all	n.a.	<0.09	n.m.	15.2 ± 9.90	8.5 ± 0.87
INOC	Initial all	n.a.	2.8 ± 0.08	n.m.	9501 ± 391	4019 ± 120

Cell		**Fe/S (mol mol^{-1})**	**ATP (nmol kg^{-1})**	**SRR[a] (mg SO_4^{2-} g^{-1} d^{-1})**	***dsrB* of SRM (·10^5 copies g^{-1})**	**16S rRNA of bacteria (·10^5 copies g^{-1})**
Sand added in the cells in the beginning						
UNIN	Initial all	n.m.	0.20 ± 0.037	n.m.	BLOD[b]	BLOD[b]
INOC	Initial all	n.m.	0.35 ± 0.034	n.m.	BLOD[c]	0.92 ± 0.26
Sand collected from the cells at the end						
UNIN	Wyoming	46.4 [3.4–211]	0.13 ± 0.075	0	0.79 ± 0.03	0.64 ± 0.13
	Indian	38.8 [7.6–129]	<0.0002	0	BLOD[c]	1.5 ± 0.10
	Bulgarian	44.3 [3.8–81.3]	0.38 ± 0.098	0.94 ± 0.260	1.0 ± 0.29	BLOD[a]
Sand collected from the cells at the end						
INOC	Wyoming	0.97 [0.1–1.8]	2.0 ± 0.13	2.0 ± 0.59	8.5 ± 5.13	1.5 ± 0.03
	Indian	23.3 [1.3–139]	2.3 ± 0.27	2.1 ± 0.40	2.9 ± 2.79	5.2 ± 0.57
	Bulgarian	6.5 [0.5–22.9]	8.1 ± 0.83	0.79 ± 0.257	51.8 ± 26.0	1.8 ± 0.26

UNIN and INOC; the sand layers of the cells were uninoculated or inoculated with microorganisms; ATP, adenosine triphosphate; SRR, sulfate reduction rate; *dsrB*, dissimilatory sulfite reductase subunit B; rRNA, ribosomal ribonucleic acid; SRM, sulfate-reducing microorganism; SD, standard deviation; n.a., not applicable; BLOD, below limit of detection; n.m., not measured.

[a]Activity of SRM in the sand was measured post-experiment as a sulfate reduction rate in a batch assay.

[b]LOD 600 copies of 16S rRNA μL^{-1} and 600 copies of *dsrB* μL^{-1} of DNA extract.

[c]LOD 6000 copies of *dsrB* μL^{-1} of DNA extract. The LOD was determined separately for each run.

Table 4. MPN of SRM in the compacted bentonite of the experimental cells after the experiment and in the original uncompacted bentonite.

Bentonite	**Cell**	**MPN of SRM (g^{-1} bentonite) (lower–upper 95% confidence interval)**
Wyoming	Uncompacted	370 (100–1300)
	UNIN	210 (72–620)
	INOC	660 (160–2800)
Indian	Uncompacted	7500 (1900–30000)
	UNIN	2900 (1100–7900)
	INOC	14000 (4100–47000)
Bulgarian	Uncompacted	3800 (100–14000)
	UNIN	2200 (750–6500)
	INOC	1200 (360–3800)

MPN, most probable number; SRM, sulfate-reducing microorganisms; UNIN and INOC, sand layer of the cells were uninoculated or inoculated with microorganisms.

Supplementary material for:

Compacted bentonite as a source of substrates for sulfate-reducing microorganisms in a simulated excavation-damaged zone of a spent nuclear fuel repository

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S1. Masses of bentonite and volumes of AGW required for the preparation of saturated bentonite blocks

The required mass of dry bentonite (m_s) was calculated according to Eq. S1, where ρ_d was the target dry density (1400 kg m^{-3}) and V was the predefined volume of the compacted bentonite block (5.027 L).

$$m_s = \rho_d \cdot V \quad \text{(S1)}$$

At full saturation, the total volume of liquid in the compacted bentonite block equaled the space not taken up by the solids (i.e., the void volume). Consequently, the total volume of liquid (V_w) at the target dry density was calculated according to Eq. S2, where ρ_s was the grain density (2670–2910 kg m^{-3}; Table 2).

$$V_w = V - \frac{m_s}{\rho_s} \quad \text{(S2)}$$

The volume of artificial groundwater (AGW) to be mixed with the bentonite (V_{wa}) was calculated according to Eq. S3, where V_{wi} was the volume of the internal water (i.e., moisture) in the bentonites.

$$V_{wa} = V_w - V_{wi} \quad \text{(S3)}$$

The mass of internal water (m_{wi}) was calculated according to Eq. S4, where *TS* was the total solids content of the bentonites (88.6–90.8%$_{wet\_mass}$; Table 2).

$$m_{wi} = \frac{m_s}{TS/100} - m_s \quad \text{(S4)}$$

Next, the mass of internal water was converted into volume by using Eq. S5, where ρ_{AGW} was the density of AGW (1006.3 kg m^{-3} at 21°C).

$$V_{wi} = \frac{m_{wi}}{\rho_{AGW}} \quad \text{(S5)}$$

AGW in excess of 5% (v/v) was added to the bentonite mixture to compensate for the volume lost during mixing and other working stages; volume loss was quantified during preliminary compaction testing (data not shown). After bentonite and AGW were mixed, the moisture content of the bentonite was checked and more AGW was added if the moisture content did not match the theoretical bentonite void volume at the target density (i.e., V_w).

S2. Swelling and re-compaction of the bentonite blocks before the experiment

S2.1 Swelling during storage and re-compaction before assembling the sand layers

Bentonite blocks were compacted for the six cells one after another and, hence, some of them were stored longer (108 days) than the others (10 days) before the experiment was started at the same time with all the cells. For starting the experiment, the assemblance of the sand layers was started with the uninoculated cell of the Bulgarian bentonite by filling the sand sleeve with sand (0.871 kg$_{dry_mass}$) and AGW (299 mL; sand layer construction described in detail in Chapter 2.3). Soon after, it was noticed that the Bulgarian bentonite had adsorbed the added AGW (65 mL on the sinter for storage and in the sand sleeve for start-up of the experiment) and swelled by 326 mL (Table S1). At this point, the unconstrained bentonites in the other five cells were also found to have swelled by 61–134 mL (Table S1) after adsorbing

the AGW added on the sinters for storage (50–65 mL). Thus, the blocks were re-compacted to the target density before assembling the sand layers for start-up of the experiment.

Table S1. Characteristics of the bentonite blocks in the experimental cells after swelling (during storage and after days 0–126) and re-compaction (days 146 and 167).

Bentonite	**Wyoming**		**Indian**		**Bulgarian**	
Object / Cell	**UNIN**	**INOC**	**UNIN**	**INOC**	**UNIN**	**INOC**
Bentonite blocks after swelling during storage of 10–108 days						
AGW adsorbed by the bentonite block in total (mL)	65	65	60	50	364[a]	60
Increase in volume of the bentonite block (mL)	90	134	61	83	326[a]	102
Dry density before re-compaction (kg m^{-3})	1380	1354	1376	1364	1302	1370
Liquid exiting the cells in re-compaction (mL)	2.3	1.4	0	0	150	0
Swelling of the bentonite blocks between days 0 and 126 of the experiment						
AGW adsorbed by the bentonite blocks in total (mL)	168	170	194	206	213	226
Increase in volume of bentonite blocks (mL)	184	190	254	272	240	328
Dry density at day 126 (kg m^{-3})	1337	1330	1317	1309	1311	1315
Re-compaction of the bentonite blocks at days 146 and 167 of the experiment						
Liquid exiting the cells in total (mL)	30	34	25	28	30	25

AGW, artificial groundwater; UNIN and INOC, sand layers of the cells uninoculated or inoculated with microorganisms.[a]Bulgarian bentonite of the uninoculated cell adsorbed both the AGW added on top of the sinter for storage (65 mL) and AGW added in the sand sleeve during assemblance of the sand layer for start-up of the experiment (299 mL).

S2.2 Quantification of changes in sulfate and DOC contents of the bentonite blocks

To quantify the effect that swelling of the bentonite blocks during the storage and re-compaction before the experiment had on the bentonites, mass balances were calculated for adsorbed and desorbed sulfate and organic carbon (DOC). The calculations described below were carried out assuming that the whole mass of the bentonite in the block was affected by swelling and re-compaction.

The amount of ambient sulfate in the bentonite blocks (6.86–7.05 kg dry weight; Table 2) was calculated based on the theoretical sulfate contents of the bentonites (1498, 1797 and 1595 mg SO_4^{2-} kg^{-1} in Bulgarian, Indian and Wyoming bentonites, respectively, calculated from SO_4-S contents in Table 1). In total 46–52 mg SO_4^{2-} (Table S2) was added to the bentonites when mixed with AGW during the saturation stage (20 mg SO_4^{2-} L^{-1} in AGW, 2.32–2.58 L used for each bentonite block) and it contributed only by 0.2–0.5% to the total reservoir of sulfate in the bentonite-AGW mixtures. The sum of ambient sulfate in bentonite and the amount of sulfate added within AGW equaled to the total amount of sulfate contained in the compacted bentonite blocks (10323–25126 mg SO_4^{2-}; Table S2).

For storage, the sinters in the six cells were soaked with AGW (50–65 mL) to prevent them from desaturation. After storage, the sand layer of the uninoculated cell of the Bulgarian bentonite was assembled by using 299 mL AGW (described in Chapter S2.1 and in Chapter 2.3). The unconstrained bentonite blocks adsorbed the added AGW and the sulfate within, which was 7.3 mg SO_4^{2-} for Bulgarian bentonite in the uninoculated cell and 1.0–1.3 mg SO_4^{2-} for the bentonite blocks in the other cells (Table S2). When the bentonite blocks were re-compacted, in total 150 mL of liquid filtrated out from the uninoculated cell of the Bulgarian bentonite and 0–2.3 mL from the other cells (Table S2). The concentration of sulfate was determined from the filtrate of the uninoculated cell of the Bulgarian bentonite (126 mg L^{-1}) and estimated for the filtrates of the both cells of Wyoming bentonite as the volume of the filtrate was too small for analysis (303 mg L^{-1}; see details in Table S2). Thus, the total amount of sulfate that became desorbed from the bentonite blocks in re-compaction was 18.9 mg SO_4^{2-} for Bulgarian bentonite in the uninoculated cell and 0–0.7 mg SO_4^{2-} for the bentonite blocks in the other cells (Table S2). In terms of the net balance, Bulgarian bentonite in the uninoculated cell desorbed in total 17.6 mg SO_4^{2-}, while the bentonite blocks in the other cells adsorbed 0.6–1.2 mg SO_4^{2-} as a result of adsorption of the added AGW and

re-compaction. These amounts were, however, very small (0.002–0.2%) when compared to the initial total amount of sulfate in the bentonite blocks (Table S2).

The ambient DOC concentration in the bentonites was calculated based on the theoretical total organic carbon (TOC) contents of the bentonites (1100, 1500 and 1500 mg TOC kg^{-1} in Bulgarian, Indian and Wyoming bentonites, respectively; Table 1) and by assuming that 0.1% of TOC was water-soluble from uncompacted bentonite (Marshall et al., 2015). The amount of DOC adsorbed by the bentonites when mixed with AGW (containing 0.141 mg DOC L^{-1}) during the saturation stage was 0.33–0.36 mg (Table S3), which represented 3–4% to the total reservoir of DOC in the bentonite-AGW mixtures. The total concentration of DOC contained in the compacted bentonite blocks was 7.9–10.9 mg (Table S3).

The amount of DOC adsorbed by the bentonite blocks during swelling was 0.051 mg DOC for Bulgarian bentonite of the uninoculated cell and 0.007–0.009 mg DOC for the bentonite blocks of the other cells (Table S3). The concentration of DOC in the filtrates collected during re-compaction was determined for the uninoculated cell of the Bulgarian bentonite (7.6 mg L^{-1}) and estimated for the filtrates of the both cells of Wyoming bentonite (10.4 mg L^{-1}; Table S3). Thus, the total amount of DOC that became desorbed from the bentonite blocks in re-compaction was in total 1.14 mg for Bulgarian bentonite of the uninoculated cell and 0–0.024 mg for the bentonite block of the other cells (Table S3). As for the net balance, Bulgarian bentonite in the uninoculated cell and Wyoming bentonite in both cells desorbed in total 1.09 mg and 0.005–0.015 mg DOC, respectively, while Indian bentonite in both cells adsorbed 0.008 mg DOC (Table S3). When compared to the initial total amount of DOC in the bentonite blocks, the amounts sorbed by Wyoming or Indian bentonites in all cells or Bulgarian bentonite in the inoculated cell were small (0.05–0.1%; Table S3). For Bulgarian bentonite in the uninoculated cell, the amount of desorbed DOC

during re-compaction was slightly higher than for the others (14% of the initial DOC; Table S3).

Table S2. Theoretical amount of sulfate in the bentonite blocks of the experimental cells initially, after swelling during storage and after re-compaction before start-up of the experiment.

Bentonite	**Wyoming**		**Indian**		**Bulgarian**	
Cell	**UNIN**	**INOC**	**UNIN**	**INOC**	**UNIN**	**INOC**
Compacted bentonites before storage						
Total SO_4 in bentonite blocks (mg)	25035	25126	12613	12660	10323	10603
> Ambient SO_4 in bentonites (mg)	24986	25074	12561	12608	10277	10555
> SO_4 added with AGW in saturation (mg)	50	52	52	52	46	48
Swelling during storage and re-compaction before start-up of the experiment						
SO_4 adsorbed by bentonite in total (mg)	1.3	1.3	1.2	1.0	7.3	1.2
> SO_4 from AGW added on sinter (mg)	1.3	1.3	1.2	1.0	1.3	1.2
> SO_4 from AGW added in sand layer (mg)	n.a.	n.a.	n.a.	n.a.	6.0	n.a.
SO_4 desorbed in re-compaction (mg)	0.7	0.4	0	0	18.9	0
> Liquid filtrated out from the cell (mL)	2.3	1.4	0	0	150	0
> SO_4 in the filtrated liquid (mg L^{-1})	303[a]	303[a]	n.a.	n.a.	126	n.a.
SO_4 net mass balance (mg)[b]	+0.6	+0.9	+1.2	+1.0	-17.6	+1.2
SO_4 net mass balance (% of initial)	0.002	0.003	0.01	0.008	0.17	0.011

UNIN and INOC, sand layers of the cells uninoculated or inoculated with microorganisms; AGW, artificial groundwater; n.a., not applicable.

[a]The concentration of sulfate could not be measured from the liquid filtrating out from the Wyoming bentonite blocks because the volume was too low for the analysis. Thus, the concentration was estimated based on the concentration of sulfate in the filtrate of the uninoculated cell of Bulgarian bentonite (126 mg L^{-1}); the concentration in the filtrate from the Wyoming blocks was assumed to be proportional to the ambient sulfate content in the bentonite, which was 1498 mg SO_4^{2-} kg^{-1} for the Bulgarian bentonite and 1595 mg SO_4^{2-} kg^{-1} for the Wyoming bentonite.

[b]The plus sign indicates that the bentonites retained some of the sulfate adsorbed from AGW after re-compaction and minus sign indicates that the bentonites lost more sulfate in re-compaction that adsorbed from the AGW.

Table S3. Theoretical amount of DOC in the bentonite blocks of the experimental cells initially, after swelling during storage and after re-compaction before start-up of the experiment.

Bentonite	**Wyoming**		**Indian**		**Bulgarian**	
Cell	**UNIN**	**INOC**	**UNIN**	**INOC**	**UNIN**	**INOC**
Compacted bentonites before storage						
Total DOC in bentonite blocks (mg)	10.8	10.8	10.8	10.9	7.9	8.1
> Ambient DOC in bentonites (mg)	10.4	10.5	10.5	10.5	7.5	7.8
> DOC added with AGW in saturation (mg)	0.35	0.36	0.36	0.36	0.33	0.34
Swelling during storage and re-compaction before start-up of the experiment						
DOC adsorbed by bentonite in total (mg)	0.009	0.009	0.008	0.007	0.051	0.008
> DOC from AGW added on sinter (mg)	0.009	0.009	0.008	0.007	0.009	0.008
> DOC from AGW added in sand layer (mg)	n.a.	n.a.	n.a.	n.a.	0.042	n.a.
DOC desorbed in re-compaction (mg)	0.024	0.015	0	0	1.14	0
> Liquid filtrated out from the cell (mL)	2.3	1.4	0	0	150	0
> DOC in the filtrated liquid (mg L^{-1})	10.4[a]	10.4[a]	n.a.	n.a.	7.6	n.a.
DOC net mass balance (mg)[b]	-0.015	-0.005	+0.008	+0.007	-1.09	+0.008
DOC net mass balance (% of initial)	0.14	0.05	0.08	0.06	13.9	0.10

DOC, dissolved organic carbon; UNIN and INOC, sand layers of the cells uninoculated or inoculated with microorganisms; AGW, artificial groundwater; n.a., not applicable.

[a]The concentration of DOC could not be measured from the liquid filtrating out from the Wyoming bentonite cells because the volume was too low for the analysis. Thus, the concentration was estimated based on the concentration of DOC in the filtrate of the uninoculated cell of Bulgarian bentonite (7.6 mg L^{-1}); the concentration in the filtrate from the Wyoming blocks was assumed to be proportional to the ambient concentration of water-soluble organic carbon in the bentonites, which was 1.1 mg DOC kg^{-1} for the Bulgarian bentonite and 1.5 mg DOC kg^{-1} for the Wyoming bentonite.

[c]The plus sign indicates that the bentonites retained some of the DOC adsorbed from AGW after re-compaction and minus sign indicates that the bentonites lost more DOC in re-compaction that adsorbed from the AGW.

S3. Amending the sand and AGW with microorganisms for the inoculated cells

S3.1 Pre-enrichment of sand with groundwater microorganisms

Prior to the cell experiment, the sand for inoculated cells was pre-enriched with microorganisms originating from Olkiluoto groundwater. The aim was to promote the

formation of an adhered microbial community (i.e., biofilm) that would acclimatize to the cell conditions (e.g., salinity).

In the pre-enrichment culture, organic matter leached from the Wyoming, Indian, and Bulgarian bentonites was used as a substrate for the microorganisms. Bentonite leachates were prepared by incubating bentonite with sterile and anaerobic AGW at a liquid-to-solid ratio of 40 L kg^{-1} for ≥7 days at 150 rpm. After incubation, the AGW-based leachates were separated from the bentonites by centrifugation (10 min at 10000 × *g*) and pooled (300–600 mL from each bentonite).

The pre-enrichment culture was prepared by mixing Olkiluoto groundwater (1400 mL; drill hole ONK-PVA06, collected in October 2017), a batch of carbon-free sand and the bentonite leachates. To increase the number of microorganisms in the culture, an additional batch of groundwater microorganisms was isolated from groundwater by filtering (1000 mL; 0.20 µm, Supor-200) and added to the medium by shaking the filter vigorously in the medium with tweezers. The pre-enrichment culture was incubated at 20 ± 2°C for 11 weeks.

After 11 weeks of incubation, the medium of the culture was refreshed. Approximately 75% (v/v) of the old medium was removed, and fresh anaerobic bentonite leachates (500 mL each of Wyoming, Indian, and Bulgarian; produced as earlier) were added to the remaining medium. A new batch of groundwater microorganisms collected on a filter from Olkiuoto groundwater (3600 mL; ONK-PVA06, collected in January 2018) was distributed to the medium as described above. The loss of some pelagic microorganisms along the removed batch of the old medium was assumed. Thus, the old medium was filtered (0.2 µm, Supor-200), and the biomass captured on the filter was redistributed to the new medium. The pre-enrichment culture was incubated in the refreshed medium for six weeks at 20 ± 2°C prior to the start of the cell experiment.

At the same time, when the medium was refreshed on week 11 of incubation, the sand was studied for the presence of sulfate-reducing microorganisms (SRM) in a batch assay to confirm that the pre-enrichment was successful. Samples of sand (10 g_{wet_mass}, n = 6) were incubated in two different media (8.4 g NaCl L^{-1}; Zamora & Malaver, 2012; DSMZ, 2017) for six weeks at 28°C. The medium in all the bottles turned black due to the formation of FeS, indicating that cultivable SRM were present in the pre-enrichment culture (Krieg, 1981).

When the sand layers of the inoculated experimental cells were set up in the beginning of the experiment, the sand from the pre-enrichment culture (4.7 kg) was separated from the pre-enrichment medium, which was saved for collecting the pelagic microorganisms at later stage (described in Chapter S3.2). The sand from the batch assay (60 g in total) was combined with the sand pre-enriched with groundwater microorganisms. Next, the combined sand batch was rinsed with sterile AGW to remove traces of media and the FeS precipitates formed during the batch assay. Then, the sand was divided in three sub-batches and inserted into the inoculated cells.

S3.2 Amendment of AGW with different microorganisms

The AGW used for saturating the sand layers of the inoculated cells was first amended with microorganisms from three sources:

1) Pure cultures of SRM: *Desulfobacula phenolica*, *Desulfobulbus mediterraneus*, *Desulfobulbus rhabdoformis*, *Pseudodesulfovibrio aespoeensis*, and *Desulfotomaculum acetoxidans* were obtained from DSMZ GmbH (IDs 3384, 13871, 8777, 10631 and 771, respectively). Pure cultures of the SRM were used in the experiment to create an abundant initial SRM community in the sand layers of the inoculated cells. The SRM were delivered from DSMZ GmbH in 10–15 mL of

specific media, from where the microbial cells were harvested without pre-culturing as described below.

2) Microorganisms of fresh Olkiluoto groundwater (3.6 L, ONK-PVA06, extracted in January 2018 and stored in gas-tight bottles at 4°C for three weeks) were used in the experiment to include a mixed community of native groundwater microorganisms in the sand layers.
3) Pelagic microorganisms of the pre-enrichment medium used to culture the sand (2.3 L; described in Chapter S3.1).

The pure cultures of SRM and microorganisms from groundwater and pre-enrichment medium were collected on sterile filters (0.2 µm, Supor-200). Residues of the specific media of the SRM pure cultures, pre-enrichment medium and groundwater were washed off from the filters with sterile AGW by filtering. Microorganisms captured on the filters were distributed in the batch of AGW to be added to the sand layers by shaking the filters vigorously in the solution with tweezers. Then, the AGW was divided in three sub-batches and inserted into the inoculated cells.

Table S4. qPCR thermal cycling protocols for amplification of *dsrB* and 16S rRNA genes.

Step	***dsrB***	**16S rRNA**
Primer pair	DSRp2060F/DSR4R[a]	Eub338F/Eub518R[b]
Initial denaturation	10 min at 95°C	10 min at 95°C
Denaturation, annealing and extension	15 s at 95°C, 30 s at 55°C, 30 s at 72°C, repeated 45×	15 s at 95°C, 60 s at 62°C, repeated 35×

qPCR, quantitative polymerase chain reaction; *dsrB*, dissimilatory sulfite reductase subunit B; 16S rRNA, 16S ribosomal ribonucleic acid.

[a]Geets et al., 2006

[b]Fierer et al., 2005

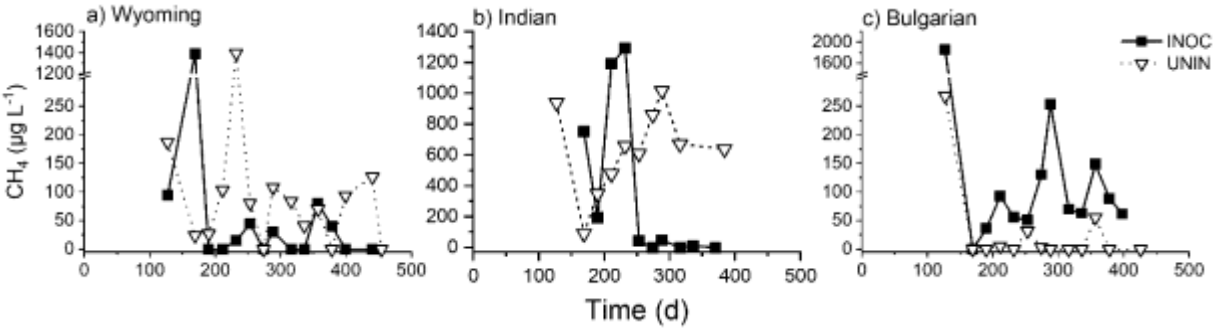

Figure S1. Concentration of dissolved methane (from day 127 onwards) of the solution in the sand layers (inoculated [INOC] or uninoculated [UNIN] with microorganisms) of the experimental cells with different bentonites (a–c).

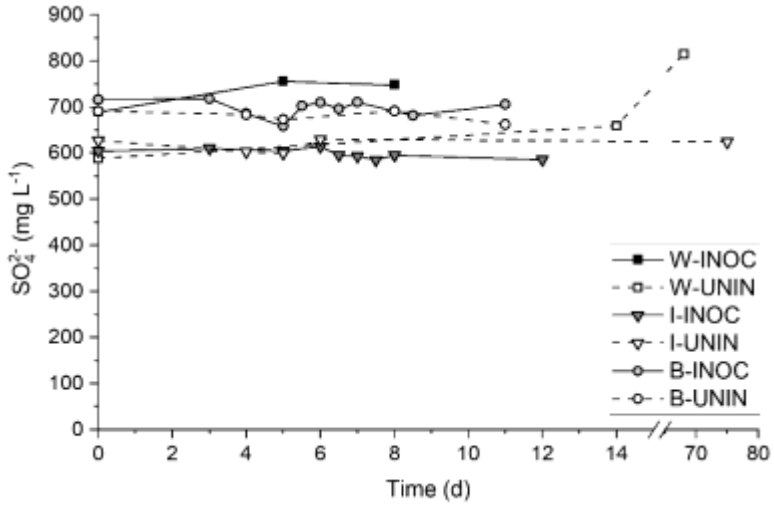

Figure S2. Concentration of sulfate in abiotic control samples of a post-experiment sulfate reduction rate batch assay (sand inoculated [INOC] or uninoculated [UNIN] with microorganisms of the experimental cells with Wyoming [W], Indian [I] and Bulgarian [B] bentonites [n = 1]).

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