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Abstract
In the context of the safety assessment of radioactive waste geological disposal in clayey formations, the evolution in time and space of clayey and metallic materials is of concern regarding their containment properties. Bacteria may induce localized corrosion processes and clay mineral alteration. Moreover, the presence of heterogeneities may favor preferential pathways or higher reactivity. Thus, the objective of the present work is to assess the influence of bacteria and heterogeneities on iron-clay reactivity. Two percolation cells are conducted in the presence of iron and argillite involving an artificial crack in biotic or abiotic conditions at 60 °C during one year. Two bacteria have been selected: a Sulphate Reducing Bacteria (SRB) and an Iron Reducing Bacteria (IRB). The reactivity of those cells is evidenced by the ongoing monitoring of water composition. First results clearly show that bacteria are able to survive in the biotic experiment. Moreover, the decrease of the flow rate observed in each cell could be explained by the clogging of the crack.

Keywords: Sulphate reducing bacteria; Iron reducing bacteria ; Argillite ; Hydrogen ; Iron ; Radioactive waste ; Tournemire

1. Introduction

The concept of high-level radioactive waste (HLW) disposal cell developed by Andra in France involves metallic materials (containers, overpacks, liner) emplaced into a geological argillaceous formation [1]. The anoxic and saturated conditions prevailing in this medium will lead to the anoxic aqueous corrosion of these metallic materials and the release of aqueous iron, which may locally induce the alteration of the favorable confining properties of the argillaceous formation (low permeability, swelling capacities and high sorption capacities).

HLW disposal cell will involve the presence of heterogeneities, such as fractures of the Excavation Damaged Zone (EDZ) or remaining void spaces between components. These heterogeneities may induce preferential pathways for solutes and thus favor non homogeneous iron/argillite interactions [2, 9].

Moreover, the presence of microorganisms, has recently been evidenced in deep clayey environment [2]. Indeed, neither the introduction of microbial species during the construction and operational phases of the repository nor the survival of bacteria after its closure can be excluded [3]. Electron donors such as hydrogen gas produced by anoxic metallic corrosion and radiolysis phenomena or biodegradable organic matter contained in argillite will be available for bacterial development [4]. The presence of

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heterogeneities may provide space for bacterial growth. It is widely accepted that microorganisms may affect the corrosion process due to their influence (directly or indirectly) on the chemical characteristics (water composition, pH, redox potential) of the metal/environment interface [5]. More specifically, SRB may induce the production of a corrosive product (ferrous sulfide), which may lead to localized corrosion [6]. The IRB reduction of Fe(III) from iron oxides present on passive layers under anaerobic conditions may also impact corrosion, in re-exposing the metal surfaces to corrosion. Finally, the changes in the oxidation state of the structural iron in clay minerals due to the IRB activity may cause changes in chemical and physical properties of clay minerals (exchange capacities, specific area and swelling pressure) [7, 8].

In this context, the present study focuses on the effects of bacteria and heterogeneities on iron/argillite interactions in conditions that may prevail in a repository after the chemical and hydraulic transients. The experimental system chosen is a percolation cell involving iron that is chosen as a representative of the metallic components and Toarcian argillite of Tournemire which is an argillaceous media with similar properties to those of the Callovo-Oxfordian argillite studied by Andra as potential host rock.

2. Materials and methods

2.1. Iron materials

Zero valent iron is introduced in the experimental system as powder and as a solid cylindrical sample. The iron powder (Goodfellow, particle size <60 µm, BET specific surface area 0.13 m²/g) allows to increase iron/argillite reactivity, while the solid iron sample, measuring 3 mm in diameter and 15 mm in thickness, is used to simulate a solid iron/argillite interface.

2.2. Argillaceous material

The argillite rock comes from the Tournemire experimental platform (Aveyron, France). Samples were collected in the Toarcian formation and conserved at 4°C. No specific anaerobic conditions were applied during storage and machining. The mean mineralogical composition of the rock is silicate phases (86 %), carbonates (10 %), pyrite (3 %) and organic kerogen (<1%) [10]. The clay fraction is composed of illite/smectite mixed layer, illite, kaolinite, chlorite, quartz and detrital K-feldspar. For each experiment, clay-rock discs were machined to obtain two half cylinders of 42 mm diameter and 30 mm length, in order to obtain an artificial crack, and thus to simulate a fracture e.g. from the EDZ.

2.3. Synthetic water solution

The composition of the synthetic pore water was similar to this of the Tournemire formation [11]. Phosphorus and nitrogen, which are nutrients necessary for the bacterial growth, were added. The water was prepared by dissolving high purity chemicals in deionised water and sterilized after preparation by filtration (0.22 µm). The final pH was 7.5 which is in good agreement with pH measured in Tournemire pore water.

2.4. Cell culturing

Two anaerobic, thermophilic and hydrogenotrophic bacteria were selected: one IRB *Thermotoga subterranea* strain *SLT 1* coming from the DSMZ German collection of microorganisms and cultures

(www.dsmz.de) [12] and one SRB *Thermodesulfovibrio hydrogeniphilus* [13]. Bacterial cultures were done in batch reactor, at 60°C under anaerobic atmosphere N_2/H_2 (40/60). A synthetic medium similar to the chemical composition of Tournemire synthetic solution was prepared. Bacterial inoculum was prepared from the bacterial culture which was washed by centrifugation (4000 rpm, 20 min) and then inoculated in Tournemire synthetic water to obtain the right volume to prepare the iron powder mixture

2.5. Experimental design

The experimental device (Fig. 1), described in [14], allows to reproduce anoxic and saturated conditions prevailing in disposal and was thus adapted to investigate iron-clay reactivity in presence of bacteria. Two percolation cells are being conducted in biotic and abiotic conditions at 60 °C during one year. The system was assembled under sterile and anaerobic conditions to avoid bacterial contamination and inhibition of bacterial activities due to the presence of oxygen. In a first step, metallic iron powder was mixed with Tournemire synthetic water for the abiotic experiment, as well as bacterial inoculum (centrifuged and washed three times) for the biotic one and then placed into a metallic ring. The argillite half cylinders were placed into an elastic membrane. Metallic and argillaceous samples were vertically confined between upper and lower drilled pistons, in addition to lateral containment applied by the elastic membrane, under water pressure (about 40 bars) in order to avoid any preferential pathway along the inner side of the cell. The cell enables water circulation by advection. Sampling solution at the iron-clay interface was made possible in order to follow bacterial development. The flow is monitored and controlled by an imposed gradient pressure between the bottom and the top of the cell.

2.6. Chemical analyses

Solution chemistry was monitored over time. Anions (Cl, PO_4 and SO_4) and cations (Na, K, Si, Sr, Mg, Fe and Ca) were measured by ionic chromatography (IC) and inductively coupled plasma-atomic emission spectroscopy (ICP-AES), respectively.

2.7. Bacterial population measurement

The microbial activity was monitored by counting cell bacteria using a direct counting epifluorescence method (Backlight). This required the use of a mixture of two fluorochromes, which stain specific genetic material of the bacteria. The microscope was equipped of a specific lamp and two filters in order to examine and count the fluorescent bacteria.

3. Results and discussion

3.1. Flow rate

As showed in Fig. 2, the flow rate decreases as a function of time. Its stabilization is observed after two months of experiment. This could be explained by the clogging of the artificial crack due to mineral precipitation or swelling of clay minerals. The flow rate of the cell (1.2 ml/day) without bacteria is about twice this measured in the biotic cell (0.7 ml/day). The Darcy's law allows to evaluate the hydraulic conductivity of the whole sample (assuming homogeneous properties) at 1.9 and 3.4 10^{-12} m/s for the biotic and abiotic cells, respectively. This is in good agreement with Tournemire argillaceous value (10^{-12} to 10^{-14} m/s) [11]. This slight overestimation could be due to the presence of the artificial crack or to mechanical perturbations during the sampling and machining of the sample.

3.2. Survival of bacterial population

A mixed IRB and SRB culture was introduced in the biotic cell, containing about 10^8 bacteria/ml. After 150 days of experiment, the bacterial population fluctuates from 2 10^7 to 3 10^7 bacteria/ml. Both IRB and SRB species are still present and alive. As showed on Fig. 4 (a), SO_4 concentration seems to be stabilized around the initial concentration in the abiotic cell whereas a significant decrease (about 50 %) in the biotic cell is observed from 2.1 $mmol.L^{-1}.d^{-1}$ during the first hundred days to 0.9 $mmol.L^{-1}.d^{-1}$. This consumption may be attributed to the SRB activities reducing sulphate into sulfide, which confirms that bacteria are able to grow under simulated disposal conditions (low permeability, high pressure and temperature conditions).

3.3. Bacterial reactivity and evolution of water chemistry

Figure 4 (b) presents the Ca concentration and pH evolution of the outlet sample in each cell. The evolution of the concentration of Ca, Mg and Sr (not shown), is quite similar in each cell. A strong decrease of those elements is observed in the biotic cell. It could result from carbonate mineral precipitation due to the increase of the pH, from 7.5 to 8.6 in the biotic experiment and to 8.2 in the abiotic one.

Silica is not present in the Tournemire synthetic solution but its concentration in the outlet sample fluctuates around 0.3 $mmol.L^{-1}$ in each cell. It may be attributed to silicate phase dissolution, such as illite or K-feldspar, which is confirmed by a small increase of K concentration in the abiotic cell. Cl is not reactive during the experiment while PO_4 and iron concentrations are below the detection limit (10 and 1 $\mu g.L^{-1}$, respectively). This may be due to the consumption of PO_4 by bacteria in the biotic experiment or its precipitation as vivianite ($Fe_3(PO_4)_2{:}8H_2O$) which has already been observed with IRB [15]. Moreover, the lack of iron in the outlet samples may also be explained by its strong immobilization as corrosion products that usually form in anoxic conditions, such as magnetite (Fe_3O_4) and/or siderite ($FeCO_3$).

4. Conclusion

The first results of biotic and abiotic experiments performed in the present work have shown that bacteria could survive in a system reproducing conditions that may prevail in HLW disposal cell (anoxic, high temperature, high pressure, nutrient poor). The reactivity of the biotic experiment is evidenced by the specific evolution of the water composition (pH, Ca, SO_4). The characterization of the solid phase (MEB, MET, µRaman, µDRX) will be carried out at the opening of the cells after about one year of experiment in order to verify and complete these hypotheses.

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Table 1. Chemical composition of Tournemire synthetic water (25 °C) in mmol L^{-1}

Na	K	Ca	Mg	NH_4^+	Cl^-	SO_4^{2-}	HCO_3^-	PO_4^{3-}
21.3	1.1	1.7	0.8	6	7.2	11.2	3.6	0.3

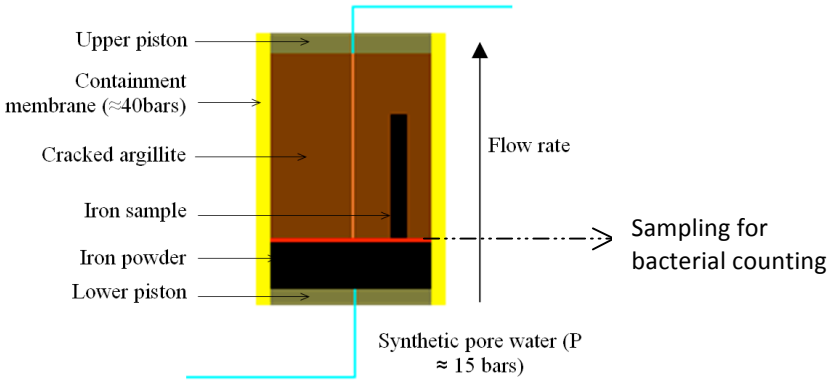


Fig. 1. Schematic view of the experimental cell

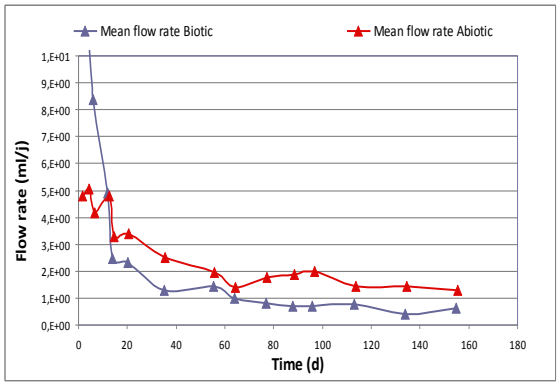


Fig. 2. Evolution of mean flow rate of each cell

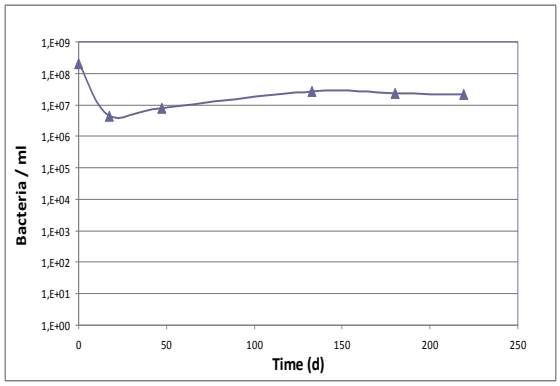
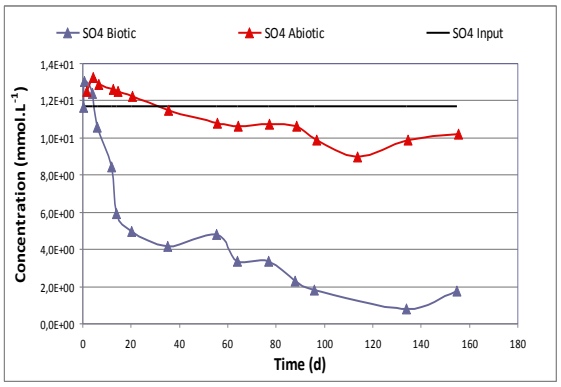


Fig. 3. Evolution of bacterial population in the biotic cell



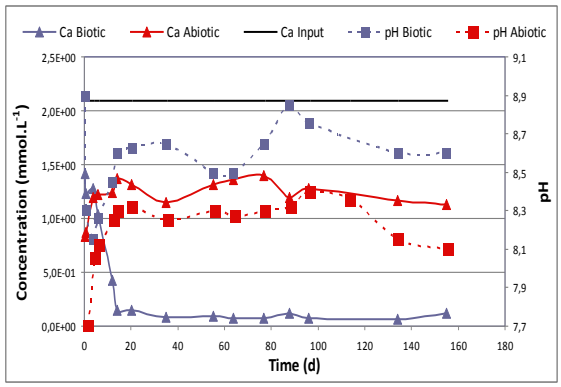


Fig. 4. Evolution of sulphate concentration (a) and calcium concentration and pH (b) in each cell