Applied Clay Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Clay Science

journal homepage: www.elsevier.com/locate/clay

Research paper

Microbial community changes induced by uranyl nitrate in bentonite clay microcosms

Margarita Lopez-Fernandez[a,*,1], Ramiro Vilchez-Vargas[b,2], Fadwa Jroundi[a], Nico Boon[b], Dietmar Pieper[c], Mohamed L. Merroun[a]

[a] Department of Microbiology, University of Granada, Granada, Spain
[b] Laboratory of Microbial Ecology and Technology, Ghent University, Ghent, Belgium
[c] Helmholtz Centre for Infection Research, Braunschweig, Germany

ARTICLE INFO

Keywords:
Bentonite
Microcosms
Microbial diversity
Uranium
Biomineralization

ABSTRACT

Deep geological repository (DGR) is one of the internationally accepted options to dispose radioactive wastes. Bentonite formations from Almeria, Spain, were selected as reference material for artificial barriers for the future Spanish repository. However, the safety of this long-term disposal could be compromised not only by physicochemical factors but also by microbial processes. The highly radioactive waste must be safely stored at least for 100,000 years for the radioactivity to decrease to similar levels to those of natural uranium. To simulate a scenario where the mobilization of radionuclides from the repository to the host formations may occur, long-term microcosms were studied. After being exposed to uranyl nitrate for 5 months, the response of the bentonite microbial community to the addition of this radionuclide was evaluated. High throughput 16S rRNA gene sequencing revealed that the structure of the microbial community after the uranyl nitrate treatment differs to that of the control microcosms. The microbial diversity was dominated by Firmicutes and Proteobacteria. Moreover, after the uranyl nitrate treatment OTUs annotated as *Paracoccus* and *Bacillus* were highly enriched. The mineralogy of bentonites was not affected by the uranyl nitrate treatment as was demonstrated by X-ray diffraction analysis. In addition, the study of uranium-bacteria interaction revealed the ability of isolates to biomineralize uranium as uranium phosphate mineral phases. Thus, the changes induced by the release of uranium in the microbial population may also affect the mobility of this radionuclide, making it less mobile and therefore less harmful for this environment.

1. Introduction

Management of nuclear waste is a serious environmental problem all over the world. A long-term disposal scenario is required for the safe storage of these hazardous wastes over hundreds of thousands of years for the radiotoxicity to decrease to levels similar to those of natural uranium and its products (Hedin, 1999). Since the 1970s, worldwide efforts have been focused on finding a safe and sustainable disposal concept for highly radioactive waste. The use of deep geological repositories (DGR) has been internationally proposed as the safest option for the disposal of these hazardous materials (IAEA, 2003). The general DGR concept is based on a multi-barrier storage system that entails encapsulating the waste in corrosion-resistant metal containers (first barrier), surrounded by a bentonite buffer (second barrier), and buried deeply within a stable geological formation (third barrier) (Ojovan and Lee, 2013). Clay formations are one of the candidate host rocks proposed for high-level nuclear waste repository. These environments have been extensively studied in Europe (Lopez-Fernandez et al., 2014; ONDRAF/NIRAS, 2001; Pedersen et al., 2017; Stroes-Gascoyne et al., 2007; Wouters et al., 2013). Additionally, bentonite clays are also a suitable material for the second barrier. Within this context, bentonite clays from Almeria, Spain, were selected as Spanish reference material for the engineering barrier after an extensive characterization of their mineralogical, geochemical and technological properties (Villar et al., 2006). However, over the last decades, the presence of microorganisms several kilometers below the surface has been demonstrated (Gold, 1992; McMahon and Parnell, 2014). Thus, safety of this long-term geological disposal could be compromised by physical and chemical factors, but also by biogeochemical activity of either indigenous or microorganisms introduced during the construction of the repository

* Corresponding author.
E-mail address: margaritalopez@ugr.es (M. Lopez-Fernandez).
[1] Present address: Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, Kalmar, Sweden.
[2] Present address: Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany.

https://doi.org/10.1016/j.clay.2017.12.034
Received 12 October 2017; Received in revised form 14 December 2017; Accepted 18 December 2017

(Meleshyn, 2011; Stroes-Gascoyne and West, 1997). The bentonite barrier performs important functions in maintaining the integrity of the metal canisters isolating the spent fuel; containing, preventing, and retarding the dispersion of radionuclides; and acting as a buffer against rock movements (SKB, 2010). Therefore, it is crucial to study the microbial population in the proposed bentonite buffer engineering barrier as microorganisms could impact the stability of the repository.

Previous studies have extensively investigated the microbial diversity in different types of commercially available bentonites such as Asha, Calcigel and Wyoming MX-80 (Bengtsson and Pedersen, 2017; Svensson et al., 2011) or Opalinus Clay (Leupin et al., 2017). The microbial diversity has also been studied in these Spanish reference bentonite clay formations (Lopez-Fernandez et al., 2015; Lopez-Fernandez et al., 2014). Microorganisms mainly affect the geochemistry of clays through three different mechanisms: reduction or dissolution of the structural clay minerals, e.g. Fe(III) (Pentráková et al., 2013); alteration of mineral surfaces by the production of siderophores and small-organic acids; and formation of biofilm on the clay mineral surface (Meleshyn, 2011). In addition, microbes are able to control the speciation and mobility of radionuclides (Newsome et al., 2014) through processes such as biosorption to the cell surface (Lloyd and Macaskie, 2000; Merroun et al., 2005), intracellular accumulation (Brookshaw et al., 2012; Suzuki and Banfield, 2004), biomineralization (Macaskie et al., 2000; Merroun et al., 2011), or biotransformations (Brookshaw et al., 2012; Lovley et al., 1993). Therefore, microbial processes occurring in the bentonite clay engineering barrier might play an important role in the mobility and migration of radionuclides in these environments.

There are several studies describing microorganisms influencing the speciation of radionuclides to be stored within a DGR, such as uranium (Lopez-Fernandez et al., 2014; Lütke et al., 2013; Lütke et al., 2012; Merroun et al., 2011), through a biomineralization process resulting in the formation of U(VI) phosphate mineral phases probably due to the activity of acid or alkaline phosphatases. However, these studies were conducted using bacterial pure cultures that did not simulate the natural conditions of the DGR of nuclear wastes. Nevertheless, there are some works studying the effect of uranium addition in the microbial diversity of soil microcosms (Geissler et al., 2009), dune sand microcosms (Handley-Sidhu et al., 2009) and sediment microcosms (Salome et al., 2013). But, the mentioned studies were not focused on clays considered as engineering barriers for DGR of radioactive wastes. Nevertheless, it is important to consider the specific scenarios where uranium may be mobilized from the underground repository to the environment. Therefore, this work aimed to 1) investigate the mineralogical and microbial changes induced by the addition of uranyl nitrate to microcosms elaborated with bentonite clay samples from Almeria; and 2) study the role of uranyl-treated bentonite bacterial isolates in the mobility of uranium.

2. Materials and methods

2.1. Sample collection and description

Bentonite clay samples were collected from two different locations of Almeria, Spain. Sample called BI was collected from El Cortijo de Archidona and sample BII was taken from El Toril. Afterwards, samples were transported on ice to the laboratory and processed immediately. Geochemical and mineralogical characterization of these two different clay samples was previously described in Lopez-Fernandez et al. (2014).

2.2. Preparation of clay microcosms

Microcosms were prepared in sterile Petri dishes using 20 g of dry crushed bentonite clay soil from El Cortijo de Archidona (BI-A, BI-B and BI-C) and from El Toril (BII-A, BII-B and BII-C). All microcosms were treated with an appropriate volume (20 ml for bentonite BI and 16 ml for bentonite BII) of the corresponding solution to saturate the bentonite. The solutions were gently and homogenously added to the microcosms, then, the microcosms were tightly closed to keep them moist over the incubation time. Sodium nitrate and uranyl nitrate solutions were prepare by adding 151 ppm of $NaNO_3$ and 300 ppm of $UO_2(NO_3)_2$, respectively, and sterilized by filtration (pore size 0.22 µm). Microcosms called BI-A and BII-A were treated with sterile MilliQ water (water controls). BI-B and BII-B microcosms were nitrate-controls, treated with a sodium nitrate solution; and microcosms BI-C and BII-C were treated with a uranyl nitrate solution and considered as uranyl-treated samples (Table 1). Water-controls (-A) were included in this study to test the effect of the long term incubation under the same experimental conditions. Nitrate-controls (-B) were added to investigate the nitrate effect on the bacterial diversity since uranyl ion was added as uranyl nitrate. The pH of the microcosms was measured as described in Lopez-Fernandez et al. (2015). The pool of microcosms was incubated at room temperature in darkness under oxic conditions, for five months.

Table 1
Bentonite and microcosm samples description: treatments, pH and incubation time.

Sample	Treatment	ppm	pH	Incubation time
BI	–	–	9.08	0 month
BI-A	Water	–	9.03	5 months
BI-B	$NaNO_3$	151	8.58	5 months
BI-C	$UO_2(NO_3)_2$	300	7.32	5 months
BII	–	–	7.86	0 month
BII-A	Water	–	7.82	5 months
BII-B	$NaNO_3$	151	7.61	5 months
BII-C	$UO_2(NO_3)_2$	300	6.73	5 months

2.3. X-ray diffraction analysis

The mineralogy of the bentonite clay microcosms used in this work was studied by X-Ray diffraction (XRD) before and after the different treatments and 5 months incubation time. The mineralogy before the treatments and incubation is considered time 0 and was earlier reported in Lopez-Fernandez et al. (2014). For the analysis after the incubation time, the diffractometer used was a Bruker D8 Advance instrument with Bruker Linxeye detector, Cu Kα radiation (λ = 1.5406 Å), a 2θ explored area of 5 to 70°, and a goniometer speed of 0.02° 2θ s^{-1}. XRD goniometer calibration was performed using a silicon standard. Powder samples were placed in zero-background silicon sample holders.

2.4. DNA extraction and Illumina sequencing

To study the microbial diversity of the bentonite clay microcosms total DNA was extracted in triplicates using the Fast Prep DNA extraction protocol described in Vilchez-Vargas et al. (2013), with some modifications. Briefly, microcosm samples (1 g) were mixed with 200 mg of glass beads and 1000 µl of lysis buffer (Tris/HCl (100 mM, pH 8.0), supplemented with 100 mM EDTA, 100 mM NaCl, 1% (wt/vol) polyvinylpyrrolidone and 2% (wt/vol) sodium dodecyl sulfate). Cells were lysed in a Fast Prep-24 instrument (40 s, 6 m s^{-1}), two times. Samples were centrifuged at 14000*g* for 5 min. Supernatants were collected in new tubes and pellets were dissolved into 1000 µl of MilliQ water and disrupt another two times (40 s, 6 m s^{-1}). This extra step was included to detach all cells from clay particles. After that, samples were centrifuged at 14000*g* for 5 min. All supernatants were washed with one volume of phenol/chloroform/isoamyl alcohol (25:24:1, v/v), pH 7. Then, samples were centrifuged at 14000*g* for 1 min and the aqueous phase was washed with one volume of chloroform. After centrifugation, nucleic acids present in the aqueous phase were precipitated with one volume of ice-cold isopropanol and 1:10 volume of 3 M sodium acetate. Finally, total DNA was washed with 80% ethanol and the pellet was dissolved into 100 µl of MilliQ water and purified