The effect of cesium ions on natural anaerobic microbial community in relation to safety of the radioactive waste repository

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Abstract

Radioactive isotopes and fission products represent a serious danger for humans and other living organisms. Radioisotope Cs-137 has been given a major attention because of long half-life, high solubility in water and similarity to potassium, a metabolically important chemical element. Interestingly, some groups of microorganisms can interact with Cs through accumulation or sorption and are suggested as excellent Cs bioremediators. In this study, the effect of different concentrations of non-radioactive Cs on the survivability of natural anaerobic bacteria was determined. The impact of Cs on bacteria was examined using molecular-biological methods and transmission electron microscopy techniques. Lower Cs concentration (0.5 mM) promoted the growth of bacteria (specifically total bacterial biomass and nitrate-reducing bacteria), while higher concentrations (1 mM) limited the bacterial growth, and 5 mM was found to be lethal for bacteria. These findings correspond well with results of TEM observations.

Key words: *cesium, anaerobic bacteria, radioactive waste repository, molecular-biological methods*

Introduction

Waste produced from nuclear power plants and other applications involving nuclear fission contains majority of radioactive material. The release of radioactive isotopes and fission products into the environment can cause a serious harm for humans and other living organisms. Major attention has been given to radioisotope Cesium-137, because of its long half-life, high solubility of Cs salts in water and its similarity to potassium which is a metabolically important chemical element (Ivshina et al., 2002). Cs-137 was also one of the main radioactive materials released from the Fukushima Daiichi nuclear power plant after the tsunami in March 2011.

Cs^+ ion can be assimilated by living organisms such as bacteria, fungi and plants even though it is regarded as a nonessential biological element (Kato et al., 2016, Gyuricza et al., 2010, White and Broadley 2000). Some bacterial species can tolerate high concentration of Cs^+ ion whereas to some it is toxic. As an example, *Cupriavidus metallidurans* strain CH34, the most Cs^+ resistant strain to date was observed to grow in the presence of 500 mM CsCl (Dekkar et al., 2014). On the contrary, the growth of common soil bacterium *Pseudomonas aeruginosa* was inhibited by ~0.45 mM Cs^+ (Kang et al., 2017). Microorganisms can take up Cs^+ either by the incorporation via K^+ transport system due to its chemical similarity to K^+ (Avery, 1995, Kato et al., 2016) or it can be adsorbed on the surface of cell walls (Lan et al., 2014). Due to such ability, microorganisms can be very useful for the removal of Cs from the contaminated sites (Komoriya et al., 2016).

Generally, the high-level radioactive waste is accepted to be finally disposed in a deep stable geological structure. This strategy entitles the waste material encapsulated in a metal canister surrounded by bentonite buffer embedded in a host rock. Nonetheless, the deep geosphere is not a sterile environment and therefore, microorganisms with diverse metabolic activity might be present in buffer material or in host rock (Masurat et al., 2010a). Moreover, microorganisms could also reach the repository site with flowing groundwater through fractures and fissure of bedrock or be introduced during the operation phase of the repository. Activity of anaerobic microorganisms under repository conditions

may influence and compromise the safety performance of the whole disposal system. Furthermore, in the radioactive waste disposal system, the only absolute barrier for radionuclides transportation is intact metal container (Masurat et al., 2010b). Consequently, any corrosion or damage of waste canister may result in release and transportation of radionuclides to the surface biosphere. In the event of canister failure, microorganisms can affect the performance of repositories by mobilizing radionuclides through the groundwater (Stroes-Gascoyne and Sargent, 1998). Hence, bioaccumulation of ions and migration of microorganisms can also help the transportation of the Cs^+. It was reported that bacterial exudates can influence the Cs^+ adsorption on clay minerals (Wendling et al., 2005), planned as backfill and buffer material. It was confirmed that sulphate-reducing bacteria (SRB) are able to decrease Cs^+ adsorption thus the mobility of Cs^+ would be enhanced (Russel et al., 2004). Therefore it is highly important to understand the interactions between Cs^+ and natural anaerobic microbial communities that will be present in deep geological radioactive waste repository.

The main aim of this study was to investigate the effect of non-radioactive Cs^+ on natural anaerobic microbial consortia from groundwater source. Particularly we focused on the effect of increasing Cs^+ concentration on sulphate-reducing and nitrate-reducing bacteria that might compromise the safety of deep geological radioactive waste repository.

Experimental part

Natural groundwater was collected at Josef Underground Research Centre, Czech Republic (source "VITA") right before performing the experiment. Preliminary characterization proved high abundance of anaerobic sulphate-reducing bacteria (SRB). A commercially available CsCl (Lach-Ner, Czech Republic) was used in the study. The concentrations of Cs^+ were 0.5 mM, 1 mM and 5 mM along with negative control without any Cs^+. The sub-samples were collected after 0, 1, 3, 6, 8 and 23 days. The experiment was performed under a strict anaerobic condition in the anaerobic glove box (Jacomex GP, France).

Live/dead staining was performed to determine bacterial viability by analyzing the membrane integrity of bacteria. Live/Dead® Baclight™ bacterial viability kit (L-7005, ThermoFisher) was used according to manufacturer's instruction. The kit contains mixture of nucleic acid binding dyes, SYTO® 9 green-fluorescent and propidium iodide, the green fluorescence for the detection of the alive and red fluorescence for detection of dead bacteria, respectively. Samples with different Cs^+ concentrations were prepared in duplicate and Live/dead staining was performed after 3 and 8 days. Each sample (10 ml) was first centrifuged at 5000 rpm for 15 min, and then the supernatant was discarded and the pellet was used for the staining analysis. 5 ul of sample (from the pellet) and 3 ul of probe was mixed and incubated under dark conditions for 15 min and analyzed under fluorescence microscope.

TEM analysis was performed on JEM-1011 microscope (JEOL, USA). Samples (30 ml) were centrifuged at 8000 rpm for 15 min and analysed by TEM without method of negative contrast coloring. Similarly to Live/dead staining, TEM was also performed after 3 and 8 days of the experiment.

The remaining volume of the samples was sterile filtered to concentrate the biomass for the further microbiological analysis. Molecular analysis was performed also at the beginning of the experiment and at day 1, 6 and 23. Likewise, the total DNA was extracted from the filters using MoBio Power Water DNA extraction kit (MoBio Laboratories, Inc., USA) following the manufacturer's protocol. The extracted DNA was quantified with a Qubit 2.0 fluorimeter (Life Technologies, USA). Quantitative polymerase chain reaction (qPCR) was employed to monitor the relative level of SRB, by detecting levels of genes for the adenosine-5'-phosphosulphate reductase apsA and dissimilatory sulfite reductase dsrA2 (Ben-Dov et al., 2007) and nitrate-reducing bacteria (NRB), by detecting relative levels of the nitrite reductase genes nirK and nirS and nitrous oxide reductase gene nosZ (Geets et al., 2007. In addition, 16S rDNA gene was used for monitoring the changes in total bacterial biomass using primer 16S rRNA (Clifford et al., 2012). Reaction mixtures were prepared as follows: per 10 µL reaction volume, 5 µL KAPA SYBR FAST qPCR Kit (Kapa Biosystems, Inc., MA, USA), 0.4 µL of 20 µM forward and reverse primer mixture (Generi Biotech, Czech Republic, IDT, US) with the addition of 3.6 µL ultra-pure water (Bioline, UK) and 1 µL of DNA. Each sample was analysed in duplicate with ultra-pure water used as a template in a negative control. The measurements were performed in duplicate using a LightCycler® 480 instrument (Roche, Switzerland) under the following reaction conditions: 5 min at 95°C for initial denaturation, followed by 45 cycles of 10 s at 95°C, 15 s at 60°C and 20 s at 72°C. Finally, a melting curve was set for 5 s at 95°C, 1 min at 65°C and final ranging from 60 to 98°C, with a temperature gradient of 40°C per 10 s. Purity of the

amplified fragment was determined through observation of a single melting peak. Crossing point values were obtained using the 'second derivative maximum' method included in the LightCycler® 480 Software. The method has different amplification efficiency for each prime, which was determined by measuring the slope of curves constructed from a serial dilution of a template DNA from five environmental internal standards. In the evaluation process, a water sample was normalized to dilution and the total amount of DNA was extracted. The relative abundance of the bacterial 16S rDNA gene and of functional genes was calculated and expressed as a fold change between two states (at a given sampling time and at an initial time) using the delta Cp method. Based on the approach used, it is necessary to consider each molecular biological marker separately and observe the trends in the relative abundance over time.

Results and discussion

Relative quantification of SRB, NRB and total bacterial biomass

The total bacterial biomass significantly increased in the sample containing 0.5 mM of Cs^+ after six days and this situation remained until the end of the experiment (Figure 1A). Similarly, bacterial abundance increased after three days in control without Cs^+. In contrast, 1mM and 5 mM of Cs^+ did not support proliferation of bacteria.

SRB did not proliferate in any Cs^+ treatment including control (Figure 1B, C). On the other hand, all three marker genes for NRB showed similar pattern: enhanced growth at 0.5 mM of Cs^+ after six days (up to 100-fold and 400-fold increase detected by nirK, nosZ and nirS genes, respectively) (Figure 1D, E, F). The positive effect of Cs^+ might be due to changes in environmental conditions as Cs^+ quickly oxidizes and interacts with oxygen-donor ligands more easily than with nitrogen-donor ligands (Avery, 1995) which might favour NRB. As a consequence, the growth of NRB could decrease the redox potential in the waste repository. Higher concentrations of Cs^+ (1 and 5 mM) were clearly toxic to NRB, most probably due to Cs^+-induced K^+ starvation and impairment of metabolic pathways comprising K^+-dependent enzymes (Avery, 1995).

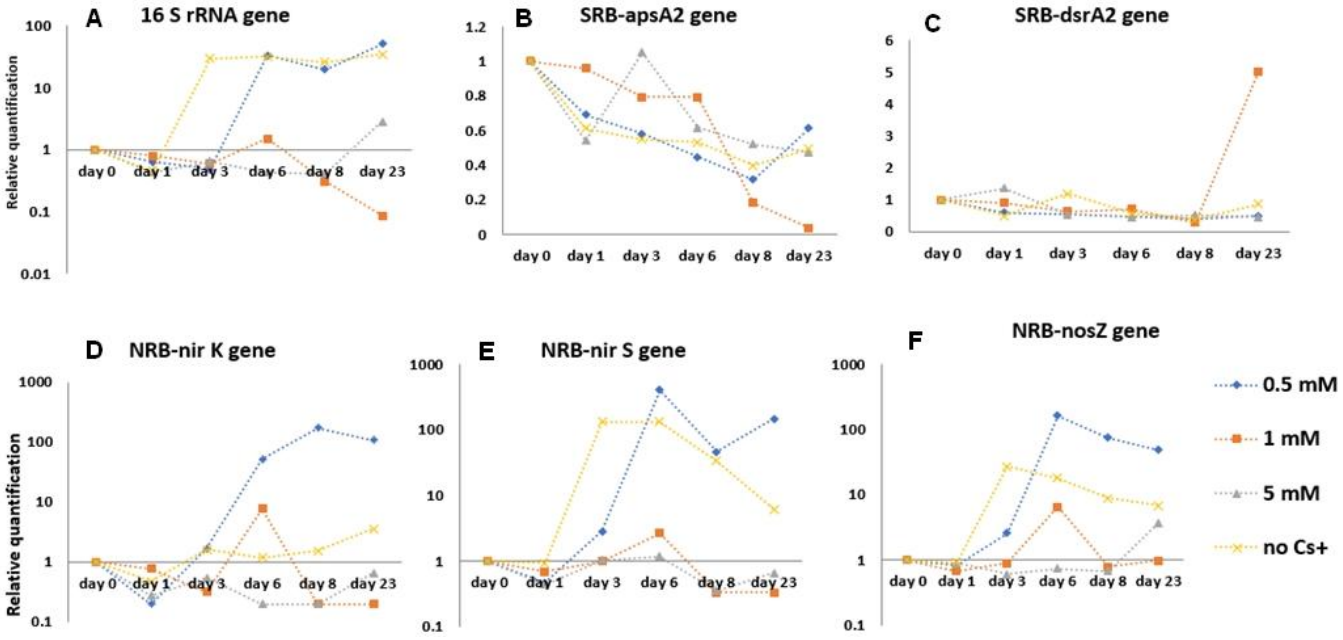

Figure 1: Relative quantification of specific gene markers in different concentration of Cs+ (0, 0.5, 1, and 5 mM) during 23-day experiment determined using qPCR. Note logarithmic scale of A, D, E, and F.

Live/Dead staining

Living bacteria were present in higher number at lower Cs^+ concentration (0.5 mM) whereas much higher proportion of dead bacteria was detected at higher Cs^+ concentration (5 mM) after three and eight days (Figure 2). The results obtained from Live/Dead cell staining analysis correspond well with the results of qPCR analysis.

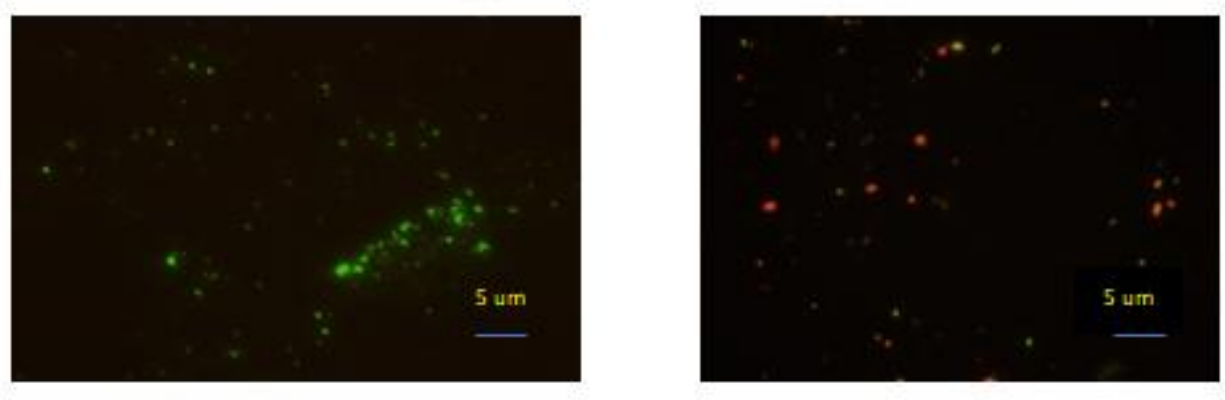

Figure 2: Live/Dead cell staining analysis - the presence of living bacteria is denoted by green fluorescence in 0.5 mM Cs^+ concentration (left) and mostly dead bacteria marked with red fluorescence in 5 mM Cs^+ concentration after 8 days.

TEM analysis

TEM results are consistent with results obtained from qPCR and Live/Dead cell staining analyses. Numerous bacterial cells were detected using TEM in 0.5 mM of Cs^+ (Figure 3). Similarly, in the 1 mM Cs^+ solution some bacteria were observed (although in lower quantity compared to 0.5 mM solution), whereas at the highest Cs^+ concentration only a few destroyed bacterial cells were found.

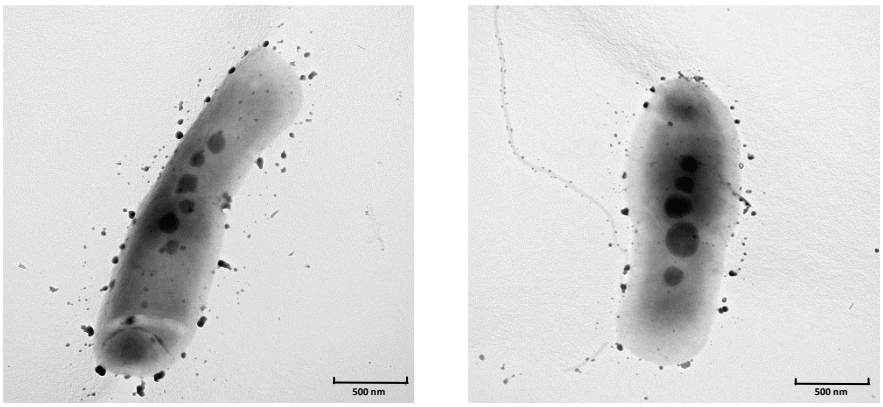

Figure 3: TEM image showing the presence of bacteria in 0.5 mM Cs^+ after 8 days.

Conclusion

We described effect of increasing concentrations of non-radioactive Cs^+ ions on anaerobic microbial consortium from natural underground water. Lower concentration (0.5 mM) of Cs^+ promoted the bacterial proliferation, particularly total biomass and NRB, while 1 mM solution limited the bacterial growth, and 5 mM of Cs^+ was found to be lethal for natural bacteria. These findings were supported by similar observations using Live/Dead cell staining, and TEM analysis. Although the higher concentration of Cs^+ displayed inhibitory effect to bacteria, lower concentration can yet induce the growth of NRB which can influence the redox conditions of the radioactive waste repository. Further study that would include measurement of physico-chemical conditions and sequencing techniques could reveal more information about influence of Cs on natural microbial communities and its relevance for safety of the radioactive waste repository.

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