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Research paper

Microbial metabolism in bentonite clay: Saturation, desiccation and relative humidity

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ABSTRACT

Within a Deep Geological Repository for used nuclear fuel storage, compacted bentonite clays are the candidate buffer due to their physical and rheological properties, and their ability to suppress microorganisms. This study focused on the potential for microbial metabolism at bentonite-air interfaces, the influence of relative humidity (RH) and the consequences of metabolic activity on bentonite. Microbial activity, determined by monitoring the concentration of evolved CO_2, was sustained at desiccated bentonite-air interfaces at 75% RH (0.6 ppm CO_2/min after 5 days of dessication) but was completely suppressed at 30% RH. Conversely, microbial survival was promoted in dry bentonite, with culturable cell survival up to 3 times higher at lower RH (30%) than higher RH (75%). It was also shown that, under water-saturated conditions, microbial sulphur reduction decreased the clay swell index of uncompacted bentonite, swelling approximately 2.7 cm/(g dry weight) less than controls. Notably, natural groundwater salinities were shown adequate to suppress all microbial activity under both saturated and desiccated conditions, confirming that a combination of high bentonite dry density and high salinity inhibits microbial activity, even in microenvironments like surface-air interfaces where swelling pressure limitations may be transiently compromised. Along with the applied need for this knowledge, this study also provided a fundamental opportunity to explore microbial activity in desiccated environments, and suggests that lower RH may promote rapid entry into a dormant cell state and thus more effective long-term adaptation.

1. Introduction

Many countries are actively developing deep geological repositories (DGR) for the long-term storage of used nuclear fuel. In Canada, the Nuclear Waste Management Organization (NWMO), mandated by the Nuclear Fuel Waste Act (2002), has implemented a site-selection program to develop a DGR in a willing, informed host community (NWMO, 2005; NWMO, 2010). As of 2014, Canada had accumulated approximately 2.5 million CANDU fuel bundles (ca. 50,000 t of heavy metal, t-hm) in reactor site storage, projected to increase to >3.4 million fuel bundles (ca. 69,000 t-hm) prior to DGR emplacement (Garamszeghy, 2010). DGR designs rely on engineered and natural barriers to isolate radionuclides. The Canadian engineered barrier system (Fig. 1) consists of copper-coated steel used fuel containers (UFC's), containing 48 CANDU bundles each, surrounded by highly-compacted bentonite (HCB) clay. The natural barrier system will be 500 m of overlying low-permeability sedimentary or crystalline host rock. The UFC is designed to provide containment for at least 100,000 years; whereas, the HCB clay and host rock will be relied upon for isolation of radionuclides to one million years.

The primary microbiological concern in the DGR is that metabolic sulphide production could cause microbiologically influenced corrosion (MIC) of the UFC's. Highly compacted Wyoming bentonite (MX-80) was the buffer initially selected and designed to physically and chemically protect the UFC's. The fact that the HCB can suppress microbial activity was discovered through research, and the HCB requirements in the NWMO design are now such that the HCB will suppress microbial activity (Fig. 1; NWMO, 2005). Water activity (a_w) is the key parameter limiting microbial survival in a DGR environment (Stroes-Gascoyne et al., 2010). The HCB compaction and resultant swelling pressure, combined with the natural salinity of groundwater, are relied upon to limit a_w and prevent MIC (Stroes-Gascoyne et al., 2010; Masurat et al., 2010). Low bentonite porosity and permeability limit transport of metabolites and microbes from the host rock (Sherwood Lollar, 2011) through the bentonite buffer by creating a diffusion-dominated environment (Stroes-Gascoyne et al., 1997; Pedersen, 2010; Hallbeck and Pedersen, 2012).

Saturated, compacted bentonite microbiology has been assessed in terms of aerobic culturable numbers and migration (Stroes-Gascoyne et al., 2010). Compaction of bentonite to a dry density of 1.6 g/cm^3, with a corresponding saturation swelling pressure of >2 MPa and a_w

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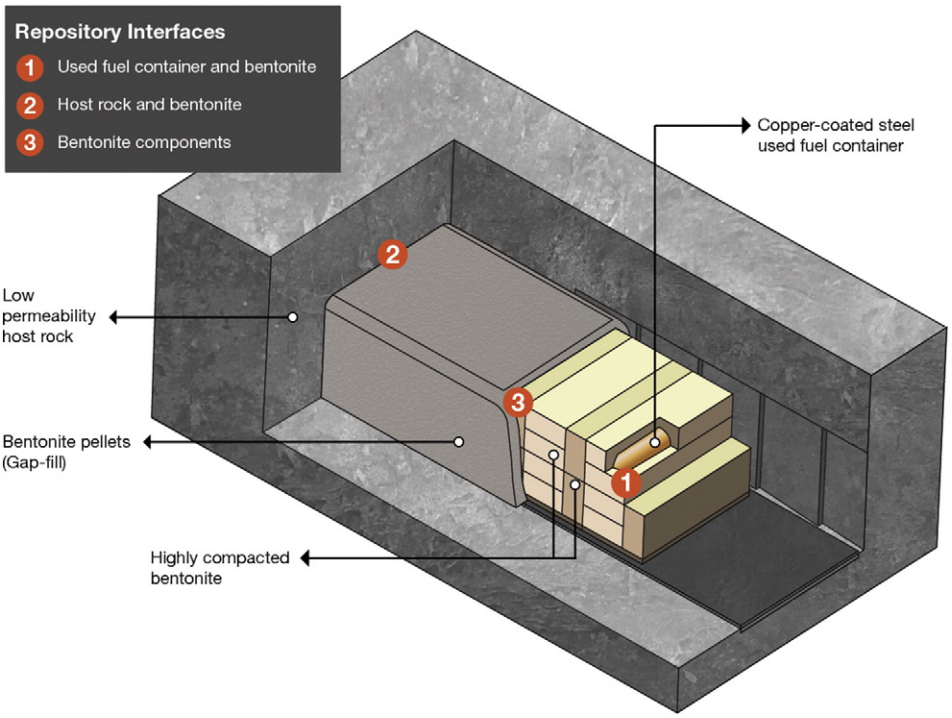

Fig 1. The Canadian engineered barrier system in a deep geological repository for used nuclear fuel storage. The current Canadian design employs copper used fuel containers as a first barrier for the prevention of radionuclide escape from CANDU fuel bundles, and highly-compacted bentonite (HCB) blocks as a buffer between the used fuel containers and the host rock. The interface between the host rock and HCB is of greatest microbiological interest.

of <0.96, limited culturable microbial numbers to background levels in as-received, powdered bentonite. These limiting parameters, combined with used nuclear fuel heat and radiation, are expected to create a virtually sterile zone in the UFC-HCB buffer interface and its immediate vicinity (Fig. 1). However, limiting parameters may not be maintained at HCB buffer-host rock interfaces due to non-homogenous swelling. This warrants further study to determine if these interfaces could provide transient microenvironments conducive to microbial activity (Pedersen, 2010; Stroes-Gascoyne et al., 2011; Wolfaardt and Korber, 2012).

Although anaerobic Sulfate Reducing Bacteria (SRB) have received the most attention, other potential microbial DGR impacts include weathering of bentonite and gas production (Mulligan et al., 2009). Biofilm formation at HCB-host rock interfaces may affect the transport of radionuclides and metabolites or provide microenvironments that promote microbial survival and activity. Stroes-Gascoyne et al. (2010, 2011) postulated the occurrence of DGR moisture gradients that would influence microbial activity. Heat from radioactive waste would drive water out of the repository, with subsequent cooling resulting in increased moisture levels. Desiccation would be expected to limit microbial activity, whereas upon saturation, clay swell pressure would additionally limit a_w and microbial metabolism. During the transition between the dry and wet phase, microbes may have access to water or water vapor without sufficient clay swell pressures to limit activity. Microbial activity in non-uniform microenvironments could lead to the production of extracellular polymeric substances (EPS) and metabolites, changing the properties of the clay matrix.

It is accepted that microbes survive desiccation due to dormancy, a cessation of metabolic processes. DGR microbiology has thus focused on characterizing microbial survival under desiccation, and metabolic impacts within saturated HCB. However, biofilms provide alternative survival strategies during desiccation (Chang et al., 2007) that may sustain metabolism at repository interfaces. As such, the overall objective of this study was to explore the potential for microbial activity at bentonite-air interfaces. The three main questions posed are:

(i) Can microbial biofilms at bentonite-air interfaces metabolize during desiccation under saline and non-saline conditions?
(ii) Does relative humidity (RH) have an effect on microbial metabolism at bentonite-air interfaces?
(iii) Does microbial activity influence the swelling ability of bentonite, a key suppressive attribute?

These questions evaluate potential impacts of microbial activity in DGR interface microenvironments, when limiting parameters may not be maintained during the heat-shrinking and moisture-swelling bentonite gradient.

2. Materials and methods

2.1. Microbes: activity and survival in desiccated bentonite

2.1.1. Isolation and identification of the aerobic culturable bentonite microbial community

Uncompacted Wyoming VOLCLAY MX-80 bentonite powder (American Colloid Company, Wyoming, USA) and compacted bentonite blocks (14.4% water, 1.61 g/cm^3 dry density, 1.88 g/cm^3 bulk density, 5 × 5 × 5 cm, crushed with a spatula for isolations) were stored in sealed bags and handled aseptically. Enrichments involved (1) direct scattering of bentonite powder (0.5 g/plate) on agar media and (2) detaching microbiota from bentonite using sonication. For detachment, 2 g bentonite (100 g/L) was suspended in 20 mL NaCl (8.9 g/L) in 50 mL Falcon tubes, and the resultant slurry sonicated (Branson B3510 Ultrasonic Bath, Danbury, CT, USA; 335 W, 40 kHz, 45 min) according to Mermillod-Blondin et al. (2001). The undiluted slurry (500 μL) was plated on agar media. Eukaryotes were enriched on full- and 10% strength Yeast Malt Agar and ¼ strength Cornmeal Agar. Prokaryotes were enriched on Tryptic Soy Agar (3 g/L) and R2A Agar (18 g/L), all at natural bentonite pH (9.0). Media recipes were according to Atlas (2010) and chemicals purchased from Sigma-Aldrich (Oakville, ON, Canada). Colonies were selected for isolation based on distinctive