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Column experiment for assessing microbial behavior around radioactive waste repositories, including migration of potentially radionuclide-accumulating bacteria

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ABSTRACT

To assess microbial behavior at anticipated repositories of nitrate-containing radioactive waste such as TRU waste, we set up an anoxic single horizontal column filled with Pleistocene sand with indigenous microorganisms as model samples. The column was supplied with artificial groundwater containing nitrate and acetate for 9 weeks (Run 1) or nitrate-amended groundwater from the same Pleistocene stratum for 6 weeks (Run 2). Bacterial communities, including culturable denitrifiers, were established in the sand bed, resulting in acridine orange direct counts per pore water of 3×10^8 cell mL^{-1} in Run 1 and 5×10^7 cell mL^{-1} in Run 2 and nitrate-reducing activity per pore water of roughly 13 mg L^{-1} d^{-1} in Run 1 and 1–4 mg L^{-1} d^{-1} in Run 2. Eh and hydraulic conductivity declined in Run 1, indicating microbial activity capable of retarding radionuclide transport. However, the ratio of bacterial cell concentration found in the effluent water (free-living bacteria) to the total bacterial concentration in sand (R_{mobile}) exceeded 2%. This finding is relevant to the increase in radionuclide transport associated with free-living cells. As a tool for quantifying this influence, we introduced an index, $K^{d,att}$ (distribution coefficient for microbes on sand particles), and calculated this value from the R_{mobile} value. By sensitivity analysis using a numerical simulation model (MINT), we then demonstrated that higher $K^{d,att}$ values would suppress the detrimental effects of the free-living bacteria. Quantification of microbial influences can be made more realistic by obtaining $K^{d,att}$ values in a column experiment and incorporating this index into radionuclide transport models.

1. Introduction

The geological disposal of radioactive waste has been implemented or is currently planned in many countries. Despite significant variation in the details of individual repository systems, a common concern is the quantification of the behavior of repository systems over very long periods of time. Given the requirement for performance assessments, it is important to consider the many potential effects of microbial activity in a waste repository (West et al., 2002). The importance of microbes in and around the repository is convincing for the following reasons: First, independent scientific work has unambiguously demonstrated life to be present in most deep geological formations investigated, down to depths of several kilometers (Pedersen, 2000). Second, over very long periods, the metabolic activity of deep subsurface microorganisms may be responsible for the slow mineralization of organic compounds and the release of products into groundwater (Madigan and Martinko., 2006). Third, such microbial activity may be stimulated in and around a repository by the release of energy sources and nutrients from waste or engineered barriers (West and McKinley, 1984; Stroes-Gascoyne et al., 1997) or by the excavation of a disposal vault (Stroes-Gascoyne and Sargent, 1998). Fourth, this microbial activity may have various effects on the transport of radionuclides, including its retardation through their association with biofilms, the precipitation of radionuclides due to changes in the geochemical environment such as pH and Eh effected by microbes, pore clogging by profuse growth of microbes in porous materials, and detrimental effects such as transport of radionuclides adsorbed by mobile microbial cells (West and McKinley, 1984; Stroes-Gascoyne and Sargent, 1998; Fox et al., 2006).

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Extensive field studies have been carried out to provide quantitative information on subsurface microbial activity. The quantitative data gathered to date on microbial abundance in deep underground test sites is summarized by Pedersen (2000), West et al. (2002), Wang and Francis (2005), and Fukunaga (2008). Microbial growth rates and activity in groundwater have been measured not just in laboratories using field samples (Krumholz et al., 2002; Horn et al., 2004), but *in situ* using submerged coupons (Pedersen and Eckendahl, 1992; Stroes-Gascoyne and Sargent, 1998), membrane chambers (Lehman et al., 2004; Nagaosa et al., 2008), or circulation systems (Hallbeck and Pedersen, 2008). To investigate microbial activity in repositories, full-scale container experiments have been carried out in Canada in an underground granitic rock research laboratory (Stroes-Gascoyne et al., 1996; Stroes-Gascyone, 2010). Hama et al. (2001) and Tuck et al. (2006) identified microbial activity affecting rock-water interactions, which would affect permeability of groundwater around repositories. There are extensive studies on the interactions between microbes and radionuclides (Francis, 1998; Lloyd and Renshaw, 2005; Behrends et al., 2012).

In Japan, the TRU waste that will be generated during the operation and dismantling of reprocessing facilities and MOX fuel fabrication facilities containing long-lived radionuclides such as C-14, I-129, and Np-237 and high level radioactive waste (HLW) are to be disposed of in stable rock deeper than 300 m. The principles of deep geological disposal are outlined in JNC (2005a) and in JAEA and FEPC (2007), focusing on passive safety measures established by multiple engineered and natural barriers. The key steps of a quantitative safety assessment involve scenario analysis characterized by FEPs (Features, Events, and Processes), model development accounting for uncertainties via sensitivity analysis or stochastic approaches, and consequence analysis, such as assessments of doses released into the biosphere. The scenario includes radionuclide transport by groundwater through a matrix of rock and fractures (JNC, 2005b). JAEA (2014) discussed future surveys of various issues, including the co-disposal of various TRU waste and HLW, evaluations of major incidents, and the development of computational methods and other advanced techniques. It also presented a disposal system concept that accounts for reversibility and recovery.

For safety assessments, both reports (JAEA and FEPC, 2007; JNC, 2005a) consider microbial activity having safety implications. For TRU waste, transport of radionuclides as complexes with microbial metabolites, the behavior of colloids, including radionuclide-accumulating microorganisms, and the production of radioactive gases such as $^{14}CH_4$ are given as examples of uncertainties (JAEA and FEPC, 2007). For HLW, the development of simulation codes, confirmation of the applicability of the codes, and databases used for associated repository design are emphasized (JNC, 2005a). JNC (2005b) introduced studies that assess the radionuclide transport associated with microorganisms as colloids and their retardation in cases in which biofilms predominate. They (JAEA and FEPC, 2007; JNC, 2005b) focused on the microbial influences on natural barriers for either crystalline rock or sedimentary rock as the targeted rock type. Methods for estimating permeability and biofilm formation of biotic columns have been established and applied to granite (Hama et al., 2001) and sedimentary rock (Harrison et al., 2011; Wragg et al., 2012). JAEA (2014) cites unsolved issues related to microbial influence, namely sorption of radionuclides by colloidal microorganisms, the sorption potential of biofilm for radionuclides, effects on the sorption potential of rock for radionuclides, and the influence of the metabolism of VBNC (viable but not culturable) microorganisms on natural barriers. While integrating the above knowledge will reduce the uncertainties associated with microbial activity, it remains unclear whether the stimulation of radionuclide transport by colloidal microorganisms (free-living bacteria) exceeds the retardation of radionuclide transport by other results of microbial activity, such as biofilm formation. Relatively little work has been done to quantify how many microbial cells would migrate as free-living cells or would be unable to move through the rock matrix in the environments around proposed repositories.

The primary goal of this study was to propose a column experimental method and to implement it using model samples, thereby adding to our knowledge regarding the applicability of simulation codes, considering microbial influences. Our column is relatively large and packed with sand. It is characterized by lateral flow and has water pools and ports to allow separate sampling of sand bed and water. These characteristics enable comparisons of total and free-living bacteria by allowing counts of bacteria in the sand bed and the water pool, respectively, as well as measurements of the hydraulic conductivity of the sand bed and water chemistry parameters such as pH, Eh, and substrate concentrations during column operation. The experiment models groundwater flow paths like those occurring in mineral filled fractures, wherein nitrate or organic matter may disperse from a repository (Humphreys et al., 1997; Crawford et al., 2006; Nazina et al., 2010). However, this study used sand and groundwater from near-surface and artificial groundwater as model samples. Microorganism preparation generally uses one of two strategies: seeding pure bacterial cultures to a sterile column, as done by Witt et al. (2001), or using indigenous microorganisms in packed sand or soil, as done by Wan et al. (2005). We took the latter approach. While this approach renders physiological analysis difficult, it allows the complex microbial communities found in the field to reappear in the column. Recent refinements in molecular biological techniques may also allow monitoring of microbial community structures.

The second purpose of this study is to apply an index, distribution coefficient for microbes on sand particles, obtained in this experiment, to sensitivity analysis using a numerical simulation model that describes bacterial growth, death, sorption to solids, and movement. This would allow use of the column experiment to help predict the amounts of migrating bacteria at the outlet of a fracture.

2. Methods

2.1. Experimental apparatus

We designed the column reactor shown in Fig. 1 and 2, after Witt et al. (1999), Hama et al. (2001), and Kanno et al. (2003). The reactor is a box made of acrylic resin having the following dimensions:

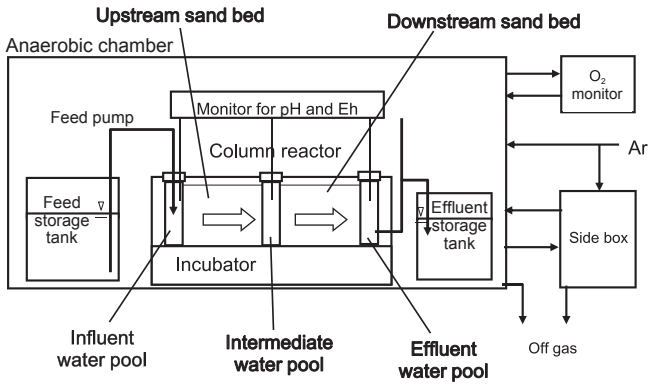

Fig. 1. Experimental apparatus placed in anaerobic chamber.