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MICROBIAL PROCESSES IN RADIOACTIVE WASTE REPOSITORY

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Abstract

Microbial processes could potentially affect the performance of a radioactive waste disposal system and related factors that could have an influence on the mobility of radionuclides are outlined. Analytical methods, including sampling of water, rock and surface swabs from a potential disposal site, are described and the quantitative as well as qualitative experimental results obtained are given. Although the results contribute to an understanding of the impact of microbial processes on deep geological disposal of nuclear waste, there is not yet sufficient information for a model which will predict the consequences of these processes.

1. Introduction

Bacteria are much more diverse in comparison with plants and animals. Among the huge diversity of bacteria there are micro-organisms capable to grow at or adapt to extreme conditions. Some bacteria grow at temperature above 100 °C, other thrive in high salinity such as 20-30 % NaCl, still others can live at pH lower than 2 or pH higher than 10 or exhibit high radio-resistance. Due to accelerated disarmament and nuclear energy activities, large quantities of radioactive waste and nuclear fuel are being placed in storage areas. The awareness that the microbial activity could potentially affect the performance of the system for geological disposal of radioactive waste gained acceptance in the early to middle 1980s, and, consequently, many countries consider to develop programmes to study and quantify microbial effects in terms of their own particular disposal concept.

Our programme concerns several major factors that may have an influence on the mobility of radionuclides in direct and indirect ways thereby being important for the safety analysis. They are uptake and transport of radionuclides by micro-organisms, diversity and distribution of subterranean bacteria in typical repository environments, environmental limitation and bacterial activity, effect of bacterial activity on the mobility of radionuclides, microbial gas production and consumption, bacterial recombination of hydrogen and oxygen from radiolysis, and microbial induced corrosion of waste canister.

The Permian Boda Claystone Formation in the Mecsek Hill area is being considered for high level waste disposal. Groundwater, technical water, rock and surface samples were collected and gas, organic acid, siderophore production, biosorption of radionuclides and radiosensitivity of isolates were studied.

2. Materials and methods

In October 1997 and 1998, samples were taken via this access tunnel from the air, groundwater, aleurolite stones and surfaces. The bore-hole-samplers were built by using carbon steel casing and stainless steel screens. Groundwater sampling for chemical analysis

was carried out in parallel with sampling for microbiological analysis. All bore-holes were sampled on numerous other occasions as well.

Sample analysis:

Sterile, 1 liter bottles were filled with water for bacteria counts. From all water samples, 500 ml was placed in an anaerobic container for anaerobic cultivation. All samples were transported to the laboratory for analysis within 4 hours.

Air samples: Fifteen open Petri dishes with Nutrient agar (Oxoid CM 82) were positioned at a sampling depth of 510 m for 20 min. Eight plates were incubated under aerobic conditions at 35°C for 4 days. Seven plates were incubated anaerobically (Gas Pak, H_2+CO_2) at 35 °C (imitating the temperature of the air in the tunnel) for 4-14 days. After incubation, all well-separated colony types, based on morphological appearance, were isolated.

Water samples: The numbers of viable mesophilic anaerobic and of mesophilic and thermophilic anaerobic bacteria were investigated by the Most Probable Number technique, using Nutrient Broth (Oxoid CM 79). One ml samples were added to 10 ml of Nutrient Broth, with 3 replicates in 4 dilutions. After incubation, 0.1 ml of inoculum from positive samples was spread on Nutrient agar plates and the individual colonies were isolated. Incubation was performed at 35°C for 5 days (mesophiles), or at 55 °C for 5 days (thermophiles). The mesophilic aerobic counts of bacteria were determined by a plate count technique on Nutrient agar (Oxoid CM 67). The samples were serially diluted and 0.1 ml was spread on the agar plates. The plates were incubated at 35 °C for 5 days. After incubation, every morphologically distinct colony was isolated.

Aleurolite (rock) samples: Ten g samples (particle size 100 μ) were shaken (125 rpm) with 90 ml of sterile physiological saline solution (0.9 %) for 30 min. After dilution, 0.1 ml was spread on Nutrient agar plates. Separate colonies of different types were isolated. Incubation was carried out at 35 °C for 5-7 days, either aerobically or anaerobically.

Surface samples: From the walls of the access tunnel, 19 samples were taken with sterile wet swabs. The swabs were suspended in 5 ml of sterile physiological saline solution and 0.1 ml was spread on Nutrient agar. Incubation was performed at 35 °C, for 5-7 days, either aerobically or anaerobically.

The radiosensitivity of the isolated spore formers was determined. Spores were produced as a surface growth (9 days at 37 °C) on Potato Dextrose agar (Oxoid CM 139). They were harvested in distilled water, washed three times by centrifugation. The suspension was then heated at 80 °C for 15 minutes to inactivate any remaining vegetative cells, cooled, washed three more times and finally re-suspended in distilled water to give 10^7 spores ml^{-1} (Tallentire and Khan, 1975). These suspensions were irradiated in air at room temperature, with exposure to 1, 2, 3 and 4 kGy. The irradiation facility was an RH–Y–30 ^{60}Co apparatus with a dose rate of 2.0167–2.0475 $kGyh^{-1}$.

After irradiation the survivors were detected on Nutrient Agar Medium (Oxoid CM 67). Incubation was performed at 35 °C for 72 h. D_{10} value is defined as the dose that reduces a given population by a factor of 10. The linear plot can be expressed mathematically as ln

S/S_0= ln n – kD, where S is the number of cells surviving treatment with dose D, S_0 is the initial number of viable cells, and k is a constant equal to the slope of the curve.

3. Results and conclusion

The quantitative and qualitative analysis of aerobic and anaerobic isolates were established. Sixty-seven air, groundwater, technical water, rock and surface samples were collected aseptically from the potential repository site. The number of aerobic and anaerobic isolates was 300. The proportion of spore-forming isolates was 50.6% among the aerobic bacteria and 59.4% among the anaerobic bacteria. There were more mesophilic aerobic and anaerobic bacteria in the technical water than in the other samples. The thermophilic anaerobic counts in the water and in the technical water (7.3×10^0-4.6×10^4 and 0.43-4.6×10^4) were higher than the aerobic counts (0-2.4×10^2 and 9.5×10^1-1.1×10^3) (Table 1).

Table 1. Mesophilic aerobic and anaerobic CFU counts of samples

Sample origin	Aerobic bacteria ml^{-1}	Anaerobic bacteria ml^{-1}
Air*	1,07-5.84x10^2	0.22-1.04x10^2
Water	0.39-1.2x10^5	0.36-3.9x10^3
Technical water	0.27-5.03x10^6	>4x10^5
Aleurolite**	2.32x10^2-2.47x10^5	0,45-9,5x10^2

* count per Petri dish
** count per gram

The gases produced by the gas-forming isolates were CO_2 and H_2 (anaerobic isolates). We tested the aerobic bacteria for the ability to produce low molecular weight Fe (III) scavenging ligands (siderophore). Almost 20 % of the bacteria showed siderophore activity and of these, almost 7 % showed a high level of activity. The highest proportions of organic acid producers in the aerobic and anaerobic isolates from the air samples were 63% and 54%, respectively (Table2).

Table 2. Organic acid production

Sample	Aerobic isolates		Anaerobic isolates	
	No.	Acid producers (%)	No.	Acid producers (%)
Air	21	63	11	54
Water	43	37	14	54
Technical water	16	33	8	25
Aleurolite	20	13	13	14
Surface	20	38	7	57

The biosorption of Cd and Cr by the isolates was studied. We found that the biomass concentration has a significant effect on adsorption. The uptake capacitiy decreases with increasing cell density. The radio-sensitivities of 93 isolates were determined. The effect of radiation is conventionally expressed in terms of the survival curve of the isolates. The values of D_{10} determined for the isolates are listed in Table 3, divided into four differently radio-resistant groups.

The D_{10} values of the aerobic isolates lay in the range 0.8-2.44 kGy, and those of the anaerobic isolates in the range 1.86-4.93 kGy. The D_{10} values of the vegetative aerobic and

anaerobic isolates were much lower: 0.11-0.57 kGy and 0.22-0.40 kGy, respectively. These results are in the same range as the D_{10} values cited in the literature (Urbain, 1986; Jay, 1992; Stroes-Gascoyne and West, 1996) for many species of micro-organisms. Among the vegetative bacteria, there were no significant differences in radio-resistance between the aerobic and the anaerobic isolates, and the minimum and maximum values of D_{10} did not deviate much from the average. It is also consistent with the results of other microbiological studies that the anaerobic spores are more resistant than the aerobic ones, and that the differences between the D_{10} values in both of the spore-forming groups are higher than those for the vegetative cells.

Table 3. D_{10} values of the isolates

Isolates	D_{10} values (kGy)
Aerobic vegetative	0.11, 0.13, 0.15, 0.15, 0.16, 0.16, 0.19, 0.21, 0.26, 0.27, 0.32, 0.38, 0.39, 0.42, 0.57 average: 0.26
Aerobic spore-former	0.80, 0.90, 0.91, 0.93, 1.00, 1.00, 1.00, 1.10, 1.12, 1.20, 1.20, 1.20, 1.20, 1.24, 1.27, 1.30, 1.32, 1.40, 1.40, 1.40, 1.40, 1.40, 1.40, 1.42, 1.46, 1.47, 1.49, 1.49, 1.50, 1.53, 1.60, 1.60, 1.60, 1.61, 1.62, 1.66, 1.67, 1.67, 1.69, 1.69, 1.72, 1.73, 1.76, 1.80, 1.81, 1.85, 1.85, 1.92, 2.06, 2.07, 2.08, 2.12, 2.44 average: 1.49
Anaerobic vegetative	0.22, 0.22, 0.26, 0.26, 0.39, 0.40 average: 0.29
Anaerobic spore-former	1.86, 2.03, 2.06, 2.18, 2.18, 2.25, 2.32, 2.33, 2.55, 2.79, 2.79, 2.95, 2.99, 3.10, 3.17, 3.33, 3.37, 3.83, 4.93 average: 2.79

Although our results contribute towards an understanding of the impact of microbial activity on the deep geological disposal of nuclear waste, we do not as yet have sufficient information to develop a model which will predict the consequences of these processes. Future investigation will focus on specific questions such as the effect of biofilms on radionuclide migration and the uptake and accumulation of cations by the isolated bacteria. These processes should be evaluated for the long term safe disposal.

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