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Microbial Issues Pertaining to the Canadian Concept for the Disposal of Nuclear Fuel Waste

Questions à examiner quant aux microbes lors du développement du concept Canadien de stockage permanent des déchets de combustible nucléaire

S. Stroes-Gascoyne, J.M. West

March 1994 mars

AECL RESEARCH

MICROBIAL ISSUES PERTAINING TO THE CANADIAN CONCEPT FOR THE DISPOSAL OF NUCLEAR FUEL WASTE

by

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QUESTIONS À EXAMINER QUANT AUX MICROBES LORS DU DÉVELOPPEMENT DU CONCEPT CANADIEN DE STOCKAGE PERMANENT DES DÉCHETS DE COMBUSTIBLE NUCLÉAIRE

par

S. Stroes-Gascoyne et J.M. West

RÉSUMÉ

EACL développe un concept de stockage permanent des déchets de combustible nucléaire dans la roche plutonique du Bouclier canadien. La Commission fédérale d'examen de l'évaluation environnementale a publié une série de directives qu'EACL Recherche doit suivre lors de la préparation de l'Étude d'impact sur l'environnement (EIE) se rapportant au concept. Les directives demandent qu'on examine, au stade de l'EIE, un certain nombre de facteurs microbiologiques et leur capacité d'affecter l'intégrité du système de barrières multiples sur laquelle repose le concept de stockage permanent.

Dans le présent rapport, on formule un certain nombre de points de vue au sujet des facteurs microbiologiques qui pourraient influencer le fonctionnement et comportement d'une installation souterraine de stockage permanent dans la roche plutonique. Les facteurs microbiologiques examinés sont, entre autres, la présence et la survie des microbes, les films biologiques, la corrosion, la biodégradation (des matières nucléaire stockées), la production de gaz, les changements géochimiques, la migration des radionucléides, la formation des colloïdes, la mutation, les agents pathogènes et la méthylation. On ne peut pas résoudre entièrement toutes les questions dans l'état actuel des connaissances. On examine brièvement les études effectuées pour mettre en évidence et améliorer les connaissances actuelles.

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MICROBIAL ISSUES PERTAINING TO THE CANADIAN CONCEPT FOR THE DISPOSAL OF NUCLEAR FUEL WASTE

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ABSTRACT

AECL Research is developing a concept for the permanent disposal of nuclear fuel waste in plutonic rock of the Canadian Shield. The Federal Environmental Assessment Review Panel has issued a set of guidelines to be used by AECL Research in preparing an Environmental Impact Statement (EIS) for this concept. These guidelines require that the EIS address a number of microbiological factors and their potential to affect the integrity of the multiple barrier system on which the disposal concept is based.

This report formulates a number of views and positions on microbiological factors that could influence the performance of a disposal vault in plutonic rock. Microbiological factors discussed include the presence and survival of microbes, biofilms, corrosion, biodegradation (of emplaced materials), gas production, geochemical changes, radionuclide migration, colloid formation, mutation, pathogens and methylation. Not all issues can be fully resolved with the current state of knowledge. Studies being performed to underscore and strengthen current knowledge are briefly discussed.

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1. INTRODUCTION

1.1 THE NUCLEAR FUEL WASTE MANAGEMENT PROGRAM

In 1978, the governments of Canada and Ontario established the Canadian Nuclear Fuel Waste Management Program (NFWMP) to ensure the safe and permanent disposal of nuclear fuel waste. AECL Research was made responsible for research and development of a concept for disposal in a deep underground engineered vault in plutonic rock of the Canadian Shield. The concept being proposed by AECL Research for the disposal of nuclear fuel waste in Canada includes the following elements (Dormuth and Gillespie 1990, Allan and Stephens 1992):

- the waste form would be either used CANDU* fuel or solidified highly radioactive reprocessing waste;
- the waste form would be sealed in a corrosion-resistant container designed to last at least 500 a;
- the containers of waste would be emplaced in, or from, disposal rooms in a vault, nominally 500 to 1000 m deep in plutonic rock of the Canadian Shield;
- access to the disposal rooms would be by shafts and tunnels;
- the waste containers would be separated from the surrounding rock by a buffer material; and
- all rooms, tunnels, shafts and exploration boreholes would ultimately be sealed such that the system would be passively safe.

The disposal vault would be a network of horizontal tunnels and disposal rooms excavated deep in the rock, with vertical shafts extending from the surface to the tunnels. It would have a layout designed to accommodate the rock structure and other subsurface conditions at the chosen site. After the vault was sealed, humans and the natural environment would be protected from contaminants in the waste by several barriers: the waste form itself, the container, the sealing materials, and the rock.

In 1981, the governments of Canada and Ontario announced that ". . . no disposal site selection will be undertaken until after the concept has been accepted" (Joint Statement 1981). The acceptability of the disposal concept will be reviewed by a Panel under the Federal Environmental Assessment and Review Process (Minister of the Environment, Canada 1989). After consulting with the public, the Panel issued guidelines to identify the information that should be provided by AECL Research, the proponent of the disposal concept (Federal Environmental Assessment Review Panel 1992). AECL Research is preparing an Environmental Impact Statement (EIS) to

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* CANDU (<u>CAN</u>ada <u>D</u>euterium <u>U</u>ranium) is a registered trademark of AECL.

provide the information required by the Panel and to present AECL's case for the acceptability of the disposal concept. This report is one of the supporting documents for the disposal concept and the EIS. It formulates a number of views and positions on microbial factors that could influence the performance of a disposal vault in plutonic rock.

1.2 METHODOLOGY FOR SAFETY ASSESSMENT

Safety assessments are used to evaluate impacts on the health of humans and non-human biota and impacts on the natural environment that could affect their health. To estimate the impacts of a disposal facility on human health and the natural environment, standard assessment practices, such as those used for nuclear generating stations, can be used for the preclosure phase. However, assessment models have to be developed for the postclosure phase, in which the main concern is the movement of contaminants from the waste to the biosphere in groundwater and the resulting radiological impacts on humans and other biota. A SYstems Variability Analysis Code (SYVAC) has been developed for carrying out a probabilistic postclosure assessment (Dormuth and Sherman 1981). This computer code traces the migration of radionuclides from the vault to the biosphere with the use of coupled, but distinct, vault, geosphere and biosphere models.

The EIS for the disposal concept differs substantially from one that would be submitted to seek approval for construction of a disposal facility at a specific site. Because of the governments' requirement that site selection cannot begin prior to acceptance of the concept (Joint Statement 1981), AECL could not design a facility for a real site and assess that disposal system to determine its suitability, as would normally be done for a site-specific assessment. Instead, reference disposal systems were specified and assessed for the preclosure and postclosure phases. The reference disposal systems are based on information from extensive laboratory and field research. The technology specified for the reference disposal facilities is either available or readily achievable.

The reference disposal facility consists of an engineered excavation (vault), at a depth of 500 m in plutonic rock, and the associated surface facilities to handle and package the nuclear fuel waste. The disposal vault consists of arrays of rooms, each several metres high and wide, connected by access tunnels for transportation of the excavated rock, waste containers and backfill materials. Fuel wastes are isolated in corrosion-resistant metal containers (ASTM Grade-2 titanium is the reference material; copper is another option in the concept). The reference emplacement scenario is emplacement in boreholes drilled in the floor of the disposal rooms (Simmons and Baumgartner 1994). Another option in the concept is emplacement of the containers in the rooms (Johnson et al. 1987).

A minimum container-design lifetime of 500 a has been specified in the reference disposal system to ensure isolation of the fuel waste during the period of high fission-product activity. The containers would be surrounded by a compacted buffer material (50 wt.% sodium bentonite and 50 wt.% silica sand) that would swell on saturation with groundwater and ensure that transport of contaminants from the containers was controlled by diffusion. Stringent quality requirements for buffer materials have been specified by Dixon et al. (1992) to ensure the required swelling potential. A 5-cm-thick compacted pure sand layer (with an overall porosity of 35%) would separate the containers from the compacted buffer material. After the waste emplacement, the rooms would be backfilled with a mixture of 75 wt.% crushed and graded host rock and 25 wt.% glacial lake clay. On completion of the vault operations (~70 a), the remaining volume, including all the shafts and exploratory boreholes, would be backfilled and sealed. Once the facility was sealed, no further actions would be required to ensure adequate isolation of the waste (Dormuth and Nuttall 1987).

The geosphere model is based on a case study of groundwater flow in the Whiteshell Research Area (WRA) (Simmons 1986), as well as an understanding of the important geochemical processes affecting radionuclide transport through groundwater in granite rock. The biosphere model (Davis et al. 1993) is a generic model and is coupled with the WRA-based geosphere model in SYVAC through an interface model.

1.3. MICROBIAL ISSUES: HISTORY AND BACKGROUND

The safe disposal of nuclear fuel waste has traditionally been evaluated from the perspective of chemists, physicists, geologists, biologists and engineers. The realization that microbial activity could potentially affect the performance of a system for the geological disposal of radioactive waste occurred in the late 1970s in the U.S.A. (e.g., Francis and Colombo 1978) and was further examined by West et al. (1982). As a result of this awareness, many countries considering radioactive waste disposal developed programs to study and quantify microbial effects in terms of their own particular disposal concept. Reviews of these studies are available (West et al. 1985, Christofi 1991, Rosevear 1991). They cover topics such as the presence of microbes in geological media (clays, sediments, salts and granites), their tolerance to extreme conditions (e.g., alkaline solutions), biodegradation of waste packages (e.g., bitumen, concrete, organic and inorganic wastes), waste container corrosion (e.g., steel, copper), microbial effects on radionuclide migration, gas production, and modelling of microbial effects. Also, a two-part, multiauthor review of microorganisms in nuclear waste disposal has been published by the journal Experientia (Experientia 1990, 1991). This latter review concludes with the statement that

> In summary, we have amassed quite a considerable amount of qualitative information from general microbial ecology and from repository-directed experiments. For a good safety analysis however, and in order to be able to model the future behaviour of a repository with the inclusion of microbial effects, we clearly need more quantitative data.

Some of the work discussed in these reviews is relevant to the AECL disposal concept in a generic sense. However, the work performed in other countries needs to be evaluated in terms of the particular disposal concept considered, because many programs are dealing with disposal of a range of different wastes, including low- or intermediate-level wastes with high organic contents. The concept being developed by AECL is for the disposal of fuel waste, which contains no organic carbon. (Used CANDU fuel contains a 5-μm-thick CANLUB coating on the inside of the fuel sheath of about half the fuel elements in each fuel bundle; CANLUB consists of 99.5% graphite and 0.5% inorganic impurities.)

The knowledge of subsurface microbiology has undergone rapid development in the past 15 a, especially in the U.S.A. Much of the research centred initially around shallow aquifers, but recently deep aquifers and other deep subsurface environments have become the subject of rapidly expanding interest (Balkwill et al. 1989, Balkwill and Ghiorse 1985, Geomicrobiology 1989, Pedersen 1993). Various important observations have been reported from microbial investigations in deep aquifers performed in the U.S. Department of Energy (USDOE) deep subsurface science program. Microbial population densities do not seem to decrease with depth in shallow aquifers (30-35 m), and significant populations are found in deeper environments. Balkwill et al. (1989), for example, found 10^5-10^8 cells per gram dry weight in transmissive aquifer sediments (primarily sands) down to depths of 265 m. The composition and size of the predominantly heterotrophic deep microflora seemed to be influenced by the physical and chemical characteristics of the environment. Hydraulic conductivity, pore size and water availability all correlated with the size of the bacterial population: in clay-confining layers of aquifer systems, far fewer cells were found ($<10^3$ cells per gram dry weight) than in the transmissive aquifers (Balkwill et al. 1989). The microorganisms found were physiologically flexible, grew on both dilute and concentrated growth media in the laboratory, and were somewhat versatile with respect to the carbon source(s) they could use. These findings are important for the assessment of the potential microbial effects in a Canadian nuclear fuel waste vault, since the vault environment would have certain similarities with deep aquifers, such as depth, nutrient scarcity and the presence of (compacted) clay.

A number of reviews carried out by, or for, AECL Research have addressed some aspects of potential microbial effects on the performance of a Canadian nuclear fuel waste disposal vault, mostly in a qualitative manner (Mayfield and Barker 1982a; Loewen and Flett 1984; Champ 1984; Brown, in preparation). Experimental work was carried out by Mayfield and Barker (1982b) to study the types and numbers of bacteria present in some candidate buffer and backfill materials. Goyette (1987) has performed a study in which the near-field biogeochemical environment of a nuclear fuel waste disposal vault was simulated (using sand and clay columns) in the laboratory, to evaluate the development of a microbial population and its effects on the transport behaviour of technetium, iodine and strontium. Brown and Hamon (1994) have reported a preliminary investigation of the types and numbers of bacteria naturally present in deep granitic groundwaters from AECL's Underground Research Laboratory (URL) near Lac du Bonnet, Manitoba, and Stroes-Gascoyne et al. (in preparation) have studied microorganisms in deep groundwater samples from the uranium deposit at Cigar Lake, Saskatchewan. A modelling study, in which the potential for microbial life in a Canadian nuclear fuel waste disposal vault was evaluated on the basis of a nutrient and energy source analysis, has also been performed (Stroes-Gascoyne 1989).

The EIS guidelines issued by the Federal Environmental Assessment Review Panel (1992) identify (in Section 5.4) a number of microbiological factors that need to be addressed in the EIS. Appendix A includes Section 5.4 of the EIS guidelines together with a list of other microbial processes that have been identified by the Panel.

The objective of this report is to formulate positions on a number of microbial issues pertaining to the disposal concept being developed by AECL Research. Positions need to be formulated on the presence, survival and potential effects of microbes during the various phases of the vault (i.e., preclosure and postclosure) and in the various compartments (i.e., vault, geosphere and biosphere) of the disposal system.

2. THE PRESENCE AND SURVIVAL OF MICROBES

2.1 PRE- AND POSTCLOSURE PHASES OF THE VAULT

Microbes can grow in any environment in the presence of liquid water if their nutrient and energy requirements are met and if they can physiologically tolerate that environment. The conditions in a Canadian used-fuel disposal vault (100°C maximum temperature at the container skin; ~12 MPa maximum pressure in the buffer/backfill; and a maximum dose rate of 52 Sv/h at the container skin (Simmons and Baumgartner 1994)) may not be extreme enough to inhibit the survival of either introduced or naturally present organisms during both the pre- and postclosure phases (Stroes-Gascoyne 1989). However, it is probable that during the preclosure phase, the environment close to the waste containers would alter so rapidly in such a variety of ways (e.g., radiation, heat, pressure) that the evolutionary changes needed in microbes for their survival in this environment would not occur fast enough. Also, survival would be limited spatially because of the compacted nature of the buffer material (which has a very small average pore size, see Section 3.1) surrounding the waste containers. Conditions would be less harsh farther away from the waste containers (i.e., in the backfill), where microbes might be able to survive.

The presence of nutrients and energy in a used-fuel disposal vault would be governed largely by two sources, both introduced during the preclosure phase. These sources are the waste and the barrier materials emplaced in the vault (Stroes-Gascoyne 1989), and any other material that was introduced during the construction, operation and closure of the disposal vault (e.g., drill water, blasting residues).

Stroes-Gascoyne (1989) performed a detailed analysis of the total nutrient and energy inventories of the materials emplaced in the reference design for a Canadian used-fuel disposal vault. Sources and sinks of nutrients as a result of groundwater movement in a sealed vault were also considered. On the basis of this analysis it was concluded that nitrogen and/or phosphorus would be the growth-limiting nutrients. A maximum microbial population size was calculated on the basis of the limiting nutrient, considering three scenarios: heterotrophy only, chemolithotrophy only, and a mixture of these two. A sequence of reactions involving components of the emplaced materials was proposed that could provide sufficient energy to sustain such a population. This report also speculated on which physiological groups of bacteria might occur in a vault environment, on the basis of organisms found in mines. However, the analysis did not take into account the possibility that not all of the nutrients and energy present might be available for use by microorganisms, nor were any reaction or growth kinetics considered, because of a lack of data. The analysis is, therefore, conservative and probably overestimates microbial population size by several orders of magnitude.

Excavation and construction of a disposal vault would inevitably introduce biological material from the surface to the subsurface. In addition, nutrient and energy sources would be brought into the excavated areas through movement of engineered materials, particularly in heavily used access tunnels. It is recognized that the uncontrolled development of a microbial ecosystem could have perturbing effects in the preclosure phase (a period of ~70 a), and these perturbations could extend into the postclosure phase. Good housekeeping of the excavated areas would therefore be necessary to control this development. Controllable factors include the quality of any water used (i.e., in drilling and cleaning operations as well as for buffer and backfill saturation), the occurrence of blasting residues, and the maintenance of quality procedures throughout all stages of the preclosure phase (i.e., excavation, waste emplacement and final sealing). The degree to which these factors need to be controlled remains to be determined, and calculations are under way to quantify the potential input of microbes, nutrients and energy into the system during the preclosure phase.

Microbes are known to occur on the walls of water-filled fuel bays at nuclear generating stations, where most used-fuel bundles are currently stored. The water in these bays reduces the radiation fields effectively, and microbial activity is, therefore, not an unexpected observation. It is presently not known whether biofilms occur on used-fuel bundles. It seems unlikely that microorganisms, if they did occur on used-fuel bundles, would survive during the transportation of the bundles in fuel transport flasks and the subsequent packaging of the fuel bundles in the disposal containers, because of the high temperatures, radiation fields and lack of water and nutrients inside the transport flasks and containers. Work is in progress to determine the occurrence of microorganisms in water from fuel bays, on used-fuel bundles during storage, and on the inside of fuel transportation flasks.

2.2. THE GEOSPHERE

The development of reliable methods for sampling groundwaters from deep geological environments and improvements in detection methodologies have advanced subsurface microbiology research considerably over the last 15 a. Work on microbial presence in deep groundwaters from granites is very limited, but has been undertaken by Christofi et al. (1983, 1984a,b, 1985), Pedersen (1987, 1989), Pedersen and Ekendahl (1990), Pedersen et al. (1991), Banks and Banks (1993) and Brown and Hamon (1994). Pedersen et al.

(1991) detected ~10^5 microbes per millilitre in Swedish granite groundwaters from depths below 800 m, and calculated from in situ experiments that 10^3 to 10^6 times more microbes could be attached on the surfaces in contact with these groundwaters (i.e., in water-conducting channels in the crystalline bedrock). Assimilation studies with CO_2 and with introduced organic compounds (such as formate, acetate, glucose and leucine) indicated in situ production of organic matter by viable populations of methanogenic, sulphate-reducing, and heterotrophic bacteria.

Initial data from current microbial investigations of groundwaters from granitic rocks of the Canadian Shield at AECL's URL show that these waters contain from 10^3 to 10^5 planktonic microbes per millilitre (Brown and Hamon 1994). The microbial concentration seems to decrease with depth and the concurring increase in total dissolved solids in the groundwaters. Microbial concentrations were determined both by direct microscopic counting and by using BART tubes (Biological Activity Reaction Tests, a recently developed commercially available field method for bacterial analysis). The BART tests actually induce growth of certain physiological groups of bacteria and derive a microbial population size from the colour change in the growth medium. They also provide a qualitative measure of the activity (and aggressiveness) of the microbial population present. The main bacterial activity in the URL groundwaters appears to be from iron-related bacteria, despite the generally low iron concentrations in the groundwater. Slime-producing and sulphate-reducing bacteria (SRB) occur in approximately 25% of the samples, but the number of SRB is low despite generally high sulphate concentrations, particularly at greater depths (Brown and Hamon 1994). However, it appears from the preliminary study by Brown and Hamon (1994) that the hydrogeochemical practice of borehole flushing at the URL prior to sampling could decrease the number of microbes detected in the groundwater samples by up to two orders of magnitude. Such a decrease could arise from several factors:

(1) The borehole may provide an altered environment in which microorganisms thrive better than in fractures in the rock.

(2) There may be some residual contamination in the boreholes from drilling, allowing more growth.

(3) Nylon sample tubes are used in borehole installations in the URL, and large numbers of microbes have been found to grow in these tubes (Stroes-Gascoyne et al., unpublished results). Flushing would remove the standing borehole waters, which may have higher microbial populations (for reasons mentioned above) than the fracture-zone waters, causing the observed decrease in microbial concentrations.

(4) Mixing of standing water in the borehole with fresh fracture-zone water may occur during water withdrawal, affecting microbial concentrations.

(5) The flow induced in the borehole and the fractures of the rock by flushing may cause sessile organisms to detach and be sampled; in addition, planktonic bacteria and particles could be filtered out or trapped in the fractures during flushing.

A further study was therefore initiated to determine the cause of the decrease in microbe concentrations during water withdrawal by flushing (at various flow rates) and sampling the zones of two boreholes that intersect an inclined fracture zone at the URL (Stroes-Gascoyne et al., in press). The results from this investigation illustrated the importance of taking the hydrogeological characteristics of the borehole and fracture zone into account when interpreting microbial concentrations in groundwaters drawn from such systems.

Vault excavation and operation could potentially disturb the adjacent geosphere, and hence its microbiological population, through the introduction of surface organisms and the alteration of both the geochemistry and nutrient and energy levels. However, the geosphere, representing by far the largest volumetric part of the entire disposal system, has an enormous capacity to buffer (i.e., dilute) any geochemical and microbiological changes occurring during the preclosure phase. The disturbing effects of vault excavation and operation are expected to extend into only a relatively limited part of the geosphere, i.e., the immediate surrounding area, for a limited time after vault sealing. Conditions here may at first favour introduced groups of microbes (e.g., aerobes) over indigenous species. However, after closure, previous conditions in the geosphere would eventually prevail in the part of the geosphere affected during the preclosure phase. These conditions are expected to be nutrient-poor (organic carbon levels of 1.8 mg/L in groundwater (Stroes-Gascoyne 1989)) and saline (up to 25 g/L total dissolved solids), with a pH between 7 and 9 and an Eh varying from -300 to +200 mV (Gascoyne 1988, Gascoyne and Kamineni 1992). Effects on the migration of radionuclides arising from the presence of microbes in the geosphere are dealt with in Sections 3.5 and 3.6.

2.3 THE BIOSPHERE

Microbes are ubiquitous in the biosphere and include algae, bacteria, fungi, protozoa and viruses. The occurrence of microbes and the size of their populations depend on physical and environmental conditions and on the availability of suitable growth substrates for providing essential nutrients and energy (Loewen and Flett 1984, Stroes-Gascoyne 1989). Many factors can be limiting, but microbes are very tolerant to adverse environmental conditions, and, as a group, have very diverse mechanisms for securing essential nutrients and energy. No one environmental factor can guarantee sterile conditions. The absence of microbes in the biosphere is a highly artificial condition that can only be brought about by deliberate sterilization. The presence of a used-fuel disposal vault deep in granitic rock of the Canadian Shield is not expected to affect the presence and survival of microbes in the biosphere, either in the preclosure or postclosure phase. The effects that biosphere microbes can have on radionuclide redistribution is discussed in Sections 3.5 and 3.6.

3. POSSIBLE EFFECTS OF MICROBES ON THE PERFORMANCE OF A DISPOSAL SYSTEM

The presence, survival and growth of microbes at any stage and in any compartment of the reference disposal system could potentially have a number of consequences for its performance. Table 1 shows possible effects that microbial activity may cause and where they may occur. Each of these effects is discussed below.

3.1 BIOFILMS

In the low-nutrient environment of a nuclear fuel waste disposal vault, microbial presence and activity would be associated with surfaces, since this is where nutrients and energy sources would be concentrated. Once

TABLE 1

POTENTIAL MICROBIAL EFFECTS IN THE VARIOUS COMPARTMENTS OF A NUCLEAR FUEL WASTE DISPOSAL SYSTEM

Microbial Effects	System Compartment		
	Vault	Geosphere	Biosphere
Biofilm Formation	+	+	+
Corrosion	+		
Biodegradation (of Emplaced Materials)	+		
Gas Production	+		
Geochemical Changes	+	+	+
Radionuclide Migration	+	+	+
Colloid Formation	+	+	+
Mutation/Pathogens	+		
Methylation	+	+	+

+ indicates in which compartment of the disposal system the microbial effect would be expected to primarily occur.

attached to a surface, microbes would produce extracellular polymers (Costerton et al. 1986), and a biofilm would develop, thus maintaining an optimum environment for growth. Biofilm growth results in biofouling when it causes adverse effects on human activities, such as well clogging. The ecosystem of a biofilm would be concerned with trapping, recycling and retaining nutrients, and thus few organisms would be lost from the system. A biofilm can vary from a monolayer of cells (0.2-1 μm thick) to a multilayer of cells and extracellular components, visible to the naked eye. It is possible that biofilms could form in any compartment of the reference disposal system (see Table 1) and during both the pre- and postclosure phases.

Biofilms could potentially form on any wet surface of a disposal vault during excavation, and could be a source of introduced nutrients when the vault is backfilled and closed. The development of such ecosystems, therefore, would need to be controlled by good housekeeping during the excavation stages (see also Section 2.1). Substantial biofilms are unlikely to form in the compacted buffer, however, because of the small pore spaces present in the highly compacted clay.

Pusch and Forsberg (1983) state that from a physical point of view, any soil, including highly compacted bentonite, can be regarded as a system of irregularly shaped capillaries. In water-saturated, highly compacted bentonite the average "capillary" diameter can be roughly estimated at 50 nm, the statistical spread being illustrated by a 90-percentile of 1 μm and a 10-percentile of 5 nm (Pusch and Forsberg 1983). Pusch et al. (1987) have estimated that the average pore size in bentonite compacted to 1.22 Mg/m^3 (the AECL effective clay dry density of the buffer; the AECL reference compaction density for the buffer is 1.68 Mg/m^3) is 0.1 to 0.5 μm, which is much smaller than would be required for a substantial biofilm. Chapelle (1993), qouting studies by Sinclair and Ghiorse (1989), Hicks and Frederickson (1989) and Chapelle and Lovely (1990), states that in aquifers, the most consistent predictor of microbial abundance is sediment texture. Sands generally contained the greatest numbers of microbes and clays contained the lowest numbers of microbes. Significantly, the diversity of bacteria also seemed to correlate with sediment type, with the greatest diversity being found in the sandiest sediments. Chapelle (1993) states that there are probably many mechanisms that contribute to this effect, but that one important factor is the small size of pore throats in clays relative to sands. The average pore throat diameter in clays, as measured by mercury injection, is less than 0.05 μm. In sands, on the other hand, average pore throat diameters are much greater, in the 2-20 μm range. Because bacteria generally have diameters from 0.1 to 1.0 μm, the small pore throats of clays must greatly restrict the ability of bacteria to move about and reproduce effectively. Similarly, helium injection into sediments has shown that 90-95% of the porosity in sands is interconnected, whereas in clays this percentage is much lower. This would tend to restrict the mobility of nutrients to active cells as well as restrict the movement of metabolic waste products away from cells. Again, this would tend to depress bacterial activity in clays relative to sands (Chapelle 1993). Furthermore, in the buffer component of the reference disposal vault, environmental conditions are quite extreme and the necessary nutrients and energy required for growth may be less readily available. Experiments are needed to confirm the expected restriction of bacterial growth and movement in the buffer. The Buffer/Container Experiment (a full-scale engineering test in which buffer materials were compacted around a heater simulating a fuel waste container) in progress at the URL will be examined upon decommissioning to assess survival and growth of microbes in such a compacted environment.

The 5-cm-thick compacted sand layer between the containers and the compacted buffer material in the reference vault design would provide more space for bacteria to grow, but this environment would be subject to the most severe conditions of temperature, radiation and nutrient scarcity, and development of abundant microbial life in this sand layer is therefore not expected.

In the backfill environment (consisting of 25% glacial lake clay and 75% crushed rock), pore sizes would be several times larger than in the buffer environment, and it is possible that biofilms could form. However, space limitations would still inhibit the development of large, complex biofilms. Moreover, the production of thin biofilms could plug pores and hence decrease the permeability of the backfill. Consequently, the formation of biofilms may be advantageous, particularly if located within the buffer and backfill materials.

Similar effects would occur in the geosphere where space for microbial growth is limited to pores and fractures. Again, in the low-nutrient environment of the geosphere, microbial activity would be associated primarily with these surfaces (Pedersen et al. 1991). Formation of biofilms might occur to the extent that they would block pores, reduce fissure size and attenuate radionuclide migration. In this context, observations from the use of microbes (particularly bacteria and yeasts ranging in size from 1-10 μm) as groundwater tracers in soils, aquifers and even fractured bedrock (Davis et al. 1985, Allen and Morrison 1973, Champ and Schroeter 1988) should be mentioned. Observations in a sand and gravel aquifer (Davis et al. 1985) indicated that microbial cells are mechanically filtered as they pass through the intergranular pore space. They appear to become trapped at the soil-water interface (e.g., of an injection well), and as the mat of cells increases, filtration becomes more effective. The breakthrough curve tends to increase first to an abrupt maximum, because the bacterial cells do not block the flow channels initially. As the filtering continues, the flow channels become clogged and the breakthrough curve decreases sharply. Similar observations were made by Champ and Schroeter (1988), who studied bacterial, particle and conservative tracer transport through fractured rock at the Chalk River Laboratories (CRL). They found that, although some bacteria and particle tracers would appear at the recovery well either with, or slightly before, the conservative tracers, most of the bacteria and particles were removed from the system through a filtering process, which in this fracture system appeared to be similar to the filtering process in a gravel aquifer.

The studies with microbial tracers indicate that microbes can move through subsurface systems. However, it should be pointed out that such tests are usually performed in situations with considerable flow, enhanced by pumping at the recovery well, and over relatively short distances. In the granitic environment of the geosphere suitable for a disposal vault, flow rates would generally be low, fractures would generally be narrow, and distances would be long. These factors, combined with a low-nutrient environment, would enhance the attachment of microbes to fracture surfaces and reduce their mobility. Moreover, formation of biofilms in the geosphere could be beneficial, because biofilms would likely sorb radionuclides, thereby reducing their migration.

Biofilms are ubiquitous in the biosphere and often fulfill essential roles, for example in the decomposition of organic materials. The effect of biofilms in the biosphere on radionuclide migration is discussed in Section 3.6.

3.2 CORROSION

Microbially influenced corrosion (MIC) of metals is either a direct reaction in which microbes use the metal as an energy source, or an indirect process, in which microbes change the environmental conditions such that chemical corrosion processes are induced or enhanced.

Microbially influenced corrosion of waste containers in a disposal vault is considered improbable in the first few hundred years after waste emplacement because of the high gamma-radiation fields and temperatures (and expected initial desiccation), arising from the used fuel, which would greatly reduce the microbial population at and near the container surface. In addition, as discussed earlier (Section 3.1), the pore sizes in the buffer material would likely be too small to permit mobility or growth of microbes. However, after a period of time, possibly as long as several hundred years, the gamma-radiation fields and high temperatures at the container surface would decrease sufficiently to enable any surviving microbial population to multiply, and thus MIC could be possible, although spatial and nutritional restrictions in the buffer would continue to exist.

The Canadian program has focused on both Grade-2 and Grade-12 titanium and on copper as container materials, because these materials have good corrosion resistance under the saline conditions typical of groundwater solutions found at depths of 500 to 1000 m in the Canadian Shield.

3.2.1 Titanium

Of the commonly used engineering metals and alloys, titanium and its alloys are the only ones for which microbially influenced corrosion has not been reported (Schutz 1991, Pope et al. 1989, Little et al. 1991). For this reason, among others, it is the commonly recommended material for many seawater and biomedical applications. Titanium has good corrosion resistance because of a stable passive film over a wide pH range. Very acidic conditions are required to destabilize this film, and, although specific microbes can produce strong acid under certain conditions (e.g., *Thiobacillus* sp.), this would not likely occur in the buffer environment because of its high pH-buffering capacity. Also, production of inorganic acid by microbes requires mostly aerobic conditions, and the vault environment would evolve relatively rapidly towards anaerobic conditions upon closure (estimated to be within 300 a (Johnson et al. 1994a)).

Many transition metals play a role in biological systems, either in redox reactions or in coordination with ligands containing electron donor groups. This can be attributed to the presence on the metal cation of an incomplete set of electrons in the d-orbitals. Titanium exists in aqueous solutions almost exclusively in the +4 oxidation state, and as a result its ions have no available d electrons; they have a closed-shell configuration, which makes titanium unavailable for biologically supported redox chemistry and coordination reactions (Wackett et al. 1989).

Titanium is not biotoxic and permits the growth of biofilms that are known to act as crevice formers on other metals (Schutz 1991, Mansfeld and Little 1991). The possibility that microbial activity in the form of biofilms might lead to the initiation of crevice corrosion can, therefore, not be discounted, despite the fact that no case of localized corrosion has ever been observed under such a biofilm formed on titanium. Crevice corrosion is the most probable failure mechanism for ASTM Grade-2 titanium under the conditions anticipated in a Canadian disposal vault and forms the basis for modelling the failure of titanium containers in the vault model (Johnson et al. 1994a). In this model, it is assumed that crevice corrosion initiates on <u>all</u> containers, because a rigorous basis for predicting the probability of initiation has not been developed, despite extensive studies. It is therefore of no consequence for the present container failure model whether or not microbial activity causes initiation of crevice corrosion on the titanium containers. It is not known if microbes could accelerate the rate of crevice propagation in titanium, because localized corrosion under a biofilm on titanium has never been observed. However, crevice propagation requires a pH within the crevice of <1 and it appears not likely, therefore, that microbial effects would be more severe than existing conditions. The rate of hydrogen absorption by the metal is the critical factor in determining the material's susceptibility to hydrogen-induced cracking. Since the rate of crevice propagation determines the rate of hydrogen absorption, microbial effects should produce insignificant amounts of additional absorbed hydrogen.

3.2.2 Copper

Copper has good corrosion resistance under simulated disposal vault conditions (P.J. King et al. 1983). Unlike titanium, however, copper can be a source of energy for microorganisms and hence is susceptible to both direct and indirect MIC. Although copper ions are generally toxic to microorganisms, copper-resistant microorganisms can form biofilms on copper surfaces under both aerobic and anaerobic conditions. Corrosion of copper in the presence of H_2S produced by anaerobic sulphate-reducing bacteria under a variety of conditions has been reported extensively (McNeil et al. 1991, Videla et al. 1989, Syrett 1981, Pope et al. 1989, Little et al. 1991, Swedish Corrosion Institute 1983). Biofilm formation is characteristic for most cases of MIC involving copper.

The current copper container failure model (King and LeNeveu 1992; King et al. 1992, in press) developed for Canadian disposal conditions considers both uniform corrosion and pitting, processes that can be affected by MIC. The rate-limiting step for uniform corrosion in this model is the diffusion of copper corrosion products (i.e., Cu^{2+}) away from the container surface into the surrounding buffer material. It is unlikely that this diffusion process would be enhanced by the presence of microbes in a vault, because of the spatial limitations in the buffer, as discussed in Section 3.1. Pitting is accounted for in the copper container failure model by using a statistical extreme-value analysis of long-term pit depths from the literature. The data used were all obtained in natural environments for long exposure times and probably would have included microbiological activity. However, there is a need to further quantify the effects of microbes on copper corrosion in the gypsum-rich clay (Avonlea bentonite) proposed as buffer material in the reference disposal vault design, because of the potential for sulfide formation by sulfate-reducing bacteria. The assumptions in the current copper container failure model may need to be reexamined as the corrosion research program continues.

The possibility of microbially induced stress corrosion cracking of copper cannot be discounted, although this process is not considered explicitly in the failure model. However, O_2 is required as an oxidant for this process and, after vault sealing, anoxic conditions within the vault should be reached within 300 a (Johnson et al. 1994a). Since microbial activity would be limited in the vault during the first few hundred years after sealing, because of the prevailing harsh conditions (i.e., relatively high temperature, pressure and the presence of gamma radiation, see also Section 3.8), microbially induced stress corrosion cracking would be an unlikely failure mechanism for copper containers.

3.3 BIODEGRADATION

In the above section we discussed why microbes would likely have a limited influence on the corrosion (and hence deterioration) of titanium and copper disposal containers. In this section, biodegradation of other engineered barrier materials, such as cements and concretes, is discussed. These materials are likely to be used as components of the sealing system for a disposal vault.

3.3.1 Concrete Materials

Biodegradation of concrete materials under aerobic conditions is a well-known process. Sulphate-producing bacteria (Thiobacillus sp.) are capable of oxidizing sulphur, sulphides and thiosulphates (S^0, S^{2-}, $S_2O_3^{2-}$) to sulphuric acid in a relatively short period under aerobic conditions. Nitrifying bacteria (Nitrosomonas, Nitrobacter) can transform ammonia into nitric acid under aerobic conditions. The inorganic acids produced attack the concrete by dissolving $Ca(OH)_2$ and CSH (calcium silicate hydrate) gel from cement (Biczok 1967). In the Canadian disposal concept, cements are used in grouts and concretes for a variety of sealing applications (Johnson et al. 1994b). During the preclosure phase, aerobic degradation of concrete surfaces could occur, and precautions should be taken to prevent this. Degradation throughout concrete materials is unlikely because the small pore size and very low hydraulic conductivity of the material prevent microbial growth.

After closure, the vault environment would evolve relatively rapidly towards anaerobic conditions, and aerobic degradation of concrete may no longer occur. There are no reports in the literature of the biodegradation of concrete materials under reducing conditions. Under anaerobic conditions some microorganisms are capable of producing organic acids, but the effects of organic acids on the high-performance concrete material proposed for use in the reference disposal vault are likely to be negligible.

Small quantities (<1 wt.%) of synthetic plastic (sulphonated naphthalene polymer, superplasticizer) might be mixed in the cement-based sealing materials to reduce the mixing water required and to improve their emplacement properties. The biodegradability of this material remains to be investigated, although it is likely to be a poor source of nutrients for microbes. The availability of the superplasticizer for microbial reactions depends on its release from the grout materials, either in bleedwaters from unset grouts or as a result of leaching of set grouts in groundwater. The first mechanism appears to be of little consequence, because of the lack of bleeding of the reference grout (Onofrei et al. 1991). The second mechanism was studied in leaching experiments by Onofrei et al. (1991); fractions of superplasticizer released were eight orders of magnitude lower than fractions of Ca^{2+} released. How much the organic content of groundwaters would be increased because of this small release of superplasticizer remains to be calculated. This will be done in conjunction with estimating the nutrient load increase as a result of vault construction and operation (see Section 2.1).

3.3.2 Other Materials

Biodegradation of other materials emplaced in the vault has been considered by Stroes-Gascoyne (1989). In this study, the compositions of the waste, the containers, and the buffer and backfill materials were assessed with regard to their nutrient and energy inventories for microbial growth. This assessment did not consider the kinetics of energy and nutrient supply, nor their actual availability for microbial use, and is, therefore, very conservative. If one assumes that the carbon (organic and/or inorganic) in the buffer and backfill materials is entirely available for microbial use, the assessment showed that nitrogen and/or phosphorus would be the limiting nutrients rather than carbon. However, some or all of the organic material present in the buffer and backfill materials may be recalcitrant (e.g., lignin, kerogen and humin) and would therefore be highly resistant to decomposition by microbes. This, combined with the generally adverse conditions in the buffer and backfill, suggests that microbial populations would probably be small, and therefore the significance of biodegradation would be small compared to other, abiological processes. The Buffer/Container Experiment in progress at the URL will be examined upon decommissioning to assess the occurrence of microbes in a compacted buffer environment (see Section 3.1).

3.4 GAS PRODUCTION

The reference disposal vault design being considered in the EIS is based on the disposal of used CANDU fuel, which contains no organics. The only organics that would be present in the disposal vault would be those introduced during the preclosure phase, from construction and operation activities, and those contained in the mined natural clays used as buffer and backfill components (see Section 3.1). Granitic groundwater also contains organic material, but the assessment of nutrients and energy in a Canadian disposal vault by Stroes-Gascoyne (1989) showed that the contribution from groundwater to the total carbon inventory is insignificant in comparison with the contributions from the buffer and backfill materials.

Gas could be produced in a disposal vault as a result of chemical and biological processes (Biddle et al. 1987). Degradation of available carbon by microbes is of concern as it can produce carbon dioxide and methane. However, it would be an unrealistic scenario to assume that all available carbon would be degraded to carbon dioxide and methane instantly. Experiments are, therefore, currently being performed at AECL Whiteshell Laboratories (WL) to determine production rates for carbon dioxide and methane from organics in the reference clays being considered for use in a Canadian nuclear fuel waste vault. Other biological processes also produce gases, including biocorrosion (e.g., sulphate-reducing bacteria produce H_2S, which plays a role in Cu corrosion, ultimately producing H_2). Johnson et al. (1994a) have calculated that the amount of H_2 produced in a Canadian disposal vault by non-biological corrosion and radiolysis processes would produce a gas phase that would occupy only 1% of the total pore space in the buffer and backfill after 40 000 a, and that effects are, therefore, unlikely to be significant in the time frame of the assessment calculations (i.e., 10 000 a). It is unlikely that microbiological corrosion catalyzed by sulphate-reducing bacteria would be more significant in terms of amounts of gas produced than the abiological processes considered by Johnson et al. (1994a).

The organic carbon content of the reference clays used for the buffer and backfill is 0.31 and 1.2 wt.% respectively (Oscarson et al. 1986; Oscarson, in press). The buffer and backfill contain 50 and 25 wt.% clay respectively, so the overall organic carbon content of buffer and backfill would be 0.15 and 0.3 wt.%. Much of this organic material would be difficult to degrade, as it has demonstrated long-term stability. The reference bentonite used in the buffer material is from the Bearpaw Formation (of Upper Cretaceous age, 75-85 Ma old) in south-central Saskatchewan, Canada (Oscarson et al. 1990). The reference backfill clay is Lake Agassiz clay, which was deposited approximately 8000 a ago (Teller 1976). Organic matter was extracted from this clay and was ^{14}C-dated; it had an age of 23 950 ± 270 a, i.e., the ^{14}C content of the extracted organic material is 5.1 ± 0.2% of modern ^{14}C levels (Sheppard et al., in preparation). This indicates that most of the organic carbon present in this clay is very old and likely recalcitrant, since, had degradation been possible, it would have occurred a long time ago. Moreover, Stotzky (1986) states that a significant fraction of the organic matter bound to the inorganic component of soils cannot be used by microorganisms. Nedwell (1987) also demonstrated that carbon available for microbial use in clays may only be 1% of the total organic carbon. However, the effect of radiation and heat on the organic material in the clay remains to be investigated; complex organic matter may be broken down into smaller segments, which may be more available for microbial use.

Microbes can also act as consumers of some gases, such as CO_2 (Bachofen 1991) and N_2. The utilization of N_2 as a source of nitrogen is called nitrogen fixation, and in this process N_2 is reduced to ammonium, which is generally not released but rapidly converted into organic matter (amino acids) by these N_2-fixing bacteria (Brock and Madigan 1991). More information on the production of gases, the balance between gas production, dispersion and consumption, and the kinetics of these processes, still remain to be elucidated. Work is in progress at WL to define boundary conditions for the various processes.

3.5. GEOCHEMICAL CHANGES

Microbial activity could influence the geochemical conditions in a vault because of microbially mediated redox processes and the production of CO_2, sulphides, acids and complexing agents that result from microbial metabolism. These products might cause changes in pH and redox potential, which, in turn, could bring about changes in radionuclide speciation, solubility and sorption, resulting in either increased or reduced migration. However, such effects are most clearly seen in nutrient-rich, shallow waste-disposal environments (Francis 1986, Francis and Dodge 1987). In the nutrient-poor vault and geosphere environments in the Canadian Shield, where the vault and geosphere materials would provide an enormous buffering capacity, these effects would be much less significant. Any microbial changes to the geochemical environment that might occur would be on a very localized scale where conditions for microbial activity were optimal, but such changes would be swamped by the overall buffering of the surrounding materials both in the vault and in the geosphere.

In the nutrient-rich biosphere, microbes are ubiquitous and thrive as an integral part of this environment. Loeven and Flett (1984) indicated several microbial processes that could cause geochemical changes to this environment and hence could affect radionuclide migration in soils. These effects include pH and Eh changes, which could influence the solubility and sorption properties of radionuclides; production of chelators that could make radionuclides more soluble; and methylation (see Section 3.9), which could also affect mobility. Those effects that could influence radionuclide migration have been incorporated implicitly in the various biosphere models used to assess the reference disposal vault (Davis et al. 1993).

3.6 RADIONUCLIDE MIGRATION

It is well known that microbes can take up metals both within and outside of their cell structure, and this may affect the migration of metals. Experiments have shown that radionuclides are similarly affected and that the degree of migration depends on the nuclide involved. The effects of microbes on radionuclide migration can be positive or negative, depending on whether or not the microbes are attached to solid surfaces. The small pore sizes of the compacted buffer and backfill materials in the vault would ensure that the migration of radionuclides away from the used fuel would occur only via diffusion in groundwater, and not as a result of microbial migration.

Microbial migration might become important, however, in the fractures of the geosphere and at the interface between the vault and geosphere, where the pore sizes would be larger than in the compacted buffer/backfill materials. In deep fractured rock environments, most microbes have been found to be attached in biofilms to fracture surfaces (Pedersen et al. 1991). These biofilms are held together by a matrix of extracellular polymers (see Section 3.1). Since both microbes and extracellular polymers can sorb or bind radionuclides, biofilms would tend to fix the radionuclides until or unless the equilibrium of the biofilm was disturbed by changes in nutrient conditions. Radionuclide migration as a result of microbial uptake would therefore probably be reduced in the geosphere, because microbes would tend to be concentrated in the protective environment of a biofilm.

In the biosphere, microbes are ubiquitous, and their effects on radionuclide migration through soils, root uptake by plants and digestion by herbivores have been implicitly considered in the biosphere models. Virtually all of the field and laboratory studies in support of the biosphere models have proceeded under natural conditions, in that microbes were allowed to function naturally. Thus microbial effects on the behaviour and transport of radionuclides have been implicitly taken into account. As an example, the transfer coefficient distributions (concentration factors for radionuclides from soil to plants, transfer rates of radionuclides from water in a lake to sediments, etc.) include variations due to microbial effects. No other treatment is therefore thought to be necessary.

3.7 COLLOIDS

The presence of colloids (groundwater particles with diameters between 1 and 450 nm (Vilks 1994, Vilks et al. 1993)), because of their sorption and transport properties, can potentially influence radionuclide solubility. Microbial colloid formation has two aspects: microbes themselves can act as colloids, or microbes can produce materials that can become colloidal.

Microbes themselves could act as colloidal particles, potentially increasing radionuclide mobility in the vault environment through sorption or uptake (thereby increasing the amount of "dissolved" radionuclides). Aerobic microbes might prevail in the vault near-field environment during the preclosure phase and for a relatively short time into the postclosure phase (up to 300 a, Johnson et al. 1994a). Aerobic microbial processes may include the oxidation of iron, producing ferric oxyhydroxide colloids, for example. Microbes can also excrete organic materials that could become colloidal, especially in a saline environment. However, the engineered barriers (i.e., the compacted bentonite/sand buffer and the clay/crushed rock backfill) would filter microbial and other colloids and prevent their migration from the vault near-field into the geosphere. A study of colloids and suspended particles in the Cigar Lake uranium deposit, Saskatchewan, was conducted by Vilks et al. (1993). The results indicated an absence of particle migration from the clay-rich zone of the deposit to the surrounding sandstone, suggesting that clay is an effective barrier to colloid migration. Sorption of radionuclides by pre-existing natural colloids of minerals and organics, or by naturally present microbes, are the only likely processes for radiocolloid formation in the geosphere. In the nutrient-poor environment of the geosphere, microbes likely survive in biofilms, which would tend to fix radionuclides and reduce their migration (see Section 3.6). Therefore, since the major fraction of microbes is expected to be attached to fracture surfaces, microbes are not likely to contribute significantly, as mobile colloids, to radionuclide transport in nutrient-poor groundwater systems.

Vilks et al. (1991) and Vilks (1994) have shown that, provided radiocolloid formation is reversible, colloid concentrations in natural granitic groundwaters have a negligible effect on radionuclide transport, even if one assumes, conservatively, that the radiocolloids travel with the velocity of groundwater. Even for irreversible colloid formation, the impact on radionuclide migration is not expected to be larger than predicted by the current range of parameter values used for radionuclide transport in the assessment models. Allard et al. (1991) performed model calculations for both reversible and irreversible sorption of radionuclides onto colloidal particles (which included microbes). They concluded that colloids have a negligible effect on radionuclide migration for reversibly sorbed radionuclides. Vilks et al. (1991) and Allard et al. (1991) also showed that, even in the case of irreversible sorption, the transport capacity of colloids for radionuclides is small, and, correspondingly, the potential radiological doses resulting from this transport are also small.

The observed colloid and suspended particle concentrations in deep groundwaters from the Cigar Lake uranium deposit are too low to have a significant impact on radionuclide migration, provided radionuclide sorption is reversible (Vilks et al. 1993). If radionuclides are irreversibly sorbed to particles they cannot sorb to the host rock and their migration can only be evaluated with an understanding of particle mobility. However, as discussed earlier, irreversible colloid formation is unlikely to have a significant impact on radionuclide migration. The study by Vilks et al. (1993) found that clay is an effective barrier to colloid migration. This implies that radionuclides sorbed on colloids formed in a near-field vault environment would not reach the geosphere, which is separated from a nuclear fuel waste disposal vault by clay-based compacted buffer and backfill materials. Studies of colloid formation and their impact in the Poços de Caldas natural analog also suggest that colloids would have no effect on radionuclide migration (Chapman et al. 1991).

Colloidal effects in the biosphere are implicitly incorporated in the various biosphere models.

3.8 RADIATION-INDUCED MUTATIONS AND PATHOGENS

Higher-than-normal background radiation levels may enhance microbial mutation or evolution. This might result in the development of organisms more tolerant to radiation, with a larger capacity to sorb radionuclides, which could result in enhanced transport.

Containers with fuel waste are highly radioactive and, in addition, are thermally hot. At the surface of the container, radiation fields of about 52 Sv/h would be present at the time of emplacement (Simmons and Baumgartner 1994); the temperature at the container surface would be a maximum of 100°C. Such conditions may not be compatible with the survival of microbes. Thus, in the immediate region of the fuel waste container, most organisms originally present at the time of emplacement would likely be killed in a matter of hours or days. The container and its immediate surroundings may therefore constitute, effectively, an "abiological" system. With increasing distance from the container surface, the radiation field would be reduced through attenuation by the absorbing material between the container surface and the region being considered. Temperatures would also be lower than at the container surface. At some distance from the container, conditions permissive for survival of microorganisms would be reached. The region between the container surface and the point in the surrounding environment where conditions were permissive for survival of living organisms would constitute a zone in which microbiological activity would not likely occur. This zone would be of the order of tens of centimetres in thickness (B. Wilkin, personal communication 1985), assuming that life could survive at a distance where the radiation field was reduced by approximately two orders of magnitude from its value (52 Sv/h) at the container surface, i.e., approximately the thickness of the buffer surrounding the containers (25 cm). The compacted buffer around the containers would likely serve to isolate the container from any surrounding microorganisms, because of the extremely small pore size of the compacted buffer material (see Section 3.1). This would restrict the reestablishment of a microbial population at the surface of the containers, even at some time in the future when the radiation and thermal conditions at the surface of the container were no longer microbiocidal.

Outside the zone of high radiation and high temperature there would be a zone wherein the level of radiation was sufficiently above natural background that there would be an increased probability of mutation (i.e., a sudden change in chromosomal DNA, or the new species resulting from this change) in any organisms present. It is possible that radiation-resistant variants would be favored in such an environment. The outside of this zone of enhanced radiation would gradually merge with the surrounding rock, where the radiation levels would not be significantly enhanced over background and where there would be no enhanced probability of mutation.

Radiation would increase the rate of mutations, but over the geological time scale of the existence of a vault, a very large number of mutations would occur naturally, i.e., in the absence of increased radiation. There are a finite number of permutations in the way in which the DNA of an organism can be rearranged and still result in a viable organism. Within the zone where the probability of mutation was enhanced (i.e. the mutation rate was increased), movement of any new mutants would be constrained by the pore size of the materials in the zone (i.e., the backfill). Thus, even if there were some new and novel mutations generated, they would likely be confined to the vault environment. The mutagenic effect of the fuel waste is not expected, therefore, to significantly affect the range of microbial activity or microbial characteristics released into the geosphere and biosphere.

It should be mentioned here that no unusual characteristics have been noted in microbes isolated from deep groundwaters of the uranium deposit at Cigar Lake in Saskatchewan. Work in progress is examining whether these microbes are involved in the redox chemistry of uranium, and if so, whether they may have evolved from an iron to a uranium redox system.

Abundant microbial life was found in the water covering the damaged reactor core at Three Mile Island. The reactor was compared to a stagnant pond in summer time, with the water at a tepid 27°C and underwater camera lights allowing for photosynthesis. Bacteria present fed on carbon-rich hydraulic fluid (ethylene glycol) that leaked from defuelling tools (Booth 1987). These conditions (liquid medium of optimal temperature and rich in organic carbon) are very unlike the conditions in a Canadian nuclear fuel waste vault, as discussed earlier.

The term "pathogen" (i.e., a disease-causing microorganism) only has relevance in relation to the effects that some organisms can have on man and such other living systems as man exploits. These effects constitute a small subset of the total interactions (competitive, synergistic, etc.) that occur naturally between species. In the subsurface environment of a waste vault the term "pathogen" has no meaning. As explained above, even if through mutation organisms were generated with potential pathogenic capacity to man, they would likely remain confined to the vault environment.

3.9 METHYLATION

Biological methylation of elements resulting in the formation of organometals and organometalloids could play a role in the mobilization of some elements, and it has been suggested that biological methylation of radionuclides could result in increased mobility of radionuclides in the environment (Loeven and Flett 1984).

Biological methylation is a common process for living organisms, and microorganisms may employ it as a means to detoxify various toxins found in their environment. Methylcobalamine, a vitamin B_{12} derivative, or S-adenosylmethionine appear to be the methyl donors in the biological methylation of a number of metals (Klein and Thayer 1990). A number of factors control microbial methylation, which is generally an anaerobic process but has also been observed under aerobic conditions.

The methylation of metals in the environment, as reported in the literature, appears to be restricted to selected main group metals, including tin, lead, mercury and germanium. Some simple methyl compounds of transition metals have been prepared in the laboratory, but usually in conditions (e.g., synthesis in non-aqueous solvents) not likely to be relevant to the vault environment, the geosphere or biosphere. The oxidation state of the metal, the required orbital rearrangement on addition of methyl groups, and the comparative strengths of the metal-carbon and metal-oxygen bonds are major factors in determining whether methylation occurs. Simple metal complexes with methyl ligands may be thermodynamically unstable with respect to formation of oxygen-metal bonds with ligands such as water or OH^-, yet the decomposition reactions may be slow. Also, if an element is complexed, either by organic or inorganic complexing agents, it may be unavailable for methylation. It appears that pH values between 4 and 5 are optimal for biological methylation, and that methylation is also temperature-dependent, presumably because of the relationship between microbial or enzyme activity and temperature. Bacteria capable of methylation require a source of organic carbon for their metabolism, and enhanced methylation in methylating systems has been observed through the addition of organic matter (i.e., soils).

Loeven and Flett (1984) have extensively reviewed the literature and concluded that there is very limited information on the biological methylation of radionuclides. Also, there appears to be no evidence in the literature for the formation of simple (alkyl) organometallic compounds of actinides that are stable in water at or above room temperature, and it seems unlikely that any methylactinide compounds can form in aqueous solution under anything but very unusual conditions (Marks 1986).

The first examples of alkyltechnetium compounds were recently reported by Herrmann et al. (1990). The compound CH_3TcO_3 is volatile, but is very rapidly hydrolyzed, and decomposes above approximately 20°C. The more stable $Tc_2O_4(CH_3)_4$ is a solid at room temperature. Considering the conditions for synthesis of these compounds, it is unlikely that methyltechnetium compounds could form in natural systems, with or without microbial assistance. Further, even if they were formed, their subsequent hydrolysis would probably be rapid.

Because of the limited microbial activity in the nutrient-poor vault and geosphere environments, the expected lack of suitable organic matter, and the instability of methylactinide and methyltechnetium compounds in aqueous solution, methylation is not expected to be an important process in a disposal vault or the geosphere.

Methylation activity has been reported in near-surface soils and sediments for a number of elements (Loeven and Flett 1984) and may affect the mobility of certain radionuclides in the biosphere. Methylation is implicitly included in the biosphere models for radionuclides such as iodine, selenium and carbon because their mobility is controlled by their sorption coefficients onto soils and sediments and by the degassing rates of their methylated and other volatile forms. The sorption and degassing rates incorporated in the biosphere models were obtained under natural conditions, in that microbes were allowed to function naturally in the experiments or natural settings from which the data were obtained (Davis et al. 1993).

4. SUMMARY

AECL Research is developing a concept for the permanent disposal of nuclear fuel waste in plutonic rock of the Canadian Shield. The guidelines issued by the Federal Environmental Assessment Review Panel require that the EIS on the disposal concept being prepared by AECL address a number of microbiological factors and their potential to affect the integrity of the multiple barrier system on which the concept is based. This report formulates a number of views and positions on the microbiological concerns expressed in the EIS guidelines.

The conditions in the vault would not be extreme enough to completely inhibit survival of microorganisms, other than perhaps in the region closest to the container where radiation fields and temperature effects would be the most extreme.

The main nutrient sources for microbes in the vault would be from the emplaced materials, especially the organics associated with the clay components of the buffer and backfill barriers. However, most of this organic material appears to be old and may be recalcitrant to microbial decomposition. The contribution of groundwater to the overall nutrient inventory in the vault would be small. Microbial life in the buffer material surrounding the waste containers would be restricted because of the compacted nature of the buffer, and perhaps because of a lack of nutrients. Limited microbial growth may be possible in the backfill materials, and such growth would likely result in plugging, thereby effectively reducing the permeability of this barrier. The geosphere is nutrient-poor, and microbial growth would largely occur in biofilms, which may have the capacity to sorb and retard radionuclides released from the waste. Microbes are an integral part of the biosphere and their effects on radionuclide migration have implicitly been taken into account in the biosphere models.

Excavation would potentially introduce microbes and nutrients from the surface to the subsurface environment of the vault. These sources would be largely controllable and the degree to which control is desirable remains to be determined.

Microbially influenced corrosion in a disposal vault is considered improbable in the first few hundred years after waste emplacement because of the high gamma-radiation fields and temperatures (and expected initial desiccation) arising from the used fuel, which would greatly reduce the microbial population at and near the container surface. The buffer would likely present an effective barrier against repopulation of regions near the container surface, because of the extremely small pore space in the buffer. Experiments are needed to confirm the restriction of bacterial growth and movement in the buffer. The Buffer/Container Experiment in progress at the URL will be examined upon decommissioning to assess survival and growth of microbes in such a compacted environment.

Microbially influenced corrosion has not been observed for titanium, but the possibility that microbial activity in the form of biofilms may lead to crevice corrosion cannot be discounted. It is assumed in the failure model for titanium containers that crevice corrosion initiates on all containers, so it is of no consequence for the container lifetime predictions whether or not microbial activity causes the initiation of crevice corrosion on titanium containers. It is not known if microbes could accelerate the rate of crevice propagation in titanium, although localized corrosion under a biofilm on titanium has never been observed.

The copper container failure model considers both uniform corrosion and pitting, processes that can be affected by MIC. The assumptions in this model take microbial effects into account implicitly, but these assumptions may need to be reexamined as the research program on MIC and copper containers continues.

In the reference disposal vault design, cements are used in grouts and concretes for a variety of sealing applications. It is not expected that microbial processes would reduce the integrity of the high-performance concrete material proposed for use in the reference concept. Neither is it likely that the minimal quantities of superplasticizer that may leach from the cements and concretes would have any significant impact on microbial growth.

Studies are being carried out to determine the rate of microbial gas formation (CO_2, CH_4) from the organics in backfill clay. The effects of radiation and heat on the potential decomposition of these organics into compounds that are more easily used by microbes remains to be investigated.

Microbial activity could result in changes to the geochemical conditions (e.g., pH, Eh) in the vault and geosphere, which might affect radionuclide speciation, solubility and sorption. However, such effects are most clearly seen in nutrient-rich shallow waste-diposal environments. In the nutrient-poor conditions expected deep in the Canadian Shield these effects would be much less significant because of the enormous buffering capacity of the vault and geosphere materials. Also, the parameter ranges used in the probabilistic assessment models are expected to cover any effects microbes may have on geochemical processes.

Methylation is a specific microbial process by which radionuclides could be made more mobile. However, studies have shown that methylactinide and methyltechnetium compounds in aqueous solution would be very unstable. This fact, combined with the expected limited microbial activity in the vault and nutrient-poor geosphere, suggests that methylation would not be an important process in a disposal vault or the geosphere. Methylation is implicitly included in the biosphere models for radionuclides such as iodine, selenium and carbon because sorption and degassing rates incorporated in the models were obtained from experiments in which microbes were allowed to thrive.

Studies of the Cigar Lake uranium deposit have shown that clays form an effective barrier against particle transport (10-450 μm). The buffer and backfill in a disposal vault, therefore, are expected to form an effective barrier against radionuclide migration from the vault to the geosphere as a result of sorption on colloids and bacteria. Consequently, migration of radionuclides to the geosphere would occur primarily by diffusion. Microbial and colloid migration might become important in fractures in the geosphere. However, in the nutrient-poor environment of the geosphere most microbes have been found to be contained in biofilms on fracture surfaces, and since such biofilms have a large capacity for metal uptake, radionuclide migration would likely be reduced as a result of the presence of biofilms. It has been shown by several groups that colloids have a negligible effect on radionuclide migration for reversibly sorbed radionuclides, and even in the case of irreversible sorption the transport capacity of colloids is small. Moreover, the current range of parameter values used for radionuclide transport in the assessment models would likely cover any impact of irreversibly sorbed radionuclides.

Radiation and high temperatures at the container surface would likely cause a zone of greatly reduced microbial activity around the containers. Repopulation of this zone, after the radiation levels and temperature had decreased, would be limited because of the restricted microbial movement in the compacted buffer material. The radiation field would be attenuated by the clay material surrounding the containers, and at some point away from the container surface the dose would be low enough that bacteria could survive. This would be the region where mutations (possibly favouring radiation-resistant species) would most likely occur. However, even if through this process some organisms with pathogenic potential to man were generated, they would likely remain confined to the vault and geosphere region.

The review and the analysis presented in this report suggest that the presence of microbial activity in the vault near-field (i.e., at the container surface and in the buffer and backfill environments) would be of limited consequence, because the prevailing conditions (limited space and nutrients; heat and radiation) would not allow the establishment of large, thriving microbial populations. A crucial argument in this discussion is the effectiveness of the clay-based barriers (especially the buffer) in limiting bacterial growth and in preventing bacterial and particle transport. Another crucial point is the suitability of the organic materials associated with the clays for microbial metabolism. Ongoing research at WL is concentrated on addressing these issues. The impact of the presence of microbial activity in the geosphere on radionuclide transport is expected to be small, because it is likely that microbes would grow largely in biofilms. Ongoing work is focused on quantifying the effects of biofilms in rock fractures on radionuclide transport. Microbes are an integral and ubiquitous part of the biosphere, and the effects of their presence have been implicitly included in the biosphere models.

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APPENDIX A

MICROBIOLOGICAL ISSUES RAISED IN THE EIS GUIDELINES

CONTENTS

A.1 MICROBIOLOGICAL ISSUES RAISED IN SECTION 5.4 OF THE EIS GUIDELINES

The following, taken from Section 5.4 (p.22) of the FEARO Guidelines (Federal Environmental Assessment Review Panel 1992), summarizes the microbiological issues that are to be addressed in the EIS.

> The EIS should address the following microbiological factors with respect to their potential to affect the integrity of the Multiple Barrier System and the release of potentially harmful substances to the surface environment:
>
> - the present state of knowledge of microbes at depth;
> - the origin of microbiological activities that are likely or possible at any stage during nuclear fuel waste storage, preparation, transport, emplacement, or in the vault and rock mass system, and the relative importance of indigenous or introduced organisms;
> - the most important sources of nutrients that may be found at any stage of the disposal concept, including those in the rock mass at the proposed vault depth, in the groundwater under expected conditions, and introduced by vault construction, loading and sealing;
> - the potential rate (ranges and uncertainties) of microbially-induced corrosion of the disposal container, including the influence of thermal loading, saline groundwater, radiolysis and gas emanations;
> - the possibilities and likelihood of enhanced microbial mutation or evolution resulting from higher than normal background radiation levels, and the potential effect of such changes on radionuclide or chemical transport and release;
> - the potential for intrinsic microbial activities at any stage from reactor to emplacement, and in the vault or the rock mass barrier, to affect the formation of radiocolloids which might influence the adsorption or movement of radionuclides and other contaminants;
> - the nature and rates of microbially mediated processes which may result in the release of radionuclides and other contaminants from a disintegrating vault, the mechanisms by which these contaminants may be modified (e.g. through methylation), the forms in which the modified contaminants may reach the surface environment, and the various possible impacts on humans and the natural environment which may result.

A.2 MICROBIOLOGICAL PROCESSES IDENTIFIED ELSEWHERE IN THE EIS GUIDELINES

(p.10-11) The EIS should discuss . . . the risks to the health of humans and human communities, and to the work site and the natural environment, that are associated with the concept for both the management and the transport of nuclear fuel waste. . . . This discussion should include . . .

- risks resulting from . . . microbiological processes.

(p.13) . . . the EIS should discuss the possible migration of radionuclides and other contaminants at all stages and through all barriers. In this discussion, the EIS should consider . . . the effects of . . . microbiota and other factors on the migration through various barriers.

(p.14-15) The EIS should describe the methods used to estimate radionuclide release and consider . . .

- the potential for the generation of gases by . . . biological processes, and the consequences of the presence of these gases;
- the potential for other biological and microbiological interactions with nuclear fuel waste;
- the possible dissolution mechanisms of nuclear fuel waste including biologically mediated mechanisms . . .

(p.19) The EIS should describe the vault sealing program, including . . .

- the effects of biofouling of the vault, and the sealing materials.

(p.20) The discussion of those properties of the rock mass and the groundwater flow system that could affect the migration of radionuclides and other contaminants should include, but not be limited to, the following:

- procedures for obtaining a representative description of the important generic . . . biological . . . properties and the variations of these properties in space and time (including ranges of values and their uncertainties); . . .
- relevant . . . biological and biochemical processes in the rock mass and groundwater flow systems that may impede or enhance the transport of radionuclides and other contaminants, and the coupling between these processes; . . .
- short-term or transient changes in the processes and properties of the rock mass and the groundwater system that may be expected due to the establishment of the disposal vault, including the effect of the biological . . . changes due to the construction and loading of the vault.

(p.21) Criteria for the rejection of a rock mass on the basis of its . . . biological properties . . . should be stated.

(p.21) The description of the generic surface environment should include . . .
- the key . . . biological processes that control the movement and concentration of radionuclides and other contaminants in the surface environment;
- the key linkages among the physical, chemical and biochemical processes in the rock mass and in the surface environment.

(p.24) For each [model] component, the . . . biological principles underlying the model . . . should be explained. . . .

The discussion of processes and mechanisms should include
- . . . biological and microbiological transformations of and interactions among all active constituents, both mobile and immobile; . . .
- the coupling among physical, hydrogeological, chemical, biochemical, and geomechanical processes and mechanisms.

(p.27-28) The EIS should discuss the use of scenario and sensitivity analyses. . . . Aspects investigated should include . . .
- the effect of variation or uncertainty in individual . . . biochemical parameters.

(p.28) The discussion of the scenario and sensitivity analyses should include . . .
- the identification of the relevant . . . biological factors to be included in a particular scenario, and the justification for rejecting other factors.

REFERENCE

Federal Environmental Assessment Review Panel. 1992. Final guidelines for the preparation of an environmental impact statement on the nuclear fuel waste management and disposal concept. Federal Environmental Assessment Review Office, 13th Floor, Fontaine Building, 200 Sacré-Coeur Blvd., Hull, Quebec K1A 0H3.

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