The potential significance of microbial activity in radioactive waste disposal

A. McCabe

Nuclear Electric, Barnett Way, Barnwood, Gloucestershire GL4 7RS (England)

Summary. Active microorganisms can exist in any proposed environment if the basic requirements for life are satisfied, i.e. a suitable temperature and pH, the presence of the necessary nutrients and water. If conditions are not favourable microbes may survive in a dormant state until a change will allow activity. In local pockets microenvironments may become established where microbial activity may increase leading to altered environmental conditions and to changes in the near-field, e.g. degradation and breakdown of barriers, gas generation and/or uptake and transport of nuclides.

Key words. Microbial activity; environmental conditions; microenvironments; gas generation; breakdown of barriers; nuclide uptake; nuclide transport.

1. Introduction

The aim of this paper is to assess the potential significance of microbial activity in radioactive waste disposal. It outlines the major factors which need to be considered in order to evaluate the importance of microbiological action. These include water and nutritional sources, environmental conditions, the establishment of microenvironments and the effect of eventual microbial metabolic by-products on the disposed waste forms.

In the design of a radioactive waste repository the main objective is to immobilize radionuclides so as to prevent their reintroduction to man's environment. The mechanisms by which radionuclide transport could occur from the waste through the near-field containment, the geosphere and back to the biosphere are presently the subject of intensive research (table 1), the objective being to delay the return to man beyond the decay period.

The safety case for a repository depends on the principle of multiple layers in which a series of essentially independent barriers are employed to delay or retard the migration of radionuclides. Implicit in this principle is the idea that if some of the barriers should fail, the remaining ones would still be sufficient to assure safety. Factors which might change the integrity of individual barriers need to be examined, however, and microbiological action is one such factor ⁸³.

2. Conditions for microbial activity

Microbiological action will be influenced strongly by the waste forms and repository designs. For example vitrified high-level waste or spent fuel will generally be encapsulated in a metal container and sealed by a backfill in a mined repository. Intermediate-level waste might consist of ion-exchange resins or evaporator sludges incorporated into a cement matrix in steel drums sealed in a mine. Low-level wastes include even more diverse forms such as contaminated paper, cloth, filters and clothing and may be solidified with concrete or bitumen.

To allow an active microbial population to develop, certain basic requirements for life have to be fulfilled, such as the presence of water, carbon sources, main nutrients, energy sources, and electron donors and acceptors.

Sources of carbon include carbon sources, particularly organic carbon, within the waste form, but also organic carbon in the backfill and groundwater and inorganic 'carbonate' present in flowing groundwater and minerals in the backfill. Nitrogen may be introduced in entrapped air, as NH₄⁺ or NO₃⁻ in the backfill or in the groundwater and in the waste itself. Phosphorus and sulfur are possibly also present in sufficient amounts.

Various chemical processes may be used as energy sources, they may proceed in either aerobic or anaerobic conditions as outlined in the sample of the oxidation of the iron of the canisters:

Table 1. Microbial influence on containment breakdown and radionuclide migration 61

Processes		
Containment breakdown	Biodeterioration of concrete, steel.	Corrosion studies, petroleum industry, extreme environments, drilling procedures.
Waste leaching	Wasteform destruction, alteration of groundwater chemistry.	Hydrogeology, water industry, extreme environments.
Migration through backfill	Biodeterioration of bentonite, bitumen, concrete etc.	Radiochemistry, petroleum industry, construction industry, corrosion studies, extreme environments.
Far-field migration	Radionuclide migration, sorption onto microorganisms, groundwater flow.	Radiochemistry, geomicrobiology, hydrogeology.
Input to geosphere	Radionuclide migration, movement through rock accelerated by resident motile microorganisms.	Drilling procedures, geomicrobiology, mining industry, speleology, hydrogeology.
Dose to man	Nuclide speciation, input into food chains, in concentrations with trophic level.	Soil science, food and water industries, ecological- energetics, landfill studies, radiobiology.

Fe + 2 H₂O
$$\rightarrow$$
 Fe²⁺ + 2 OH⁻ + H₂ Anaerobic
3 Fe + 4 H₂O \rightarrow Fe₃O₄ + 4 H₂ (anoxic)
Fe + H₂O + $\frac{1}{2}$ O₂ \rightarrow Fe²⁺ + 2 OH⁻ Aerobic
3 Fe + H₂O + $\frac{3}{2}$ O₂ \rightarrow Fe₃O₄ + H₂ (oxic)

In practice the corrosion process would probably be very complex and within the near-field area, zones of both oxic and anoxic corrosion resulting in the formation of both Fe(II) and Fe(III) salts would occur, depending on variations in E_h and pH. Oxic corrosion, however, is fast while anoxic corrosion occurs only slowly, i.e., the kinetics of reaction are determined by the rate of supply of oxygen which comes from entrapped air, groundwater flow or radiolysis.

Of all the outlined requirements for life, energy, water and carbon availability are of the most importance ^{1, 13, 54}. The amounts of carbon required are such that if this requirement is met then it is likely that the smaller amounts of the other elements needed will also be present in sufficient quantities.

Analysis of calcium montmorillonite (Fullers's Earth) from a site in Oxfordshire containing up to 10 % organic material indicate that a carbon-rich environment supports a wide range of microbial groups, e.g., aerobic heterotrophs, iron-precipitating heterotrophs, denitrifiers, sulfur oxidizers and sulfate-reducing bacteria. In contrast it has been shown that Boom clay, another potential backfill material, recovered aseptically is practically microbe-free ¹⁸, in spite of total carbon and nitrogen values of 1.25 % and 0.078 % dry weight, respectively. Organic material in these clays is often of high molecular weight and is degraded only slowly under anaerobic conditions. However radiolytic processes may enhance microbial breakdown and make more organic carbon available for growth.

In certain environments the nutrient concentration may be limiting for microbial activity and only oligotrophic organisms may slowly develop ^{27,48,64}. Such a population may be able to solubilize precipitated and insoluble substrates and change the conditions to allow less specialized species to develop.

A restricted range of organisms, the chemolithotrophs ^{1, 13, 54}, use carbonate as their carbon source. They often require an acidic environment. The probability of such organisms exhibiting significant levels of microbial activity in repositories has been regarded as negligible ³².

A water activity of greater than 0.6–0.7 is required for life ^{35,74}. However, if water activity falls below this value microorganisms may survive as spores or in another dormant state over periods of unfavourable conditions. The longevity of spores has been demonstrated from occupational debris from beneath the Roman fort Vindolanda, and lake sediments cored from Seamere in East Anglia ^{32,81}, where up to 104 viable endospores/g dry wt from *Thermoactinomycetes* in layers of bracken and straw litter sandwiched between clay were found.

In a cement matrix water comprises up to 20 % of the volume at the beginning and 10 % after three months and is present free in pores or the waste material. Within these regions microbial activity is greatly stimulated.

An environmental restriction may also be given by the presence of heavy metals. Some microorganisms have developed mechanisms to cope with high concentrations of such heavy metals and the possibility exists that such abilities are genetically transferred relative easily among microorganisms ^{23, 28-30, 51}.

3. Repository conditions and microbial activity

Constraints on biological activity may be a consequence of the lack of any of the basic requirements of life. However the various environmental conditions likely to exist in a repository may also restrict microbial activity.

The pH greatly influences microbial growth. Only few organisms are known to be capable of surviving at a pH of above 11.5; however, populations from natural alkaline environments have rarely been investigated 34,38,50 . Microorganisms have varying E_h requirements. While some such as Nitrosomonas sp. and Nitrobacter sp. need a high E_h and may not survive as the redox conditions become reducing, others such as methanogens must have an extremely reducing environment for growth. Upon closure, conditions in a repository would slowly change from oxic to anoxic. Later saturation with water would maintain a low E_h by limiting the diffusion of free oxygen 90 .

Temperature is a major factor controlling metabolic activities, however microbes are known to grow and reproduce at temperatures < 0 °C^{16,37} while others exist and multiply above 100 °C^{22,75,83}.

Increasing pressure slows down the activity of most microorganisms and may cause death at -20 MPa. However a number of species can live up to 180 MPa 44,57,58,65.

Microorganisms vary widely in response to radiolytic effects. *Micrococcus radiodurans* has been found to withstand a single dose of 5×10^3 Gy 11,21,66 microorganisms have even been observed that survived up to 10 Sv/h in the Three Mile Island reactor core.

Often microbes appear to be intolerant to hostile conditions if delivered without pre-conditioning, but will adapt if the conditions are altered gradually. Such a situation is probably more realistic when considering a repository under construction and during operation. Parameters would slowly change as waste emplacement takes place over a period of time. After repository closure regions of optimum conditions would be set up allowing adaption to occur at certain points within the near-field. Thus a repository environment could provide an opportunity for microorganisms to adapt to and hence exploit this novel ecological niche.

The availability of oxygen will effect the types of microorganisms present in the repository. Microorganisms introduced will use oxygen as the electron acceptor. On closure the area becomes oxygen-deficient after some time and facultative aerobes will predominate, such as denitrifying bacteria or sulfate reducers 76, 79, 84, 89. When all sources of bound oxygen have been consumed then anaerobic species will take over. Many natural clays contain high concentrations of reduced sulfur compounds such as sulfides, thiosulfates and tetrathionate. Should a microenvironment of low pH exist then various major groups of microbes are able to oxidize these compounds, assuming electron acceptors are present, i.e. filamentous bacteria like Beggiatoa or bacteria of the genus Thiobacillus. Radionuclide release and decay in the groundwater environment may facilitate aerobic conditions through radiolytic processes generating oxidizing species such as hydrogen peroxide or superoxide radicals. These microorganisms produce H₂SO₄ as an end-product, which may aid the solubilization of radionuclides. Complexation with organic acids (humic or fulvic) formed by microbes in the surrounding clay might enhance its movement 8,9.

As a result of oxygen depletion and the use of alternative electron acceptors, such as nitrate, sulfate and carbon dioxide, the E_h of the ecosystem is reduced $^{32,\,33,\,62,\,90}$. This results in multivalent metal ions existing in their more soluble reduced states. On the other hand, fermentation by anaerobes may generate carbonate and sulfide, both of which will precipitate many heavy metal ions.

Anaerobic metabolism may have an important effect on radionuclide mobility. During fermentation degradation is usually incomplete and various organic compounds may accumulate, such as alcohols, acids and partially oxidized aromatic compounds. These substances may complex with cationic nuclides which would in the absence of microbial activity be stabilized into water-insoluble states ^{8,9,17,60,73}.

The development of anoxic conditions in trench leachates as studied at Maxey flats 20 can be attributed to microbial degradation of organic matter present in the buried wastes. During the aerobic and later anaerobic degradation of organic matter, decomposition products build up, such as CO₂ and ammonia increasing alkalinity, while dissolved oxygen, sulfate and nitrate are consumed. In flowing water the alkalinity and the sulfate concentration in the trenches may serve as a measure of the extent of microbial degradation dependent on the quantity and reactivity of the organic waste materials 24, 45, 87. Preliminary analysis of Maxey flats leachate data indicates that siderite, calcite, dolomite, rhodochrosite and iron sulfides may be controlling the Fe⁺², MN+2, Ca+2, Mg+2, carbonate and sulfide concentrations in the leachate. Therefore in relatively closed systems the effects of solubility constraints should be considered in interpreting quantitatively sulfate, sulfide and alkalinity data in terms of microbial decomposition processes 26, 52

Given the wide range of conditions in which microbes can adapt it is not surprising that such organisms are found in geological formations. The literature on this subject is rather limited due mainly to the difficulties of obtaining suitable samples, avoiding contamination with surface organisms, and culturing the species ^{32, 33, 44}.

4. Potential microbial effects on near-field containment

From the viewpoint of repository safety assessment, a number of possible consequences of microbial activity require evaluation.

Breakdown of containment

Table 1 outlines the influence of microbial activity on containment breakdown, near-field effects and radionuclide migration to the far-field.

Direct attack mechanisms

Degradation of inorganic materials by microorganisms is slow unless organic rich materials are available, either in the waste itself or as encapsulant.

The engineering lifetime of a 1-m-thick reinforced concrete slab could be as short as 380 years for a concrete based on ordinary Portland cement, but is likely to be in the range 500–1000 years for sulfate-resisting Portland cement (SRPC). The pH at the surface is reduced in stages from approximately 13 at the beginning to that of groundwater as Na, K, Ca and Si are removed from the system.

For a low-level waste repository leaching effects will largely determine the rate of concrete degradation. The action of microorganisms may result in fissuring, void formation and/or gas generation.

Concrete will be attacked by species of *Thiobacilli* obtaining energy from the oxidation of sulfur compounds, producing sulfuric acid as a by-product which increases concrete corrosion by establishment of a low pH microenvironment. H₂SO₄ also attacks the physical structure of the concrete encasement by reacting with calcium hydroxide to produce calcium sulfate resulting in a disruption of the concrete matrix in the surface layers, furthermore the acid may react with aluminates to produce calcium sulfoaluminate (ettringite).

Bitumen is used for encapsulation of low- and intermediate-level radioactive wastes. Bitumen consists of a mixture of organic substances, particularly hydrocarbons and has excellent coating and waterproofing qualities which make it highly suited for the disposal of waste in a repository.

Degradation of bitumen can take place under both aerobic and anaerobic conditions depending on the organisms involved ^{15,71,85,88}. It appears that no single organism is capable of completely breaking down bitumen. The end-products are H₂O, CO₂, H₂, CH₄ and other low-molecular-weight compounds. The most widely-known microbes which preferentially attack bitumen are *Pseudomonas* species ^{14,88}.

Among the metals used for containers, copper is thermodynamically stable in pure water, thus corrosion of copper in the groundwater will be determined by the supply of corrosive substances dissolved in the water, dissolved oxygen and, for reducing groundwater conditions, dissolved sulfide ⁶¹.

Under anaerobic conditions sulfate-reducing bacteria, e.g. species of *Desulfovibrio* and *Desulfotomaculum*, corrode iron ⁴⁹. They produce H₂S as a metabolic product in sulfate and organic-rich soils. It is conceivable that the end-product could then permeate to a localized area of oxidizing conditions, become converted into sulfuric acid and subsequently attack the concrete trench walls. However corrosion products, i.e. gypsum and ettringite are relatively insoluble.

When moist clean iron surfaces react with $\rm H_2O$ and quickly become depolarized, further reaction is inhibited. Sulfate-reducing bacteria remove this layer by the action of a hydrogenase and produce iron hydroxides and iron sulfide corrosion products. Also areas subjected to alternating aerobic and anaerobic conditions corrode faster than in entirely anaerobic environments.

Physical disruption includes effects such as fissuring and void formation. The first could affect the containment material, i.e., in concrete size changes due to microbial activity may result in cracks developing leading to accel-

eration of water movement through the repository resulting in transport of radionuclides away from the waste form. Void formation will occur as a result of the conversion of solid materials to gas. By many types of fermentations gaseous end-products, i.e., CO_2 , H_2 and CH_4 are liberated.

Gas generation could also be a mechanism for the direct release of radionuclides from the waste site. The gaseous species included ³H, ¹⁴CH₄, ³HCH₃, ¹⁴C and tritiated higher hydrocarbons and ¹⁴CO₂ ⁶⁸.

Radionuclide uptake

Microbes show the ability to concentrate trace metals from solution by either active uptake into the organism or surface absorption onto outer membranes (table 2). Uranium accumulates externally on the surfaces of, e.g., Saccharomyces cerevisiae cells. The rate and extent of accumulation is subject to environmental parameters, such as pH, temperature and interference by certain anions and cations; e.g., reducing positive charge on the cell wall of S. cerevisiae components by chemical treatment can enhance metal uptake ⁷⁷. Phosphate groups in the cell wall of S. cerevisiae have been implicated as sites of uranium complexation ⁷² as well as the carboxyl groups of the peptidoglycans in the cell walls for divalent metal complexation. The principal binding site of uranium and

Table 2. Microbial mechanisms for metal extracting/concentrating/recovery

Microbes	Metals removed	Method	
Thiobacillus Sulfolobus	Iron (sulphur)	Oxidation	56
Sphaerotilus Leptothrix Hyphomicrobrium Gallionella	Iron, manganese	Oxidation	12
Spirogyra Oscillatoria Rhizoclonium Chara	Molybdenum, selenium, uranium, radium	Oxidation	12
Desulfovibrio sp.	Mercury	Reduction	12/86
Scenedesmus Synechococcus Oscillatoria Chlamydomonas Euglena	Nickel	Surface ion-exchange	86
Saccharomyces cerevisiae Rhizopus arrhizus	Uranium, cesium, radium	Surface ion-exchange	12/77
Penicillium digitatum	Uranium	Surface ion-exchange	31
Ustilago sphaerogena	Iron	Surface chelation	25
Aspergillus niger	Aluminium	Surface chelation	47
Cyanidium caldarium	Iron, copper, nickel, aluminium, chromium	Surface precipitation	86
Staphylococcus aureus Escherichia coli	Cadmium, zinc (arsenate), (arsenite) antimony	Chemiosmotic efflux	23/67
Anabaena variabilis Pseudomonas aeruginosa	Potassium, rubidium, uranium, cesium, radium	Chemiosmotic efflux, intracellular trap	77
Synechococcus	Nickel, copper, cadmium	Intracellular trap	86
Clostridium cochlearium	Mercury	Biomethylation	86
Pseudomonas sp.	Tin	Biomethylation	36

thorium on *Rhizopus arrhizus* cell walls is the chitin component ^{5-7, 55, 80, 82}.

Transmission electron microscopy has shown that Sulfolobus attaches to elemental sulfur via adhesive pili of polysaccharide polymer material bridging between the organism and its substratum and help to assure strong attachment. Leaching organisms including Thiobacilli also appear to be bridged to the ore as their energy source. Laboratory studies of *Pseudomonas*, a bacterium involved in uranium recovery, have indicated that organism attachment is mediated by polymer material 12, 46. Heavy metal binding to metallothionein proteins may account for 238U, 137Cs and 226Ra in Pseudomonas aeruginosa to the extent of 56 % of the dry weight of the cells; however only 44 % of the cells within a given population possess visible uranium deposits when examined by electron microscopy. Metabolism is not required for metal uptake which occurs so fast that the transitions between initial and equilibrium stages cannot be observed.

Little is known about the microbial accumulation of radium. Jilsk et al. ⁴² observed the removal of ²²⁶Ra from a waste stream by chemically derivatized mycelia of *Penicillium chryogenum* and furthermore it has been found that 95% of the ²²⁶Ra was removed during three months growth of denitrifying microorganisms.

Plutonium has been found to be highly toxic to microorganisms although rather because of radiation than of toxic effects. However highly resistant bacteria, fungi and actinomycetes have been isolated from soil which are capable of incorporation plutonium in their cells and altering its form in the cell and in solution. The resulting soluble plutonium complexes exhibit a range of mobilities in soil 8-10, 49, 63, 69.

Microbial metabolism may alter groundwater chemistry leading to large changes in E_h , pH and concentrations of organics, thus affecting chemical corrosion of materials, nuclide speciation, sorption processes etc.

Under anaerobic conditions incomplete degradation of organic material may result in the addition of low concentrations of organic by-products of the groundwater (tables 3 and 4) which could influence the solubility of particular nuclides ^{17, 60, 70}.

Much of the information obtained on microbial accumulation of heavy metals is from the mining industry, i.e. lead or uranium from uranium mill tailings 4,52. Its uptake is more likely to be governed by physico-chemical factors than by active cellular processes. Justyn and Stanek 43 reported that the filamentous algae, Cladophora, Oedogonium and Rhizoclonium accumulate uranium by binding the ion to cellular surfaces. Chlorella will absorb uranium intracellularly 39 but this uptake is probably independent of cellular metabolism since the uranium accumulated by the living cell can be removed by washing the cells with EDTA. Heat-killed Chlorella will also accumulate large quantities of uranium intracellularly 53.

Table 3. Compounds identified in trench, leachates from Maxey Flats and West Valley disposal sites 20

and west valley disposar sites		
Acids	Diacetone alcohol	
Benzoic acid	Dibutyl ketone	
Butanoic acid	Fenchone	
Decanoic acid	Methyl ethyl ketone	
2-Thylhexanoic acid	Methyl isobutyl ketone	
Hexanoic acid		
Hydroxybenzoic acid	Amines	
3-Methoxy-4-hydroxybenzoic acid	Aniline	
2-Methylbutyric acid	Cyclohexylamine	
3-Methylbutyric acid	Dicyclohexylamine	
2-Methylhexanoic acid	Methyldicyclohexylamine	
2-Methylpentanoic acid	1/10th y taloy of only talling	
3-Methylpentanoic acid	Aromatic hydrocarbons	
2-Methylpropionic acid	Benzene	
Nonanoic acid	Biphenyl	
Octanoic acid	Dimethylnaphthalene	
Pentanoic acid	Naphthalene	
Phenylacetic acid	Toluene	
Phenylbutyric acid	Xylene (isomers)	
Phenylhexanoic acid	F /	
Phenylpropionic acid	Esters	
Toluic acid (isomers)	Butyl phthalate	
47 1 7	Diethyl phthalate	
Alcohols	Several unidentified phthalates	
Borneol	Tributyl phosphate	
2-Butanol	Triethyl phosphate	
Cyclohexanol	Triphenyl phosphate	
2-Ethylhexanol		
3-Ethylhexanol	Ethers	
2-Hexanol	Anisole	
3-Hexanol	Bis(2-chloroethyl)ether	
2-Methyl-2-butanol	Bis(2-chloroethoxy)methane	
Methylcyclohexanol	Bis(2-ethoxyethyl)ether	
Octanol	1,1-Diethoxyethane	
2-Phenylcyclohexanol	1,1-Diethoxy-2-chloroethane	
(Propylene glycol)n	1,4-Dioxane	
α-Terpineol	Tetrahydrofuran	
3,3,5-Trimethylcyclohexanol	Tripropylene glycol methyl ethe	
Aldehydes and ketones	Phenols	
p-Hydroxybenzaldehyde	Cresol (isomers)	
Paraldehyde	Octylphenol	
Vanillin	Phenol	
Acetovanillon	4-t-Butylphenol	
Camphor	Tetramethylbutylphenol	

Table 4. Anaerobic degradation of organic compounds present in Maxey Flats leachate sample by a mixed culture of bacteria 20

Compound	Initial concentration (mg/l)	Percent change
2-methylpropionic acid	5.9	- 2
2-methylbutyric acid	20.6	-52
3-methylbutyric acid	9.9	0
Valeric acid	5.5	+27
2-methylpentanoic acid	-	-24
C ₆ acid (unidentified)	→	-27
Phenol	1.1	-27
Hexanoic acid	5.1	-45
2-methylhexanoic acid	3.0	-10
Cresol (isomers)	3.9	-21
C ₈ acid (unidentified)	_	-13
Benzoic acid	1.9	-26
Octanoic acid	1.9	-21
Phenylacetic acid	3.8	-50
Phenylpropionic acid	9.3	53
Phenylhexanoic acid	_	-11
α-Terpineol	0.26	-12
Tributylphosphate	0.24	0

One of the concerns with microbial attack is that the radionuclides might be chelated by the microbes or their metabolic by-products. Then the radionuclides could be transported quickly through porous media 41. An obvious consideration is the transport of any fine material, diameter less than 1 um, via adhesion to the surfaces of mobile microbes. The surface activity of many materials which might retard mobilized radionuclides could be considerably reduced by coverage with microbes since sorption sites are thus unavailable 19, 59, 72. In addition, bacteria capable of living in subterranean oil deposits have been shown to produce surfactant agents which could increase the rate of wetting of backfill barriers, e.g. bentonite, although organisms have also the ability to plug porous formations. It has been found that microbial materials including extracellular excretions have a potential to sorb nuclides reversibly 19,40. Microbial activity also exerts an indirect control on nuclide release and migration through the involvement in redox and acid base reactions. Changes in the oxidation state of elements can profoundly affect the uptake of metals by living cells. Ionic charge, radius and affinity for organic ligands as well as metal concentration, availability and solubility are important limiting chemical parameters. Changes in pH, temperature, competing ions present, and surface-active interfering substances such as humic acids, clays and proteins can all have an effect on the final net charge of an inorganic metal complex. Alterations in charge influence cellular metal transport involved in metabolic pathways and consequently energy-driven uptake systems. Oxidation and reduction mechanisms enzymatically or intracellularely capture energy while transforming the metal, e.g. iron oxidation with resulting cellular surface oxide precipitation is a very effective energy obtaining detoxification mechanism 78.

5. Formation of microenvironments and changes in the microbial population

Microenvironments could occur with totally different chemistry to the general system being formed by particular organisms. In the presence of concrete, which will impose a pH > 10.8, it is unlikely that microorganisms requiring neutral or acidic conditions will become established. On the other hand, organic material in close contact with concrete could contain within them areas of low pH, i.e. microenvironments. Furthermore, alkalophilic organisms may lower the surface pH of small areas on the concrete. Once established such a microbial colony would maintain the pH of its microenvironment within the optimum range.

The tolerance to environmental stress factors such as extreme temperature, pH, osmotic conditions, pressure, radiation or nutrition varies greatly among the species investigated. Little is known on combinations of stress factors. Although microorganisms appear intolerant to extreme conditions delivered with no pre-conditioning,

this may change by gradually increasing the stress factor in order to allow adaptation to take place. Such a situation is probably more realistic when considering a repository under construction and during operation. Temperature and radiation would slowly increase as waste emplacement takes place over a period of time. After closure a temperature/radiation gradient would be set up allowing adaptation to occur in the waste and the nearfield. The new environment could provide an opportunity for microorganisms to exploit a novel ecological niche. Many of the so-called extreme environments such as deep sea sediments, ice cores, vulcanic hot springs, subsurface soil or salt mines have not been investigated in great detail and as such studies always reveal new organisms with new metabolic capabilities, it would not be astonishing to see a new specific population of microorganisms evolve in such waste repositories over a longer time scale. In probably all environments one species of microorganism depends upon others, maybe for its source of an important nutrient, e.g., the carbon source or a vitamin, a specific hydrogen donor or acceptor, or for the removal of some metabolic products. Laboratory experiments using mixed cultures are rarely reproducible due to the lack of knowledge of the dynamics of the system. In contrast results of pure culture studies cannot be used as a basis to model the complex situation in the environment of a repository.

The changing conditions over time in the repository would induce a succession in population; first a heterotrophic aerobic population would be established. After exhaustion of oxygen a facultative and then possibly an obligate anaerobic population would develop. One of the concerns of an evaluation of microbial content in deep repositories has been the availability of carbon. Since every organism is part of a food chain, except for chemolithotrophs using CO₂ as carbon source, the best source of nutrients is another organism.

6. Radionuclide transport in colloidal form

Colloidal systems are abundant in the bio- and geosphere. It is therefore worthwhile to consider their role in the geological environments surrounding future nuclear waste repositories.

Colloidal particles are in the size range 1 nm to 1 μ m and microorganisms fall also within this range. Therefore should such organisms transport radionuclides they will act as colloidal particulate.

Pseudocolloids are particulate aggregates of clay, silica, glass, soil, hydroxides, microbes etc. onto which trace elements have been sorbed. This may occur altering their properties significantly. These colloidal 'carriers' exist only under certain conditions which indicates that they may not primarily interact with exposed solid surfaces. Such pseudocolloids play an important and dominating role in the transport of actinides in their lower oxidation

states under conditions where open channels are available for water flow.

Microbes act as pseudocolloids in that they collect radionuclides either on their surface (extracellular accumulation) or within themselves (intracellular accumulation) and transport the material from the near-field.

Thermodynamically stable colloids constitute chemically stable compounds, such as macromolecular complexes and polymeric hydroxides in the low pH range, e.g. Pu(IV), carry net charges and interact with solid surfaces in various ways³.

Metastable colloidal species constitute aggregates formed in the course of precipitation or coagulation. Although they are not thermodynamically stable they exist for a long time due to coulombic repulsion forces. The formation of a neutral (or anionic) hydroxide and the subsequent formation and, with time, crystallization of tri- and tetravalent actinide hydroxides could lead to an intermediary formation of metastable colloids. These sorb similar to hydrolyzed species in true solution, i.e. maximal at the pH where neutral species dominate and with a reduced sorption for anionic species.

In order to quantify the role of colloidal transport, the content of natural colloids in granite groundwaters has been measured in Sweden²; the concentration detected was less than 1 mg/dm³. If the natural colloid content remains at this low level even after waste emplacement then transport by this route will be small. However should this level be augmented by additional pseudocolloidal processes by microbial action, its contribution is no longer negligible and demands quantification during further work.

7. Conclusion and consequences of microbial activity in nuclear waste depositories

Microbes may affect the repository either by attack from outside or from within the waste.

External microorganisms could possibly degrade the backfill and canister materials but pH and nutritional requirements militate against the significance of such action.

When cement is used extensively, the ability of microorganisms to degrade containment materials is curtailed by the high pH imposed. The possibility of establishing pH microenvironments wherein a localized acidic regime may become established may not be neglected.

Restriction of nutrient sources in the near-field, particularly of carbon, acts as a powerful constraint on microbial activity. Therefore if the carbon content of the repository site and the backfill is kept low, microbial activity will be curtailed.

The nature of the backfill will have profound microbiological consequences. Calcium montmorillonite (Fuller's Earth) and water samples contain microorganisms of important microbial groups, e.g., aerobic heterotrophs, iron-precipitating heterotrophs, denitrifiers, sulfur oxi-

dizers and sulfate-reducing bacteria. The use of such clay materials to backfill and seal deep repositories will introduce nutrients. On the other hand compacted clay will restrict water movement and any additional nutrient input.

Microbial activity is stimulated by the addition of extra organic carbon and thus biomass will increase. Carbon dioxide produced could support autotrophs. Other necessary nutrients, e.g., phosphorus nitrogen and sulfur are also available in sufficient amounts.

A number of factors are important to evaluate microbial activity within the waste form itself and its consequences. Water as a prerequisite for microbial activity will not be adequately present through a cement monolith. However, local redistribution of water within the waste form, evaporation/condensation could cause local areas of moisture.

Since a suitable carbon source is necessary for microbial activity, exclusion of organic carbon from the waste may limit microbiological activity to specific oligotrophic species.

High pH, particularly a pH > 10, will effectively restrict microbial activity. In some waste forms, however, microenvironments might become established.

Oxygen availability, entering e.g. the groundwater will be an important determinant for microbial life since the waste will probably contain both aerobic and anaerobic organisms.

It thus appears that the biodegrading activity of microorganisms present outside of the waste form is small if high pH materials are used. However, microbial activity occurring within the waste form requires further evaluation. Experiments modelling repository conditions should give more information on microbial activities, effect on fissuring and void formation.

All of the above experimental studies have been carried out in ideal nutrient media which is unrealistic for a waste repository where the availability of nutrients will be limited. Repeating experiments in realistic conditions is necessary to ascertain tolerances in these circumstances.

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Disposal of low- and intermediate-level waste in Switzerland: Basic aspects of potential relevance to microbial effects

B. Knecht, I. G. McKinley and P. Zuidema

Nagra, Parkstr. 23, CH-5401 Baden (Switzerland)

Summary. Current projects for the disposal of low- and intermediate-level radioactive waste in Switzerland are based on the concept of a horizontally accessed repository under a hillside. Most of the waste to be disposed of in such a repository is operational and decommissioning waste from nuclear power plants and wastes from medicine, industry and research. This waste is generally solidified in cement and placed in steel drums or concrete containers. Once the repository caverns have been filled with waste, they will be backfilled with a porous mortar to allow gases, produced by the anaerobic corrosion of steel and by microbial degradation of organic material, to escape from the near field. Valanginian marl, which is one of three envisaged options for the host rock, is characterized by a high carbonate content, up to 75% in some locations. The organic content of the marl is between 1% and 2%, while pyrite is present in concentrations up to 5%. The groundwater is reducing, and its pH tends to lie in the neutral to slightly alkaline range. Potentially important microbial effects on the long-term performance of the system are microbial degradation of barrier materials and organics, the effect of microorganisms on sorption, and their role as catalysts.

Key words. Nuclear waste; microbial effects; cement; marl; ion exchange resins.