AN ANALYTICAL OVERVIEW OF THE CONSEQUENCES OF MICROBIAL ACTIVITY IN A SWISS HLW REPOSITORY

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December 1985

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This report was prepared as an account of work sponsored by Nagra. The viewpoints presented and conclusions reached are those of the author(s) and do not necessarily represent those of Nagra.
SUMMARY

Microorganisms are known to be important factors in many geochemical processes and their presence can be assured throughout the envisaged Swiss type C repository for HLW. It is likely that both introduced and resident microbes will colonise the near-field even at times when ambient temperature and radiation fields are relatively high. A simple quantitative model has been developed which indicates that microbial growth in the near-field is limited by the rate of supply of chemical energy from corrosion of the canister.

Microbial processes examined include biodegradation of structural and packaging materials, alteration of groundwater chemistry (Eh, pH, organic complexant concentration) and direct nuclide uptake by microorganisms. The most important effects of such organisms are likely to be enhancement of release and mobility of key nuclides due to their complexation by microbial by-products. Resident micro-organisms in the far-field could potentially act as 'living colloids' thus enhancing nuclide transport. In the case of flow paths through shear zones (kakirites), however, any microbes capable of penetrating the surrounding weathered rock matrix would be extensively retarded.

Although many gaps exist in the understanding of relevant geomicrobiological processes, research priorities can be assigned by considering the applicability of data produced to repository safety assessment. It is concluded that microbial processes are unlikely to be of significance for HLW but will be more important for low / intermediate waste types. As data requirements are similar for all waste types, results from such studies would also resolve the main uncertainties remaining for the HLW case. Key research areas are identified as characterisation of a) nutrient availability in the near-field, b) the bioenergetics of iron corrosion, c) production of organic by-products, d) nuclide sorption by organisms and e) microbial mobility in the near- and far-field.
Site specific studies are recommended but sampling must be coupled to evaluation of the tolerance of important microbial groups to expected repository conditions. All this work must be carefully coordinated in an integral research programme as many of the important processes involved are interrelated.
ZUSAMMENFASSUNG

Mikroorganismen spielen bekanntlich eine wichtige Rolle in geochemischen Prozessen, und ihre Anwesenheit in einem potentiellen Endlager für stark radioaktive Abfälle in der Schweiz kann als gesichert angesehen werden. Es ist wahrscheinlich, dass, neben den bereits vorhandenen, auch von außen eingeführte Mikroben das Nahfeld bevölkern werden, und dies selbst dann, wenn die vorherrschende Temperatur und die radioaktive Strahlung relativ hoch sind. Ein einfaches quantitatives Modell wurde entwickelt, welches zeigt, dass das Mikrobenwachstum im Nahfeld durch die Zuführungsrate chemischer Energie aus der Behälterkorrosion limitiert wird.


Obwohl viele Lücken im Verständnis relevanter geomikrobieller Prozesse bestehen, können Forschungsprioritäten gesetzt werden, indem die Anwendbarkeit der vorhandenen Daten auf die Sicherheitsabschätzungen von Endlagern überprüft wird. Es wird gefolgt, dass mikrobielle Prozesse für hochradioaktive Abfälle wahrscheinlich insignifikant sind, für mittel- und schwachaktive Abfälle dagegen mehr Bedeutung erlangen werden. Da die Erfordernisse an solche Daten für alle Abfallsorten ähnlich sind, können die Ergebnisse aus experimentellen Studien auch für die Behebung von Unsicherheiten bei der Sicherheitsabschätzung von

Feldversuche werden zwar empfohlen, die Probeentnahme muss jedoch mit der Erforschung der Widerstandsfähigkeit wichtiger Mikrobengruppen, die unter Endlagerbedingungen zu erwarten sind, gekoppelt werden. Die gesamte Arbeit muss sorgfältig in ein vollständiges Forschungsprogramm integriert werden, da viele Prozesse miteinander verknüpft sind.
Les micro-organismes sont connus pour être d'importants facteurs dans de nombreux processus géochimiques et leur présence peut être assurée dans tout le dépôt suisse de type C envisagé pour DHA. Il est vraisemblable que les microbes tant extérieurs qu'intérieurs coloniseront le champ proche, même lorsque la température ambiante et les champs de radiation seront relativement élevés. On a mis au point un simple modèle quantitatif qui indique que le développement microbien dans le champ proche est limité par le taux d'énergie chimique produite par corrosion du conteneur.

Les processus microbiens examinés comprennent la biodégradation de matériaux structurels et d'emballage, l'altération de la chimie des eaux souterraines (Eh, pH, concentration de complexant organique) et l'absorption directe de nucléides par des micro-organismes. Les effets les plus importants de ces organismes sont vraisemblablement l'augmentation du relâchemnet et de la mobilité de nucléides-clés suite à la complexation de ces derniers par des sous-produits microbiens. Les micro-organismes intérieurs au champ éloigné pourraient éventuellement jouer le rôle de "colloides vivants", augmentant ainsi le transport des nucléides. Mais en cas de chemins d'écoulement à travers des zones de cisaillement (kakirites), tout microbe capable de pénétrer dans la matrice de la roche altérée environnante serait largement retardé.

Bien que la compréhension de processus géomicrobiologiques soit encore très lacunaire, on peut établir une certaine priorité dans les recherches, compte tenu de l'applicabilité des données obtenues à la considération de la sûreté des dépôts. On parvient alors à la conclusion que les processus microbiens sont vraisemblablement de peu d'importance pour les DHA, mais qu'ils joueront un plus grand rôle pour les catégories de déchets de faible et moyenne activité. Vu que toutes les catégories de déchets nécessitent des données similaires, les résultats de ces études permettraient donc de résoudre les principales incertitudes qui subsistent dans le cas des DHA. Les zones importantes de recherches sont: a) la disponibilité des substances d'alimentation
du champ proche, b) la puissance bioénergétique de la corrosion du fer, c) la production de sous-produits organiques, d) la sorption de nucléides par des organismes et e) la mobilité microbienne dans les champs proche et éloigné.

Des études spécifiques à un site sont conseillées, mais il faut joindre à l'échantillonnage l'évaluation de la tolérance d'importants groupes microbiens dans les conditions de dépôt excomptées. Tout ce travail doit être soigneusement coordonné en un programme de recherche intégral, nombre des importants processus concernés étant interdépendants.
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1. INTRODUCTION

A recent safety analysis of proposals for high-level nuclear waste (HLW) disposal in Switzerland identified geomicrobiology as an area particularly lacking in quantitative treatment (Nagra, 1985, Vol 5). Although several reviews of the possible role of microorganisms in deep geological repositories have been published in recent years (e.g. West et al., 1982a; Mayfield and Barker, 1982a; West and McKinley, 1984, Bachofen and Luescher, 1984, Champ, 1984), they have tended to be very general and completely qualitative. In this report, possible microbial processes which could affect repository performance are described and work on these topics to date reviewed. Some approaches to quantitative modelling of microbial influences are presented and areas particularly requiring more detailed research are identified.

This report is primarily focused on current Swiss concepts for a HLW repository but, as many of the processes involved are common to any deep geological disposal operation, some reference is also occasionally made to a potential repository for Low/Intermediate Level Waste (ILW). Current concepts of these two repository types are now briefly summarised based on the recent "Projekt Gewaehr 1985" study (Nagra, 1985).
1.1 Swiss Disposal Concepts

i) Final Repository For Low and Intermediate Level Waste - Type B.

This repository type will contain reactor operational waste, reprocessing technical waste, decommissioning waste as well as medical, industrial and research waste. Three separate site investigation programmes are envisaged and applications for these have been made. The three sites are in contrasting geological formations namely, anhydrite, marl and crystalline rock. All three sites are considered to be geologically stable and water-free, or sufficiently water impermeable to allow a repository structure. The detailed repository design will depend on the rock type in question but will consist of rock caverns with an overburden of 100-600 m.

Within the repository the radioactive wastes will be mixed and solidified in either concrete, bitumen or resin according to the waste type (Fig. 1.1). This will generally be sealed inside 200 l steel drums. The steel drums are then stacked into large concrete containers (4.78 m (l) x 2.18 m (w) x 2.08 m (h)) and all the remaining space inside this chest filled with liquid cement. The containers are stacked in the caverns in their final storage positions by remote control. The caverns for the marl site are designed to have a cross-sectional area between 162 m² - 183 m² the inner surface of which will be lined with concrete to a thickness of 60 - 90 cm. Subsequent to the final emplacement of the containers in the caverns, the entire cavern will be filled with a special concrete containing additives promoting more elastic properties. The caverns will be infilled sequentially by remote control to minimise the radiation doses to personnel. The total length of caverns envisaged for the marl site is 4660 m. The quantities of waste and packaging materials involved are summarised in Table 1.1.
Safety barrier system for low- and intermediate-level waste

Solidification matrix (cement, bitumen, polymers)
- Restricts release

Container with infill (concrete/cement)
- Restricts water penetration
- Restricts release (diffusion)

Storage caverns with lining and backfilling (concrete/special concrete)
- Restricts water penetration
- Delays beginning of release (diffusion break-through time)
- Restricts release (diffusion)
- Provides favourable chemistry (pH)

Geosphere:
- Long water-flow times
- Additional retardation of radioactive material transported in water (sorption, matrix diffusion)
- Long-term stability of hydrogeological conditions with respect to climatic and geological changes

Repository zone
- Limited water supply
- Favourable chemistry (redox-potential)
- Geological long-term stability

**Fig. 1.1:** The system of safety barriers for low and intermediate level waste.
Table 1.1: Inventory of materials in a type B repository

<table>
<thead>
<tr>
<th>Inventory (tonnes)</th>
<th>Operational waste</th>
<th>Reprocessing waste</th>
<th>Decommissioning waste</th>
<th>MIR* waste</th>
<th>Total</th>
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<tr>
<td><strong>Solidification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cement</td>
<td>54730</td>
<td>9120</td>
<td>118280</td>
<td>8410</td>
<td>190500</td>
</tr>
<tr>
<td>Bitumen</td>
<td>300</td>
<td>3100</td>
<td>-</td>
<td>-</td>
<td>3400</td>
</tr>
<tr>
<td>Resin</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td><strong>Waste</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steel</td>
<td>2800</td>
<td>23600</td>
<td>39700</td>
<td>-</td>
<td>66100</td>
</tr>
<tr>
<td>Al/Zn</td>
<td>-</td>
<td>600</td>
<td>6</td>
<td>-</td>
<td>600</td>
</tr>
<tr>
<td>Salt</td>
<td>300</td>
<td>1800</td>
<td>8500</td>
<td>-</td>
<td>10600</td>
</tr>
<tr>
<td>concentrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>200</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>300</td>
</tr>
<tr>
<td>Glass</td>
<td>400</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>400</td>
</tr>
<tr>
<td>Ion exchange resin</td>
<td>12200</td>
<td>100</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Concrete</td>
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<td>-</td>
<td>4300</td>
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<tr>
<td>Resin</td>
<td>5</td>
<td>600</td>
<td>3000</td>
<td>-</td>
<td>3600</td>
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<tr>
<td>Other organic</td>
<td>0</td>
<td>1</td>
<td>-</td>
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<td>1</td>
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<tr>
<td>materials</td>
<td></td>
<td></td>
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<tr>
<td>Other solid</td>
<td>3000</td>
<td>2400</td>
<td>3300</td>
<td>-</td>
<td>8700</td>
</tr>
</tbody>
</table>

* Medicine, Industry, Research operational waste.
ii) Final Repository for High Level Waste - Type C.

The type C repository will largely contain high level waste resulting from the reprocessing of spent fuel. It will also contain intermediate level activity waste that is not emplaced in a type B repository due to its actinide content, for example fuel rod hulls, endcaps and cladding.

For the Swiss case the repository is assumed to be excavated in crystalline host rock in the north of Switzerland at a depth of about 1300 m depending on the local geology. The crystalline rock is overlain by a series of younger sedimentary rocks which provide an additional geological barrier.

The system of safety barriers for high level waste is shown in Fig. 1.2. The repository itself would comprise of a series of parallel galleries in which the waste packages would be emplaced. The galleries are envisaged to be 3.7 m diameter in the centre of which the waste packages will be placed horizontally. The waste package will contain the vitrified high level waste (borosilicate glass) encapsulated in 25 cm thick cast steel (or iron) canisters. The galleries containing the canisters are then completely backfilled with a buffer of highly compacted bentonite clay. In its "dry" compressed state the bentonite can be emplaced as machined blocks designed to completely fill the annulus between the packages and the rock. As the bentonite buffer and backfill absorb water from the surrounding rock, they expand to completely fill all the voids, forming an homogeneous and impermeable barrier between the rock and waste packages. For safety analyses the repository is assumed to contain 5895 canisters.

Other waste types, i.e. fuel rod hulls, endcaps and cladding are sealed in bitumen and concrete in steel drums and emplaced in large silos adjacent to the HLW tunnel system (Fig. 1.3). The quantity of activity in this waste will be very much less than that in the HLW packages.
A diagrammatic layout of the type C repository is shown in Fig. 1.4.

1.2 Outline of the report

The rest of this report comprises five chapters describing microbial processes, current work being undertaken throughout the world, basic modelling concepts and finally recommendations for future work. A glossary of biological, geological and waste management terminology is appended to help non-specialists reading this and the other reports referenced herein.
The system of safety barriers for high-level waste

Glass matrix (molecular distribution)
- Restricts release

Steel canister (corrosion-resistant)
- Retards water penetration
- Provides favourable chemistry

Bentonite-clay (compacted, capable of swelling)
- Restricts water penetration
- Delays commencement of release (diffusion break-through time)
- Restricts release (diffusion)

Sedimentary overburden
- Long water-flow times
- Additional retardation of radioactive material transported in water (sorption, matrix diffusion)
- Long-term stability of hydro-geological conditions in view of climatic and geological changes

Geosphere
- Limited water supply
- Favourable chemistry
- Geological long-term stability

Host rock

Repository zone

Fig. 1.2: The system of safety barriers for high level vitrified waste.
Safety barrier system for α-containing intermediate-level waste

- Solidification matrix (cement, possibly bitumen)
  - Restricts release

- First backfilling (special concrete)
  - Restricts water penetration
  - Restricts release (diffusion)

- Bentonite backfilling
  - Restricts water penetration
  - Delays beginning of release (diffusion break-through time)
  - Restricts release (diffusion)

- Sedimentary over burden
  - Geosphere
    - Long water-flow times
    - Additional retardation of radioactive material transported in water (sorption, matrix diffusion)
    - Long term stability of hydrogeological conditions with respect to climatic and geological changes

- Host rock
  - Repository zone:
    - Limited water supply
    - Favourable chemistry
    - Geological long-term stability

Fig. 1.3: The system of safety barriers for high alpha-content waste.
Fig. 1.4: Diagrammatic layout of a type C repository for HLW.
2. MICROBIAL PROCESSES

2.1 Degradation of structural materials

The primary containment of radioactive wastes in a geological repository is provided by the system of engineered safety barriers. The nature of the total waste package largely depends on the type of waste being considered; for the Swiss case reprocessed HLW will first be immobilised in a glass matrix and encapsulated in a cast steel (or iron) container which will then be sealed by a backfill of bentonite. This system is primarily aimed at preventing or minimising groundwater penetration and retarding any subsequent radionuclide removal. A realistic lifetime for the package is normally calculated based on a knowledge of the chemical, physical and thermodynamic properties of these materials and their surroundings. These calculations ignore the possibility of microbial activity which may potentially affect the integrity of the total waste package and subsequent release and migration of the radionuclides.

In this chapter the processes involved in microbial degradation of the major structural materials are discussed. Emphasis is placed upon those materials which comprise the largest bulk of the repository although other materials which may be present in small quantities are mentioned briefly. The next chapter expands on these processes describing more recent experimental work that has been conducted on these topics. The structural materials of most importance are metal, glass and backfill. Hulls, endcaps and cladding from the spent fuel rods will also be emplaced in the HLW repository, together with other high actinide content waste. These materials will be enveloped in a matrix of either concrete or bitumen and sealed in steel drums which in turn are placed in concrete. An option exists for direct disposal of spent fuel without reprocessing although this has not been examined in detail. In this case the spent fuel, occurring as uranium dioxide pellets, is likely to be sealed in a thick copper canister. This supplies a further source of metal for potential degradation. At the end of the section a number of miscellaneous materials are briefly
2.1.1 Metals

As previously discussed, metals will comprise a major component of the structural material in a HLW repository. This will include the canisters (iron, steel or copper) as well as endcaps and hulls (made of specialist steel). These metals will all be targets for corrosion.

Enhancement of metallic corrosion by microbial activity was first recognised by Gaines in 1910 (Moses and Springham, 1982). Such corrosion can occur under both aerobic and anaerobic conditions and involves a wide variety of species. Aerobic conditions will prevail during repository operation and immediately after closure whilst pockets of trapped air remain prior to bentonite swelling. In addition to this, small oxic micro-environments may result from radiolytic splitting of water (this is discussed further in Chapter 4). Anaerobic conditions are, however, expected to predominate over most of the region and timespan considered and are discussed in more detail.

2.1.1.1 Anaerobic Corrosion

Sulphate reducing bacteria (SRB) have been identified as the most frequent contributors to anaerobic corrosion and have been implicated in cases of failures of oil-well casing (9 mm steel pipe) at depths between 270-2100 m (Doig and Wachter, 1951). This phenomenon is very widespread and Booth (1964) has estimated that, in the United Kingdom, at least 50% of failures of underground pipes are due to microbial action.

The mechanisms of anaerobic corrosion have been extensively studied (see descriptions by e.g. Moses and Springham, 1982;
Booth, 1971; Ehrlich, 1981) but are still not clearly understood. Many theories have been put forward but only two will be discussed briefly.

a) Von Wolzogen Kuehr and Van der Vlugt (1934) were the first to propose a mechanism of corrosion of iron pipes by SRB. They suggested that the bacteria oxidised a protective layer of hydrogen using the enzyme hydrogenase bringing about cathodic depolarisation by the following reaction series:

1. $4 \text{Fe} \rightarrow 4 \text{Fe}^{2+} + 8 \text{e}^-$ (at the anode)
2. $8 \text{H}_2\text{O} \rightarrow 8\text{H}^+ + 8\text{OH}^-$
3. $8\text{H}^+ + 8\text{e}^- \rightarrow 8\text{H}$ (at the cathode)
4. $\text{SO}_4^{2-} + 8\text{H} \rightarrow \text{S}^{2-} + 4\text{H}_2\text{O}$ (cathodic depolarisation by bacteria)
5. $\text{Fe}^{2+} + \text{S}^{2-} \rightarrow \text{FeS}$
6. $3\text{Fe}^{2+} + 6\text{OH}^- \rightarrow 3\text{Fe(OH)}_2$

Sum: $4 \text{Fe} + \text{SO}_4^{2-} + 4\text{H}_2\text{O} \rightarrow 3\text{Fe(OH)}_2 + \text{FeS} + 2\text{OH}^-$

Evidence from a number of experiments has tended to favour this theory (eg. Horvath and Solti, 1959) but the mechanism suggested above would predict a molar ratio between iron corroded and ferrous sulphide produced of 4:1. In reality, ratios vary from 1:1 to 48:1 (Moses and Springham, 1982). Thus the mechanism must be more complex than this theory anticipates.

b) Booth et al. (1968) suggested that the ferrous sulphide produced by the bacteria may, in itself, cause cathodic depolarisation. They conducted a series of experiments without bacteria using chemically prepared ferrous sulphide which seemed to provide confirmation for this view.

Other mechanisms are probably also involved but it is thought that the two above are the most important.

The copper in copper ores does not appear to be directly used as an energy source by even the autotrophic microbes (Zajic, 1969). This
is probably explained by the fact that oxidation from Cu\(^+\) to Cu\(^{2+}\) does not provide a high energy yield and that copper is toxic to some biological systems (Ehrlich, 1971). Generally, if the copper is associated with sulphide, iron or even arsenic then it will be subject to attack. Under normal conditions in the absence of catalysts and with temperatures below 200 °C, the oxidation of copper by sulphate is negligibly slow even over geological timescales. However, microorganisms (SRB) are known to catalyse the reduction of sulphate whereby the sulphide formed can react with copper (Swedish Corrosion Research Institute, 1983)

\[
2\text{CH}_3\text{CHOH COOH(aq)} + \text{HSO}_4^-\text{(aq)} \rightarrow 2\text{CH}_3\text{COOH(aq)} + \text{HS}^-\text{(aq)} + 2\text{CO}_2\text{(g)} + 2\text{H}_2\text{O}
\]

In order to achieve this reaction the organisms require access to degradable organic matter and this determines the reaction rate.

### 2.1.1.2 Aerobic Corrosion

Aerobic corrosion can be used as an energy source by certain chemolithotrophic groups. For example, *Thiobacillus* spp are capable of oxidising sulphur compounds. These sulphur compounds can be in a variety of forms, eg. iron sulphides, copper sulphides etc. For a detailed list of substrates see Ehrlich (1981). Simplistically the reaction can be summarised as

1. \(S^2^- + 2O_2 \rightarrow SO_4^{2-}\)
2. \(S + H_2O + 3/2O_2 \rightarrow SO_4^{2-} + 2H^+\)
3. \(S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+\)

It can be seen that sulphuric acid will be produced as an end product which will enhance corrosion.

Iron can be enzymatically oxidised by microorganisms and thus act
as an energy source. Most of the groups involved are acidophiles, e.g. (Thiobacillus ferrooxidans, Sulfolobus) and ferrous iron is less susceptible to autoxidation below pH 5. However, Gallionella and leptothrix can oxidise iron at neutral pH but require a partially reduced environment for this activity. They may also passively precipitate iron oxide extracellularly.

Iron can also be oxidised non-enzymatically by microbes altering the pH and/or redox potential of the environment. This can be the consequence of e.g. various processes such as photosynthesis, ammonia production etc.

2.1.2 Glass

It is anticipated that HLW will be vitrified before encapsulation in the cast steel canister.

Microbial growth on glasses is a well known phenomenon, e.g. in the tropics where fungi such as Aspergillus spp and Penicillium spp grow on optical instruments. The glass can be etched as a result of fungal growth (Dade, 1958). Nutrients for fungal growth are generally derived from organic material adhering to the glass surface (Hutchinson 1946) but glass etching may occur where nutrients are present in the glass itself (Prod'homme, 1965). For the case of the borosilicate glass waste matrix, no organic material would be expected on its surface and the only nutrient present in any quantity is phosphorus. As phosphorus is unlikely to be a limiting factor for microbial growth in this region (cf. chapter 4) direct attack of the glass seems unlikely.
2.1.3 Bentonite

The backfill material envisaged for a Swiss HLW repository is bentonite (sodium montmorillonite). To date little microbiological work has been carried out on bentonites and it is therefore difficult to evaluate potential effects. The limited work which has been undertaken (Mayfield and Barker, 1982b, Philp et al., 1984) is described in chapter 3. SRB and Thiobacillus spp are the most significant groups isolated, both of which are implicated in biodeterioration and could also affect near-field geochemistry.

Montmorillonite is chemically unstable in the near-field environment and will be gradually converted into illite. This process is known to be very slow in natural geologic systems but may well be influenced by microorganisms (e.g. Eckhardt, 1985; Krumbein, 1985). Even if it was catalysed by microbial processes, however, this conversion would still be subject to the inherent limit set by the rate of supply of K+ ions required which, in itself, ensures barrier stability for > 10^6 y (Chapman et al., 1984; McKinley, 1985). Physical degradation of backfill performance could, however, be considered possible. The joints between the compacted bentonite blocks will provide preferential water inflow routes in the early stages of resaturation of the near-field. These could provide preferential sites for microbial growth thus introducing possible short-circuits through the bentonite buffer. Experimental studies of the swelling which occurs as bentonite saturates indicate that such gaps should be well sealed.

2.1.4 Concrete and Cement

In a type C repository concrete is extensively used as an immobilisation matrix and constructional material for the high actinide waste silos. Significant quantities will also be distributed throughout the repository in shaft and tunnel seals (e.g. Studer et al., 1984). In a type B repository, cement is the dominant component of the entire material inventory (c.f. table 1.1). Various special cements and concretes will be used for different purposes and their susceptibility to biodegradation may vary considerably. In this
section, however, the degradation of "concrete" will only be described in rather general terms due to a lack of data on most of the more specialist formulations.

Under aerobic conditions concrete is potentially open to attack from the action of sulphur oxidizing bacteria, in particular the *Thiobacillus* spp. Fresh concrete has a pH in the region of 12-13 which gradually falls to around pH 9 as a result of the influx of carbon dioxide and the leaching of alkalis. At this point the *Thiobacilli* are able to colonize the concrete, oxidizing thiosulphate to polythionate and elemental sulphur. The different species of *Thiobacilli* have specific pH and substrate requirements so that they colonize in succession as conditions become favourable. Hence *T. thioparus* will probably be one of the first to appear when the pH is still high (about 9) but becomes inactive around pH 5. Only at this point are species such as *T. concretivorus* and *T. ferrooxidans* able to survive, reducing the pH even further with their metabolic production of sulphuric acid from the oxidation of elemental sulphur. This sulphuric acid destroys the concrete.

Experimental analysis of microbial degradation of concrete under anaerobic conditions appears to be very limited. *T. denitrificans* can oxidise elemental sulphur anaerobically with the aid of nitrate (Ehrlich, 1971) producing $\text{SO}_4^{2-}$. The optimal pH for this is in the region 6-8.4, therefore this organism may function under repository conditions. Many bacterial photoautotrophs are also able to oxidise reduced forms of inorganic sulphur but will be irrelevant in a repository because light is absent.

2.1.5 Bitumen

Bitumen consists of a mixture of organic substances, particularly hydrocarbons, and is biodegradable (e.g. Bachofen et al., 1984). Degradation of the bitumen can take place under both aerobic and anaerobic conditions depending on the organism involved. It appears
that no single organism is capable of completely breaking down bitumen (Bachofen et al., 1984; Bachofen and Luescher, 1984), however, the action of one organism on a particular component may result in intermediary products that supply a suitable carbon source for other organisms. The end-products of bitumen degradation are $H_2O$, $CO_2$, $H_2$, $CH_4$ and other low molecular weight compounds (Bachofen et al., 1984). For a more detailed review of the literature concerning the role of microorganisms in bitumen breakdown see Zobell and Molecke (1978), and Bachofen et al. (1984).

Microbes have been found in a wide variety of crude oils and oil products and are known to live in oil emulsions and jet fuels (Davis and Updergraaf, 1954, Zajic, 1969). *Thiobacillus* spp. oxidise $Fe^{2+}$ to $Fe^{3+}$ and are indigenous to bitumenous coal regions. Such or similar organisms would be likely to thrive in bitumen and cause both decrease in pH and possible gas ($H_2S$) formation.

2.1.6 Miscellaneous

Materials such as ion exchangers, resins and cellulose would also be incorporated into a type B repository. These will also be subject to microbial attack.

Cellulose is a polysaccharide which is the major structural component of plant cell walls. Cellulose is highly resistant to acid hydrolysis but many microorganisms are known which are capable of breaking it down. For example, the rumen bacteria that reside within the gastro-intestinal tract of cattle and other ruminants are capable of this hydrolysis under anaerobic conditions. Resultant sugars from this hydrolysis may serve as a carbon source for other organisms.

A considerable literature exists on the biodegradation of a wide range of petrochemically based products in landfill situations (Rees, 1980). The products of such degradation include $H_2$, $CH_4$ and a wide range of organic chemicals which may act as extremely efficient
chelating or complexing agents (Francis et al., 1980a; b; Rees, 1980; Francis, 1982). Again the products of this degradation may serve as organic carbon sources.

2.2 Release and Transport

Microorganisms will only come into direct contact with waste radionuclides once the complete integrity of the system of engineered barriers has been lost. For the reprocessed waste in a HLW repository this will occur after mechanical failure of the steel canister which is expected > 1000 years after emplacement.

At present, the exact mechanism of radionuclide uptake by microorganisms remains unknown. The radionuclides may either be actively taken up into the interior of the organism or remain sorbed onto the exterior surface. Active uptake of radionuclides (particularly transition metals and actinides) within the cell may be limited for many small unicellular microorganisms and most "uptake" is due to surface adsorption (e.g. Azam, 1984; Fisher, 1984). Nevertheless, direct incorporation of important nuclides such as Tc or I can certainly occur to significant extents (e.g. Bors et al., 1984; Strack and Muller, 1984; Vandecasteele et al., 1984). The crucial point however, is the association of the radionuclide with the organism. This is particularly demonstrated for the case of metal precipitating organisms such as Gallionella sp in which sorption may occur not onto the cell wall itself but rather onto associated extracellular precipitates formed as part of the microbial metabolic process.

Transport of the radionuclides associated with microorganisms will depend on both the population size and their mobility. Some microorganisms are mobile, possessing flagella, and sorption onto such motile species would facilitate transport in certain formations where water flow occurs in open fissures or large diameter pores. Evidence for microbial mobility is limited but speeds could vary from
micrometers to centimeters a day (Rose 1968). Myers and McCready (1966) have demonstrated that Serratia marcescens can penetrate cores of sedimentary rock to a depth of about 36 cm in 84 hours. The movement of microorganisms that are not self-propelling will be influenced by their morphology and the physical characteristics of the environment. For example, small near spherical organisms will be more suited to penetrating small pores and channels in the media than those that tend to form clumps, chains or produce extracellular sheaths or slimes. For similar reasons the physical nature of the environment will be critical, e.g. the larger the water flow velocity, the more probable that microorganisms may be carried along in it. The nature of the rock surface must also be considered as it may provide a suitable niche for microbial attachment if nutrients or energy sources were available. Clumping of organisms may then follow which effectively blocks small pores and fissures thus inhibiting further migration. The presence of net charges on surfaces (e.g. clays) would produce a similar affect. The sorption of microorganisms onto surfaces is reviewed in detail by Bitton and Marshall (1980).

Absorbed microorganisms are not necessarily immobile and if, for example, a concentration gradient of nutrients away from, or along, the surface builds-up, microbes will be encouraged to move along it in a process termed chemotaxis (e.g. Chet and Mitchell, 1976).

Microbes could thus have both positive effects on radionuclide transport (e.g. inhibition by fissure/pore blockage) and negative effects (e.g. sorption onto motile/mobile groups moving towards a nutrient source or simply flowing with the groundwater). In fact, given that diffusion into the porosity of the rock matrix is an important retardation mechanism, clogging of pores by organisms could potentially be favourable if the entire rock porosity is affected but negative if it affects only the smaller points of access into the non-flowing porosity of the rock.
2.3 Effects on groundwater chemistry

Many microbial processes discussed in previous sections will influence the ambient chemistry of groundwater in the far-field or pore water in the near-field. Most obviously, organisms which produce mineral acids as by-products or catalyse metal oxidation reactions will have dramatic effects on the overall Eh/pH regime of the groundwater but other by-products such as organic acids may have even more important effects.

The direct effects of organisms on material degradation have been previously considered but the associated chemical changes in groundwater may have a major influence in the release and transport of radionuclides. The solubility of many important nuclides and their chemical form in solution can be greatly altered by relatively small changes in Eh/pH conditions, carbonate content, concentrations of inorganic ligands (e.g. $SO^{2-}$, $PO^{3-}$) and, in particular, the concentration of organic complexants (e.g. Schweingruber, 1983; Allard, 1985). The solubility of these nuclides is an obvious constraint on their maximum release rate into solution in a low water flow environment. Additionally, the extent of their retardation can be greatly influenced by their exact speciation in the aqueous phase.

Thermodynamic models are used to predict nuclide speciation under particular groundwater conditions but, as yet, such models are incapable of handling microbial processes.
3. REVIEW OF CURRENT WORK

3.1 Sampling

To date a number of different approaches have been adopted for sampling microorganisms in potential host rock formations. This arises because microorganisms found in a repository may have different origins: firstly, they were already resident in the formation (autochthonous microorganisms) or secondly, they were introduced as contaminants during excavation and operational procedures (allochthonous microorganisms). Consequently, one sampling approach has been to isolate the autochthonous microorganisms from the geological formations. This has been successfully achieved by a number of groups (e.g. Wilson et al., 1983) studying microbes in shallow formations of unconsolidated geological materials such as clays. In this case the cores can be subsampled aseptically for subsequent microbial analysis. However, considerable problems are encountered as soon as the work is carried out in deep formations because aseptic conditions cannot be easily maintained.

Work is currently being conducted in Italy on Plio-Pleistocene blue clays from Orte to isolate and identify in-situ organisms. This programme is based on the assumption that the organisms identified will be those most suited to the prevailing conditions and hence most likely to affect a disposal system. Working on similar lines Christofi et al. (1985) in their preliminary analyses could detect no microbial activity in a Boom clay extracted from a depth of about 220 m using techniques similar to those of Wilson et al. (1983).

Borehole drillings yield both core and groundwater for microbial sampling, however, results from the latter must be interpreted with caution as microorganisms are likely to adhere to surfaces in aquifers so that groundwater counts may significantly underestimate the true microbial population. In addition to this, the drilling fluids used will contaminate the groundwater compounding the problem of maintaining aseptic conditions. This point is illustrated by analysis of water
samples taken from Harwell (Oxfordshire) and Altnabreac (Caithness) test site boreholes (Christofi et al., 1983). Microbes observed in initial water samples were shown to be contaminants, and water considered to be truly representative of the formation contained no determinable microbial content using current protocols. Studies in France under the MIRAGE (Migration of radionuclides in the geosphere) project have demonstrated the presence of \(~1000\) autochthonous organisms per litre in sampled groundwaters from a depth of over \(900\) m (Chapman, 1984). The samples were obtained using a special pressurised water sampling system. It is understood that problems have been encountered with the sampling equipment but further results may be available soon.

The alternative approach is to sample mines and caves found in formations similar to the repository host rock disregarding the origin of the microorganisms found. This is based upon the assumption that conditions in mines/caves will resemble those in the repository during its construction and operational phase. At this time many microorganisms are likely to be introduced and these will be as significant as those already present naturally. Sampling in mines has become more favoured in recent times (Christofi et al., 1984, 1985) as it is easier to undertake than the borehole sampling and gives a wider choice of samples. Aseptic techniques need not be adhered to whilst sampling and can be limited to the more readily controlled environment of the laboratory. As a result, this technique of gauging microbial content is less expensive than borehole techniques and probably yields more realistic information.

Perhaps the largest sampling programme of potential host rock formations so far undertaken has been by the British Geological Survey (BGS) in cooperation with Napier College, Edinburgh within the MIRAGE framework. It has involved sampling from several sites in Europe: Cornwall and Derbyshire in England (Granite and Limestone); Mol, Belgium (Boom Clay); Asse, FRG (salt); Konrad, F.R.G. (iron ore in limestone;) and Stripa, Sweden (granite). From the results so far it is apparent that those mines considered to be "dry" (Asse, Stripa) have negligible microbial growth on rock surfaces. However, the
physical and chemical conditions of the different samples are as diverse as their microbial content. In general the number and types of micro-organisms increased as the salinity of the water samples decreased so that large populations were present in the least saline waters of Stripa.

Samples from the mines in the FRG and Belgium contained small or zero populations of bacteria as determined by culturing techniques. In samples where micro-organisms were detected, the groundwaters were generally organic carbon deficient thus limiting heterotrophic activity. Heterotrophic activity was not detected in these natural samples without nutrient additions although oligotrophic bacteria may be present but undetected by present techniques. For more details of sampling methods and results see Christofi et al., 1985.

The potential host rock for a HLW repository in Switzerland is granite and Nagra has initiated its own microbiological sampling programme of groundwaters from the Grimsel test site. This is an underground rock laboratory situated within granitic formations in the Swiss Alps. The microbial sampling has been carried out by Napier college, Edinburgh (Milner and Christofi, 1985) and is now described as an example of a sampling protocol and likely results. Table 3.1 summarises the routine analyses conducted on the samples to determine the presence of different groups of organisms. In addition to this heterotrophic bacterial activity was determined using carbon dioxide evolution in both natural groundwater samples and samples amended with an organic carbon source. The autotrophic bacterial activity was determined by measuring bicarbonate uptake. Finally a range of biochemical and morphological tests were carried out to identify the aerobic heterotrophic bacteria present in the groundwater samples.
Table 3.1

Routine microbiological analyses

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Procedure</th>
<th>Media/References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Aerobic heterotrophic bacteria</td>
<td>Spread plate; concentration on membrane filters</td>
<td>CPS (a) plus nitrate (agar plates)</td>
</tr>
<tr>
<td>2. Anaerobic heterotrophic bacteria</td>
<td>Spread plate; concentration on membrane filters; incubation under H₂/CO₂</td>
<td>CPS (a) plus nitrate</td>
</tr>
<tr>
<td>3. Metallo-precipitating non-oxidising bacteria (MPNB)</td>
<td>Spread plate; Most probable number (MPN)</td>
<td>Ferric citrate (b)</td>
</tr>
<tr>
<td>4. Fungi</td>
<td>Spread plate</td>
<td>SDA</td>
</tr>
<tr>
<td>5. Thiobacillus, spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) T.denitrificans</td>
<td></td>
<td>Lieske's (see (a))</td>
</tr>
<tr>
<td>(ii) T.thioxidans</td>
<td></td>
<td>Waksmans &amp; Starkey's (see (a))</td>
</tr>
<tr>
<td>(iii) T.thioparus</td>
<td>Enrichment/Isolation</td>
<td>Starkey's (see (a))</td>
</tr>
<tr>
<td>(iv) T.ferrooxidans</td>
<td></td>
<td>9K (c)</td>
</tr>
<tr>
<td>6. Sulphate Reducing bacteria (SRB)</td>
<td>Roll tube-quantitative Agar deep-qualitative Overlay-quantitative</td>
<td>(d)</td>
</tr>
<tr>
<td>7. Iron oxidising bacteria</td>
<td>Phase contrast microscopy Specific staining Enrichment</td>
<td>(f)</td>
</tr>
<tr>
<td>8. Denitrifying bacteria</td>
<td>MPN</td>
<td>CPS (a) plus nitrate; liquid medium (CPSN)</td>
</tr>
<tr>
<td>9. Total microorganisms</td>
<td>Concentration: staining, bright field and epifluorescence microscopy, electron microscopy (SEM)</td>
<td></td>
</tr>
</tbody>
</table>

(a) Collins (1963); (b) Clark, Scott and Bone (1967); (c) Silverman and Lundgren (1959); (d) Hungate (1968); (e) Abdollahi and Nedwell (1979); (f) Meyers (1958) and (g) Grainge and Lundgren (1969); Meiklejohn (1953).

CPS - Casein - Peptone - Starch
CPSN - Casein - Peptone - Starch - Nitrate
SDA - Sabouraud's dextrose agar
The results demonstrated the presence of microorganisms in the groundwater samples, with aerobic heterotrophic bacteria being the most important group (Table 3.2). It must be remembered that these methods only demonstrate growth of those organisms suited to the media composition and the incubation conditions employed. Therefore, the viable counts will underestimate the total number of microorganisms. These problems relating to the selection of a medium capable of stimulating the growth of all the microorganisms present are difficult to resolve when examining samples of diverse origin. The bacterial activity measured in the unamended groundwaters was low, but when a yeast extract was added (organic carbon source) all the samples exhibited a very much increased level of activity. This suggests the heterotrophic bacteria were limited by organic carbon availability or requirements for other unidentified substances in the yeast extract. Autotrophic bacteria were also shown to be active in the Grimsel groundwater although the level of activity depended on the samples.

Cultivation and identification of isolated bacteria proved to be quite problematic. Many bacterial isolates failed to grow on CPSN after primary isolation. In addition, some bacterial isolates which grew well on CPSN grew only slowly or not at all on nutrient agar. This suggests that oligotrophic bacteria formed a relatively high proportion of the groundwater bacterial populations.

In conclusion it would appear that sampling mines and caves found in similar formations to the proposed repository host rock provides the most useful information about microorganisms likely to be encountered in a repository. The in-situ sampling and isolation of purely allochthonous microorganisms is of more academic interest, more difficult and more expensive.
Table 3.2

Microbiology of Grimsel Samples

Viable counts and the occurrence of different groups of organisms culturable from two separate groundwater samples at 27°C (from Milner and Christofi, 1985).

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Sample M.D.</th>
<th>Sample L.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic heterotrophic bacteria CFS agar (CFU ml⁻¹)</td>
<td>3.6 x 10²</td>
<td>9 x 10⁴</td>
</tr>
<tr>
<td>Anaerobic heterotrophic bacteria (CFU ml⁻¹)</td>
<td>8.0 x 10¹</td>
<td>8.6 x 10⁴</td>
</tr>
<tr>
<td>Aerobic heterotrophic sporeforming bacteria (CPU ml⁻¹)</td>
<td>ND</td>
<td>3.4 x 10¹</td>
</tr>
<tr>
<td>Anaerobic heterotrophic sporeforming bacteria (CPU ml⁻¹)</td>
<td>ND</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Heterotrophic iron-precipitating bacteria (CFU ml⁻¹)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Fungi (fungal CFU ml⁻¹)</td>
<td>ND</td>
<td>4.6 x 10¹</td>
</tr>
<tr>
<td>Thiobacillus sp.</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sulphate-Reducing Bacteria</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Iron-oxidising bacteria</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Denitrifying bacteria (ml⁻¹)</td>
<td>ND</td>
<td>4.9 x 10⁴</td>
</tr>
<tr>
<td>Nitrifying bacteria</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total count of bacteria (ml⁻¹)</td>
<td>4.3 x 10⁴</td>
<td>5.1 x 10⁵</td>
</tr>
</tbody>
</table>

KEY
---
ND none detected
+ indicates presence of microorganisms in enrichment culture
CFU colony forming unit
M.D. main access tunnel
L.D. excavation test tunnel.
3.2 Extreme Environments

The environment of a HLW repository is relatively hostile to any life forms because of the radiation and temperature fields produced by the radioactive material, the toxic metals present and the pressure of the overburden. As a result it has generally been assumed that no microbial activity could be sustained in such an environment. More recently this view has been shown to be unjustified as a variety of organisms have been identified capable of tolerating these extremes of environment (West et al., 1982a,b; Mayfield and Barker, 1982a, West and McKinley, 1984). Table 3.3 lists some examples. Such tables are continually being reviewed as new discoveries are made such as the claim that black smoker bacteria live in hot sulphurous water vents on mid-oceanic ridges and grow at $250 \, ^\circ C$ and 265 atm (Baross and Deming, 1983). Before this, 120 $^\circ C$ was considered the upper temperature limit to life due to protein instability (Walsby, 1983). These temperatures and pressures are well in excess of the maximum values predicted in the bentonite backfill (about 140 $^\circ C$, 130 atm) of the Swiss reference HLW repository (Nagra, 1985). In addition, this 140 $^\circ C$ maximum is confined to the bentonite nearest the canister and is expected to be of relatively short duration ($< 10$ years). The outer areas of bentonite are always less than 100 $^\circ C$.

Radiation tolerance has also been demonstrated in a number of microorganisms. For example, Micrococcus radiodurans can survive a single dose in excess of $5 \times 10^5$ rad (Nasim and James, 1978). More recent work conducted in Belgium has detected a variety of microorganisms in the primary cooling system of a nuclear reactor which has a neutronic flux of $5 \times 10^{14}$ neutrons/cm$^2$.sec (Mergeay et al., 1984).

Although some of the organisms described above are unusual and may be found in peculiar environments, they do demonstrate the ability of prokaryotes to evolve to extremely hostile conditions. For this reason a number of experimental programmes have been set-up in which microorganism isolated in the sampling programmes described in the previous section are subjected to the environmental extremes likely to be encountered in a repository. The objectives are to assess their
ability to survive or evolve in these situations and, if possible, identify the factors limiting growth. Such an experimental programme has recently been embarked upon in Great Britain by BGS in collaboration with both Napier College and Loughborough University and some general results from this are described at the end of the section. Other tolerance studies in relation to radioactive waste are rather limited. Work has been conducted at Los Alamos scientific laboratory shallow burial sites in relation to low level radioactive waste. In this work Barnhart et al. (1980) found that the soil bacteria had developed greater resistance to gamma- and beta-radioactivity than those found in uncontaminated soil. These bacteria were resistant to 50 krad gamma-radiation but none survived at 75 krad. Buckley et al. (1985) have examined the radiotolerance of the Pseudomonas cultures used for their bitumen biodegradation studies and found that none were capable of growth at doses exceeding 75 krad. All these bacterial isolates were less radioreistant than Microoccus radiodurans (cf. table 3.3). Work is also being carried out in other places on the tolerance of microorganisms to extreme environments but they tend not to be relevant to a Swiss HLW repository, for example very high salt concentrations etc.
### Table 3.3

Tolerance of microbes to extreme environments
(updated from West at al., 1982a)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Examples of organism</th>
<th>Limit of growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>High temperature</td>
<td>'Black smoker' bacteria</td>
<td>250°C (at 26.5MPa)</td>
</tr>
<tr>
<td>Low temperature</td>
<td>Sporotrichum carnis</td>
<td>- 20°C</td>
</tr>
<tr>
<td>High pH</td>
<td>Nitrobacter spp</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Nitrosomonas spp</td>
<td></td>
</tr>
<tr>
<td>Low pH</td>
<td>Thiobacillus ferrooxidans</td>
<td>0</td>
</tr>
<tr>
<td>High salinity</td>
<td>Halobacterium halobium</td>
<td>50% salt by wt</td>
</tr>
<tr>
<td>Low salinity</td>
<td>Salmonella oranienburg</td>
<td>70 ppb dissolved salts</td>
</tr>
<tr>
<td>High pressure</td>
<td>Vibrio desulfuricans (Desulfovibrio desulfuricans)</td>
<td>180 MPa</td>
</tr>
<tr>
<td>Radiation</td>
<td>Micrococcus radiodurans</td>
<td>Single dose 5 x 10⁵ rad</td>
</tr>
<tr>
<td>Chemical Toxins</td>
<td>e.g. *PbCl₂</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td></td>
<td>* CuSO₄</td>
<td>Pseudomonas C-1</td>
</tr>
<tr>
<td></td>
<td>* Ehrlich (1978)</td>
<td></td>
</tr>
</tbody>
</table>
As an illustration, some preliminary results from research carried out by BGS are described in more detail (cf. West and Arme, 1985). The microorganisms selected for study were the SRB, *Thiobacillus ferrooxidans* and *T. thiooxidans* isolated from various sites, e.g. Matlock, Derbyshire. Some results from the temperature tolerance studies are given in Figs. 3.1-3.3. From these it is obvious that SRB from Matlock are thermophilic when considering results obtained from epifluorescence microscopy. Adenosine triphosphate (ATP) results, showing a rise followed by a rapid decline in ATP measured, are deceptive. The morphology of the culture changes with time and the cells become much smaller. It is likely that, had the experiment continued, the ATP amounts would have steadily risen as the population numbers increased. *T. ferrooxidans* temperature tolerance (Fig.3.3) is more complicated with population swings in the first 3 days. Following this the trends are more obvious with decline at 60 °C and increases at 40 °C and 30 °C. Temperature did not affect the viability of the micro-organisms as culture-on demonstrates. Pressure/temperature experiments have been carried out on Matlock SRB and *T. ferrooxidans* at 80 °C and 30 MPa using Dickson type autoclaves (Siegfried et al, 1979). The SRB results are not fully complete but their ability to tolerate these conditions is evident from the raw data available. *T. ferrooxidans* did not tolerate such conditions. Measurement of populations in all experiments is via epifluorescence microscopy and ATP estimation.

In conclusion these types of experiment are useful in ensuring the likely significance of microorganisms identified as potentially important from other experiments. They also help to quantify the extent of microbial activity anticipated under such conditions.
**Matlock SRB. Temperature tolerance using epifluorescence microscopy**

**Key:**
- Incubation at 50°C or 30°C
- After incubation at 30°C for a further 30 days

---

**Fig. 3.1: Temperature tolerance curve**
(Matlock SRB/epifluorescence microscopy)
Fig. 3.2: Temperature tolerance curve (Matlock SRB/ATP assay)
**Fig. 3.3:** Temperature tolerance curve

*(Thiobacillus ferrooxidans/ epifluorescence microscopy)*
3.3 Biodegradation

The containment of radioactive wastes in a HLW repository is provided by a system of engineered barriers which aim to prevent, or at least minimise, groundwater penetration and retard subsequent radionuclide removal. These barriers are described in chapter 1 and their expected lifetimes ignore any possible effects of microbial degradation. A number of experimental biodegradation programmes exist to quantify such microbial influences on repository performance. The studies tend to concentrate on the individual structural materials in the repository although some long term integrated experiments, for example using mild steel in contact with bentonite backfill injected with SRB and synthetic groundwater, are being performed.

Napier college in conjunction with AERE Harwell are currently looking at microbial corrosion of mild steel under simulated repository conditions. The work involves SRB and is planned to extend from current batch systems to more realistic flow-through conditions. A large amount of work on steel corrosion by microorganisms has also been conducted by the oil industry. One problem, however, is the difficulty in accessing this data.

Studies of bitumen degradation are being carried out at the University of Zuerich for Nagra. So far, an experimental system has been set up using various natural populations in a bitumen suspension but no definitive results are available to date. Work has also been carried out in Canada by Buckley et al. (1985) on bitumen degradation and the subsequent release of radionuclides sealed within it. Species of the genus Pseudomonas were used, which was extracted from tailings ponds of bitumen plants in Alberta. Every effort was made to achieve optimal growth conditions in the experiments since the rate of attack of bitumen is slow even under optimal conditions, e.g. 
\[ 0.025 \text{ mm depth of bitumen affected after 3 years contact (Buckley et al., 1985). } \]

Despite this, no differences in radionuclide release rates from the bitumen were found compared with the control which contained no microorganisms. From this it was concluded that microorganisms will not compromise the safety and integrity of bituminized wastes.
Work on concrete degradation also identifies *Thiobacillus* spp. as important. This does not appear to have been studied under expected repository conditions and requires investigation.

### 3.4 Nutrient Availability and Energetics

The bentonite backfill (mainly sodium montmorillonite) in the repository is expected to provide a major source of nutrients to a microbial population, however very little relevant microbiological work has been reported on this material. A similar material, Fuller's Earth (calcium montmorillonite), has received considerable attention in the U.K. (Philp et al., 1984). In this study a large number of different microbial groups were shown to be present but their activity was limited by the availability of organic carbon and possibly phosphorus. The Fuller's Earth was obtained from a surface quarry which probably accounts for the high natural population.

Mayfield and Barker (1982b) have reviewed a large range of potential backfill materials and identified organisms present using basic microbiological techniques. In the light of more recent work (e.g. Philp et al., 1984) it is likely that alternative plating techniques would have greatly increased the sensitivity of this work. No detailed experimental work on nutrient availability or energetics in relevant conditions is known and this is an important area which requires more research.

### 3.5 Release and Transport

Microbial influences may directly affect release and transport of radionuclides from a repository. These processes have been summarised in chapter 2. Microbial activity may also affect radionuclide mobility indirectly as a result of changes in pH, Eh etc., and hence differences in speciation.
A limited number of studies are in progress on this subject in relation to radioactive waste. Additional relevant work can be found scattered throughout the literature in references to other areas of research, e.g. radionuclide uptake by algae in the marine and aquatic environments.

Pilot studies undertaken by BGS have demonstrated that micro-organisms are capable of taking up Cs-137 and Ce-139 (West et al., 1982c). Subsequent experiments, as yet unpublished, demonstrate the same capability with sulphate reducing bacteria and Sulfolobus, a thermophilic sulphur oxidiser. Work in the Netherlands under CEC contract (Chapman, 1984) has focused on the uptake of Np by microorganisms in glauconitic sands overlying salt domes. In the USA work has concentrated on the shallow burial sites for low and intermediate waste. Under these situations microorganisms definitely influence the mobility of metals and present problems for waste management. However, many of these problems result from the high organic content at the waste sites. At Chalk River National Laboratories in Canada, Buckley et al. (1985) could find no microbiologically enhanced release or mobility of radionuclides in bituminized waste. The influence of micro-organisms and their by-products on the mobility of plutonium and strontium in tuff is being carried out at Los Alamos (e.g. Wolfsberg and Vaniman, 1983).

Although the geological setting is not particularly appropriate, the studies of radionuclide migration from buried blocks of waste glass at Chalk-River, Canada, have been quite illuminating. Initial studies of the migration of Cs and Sr from this source indicated discrepancies between laboratory and field evaluations of Cs mobility (Walton and Merritt, 1980) which was initially interpreted in terms of colloids. Further analysis indicated that a major contributing factor was uptake by microorganisms which was backed up by laboratory studies (Champ and Merritt, 1981). Microbial effects have subsequently been shown to also greatly increase plutonium transport in core column studies (Champ et al., 1982).
3.6 Modelling

In order to put the work previously described in this chapter into context, quantitative models of the processes involved must be developed. As is evident from the discussion above, much basic, background data on likely microbial populations and their effects are missing or poorly defined. Thus, although a number of fairly sophisticated models of microbial ecology in soils have been developed (e.g. Frissel et al., 1984), lack of appropriate data preclude their application to deep geological systems. Nevertheless, some constraints on the likely (or maximum) magnitude of effects from this source can be determined by purely physico-chemical models.

Work in this field has, to date, concentrated on evaluation of population sizes and activity levels in the near-field based on simple assumptions about available nutrients and energy sources. Such models have been developed for both the reference Swiss HLW disposal concept (McKinley et al., 1984a;b) and for a possible U.K. disposal option (West et al., 1984). In both these studies, available organic carbon was identified as the main limiting factor. The models involved were, however, extremely simplistic and, in particular, available energy may be greatly overestimated and might act as a much more severe constraint in real life.

No rigorous models of microbial processes in the far-field appear to have been reported. Simple scoping calculations indicate, that significant perturbation of radionuclide transport could result from this source (West et al., 1982a).

In the next chapter the development of quantitative models for both the near- and far-field of a Swiss HLW repository is considered in detail.
4. QUANTITATIVE MODELLING OF MICROBIAL EFFECTS

4.1 Introduction

Any quantitative evaluation of the consequences of microbial activity on the performance of a HLW repository must include models of both microbial population dynamics and relevant perturbing processes. A general characteristic of potential host-rock formations is a very low groundwater flow rate and thus, if present, microbial populations are expected to be severely limited by the rate of supply of nutrients and energy sources.

The emplacement of a large number of materials such as the waste itself, metal canisters, bentonite and concrete backfills etc. will, however, completely alter groundwater chemistry with additional perturbations from the effects of radiogenic temperature increases and radiation fields. Processes affecting repository performance also differ between the repository affected "near-field" and the undisturbed "far-field" and hence in the following sections these regions are considered separately.

4.2 The near-field

A repository containing 5895 waste canister is considered and the material inventory in the engineered barriers surrounding each canister is summarised in table 4.1. The near-field, shown diagrammatically in Fig. 4.1, also includes the mechanically damaged rock around the tunnel which is considered to extend about 4 m beyond the drift wall.
Table 4.1

Material inventory in the near-field (per canister)

<table>
<thead>
<tr>
<th>Material</th>
<th>Volume (m³)</th>
<th>Mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>0.15</td>
<td>405</td>
</tr>
<tr>
<td>Steel fabrication container</td>
<td>0.01</td>
<td>75</td>
</tr>
<tr>
<td>Fabrication void</td>
<td>0.03</td>
<td>-</td>
</tr>
<tr>
<td>Canister</td>
<td>0.9</td>
<td>6.5x10³</td>
</tr>
<tr>
<td>Backfill</td>
<td>52.8</td>
<td>-</td>
</tr>
<tr>
<td>a) Bentonite</td>
<td>32.7</td>
<td>8.8x10⁴</td>
</tr>
<tr>
<td>b) Pore space (water filled)</td>
<td>20.1</td>
<td>2.0x10⁴</td>
</tr>
</tbody>
</table>
Fig. 4.1: Reference HLW disposal geometry (dimensions in metres)
The chemical environment in the near-field will be dynamic, continuously evolving with time, but three distinct episodes might be recognised:

i) **Post emplacement, unsaturated backfill.** When emplaced, the backfill will be dry (water content about 7-10%) with most of the intergranular porosity filled with air. The bentonite will then be subject to fluxes of water from the surrounding rock and heat from the canister which, due to coupling of these processes, result in a complex saturation/temperature profile.

ii) **Saturated backfill, canister intact.** After a period of time the backfill will reach equilibrium saturation, the initial oxygen in the entrapped air will be displaced or used up in corrosion processes and a "steady state" thermal profile from the canister to the drift wall will be established which will alter only very slowly as the thermal output of the canister decays.

iii) **Post canister failure.** In a low-flow environment the corrosion rate would probably be very slow, but eventually the canister will fail and groundwater will contact the waste-glass matrix. The near-field chemistry will then be altered due to both hydrothermal reactions with the glass and alpha-radiolysis.

Even within each of these periods, considerable spacial variations in chemistry along the flow path (eg. upstream and downstream of the canister) will exist.

Given that the near-field environment is not sufficiently hostile to prevent microbial life (cf. chapter 3), the maximum extent of such contamination must be assessed. For this purpose constraints on the steady state biomass and level of metabolic activity which could be supported in the near-field are considered - free organic (or "available" inorganic) carbon and other important nutrients (N,P,S) as absolute limits on possible biomass and chemical energy as a limit of metabolic activity.
4.2.1 Nutrient sources in the near-field

Possible sources of carbon and the other major nutrients in a repository situation are:

1. The waste matrix
2. Bentonite minerals
3. Trapped air in bentonite

The mineralogy of the bentonite backfill (commercially available MX-80) is given in table 4.2 and the composition of reference Swiss deep groundwater is given in table 4.3. Of the elements considered, C, N, S and P, only the latter has a significant concentration in the borosilicate glass where P$_2$O$_5$ comprises about 0.3% of the total glass weight. No direct measurement of N or P in bentonite appears to have been reported but, for the following calculations, values of 400 ppm and 0.1% will be assumed based on values for sedimentary rocks (Wedepohl, 1978). Trapped air in the bentonite will be at the ambient mine pressure during emplacement which will be conservatively assumed to be 2 atmospheres. The concentration of relevant components of air are N$_2$ 78%, O$_2$ 21% and CO$_2$ 0.05% by volume. The initial pore water content of the bentonite is conservatively ignored.
### Table 4.2

Mineralogy of MX-80 Bentonite (from Mueller-Vonmoos and Kahr, 1983)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montmorillonite</td>
<td>75</td>
</tr>
<tr>
<td>Quartz</td>
<td>15.2</td>
</tr>
<tr>
<td>Mica</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Feldspar</td>
<td>5-8</td>
</tr>
<tr>
<td>Carbonate</td>
<td>1.4</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Pyrite</td>
<td>0.3</td>
</tr>
<tr>
<td>Other minerals</td>
<td>2</td>
</tr>
<tr>
<td>Organic carbon</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Table 4.3

Reference groundwater composition (from Schweingruber, 1984)

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>1.76x10⁻¹</td>
</tr>
<tr>
<td>K⁺</td>
<td>1.15x10⁻³</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1.07x10⁻⁴</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>2.17x10⁻²</td>
</tr>
<tr>
<td>Sr²⁺</td>
<td>2.40x10⁻⁴</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>8.06x10⁻⁶</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>5.64x10⁻⁵</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>1.10x10⁻⁵</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>1.87x10⁻¹</td>
</tr>
<tr>
<td>F⁻</td>
<td>1.90x10⁻⁴</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>1.62x10⁻²</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>1.90x10⁻⁶</td>
</tr>
<tr>
<td>CO₃⁻</td>
<td>1.91x10⁻³</td>
</tr>
<tr>
<td>SiO₂⁻</td>
<td>2.83x10⁻⁴</td>
</tr>
</tbody>
</table>

Temperature 55 °C
Dissolved organic carbon 0.8 mg/l
pH 6.78
Eh -230 to -60 mV
From the raw data presented, the total inventory of each of these elements can be calculated for each of the relevant sources (Table 4.4a).

Assuming a water supply of 0.7 litre/canister-year, which is the conservative reference value for an envisaged Swiss repository (Nagra, 1985), the nutrient inventory in the annual water flux to the outside of the bentonite barrier is also given in Table 4.4a along with the integrated supply from this source over a period of $10^6$ y. One additional component not considered in this table is the "organic carbon" which is present in both the backfill and the inflowing groundwater. The chemistry of such organic carbon is not defined but it will be assumed to be equivalent to the "average composition" of a microbial culture (Stanier et al., 1977) as given in table 4.5.

The organic carbon inventory in the backfill is $3.5 \times 10^5$ g/canister which corresponds to $1.5 \times 10^4$, $3.5 \times 10^3$, $3.4 \times 10^2$ and $1.1 \times 10^2$ moles of C, N, P and S respectively. The annual supply of organic carbon in groundwater amounts to $5.6 \times 10^{-4}$ g/canister which can be similarly apportioned to $2.3 \times 10^{-5}$, $5.6 \times 10^{-6}$, $5.4 \times 10^{-7}$ and $1.8 \times 10^{-7}$ moles of C, N, P and S. These data are summarised in table 4.4b.
Table 4.4

Elemental inventories in the near-field.

a) "Inorganic"

<table>
<thead>
<tr>
<th>Source</th>
<th>C</th>
<th>S</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass Matrix</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>17.1</td>
</tr>
<tr>
<td>Bentonite minerals</td>
<td>2.0x10^4</td>
<td>4.4x10^3 *</td>
<td>2.5x10^3</td>
<td>2.8x10^3</td>
</tr>
<tr>
<td>Trapped Air</td>
<td>9.0x10^{-1}</td>
<td>–</td>
<td>2.8x10^3</td>
<td>–</td>
</tr>
<tr>
<td>Groundwater: annual input</td>
<td>1.3x10^{-3}</td>
<td>1.1x10^{-2}</td>
<td>7.7x10^{-6} +</td>
<td>1.3x10^{-6}</td>
</tr>
<tr>
<td>integral over 10^6 y</td>
<td>1.3x10^3</td>
<td>1.1x10^4</td>
<td>7.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* using only data for NH_4^+. Dissolved nitrogen gas could contribute a further 1.9x10^{-3} moles of N per year.

b) "Organic"

<table>
<thead>
<tr>
<th>Source</th>
<th>C</th>
<th>S</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bentonite</td>
<td>1.5x10^4</td>
<td>1.1x10^2</td>
<td>3.5x10^3</td>
<td>3.4x10^2</td>
</tr>
<tr>
<td>Groundwater: annual input</td>
<td>2.3x10^{-5}</td>
<td>1.8x10^{-7}</td>
<td>5.6x10^{-6}</td>
<td>5.4x10^{-7}</td>
</tr>
<tr>
<td>integral over 10^6 y</td>
<td>2.3x10^{-1}</td>
<td>1.8x10^{-1}</td>
<td>5.6</td>
<td>5.4x10^{-1}</td>
</tr>
</tbody>
</table>
Table 4.5

Chemical composition of microbial cells
(from Stanier et al., 1977)

<table>
<thead>
<tr>
<th>Element</th>
<th>% (dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>50</td>
</tr>
<tr>
<td>O</td>
<td>20</td>
</tr>
<tr>
<td>N</td>
<td>14</td>
</tr>
<tr>
<td>H</td>
<td>8</td>
</tr>
<tr>
<td>P</td>
<td>3</td>
</tr>
<tr>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>1</td>
</tr>
<tr>
<td>Na</td>
<td>1</td>
</tr>
<tr>
<td>Ca</td>
<td>0.5</td>
</tr>
<tr>
<td>Mg</td>
<td>0.5</td>
</tr>
<tr>
<td>Cl</td>
<td>0.5</td>
</tr>
<tr>
<td>Fe</td>
<td>0.2</td>
</tr>
<tr>
<td>rest</td>
<td>~0.3</td>
</tr>
</tbody>
</table>
4.2.2 Energy availability in the near-field

Chemolithotrophic organisms, which obtain energy from chemical reactions in the absence of light and build organic compounds from inorganic substrates, would form the base for the food chain in the near-field. Within this region, the major chemical process which could be harnessed as an energy source would be the oxidation of the steel canister. Evaluation of the stoichiometry, thermodynamics and kinetics of iron corrosion in a reducing groundwater environment is very difficult even if only inorganic processes are considered. Generally, therefore, the anoxic corrosion processes can be represented as

\[
\text{Fe} + n\text{H}_2\text{O} \rightarrow \text{Fe}_n\text{O}_m + n\text{H}_2
\]

and

\[
\text{Fe}_m\text{O}_n + m\text{H}_2\text{O} \rightarrow \text{Fe}_m\text{O}_{(m+n)}\text{H}_2^{(m-n)} + \chi\text{H}_2
\]

with varying \(m, n\) and \(\chi\)

for the formation of iron oxides and their subsequent hydration (Heusler, 1985). This situation is further complicated by perturbing effects such as pH changes in the near-field, build up of hydrogen pressure and reactions with other solutes such as sulphide (McKinley, 1985). For the sake of calculation it will be assumed that the thermodynamically stable product of the oxidation under these conditions is magnetite \((\text{Fe}_3\text{O}_4)\) and the net reaction can be represented as:

\[
3\text{Fe}^{(s)} + 4\text{H}_2\text{O} \rightarrow \text{Fe}_4\text{O}_3 + 4\text{H}_2^{(g)}
\]

Using the thermodynamic data in table 4.5, the net free energy change in this reaction at STP is calculated as \(-64\) kJ/mol of magnetite which is equivalent to \(-21\) kJ/mol of Fe oxidised. As the canister mass corresponds to about \(1.2 \times 10^5\) moles of Fe, the free energy produced by total canister corrosion would be about \(2.5 \times 10^9\) J.

Two other reactions which should be considered as possible energy sources are Fe corrosion by initial trapped oxygen and by
radiolytically produced oxidant. The former reaction can be written as

\[ 3\text{Fe} + \text{H}_2\text{O} + \frac{3}{2}\text{O}_2 \rightarrow \text{Fe}_3\text{O}_4 + \text{H}_2 \]

for which the free energy of reaction is about \(-775\) kJ/mol \(\text{Fe}_3\text{O}_4\) \((-258\) kJ/mol of Fe oxidised). From the data presented in section 4.2.1, the content of oxygen in the trapped air can be calculated to be \(3.8 \times 10^2\) moles and hence the maximum free energy from this source would be about \(2.0 \times 10^8\) J/canister. The production of oxidants in the near-field, due to the radiolysis of water, will only occur to a significant extent after canister failure (mechanical failure after > 1000 y) by which time outer surfaces of the canister would have been at least partially oxidised. Assuming the radiolytic oxidant to be entirely in the form of \(\text{H}_2\text{O}_2\), a typical reaction with the canister corrosion products could be

\[ 2\text{Fe}_3\text{O}_4 \text{ (magnetite)} + \text{H}_2\text{O}_2 \rightarrow 3\text{Fe}_2\text{O}_3 \text{ (hematite)} + \text{H}_2\text{O} \]

which has a free energy of reaction of about \(-326\) kJ/mol. Unlike the previous reactions, for which the kinetics are very difficult to even estimate, the rate of radiolysis is reasonably well defined. For realistic assumptions of exposed waste surface area, \(\text{H}_2\text{O}_2\) production over the period from canister failure (at 1000 y) to \(10^6\) years after emplacement is calculated to be about 380 moles which, neglecting the fall-off in radiation dose rate with time, can be averaged to about \(3.8 \times 10^{-4}\) moles/year. The annual energy production from this source would thus be about 120 J/canister.
Table 4.6

Thermodynamic data for iron and possible corrosion products
(from Stumm and Morgan, 1981)

<table>
<thead>
<tr>
<th>Species</th>
<th>$G_f^O$ (kJ mol$^{-1}$)</th>
<th>$H_f^O$ (kJ mol$^{-1}$)</th>
<th>$S$ (J mol$^{-1}$K$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe (metal)</td>
<td>0</td>
<td>0</td>
<td>27.3</td>
</tr>
<tr>
<td>Fe$^{2+}$ (aq)</td>
<td>-78.87</td>
<td>-89.10</td>
<td>-138</td>
</tr>
<tr>
<td>Fe$^{3+}$ (aq)</td>
<td>-4.60</td>
<td>-48.5</td>
<td>-316</td>
</tr>
<tr>
<td>FeS$_2$ (pyrite)</td>
<td>-160.2</td>
<td>-171.5</td>
<td>52.9</td>
</tr>
<tr>
<td>FeO (s)</td>
<td>-251.1</td>
<td>-272.0</td>
<td>59.8</td>
</tr>
<tr>
<td>Fe(OH)$_2$ (precip)</td>
<td>-486.6</td>
<td>-569.0</td>
<td>87.9</td>
</tr>
<tr>
<td>$\alpha$Fe$_2$O$_3$ (haematite)</td>
<td>-742.7</td>
<td>-824.6</td>
<td>87.4</td>
</tr>
<tr>
<td>Fe$_3$O$_4$ (magnetite)</td>
<td>-1012.6</td>
<td>-1115.7</td>
<td>146</td>
</tr>
<tr>
<td>$\alpha$FeOOH (goethite)</td>
<td>-488.6</td>
<td>-559.3</td>
<td>60.5</td>
</tr>
<tr>
<td>FeCO (siderite)</td>
<td>-666.7</td>
<td>-737.0</td>
<td>105</td>
</tr>
<tr>
<td>H$_2$ (g)</td>
<td>0</td>
<td>0</td>
<td>130.6</td>
</tr>
<tr>
<td>H$_2$O (l)</td>
<td>-237.18</td>
<td>-285.83</td>
<td>69.91</td>
</tr>
<tr>
<td>H$_2$O$_2$ (aq)</td>
<td>-134.1</td>
<td>-181.1</td>
<td>144</td>
</tr>
<tr>
<td>O$_2$ (g)</td>
<td>0</td>
<td>0</td>
<td>205</td>
</tr>
</tbody>
</table>
4.2.3 Quantitative evaluation of possible population and activity levels

The model required to quantify possible microbial populations and their level of metabolic activity is shown diagrammatically in Fig. 4.2. In the previous sections the magnitude of both organic and inorganic nutrient sources and available energy have been derived. The main problems, however, are deriving a realistic representation of the very dispersed nature of the nutrient source, quantifying the rate of energy supply from canister corrosion and assessing the efficiency of microbial utilisation of both nutrients and energy. From the start it should be emphasised that, as far as is known, no laboratory or field studies of the energetics of such a microbial community have been attempted. The calculations presented are based on studies of much less extreme environments (e.g. Thauer and Morris, 1984) and must be regarded as somewhat speculative. Nevertheless, such quantitative analysis must be attempted in order to appraise the significance of the microbial processes discussed in previous chapters in a waste management context.
Fig. 4.2: Energy and mass flows in the near-field.
As a first step to linking nutrient and energy fluxes, a set of background data for 'average' anaerobic chemotrophic bacteria are taken from the review of Thauer et al. (1977):

a) 0.1 mole of ATP (adenosine triphosphate) is required to synthesise 1g (dry) of bacterial cells (catabolism)

b) the 'maintenance energy' for such cells for movement, osmosis etc. is 0.1 mol ATP/1g (dry) of cells (anabolism)

c) the energy required for the production of the ATP within the cell (ie. ADP + P --> ATP) is 32 kJ/mol

d) the microbial efficiency of chemical energy utilisation for the production of ATP is 10%

Combining this information gives an external chemical energy requirement of 64 kJ/g (dry) of bacterial cells. This calculation is probably reasonably valid for 'organic' nutrient sources but additional energy input is required where the nutrient has to be 'fixed' from the gaseous phase or extracted from a solid matrix (termed microbiological mineralisation). The treatment of maintenance energy is particularly simplistic and ideally should be related to levels of metabolic activity by introducing a time factor. At present, however, there seems to be insufficient data to allow more realistic modelling of the bioenergetics of very low activity geomicrobial communities.

To put these numbers in context, it can be noted that conversion of all available 'organic' nutrients in the backfill (3.5x10^5 g/canister) to biomass would require about 2.2x10^{10} joules which is about an order of magnitude greater than the energy produced by anoxic corrosion of the entire canister. On a 'global inventory' scale, therefore, it would appear that energy is likely to be more limiting than available nutrients although it must be remembered that the calculations above do not take into account the very dispersed nature of the nutrient source (spread over a volume of about 50m^3 around each canister).

For the sake of model development, it will be assumed that the entire inventory of Fe in the canister is oxidised to Fe_3O_4 in the time
period considered \((10^6 \text{y})\). This corrosion initially proceeds very quickly to use up all trapped oxygen and then proceeds at a constant rate with both anoxic corrosion and, following mechanical canister failure, reaction with radiolytic oxidant proceeding simultaneously. All chemical energy released is assumed to be usable by resident microbes. From the previous section, the initial energy 'pulse' is taken to be \(2.0 \times 10^8 \text{ J/canister}\) followed by \(120 \text{ J/canister/y}\) from radiolysis. The amount of Fe corroded by these mechanisms is \(7.6 \times 10^2\) and \(2.9 \times 10^2\) moles respectively (the latter case is based on the stoichiometric reaction \(3\text{Fe} + 4\text{H}_2\text{O}_2 = \text{Fe}_3\text{O}_4 + 4\text{H}_2\text{O}\)). The remnant canister mass \((1.15 \times 10^5\) moles\) is thus assumed to corrode anoxically over \(10^6 \text{y}\) releasing \(2.4 \times 10^3 \text{ J/year}\). The annual energy supply from both anoxic corrosion and radiolysis is thus about \(2.5 \times 10^3 \text{ J/canister}\).

The energy data given above can thus be translated into equivalent amounts of dry biomass produced, to give an initial production of \(3.1 \times 10^3\) g followed by an annual rate of about \(3.9 \times 10^{-2}\) g/y. In order to express these dry weights as a number of organisms, it is assumed that the water content of such bacteria is \(99\%\) and that a typical volume for each organism would be \(1.5 \times 10^{-13}\) ml (Lundgren et al., 1972). The initial production (in \(\sim 50 \text{ m}^3\) around a single canister) thus corresponds to \(2.1 \times 10^{18}\) organisms followed by a 'steady-state' of \(2.6 \times 10^{13}\) organisms/year. Both the water content and volumes assumed are very conservative. E. Coli, for example, has a dry weight of \(\sim 2.5 \times 10^{-13}\) g/cell implying that the cell numbers may be overestimated by more than 2 orders of magnitude. To put these numbers in context it may be noted that a typical soil (which is a very active microbial environment) would contain about \(10^{14}\) organisms/m\(^3\) (Cawse, 1975).

The calculations above yield maximum possible values. In reality

\* This very simplistic assumption is rather unrealistic. Subsequent to drafting this report, a more realistic model considering \(\text{SO}_4\) in groundwater as a biologically useable electron acceptor has been developed (EIR TM-45-85-28, TM-45-85-29) but the energy production rate was very similar (\(\sim 2\text{ kJ/canister/year}\)).
much of the corrosion would probably proceed completely inorganically with all chemical energy lost as heat. Also, the dispersed nature of organic nutrient sources and the continual loss of organic by-products would necessitate utilisation of inorganic nutrients at a considerable energy penalty. Thus, although these values will be used for subsequent calculations, it would probably be more realistic, and yet still conservative, to decrease them by one or two orders of magnitude.

4.2.4 Quantification of microbial effects in the near-field

The microbial processes of major concern within the near-field are:

a) canister corrosion
b) glass leaching
c) alteration of gross pore-water chemistry (Eh/pH)
d) supply of organic by-products as potential complexants
e) physical disruption of the bentonite buffer
f) direct uptake of nuclides (mobilisation/immobilisation)

The quantification of canister corrosion is considered in detail in the previous (energetics) section. As discussed in chapter 2, observed biodegradation of glass occurs due to high local concentrations of aggressive organic by-products. By the time of canister failure only relatively low levels of metabolic activity are predicted with most being concentrated at or near corroding metal surfaces. Qualitatively, therefore, direct microbial degradation of glass would not be expected to be significant.

The main gross effects on groundwater chemistry due to microbial activity would be alterations of redox conditions (Eh) and pH. The concept of overall redox equilibrium in groundwater which can be specified by a system Eh value is commonly used in chemical speciation/solubility modelling (e.g. Schweingruber, 1983; Allard, 1985)) but its reality in natural groundwaters is questionable.
(Lindberg and Runnels, 1984). Such redox disequilibria would certainly be expected in microbially contaminated systems but its quantitative evaluation is not possible at current levels of sophistication of geochemical modelling. Although the overall oxidation reactions discussed in the previous section cause neither loss nor gain of protons and hence would not affect pH, intermediate corrosion reactions may result in production of OH$^-$ while coupled reactions may result in acidic by-products. Again, however, these processes are too complex to be quantitatively modelled by current techniques.

The main importance of organic by-products is their potential role as complexing ligands for nuclides released from the waste matrix. Assuming that the effects of the initial population peak can be neglected by the time of canister failure, the maximum release rate of such materials equals the rate of cell production in a steady-state system (3.9x10$^{-2}$ g/canister/y). In fact much of the organic constituents of the cell will be re-cycled - possibly with high efficiency in such an ecosystem - but for the sake of calculation it will be assumed that about 50% is lost (2x10$^{-2}$ g), all in the form of 'organic acids' which are very effective complexing agents. Such organic acids would range in size from small carboxylic acids (e.g. acetic acid) to massive humic and fulvic acids (molecular wt. ~5x10$^5$ daltons). For the models, an average molecular weight of these species of 10$^4$ daltons is assumed, yielding a release flux of 2x10$^{-6}$ moles/year of these ligands for each canister. Strongest complexation of these ligands is likely to occur with the actinides, many of which are released at concentrations below that expected from the corrosion rate of the waste matrix due to their inherently low solubility (Tab. 4.7). Complexation of some of these elements (e.g. U, Np) by organic acids could increase their solubilities and hence their release rates by about two orders of magnitude.

* Such a flux could also be derived assuming that only smaller organics with average mw = 200 daltons, corresponding to 1% of the total cell mass, are mobile in the bentonite pore water. Larger organics would be retarded or immobilised in the backfill and might compete with mobile complexants for available cations.
Table 4.7
Actinide release rates 1000 years after emplacement (per canister)

<table>
<thead>
<tr>
<th>Isotope</th>
<th>content (mol)</th>
<th>Matrix breakdown controlled release rate</th>
<th>Solubility limited release rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cm-245</td>
<td>3.5E-3</td>
<td>6.7E-8</td>
<td>3.2E-5</td>
</tr>
<tr>
<td>Am-241</td>
<td>2.7E-1</td>
<td>5.3E-6</td>
<td>1.5E-5</td>
</tr>
<tr>
<td>Np-237</td>
<td>3.6E0</td>
<td>7.0E-5</td>
<td>1.4E-9</td>
</tr>
<tr>
<td>U-233</td>
<td>1.0E-3</td>
<td>2.0E-8</td>
<td>2.1E-13</td>
</tr>
<tr>
<td>Th-229</td>
<td>2.1E-6</td>
<td>4.0E-11</td>
<td>4.2E-10</td>
</tr>
<tr>
<td>Cm-246</td>
<td>3.4E-4</td>
<td>6.7E-9</td>
<td>3.3E-6</td>
</tr>
<tr>
<td>Pu-242</td>
<td>2.3E-2</td>
<td>4.4E-7</td>
<td>3.3E-9</td>
</tr>
<tr>
<td>U-238</td>
<td>8.0E0</td>
<td>1.6E-4</td>
<td>1.9E-9</td>
</tr>
<tr>
<td>U-234</td>
<td>1.3E-2</td>
<td>2.5E-7</td>
<td>2.8E-12</td>
</tr>
<tr>
<td>Th-230</td>
<td>4.9E-5</td>
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<tr>
<td>Ra-226</td>
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<td>Am-243</td>
<td>3.6E-1</td>
<td>6.9E-6</td>
<td>2.1E-5</td>
</tr>
<tr>
<td>Pu-239</td>
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<td>6.0E-6</td>
<td>4.3E-8</td>
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<tr>
<td>U-235</td>
<td>1.5E-1</td>
<td>2.8E-6</td>
<td>3.3E-11</td>
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<tr>
<td>Pa-231</td>
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<td>3.7E-11</td>
<td>1.2E-8</td>
</tr>
<tr>
<td>Pu-240</td>
<td>2.0E-1</td>
<td>3.8E-6</td>
<td>2.8E-8</td>
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<tr>
<td>U-236</td>
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<td>1.6E-6</td>
<td>1.9E-11</td>
</tr>
<tr>
<td>Th-232</td>
<td>5.3E-6</td>
<td>1.1E-10</td>
<td>1.1E-9</td>
</tr>
</tbody>
</table>
Physical disruption of the bentonite buffer ($\approx 9 \times 10^4$ kg, dry) would only be expected if very large amounts of biomass were distributed throughout the backfill area. On the basis of the calculations in the previous sections ($\approx 30$ mg dry weight/year production), this can be effectively discounted.

Direct uptake can be simplistically quantified by means of a 'partition coefficient' which is the ratio of equilibrium concentration of that nuclide in the organism (e.g. moles/g (dry)) to its concentration in the aqueous phase. This partition coefficient is nuclide dependent but it may be orders of magnitude higher for the organisms than for surface sorption on the bentonite itself (cf. West et al., 1982c). The consequences of such uptake are critically dependent on the extent of microbial mobility in the bentonite. As the main energy source is located at the canister surface, it is reasonable to expect that little movement from this region would occur and hence uptake would provide a net benefit to containment. The relative quantity of bentonite as compared to the steady state biomass ($\approx 10^9:1$ by weight) ensures that this effect is negligibly small. If the microorganisms were mobile, however, they could be regarded as 'organic colloids' and their release to the far-field could be very important. The size of microbes is unlikely to be significantly less than 1 µm (typical for oceanic metal-depositing bacteria - Cowen and Silver, 1984) and more realistically may be 3-10 µm in the groundwater environment. Given the average pore size in saturated, high density bentonite is about 50 nm (Pusch and Forsberg, 1983), however, it is probably reasonable to discount this process although it is acknowledged that uncertainties remain in the description of the pore structure of compacted bentonite (Pusch et al., 1985).
4.3 The far-field

The far-field is that part of the groundwater flow path between the repository affected near-field and accessible aquifers from which nuclides are released into the foodchain. For the Swiss reference case (Nagra, 1985), the most significant parts of the far-field are shear-zones (kakirites) through the host rock (middle crystalline). These form the main route for transport of released radionuclides from the repository to the overlying formations (upper crystalline, sediments) which are modelled to be porous aquifers (Fig. 4.3). Flow in these kakirites is rather poorly specified but is assumed to occur in a network of "pipes" which are partially quartz lined. Nuclide retardation during transport occurs due to sorption on the surfaces of these pipes and, more importantly, diffusion out of the water-carrying pipes into the porous, weathered matrix of the kakirite itself with accompanying sorption.

Possible microbial processes which would affect the "inorganic" transport model are:

i) Alteration of water chemistry and rock surfaces

ii) Radionuclide uptake on mobile/motile organisms in the kakirite "pipes"

iii) Radionuclide uptake by organisms immobilised on surfaces on the pipes or in the kakirite matrix

iv) Clogging of pores in the pipe walls by microbes.
Fig. 4.3: Schematic illustration of radionuclide migration paths in the far-field
Over the timescales involved, it is reasonable to assume that groundwater chemistry represents a steady-state composition. The reference groundwater chemistry defined for the safety analysis (Table 4.3) is based on field measurements and thus tacitly includes any microbial contributions. These would not be expected to alter assuming no significant changes occur within the geosphere. If microbes do play a significant role in defining the chemistry of deep groundwaters, it may limit the applicability of the equilibrium thermodynamic approach to the modelling of such systems. There are, indeed, strong indications of non-equilibrium in low temperature groundwaters (e.g. Lindberg and Runnells, 1984) but the extent to which this is caused by microbes does not directly affect the safety analysis.

The effect of microbes on radionuclide transport processes in the far-field must be examined in a more quantitative manner. Although the approach is rather crude, in order to obtain an idea of the possible magnitudes involved, it will be assumed that all "dissolved organic carbon" in the reference water (0.8 mg/l) actually corresponds to mobile microbes. Numbers derived will, at least, be conservative, if not extreme.

The value above is equivalent to a 'net weight' of 80 mg/l (assuming 99% water content) which equates to a population of about 5x10^{11} organisms/litre (for an average volume of 1.5x10^{-13} ml/organism). In the transport models used (Nagra, 1985), it is assumed that flow in the kakirite zones occurs in 1 cm diameter quartz lined tubes. Assuming simple tubes, the available surfaces area is 0.4 m^2 per litre of contained groundwater. Assuming microorganisms have an average diameter of 1 \mu m, approximately 4x10^{11} organisms would be required to effect complete surface coverage. Hence, inventories of organisms in the liquid and solid phases could potentially be of similar orders of magnitude.

As no qualitative difference between organisms on surfaces and in solution is expected and taking nuclide uptake onto (or into) organisms to be reversible, a partition coefficient for any nuclide (\(K_a\), defined as concentration per unit area of surface / concentration per unit
volume of solution] of about 1-2 m can be calculated. This type of calculation is very uncertain but, fortunately, surface sorption on the quartz tubes is not very important from the safety analysis viewpoint as such tubes are porous and solute penetrates the surrounding kakirite. The kakirite has a porosity of 3.3% which, given the rather large grain size of such rock, implies the internal matrix is likely to be directly accessible to organisms of ~\mu m dimensions. Taking the average pore radius in the matrix to be 10 \mu m, the available surface area would be about 10^5 \times higher than in the 'tubes' and hence an enormous amount of retardation would be expected. This conclusion would hold whether there was extensive microbial cover over the available surfaces or not, as long as the microbial uptake process was reversible.

As far as reversibility is concerned, most microbial uptake probably occurs onto external surfaces where continuous exchange between solution and surface (dynamic equilibrium) would be expected. For active organisms, incorporation of nuclides into the cell is possible, but as the lifetime of such organisms is likely to be \sim months, recycling of biological material following death would cause nuclide release. Even for the unexpected case of irreversible uptake by long-lived organisms, as long as the organisms themselves sorbed onto surfaces and could access the large internal volume of the kakirite, very extensive retardation would occur.

It should be emphasised that the calculations above are extremely simplistic and are based on model characteristics of the flow path rather than more realistic values. At present, however, a more detailed treatment is not justified by the data available.

Finally, the near-field calculations show the release of nuclides complexed with organic by-products is very important from a safety point of view. Such complexes may be expected to be less strongly retarded by sorption onto rocks along the flow path than the equivalent nuclide with inorganic ligands. Organic ligands are also likely to be utilised by any microbes present in the far-field. This would cause either direct uptake of the nuclide or its liberation in a form which
may be more strongly retarded. Thus this factor should be taken into account in any 'integral' model of microbial effects in the far-field.
5. RESEARCH NEEDS AND PRIORITIES

5.1 Overview

The preceding chapters have summarised the current state of knowledge in geomicrobiology with particular reference to the effects of microbial contamination on the integrity and performance of a deep geological repository for nuclear waste. For the specific case of the reference Swiss HLW repository, an attempt to put the data in context by the use of quantitative models is described in chapter 4. This analysis, and the discussion in chapter 3, highlight how much very basic geomicrobiological data are either poorly defined or missing. In order to be specific about research needs, however, it is worth considering the application of the data produced. From the nuclear waste management perspective, the central component in repository evaluation is the safety-analysis model chain (Fig. 5.1). At present, the sub-models in this chain are generally simplistic but conservative. The main requirement from microbiological studies is quantitative input into any of these models where microbial activity could conceivably cause the current "inorganic" models to be "non-conservative".

Following through the model chain shown on Fig. 5.1, significant microbial input on either regional or local hydrogeology can be immediately discounted because of the very large scales (tens to hundreds of km) over which these processes occur. Although the near-field hydrology could be altered (eg. by microbial precipitates clogging pores), the extent of this effect is anticipated to be very small compared with purely inorganic effects which are not, as yet, incorporated into near-field models.
Fig. 5.1: Safety analysis model chain (high level vitrified waste).
Near-field chemistry, canister corrosion, release from the waste matrix, chemical speciation and nuclide transport in the near- and far-field could all be significantly altered by microbial processes which are not considered in present models. The final "Biosphere" model of radionuclide distribution throughout the food chain, which yields the final dose to man, is also affected by microbial processes. Although not specifically included in present models, the empirical nature of the data used tacitly includes the microbiological activity in the surface environment.

Future research requires improved data on microbial influences on canister/glass corrosion, groundwater chemistry and nuclide transport. These general areas can be specified in more detail and assigned priorities based on the calculations in the previous chapter:

i) Canister corrosion. Present corrosion models are empirical (based on laboratory experiments) and very conservative when compared to observations of natural geological or archeological analogues (Chapman et al., 1984). Unless such models were made more realistic, it is unlikely that an explicit account of microbial effects would be required for the calculation of canister lifetime. Studies of the by-products and energetics of microbial corrosion of steel are required, however, to provide input into the near-field models.

ii) Waste-matrix. Like the corrosion model, that of degradation of the waste matrix is empirical and conservative and, in its present state, explicit inclusion of microbial effects is not required. Two poorly defined areas of input for near-field microbiological models are realistic rates of phosphorus release from the waste matrix and production of radiolytic oxidant. Both of these would be investigated by conventional "inorganic" experimental studies and neither is likely to be critically important.

iii) Near-field chemistry / Chemical speciation. These models are inextricably linked and are extremely susceptible to microbial perturbations. The key output from these models are the limiting
elemental concentrations and chemical forms of released nuclides. Both of these could be altered considerably by the by-products of microbial metabolism. The main limitation to date is the relatively low level of sophistication of current, purely inorganic chemical models. Although incorporation of quantitative data on complexation between organic by-products and key-nuclides is required, it must be matched by more basic improvements, for example in techniques of modelling redox conditions.

iv) Transport. Key assumptions in the previous chapters are that microorganisms cannot move through the porosity of the compacted bentonite but could freely access the porous matrix of the kakirite. Both these assumptions need to be checked by either field or laboratory experiments as, if invalid, the effects on resultant nuclide releases could be very large. Further study of nuclide uptake by microbes and microbial mobility in the far-field is required.

5.2 Microbial processes in perspective

A general impression gained from this study is that although microbial processes cannot be discounted for HLW, the probability that they could significantly affect repository safety is very small. Work in progress suggests that more realistic, yet still conservative, modelling would allow most processes identified above to be discounted. It seems that microbiological effort could be more valuably directed towards the low/intermediate level waste situation (in both type B and C repositories). As the type of data required for these waste types is essentially the same as for HLW, it should also allow resolution of the main uncertainties still involved.
5.3 Specific Experimental Recommendations

Combining the needs of the previous sections with the practicalities and limitations discussed in chapter 3, specific recommendations on the format of future experimental programmes can be advanced.

a. The role of allochthonous (introduced) microbes will be as important as that of autochthonous (resident) groups. Work should therefore concentrate on sampling without regard to their origin.

b. It is evident that certain functional groups, e.g. SRB, are of more importance to waste disposal than others. Hence their presence should be ascertained as a first priority.

c. Sampling from mines/caves in relevant formations is more informative than studies of boreholes.

d. Microbial populations vary with site thus site-specific studies will have to be initiated at any prospective location.

e. It follows from (d) that tolerances for each group at each site must be thoroughly investigated in view of their specificity. Similarly their sorption properties, effects on geochemistry and mobility should be assessed.

f. A crucial factor for modelling potential microbial growth will be deriving an accurate nutrient budget for a site. This will involve examining host rocks, backfill etc. using a range of analytical techniques.

g. In conjunction with (f), the local bioenergetics of the environment (efficiency, rates with respect to environmental adversity) also require quantification. Such studies will provide valuable input data for modelling.
h. Biodeterioration of particular structural components should be examined with respect to the particular isolates identified at a site. Work should be concentrated on low/intermediate waste systems. However, some of this work will be generic and would help elucidate actual biodeterioration mechanisms and energetics.

The above points indicate that any site specific study must be carefully planned in order to provide relevant and appropriate information required for modelling purposes. Models also require development and, in particular, a study like that herein focussed specifically on low/intermediate waste would be valuable.
6. CONCLUSIONS

On the basis of this report, the main conclusions of relevance to safety assessment of a Swiss HLW repository are:

i) The repository will contain active populations of microorganisms for at least a limited time period.

ii) Such organisms will cause some biodegradation of engineered barriers, alteration of groundwater chemistry and will sorb radionuclides.

iii) The most important constraint on microbial activity in the near-field is probably available energy.

iv) Radionuclide complexation by organic by-products is likely to have the largest consequence from a safety analysis viewpoint.

v) Improvement of the level of sophistication of current release and transport models is required before the effects of microbial processes could be incorporated.

vi) In general, microbial effects on safety seem to be very small and future effort should focus on low/intermediate level waste.

Specific recommendations for future research are:

i) Emphasis must be placed on ‘mixed’ source microbes from mines/caves in relevant formations.

ii) Site specific studies are necessary for an accurate evaluation of nutrient availability, migration properties, biodeterioration, bioenergetics and tolerances.

iii) Any experimental plans must be carefully coordinated to yield maximum information for both the modeller and the microbiologist.

iv) Work should concentrate on low/intermediate level waste types.
7. ACKNOWLEDGEMENTS

The beautiful, secluded location provided by P. Townend and his staff at Hotel Waldheim (organised by A. Kriesten of Nagra) provided an ideal environment for the preparation of this report which was greatly appreciated. Meticulous typing by S. Wittke is also gratefully acknowledged. The text has been greatly improved thanks to the comments of J. Hadermann, C. McCombie, F. van Dorp, R. Bachofen, E. Stoll, W. Jeschki and H. Wanner. N.A. Chapman, H.P. Alder and H. Flury are thanked for their interest in this work and Nagra for financial support.
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APPENDIX

Glossary of microbiological and waste management terminology

This glossary is expanded from an original presented by West et al. (1982b). More extensive background on geomicrobiology can be obtained from the annotated bibliographies of West et al. (1982b) and West and Arme (1984) while the Swiss disposal programme is described in detail by Nagra (1985).

**Actinide.** One of the group of elements from Actinium to Lawrencium in the periodic table. Some of these are important components of nuclear waste.

**Activation product.** Radionuclide produced from a stable element by the capture of one or more particles (usually neutrons).

**ADP (Adenosine diphosphate).** Co-enzyme, involved in energy transfer in living organisms.

**Adsorption.** Attachment of organic (including micro-organisms) and inorganic particles to larger particles or surfaces.

**Aerobe.** Organism capable of respiration in presence of free oxygen (gaseous or dissolved) which is used as an electron acceptor.

**Agar.** Polysaccharide derived from certain red algae and used for gelling culture media.

**Alga (pl. algae).** Simple, photosynthetic plants. Plant body unicellular or multicellular, non vascular. Occur in marine or fresh water or in damp situations.
Allochthonous. Has its origin from an environment other than that in which it is found.

Anabolism. See metabolism.

Anaerobe. Organisms which live in the absence of free oxygen (gaseous or dissolved). In an aerobic respiration energy is liberated by breakdown of compounds without consumption of oxygen using substances other than oxygen as electron acceptors.

Anhydrite. An evaporite mineral, CaSO₄, found in sedimentary rocks.

Anoxic. In the absence of free oxygen.

Antibiotic. Substance, generally of biological origin, which inhibits growth or reproduction of particular species, e.g. the fungus Penicillium produces penicillin which is toxic towards many types of bacteria. It can also be artificially produced by man.

Archaebacteria. Bacterial group, including some methanogens, which have a unique cell and plasma membrane structure and a unique type of ribosomal RNA that distinguishes them from all other bacteria (eubacteria).

Asphalt. Bitumenous resin formed by evaporation of the volatile components from oil.

ATP (Adenosine triphosphate). Co-enzyme providing common source energy for different cellular functions. A phosphate grouping of ATP can be transferred to other substances by enzyme action simultaneously releasing energy. Formed from ADP by using light energy in photosynthesis and by means of the energy from catabolic processes.

Autoantagonism. Production of substances by organisms which inhibit their own growth.

Autochthonous. Originating from environment in which found; endemic.
**Autotroph.** Organism obtaining energy in an entirely inorganic medium using light or chemical energy for assimilation of CO\(_2\) (see photolithotroph, chemolithotroph).

**Auxotroph.** Organism requiring organic growth factors.

**Backfill.** When the waste package is emplaced in a repository, the material used to seal all remaining voids is called backfill. In the Swiss HLW repository this would comprise highly compacted bentonite.

**Bacteria (singular bacterium).** Group of ubiquitous unicellular or multicellular, microscopic, prokaryotic organisms which can be motile or non-motile. They can be autotrophic or heterotrophic, aerobic or anaerobic. Single cells appear as rods, spheres (coccis) or spirals.

**Barotolerant.** An organism that can grow at high pressure albeit at a lower rate than growth at atmospheric pressure.

**Barophile.** An organism preferentially growing at high pressure rather than atmospheric pressure.

**Basophile.** Micro-organism living preferentially in alkaline conditions.

**Bentonite.** A special assemblage of clay minerals formed by the weathering of volcanic rocks. Its most important component is montmorillonite from which it derives its swelling properties.

**Biomass.** Mass of biological material present.

**Biosphere.** The portion of the earth inhabited by living organisms.

**Bitumen.** Naturally occurring tar-like hydrocarbon mineral of indefinite composition.
Blue Green Algae. Cyanobacteria. Members are prokaryotic, many shape formations. Wide distribution: marine, freshwater, hot springs, arctic water, soil, mud, living with fungi (lichens).

Catabolism. See metabolism

Cell wall. Limiting layer of plant and most bacterial cells.

Cement. A complex alumino silicate which when powdered, reacts with water to form a hard, rigid solid. A range of physical and chemical properties can be obtained by varying chemical composition and mode of preparation.

Chelation. Formation of a complex by a ligand which completely envelopes the central unit.

Chemolithotroph. Organism obtaining energy from oxidation of inorganic substances independent of light, and building organic compounds from inorganic substrates (also termed chemoautotroph).

Chemoorganotroph. Organism obtaining energy from oxidation of organic substances independent of light, and building organic compounds from organic or inorganic substrates (also termed chemotroph).

Chemotaxis. Movement in which stimulus is provided by a gradient of chemical concentration.

Cladding. The metal container holding the fuel pellets used in nuclear reactors which becomes very radioactive during reactor operation and is removed prior to reprocessing.

Coccus (pl. cocci). Globular organism.

Colloid. A stable suspension of small particles, usually in the nm to μm size range.
Colony Counting. Method of microbial enumeration by counting colonies (visible aggregates of microbes) grown from single organisms dispersed on a gelatinous growth medium.

Complex. A composite molecular species formed of several distinct sub-units. Generally a central metal ion surrounded by a number of small molecules or ions which are termed ligands.

Concrete. A material prepared by mixtures of cement and aggregates (e.g. sand, gravel) which has generally superior properties to the cement paste alone.

Crystalline rock. A term used loosely to imply an igneous or metamorphic rock as opposed to a sedimentary rock. Typically granite or gneiss in the Swiss case.

Culture on. Inoculating fresh medium with a microbial culture to test for viability.

Cytoplasm. All protoplasm of a cell excluding nucleus. Transparent viscous fluid containing various intracellular structures.

Daughter. Radionuclide produced by decay of another (parent) nuclide.

Decommissioning. The process of closing and totally dismantling a reactor.

Detritus. Particulate organic material which is only partly disintegrated.

Direct Count. Microscopic enumeration of micro-organisms collected on slides or filters (cf. Fluorescent microscopy).

D.O. Dissolved oxygen.
D.O.C.  Dissolved organic carbon.

Drift. Horizontally mined tunnel.

Endcaps. The ends of fuel rods which are cut-off prior to reprocessing.

Endemic. Occuring in a natural habitat.

Endospore. Under some conditions certain bacteria produce an internal resting cell called an endospore. Normally only produced under unfavourable growth conditions and give protection to the genetic material. When favourable conditions return it will grow into a new cell. Not a form of reproduction.

Enrichment. Culture method that selects for desired organism(s) by providing special nutrients and/or physical conditions that favour its (their) development. Term is also used for the process by which fissile U-235 is concentrated during nuclear fuel fabrication.

Enzyme. A protein which acts as a biochemical catalyst.

Eukaryote (Organism or cell). Having nucleus separated from cytoplasm by a nuclear membrane and genetic material borne on a number of chromosomes consisting of DNA and protein. Eukaryotic cells are unit of structure in all organisms except bacteria and blue green algae.

Euryhaline. Tolerating a wide salinity range.

Eurythermal. Tolerating a wide temperature range.

Evolution. Cumulative change in the characteristics of populations occurring in the course of successive generations related by descent. The rate at which evolutionary adaption to particular environments occurs may be influenced by external factors, e.g. temperature, radiation, mutagenic chemicals etc.
Facultative. Able to adapt to alternative environments, e.g. facultative anaerobe, facultative halophile.

Fastidious. Organism requiring narrow ranges of environmental conditions for successful growth.

Fission product. Radioactive nuclide produced by the nuclear fission process.

Flagellum (pl. flagella). Fine long thread (about 0.25 μm thick and up to several hundred μm long) projecting from cell. Capable of a lashing or undulating movement. Widely distributed. Simple flagella possessed by some bacteria.

Fluorescent Microscopy. Light microscopic examination of organisms stained with specific fluorescent dyes. Biological material (e.g. microbes) is made to fluoresce using light of certain wavelength to excite the dye used. A common method is incident light fluorescent (epifluorescent microscopy).

Fullers' earth. An aluminium poor montmorillonite clay.


Geomicrobiology. Study of role that microbes play or have played in specific geological processes.

Gram Stain. Differential staining technique for bacteria.
Halophile. Organisms which grow preferentially in high salinities.

Heteroantagonism. Production of substances by an organism which inhibit other organisms, e.g. antibiotics.

Heterotroph. Organism using organic carbon material from environment as source of carbon (and energy).

HLW. Abbreviation for high level waste.

Hulls. Metallic structure which, together with the fuel pellets and cladding, comprise the fuel rod assemblages used in nuclear reactors.

Illite. A general term for a group of triple-layer, mica-like, clay minerals.

ILW. Abbreviation for intermediate level waste.

Indigenous. (Of organisms) native to a particular area, not introduced.

Iron bacteria. Bacteria facilitating the precipitation of iron through oxidation of Fe$^{2+}$ to Fe$^{3+}$ (M.O.B. - Metallo-oxidising bacteria). Various micro-organisms which are unable to oxidise iron can also precipitate iron through cleaving of organic ferric compounds (M.P.N.B. - Metallo precipitating non-oxidising bacteria).

Kakirite. Disturbed or crushed zone in the crystalline host-rock considered for the Swiss HLW repository. Such zones provide an important potential pathway for nuclide transport through this formation.
**Lichen.** Symbiotic relationship between a fungus and an alga (usually a blue green alga). Live on tree trunks, walls, exposed rock etc.

**Marl.** A calcareous (carbonate containing) mudstone

**Mesophile.** Organism with optimum growth in moderate temperatures.

**Metabolism.** Chemical processes occurring within an organism (or part of one). These involve breaking down of organic compounds (catabolism) coupled to energy production and building up organic compounds (anabolism) using energy from catabolism or, with autotrophs, energy from external non-organic sources.

**Methane-forming Bacteria.** (Methanogens). Bacteria capable of converting \( \text{CO}_2, \text{H}_2 \) and acetate to \( \text{CH}_4 \) (methane) under highly reduced conditions.

**Microbe.** Microscopic organism (virus, bacteria, some fungi, some algae).

**Microbial Biogeochemistry.** The study of microbially catalysed reactions and their kinetics, with emphasis on environmental mass transfer and energy flow.

**Montmorillonite.** A member of the smectite group of clay minerals with extensive ion exchange properties and swelling capacity during water uptake.

**Mould.** Any superficial growth of fungus.

**Mutation.** Sudden change in DNA. If a mutation occurs it can produce an inherited change in the characteristics of the organism.

**Myxotrophy.** Combining two or more means of carbon assimilation.
Obligate. Able to function only in a specific environment e.g. obligate aerobe.

Oligotrophs. Organisms adapted to life in nutrient-poor environments.

Overburden. Thickness of rock cover above a point.

Oxic. In the presence of free oxygen.

Package. For Swiss HLW the waste package comprises the vitrified waste itself, the glass fabrication container and the thick cast steel canister.

Photolithotroph. Organism obtaining energy from light, and building organic compounds from inorganic substrates (also termed photoautotrophic).

Photoorganotrophs. Organisms obtaining energy by chemical reactions dependent on light, and building organic compounds from organic substrates.

Photosynthesis. In green plants, synthesis of organic compounds from water and carbon dioxide using energy absorbed by chlorophyll from sunlight. Photosynthetic bacteria are mostly anaerobic obtaining hydrogen from, e.g. hydrogen sulphide, organic substances.

Phototaxis. Movement in response to light.

P.O.C. Particulate organic carbon.

Prokaryote (Organism or cell). Having genetic material in form of simple filaments of DNA and not separated from the cytoplasm by a nuclear membrane, e.g. bacteria, blue green algae.
**Psychrophile.** Organisms with low optimum growth temperatures (≤20°C).

**Psychrotolerant.** Organism growing at low temperature but preferring higher temperatures (above 20°C).

**Reprocessing.** Treatment of spent fuel to recover unused fissile materials and separate out waste radionuclides.

**Resin.** A rigid hydrocarbon or inorganic plastic with a glassy structure.

**Respiration.** Oxidation of organic carbon compounds to CO₂ and water with various substances utilised as terminal electron acceptors e.g. O₂ used in aerobic respiration, NO₃⁻, SO₄²⁻ etc. used in anaerobic respiration.

**Saprophyte.** Organism which obtains organic matter in solution from dead and decaying tissues of plants or animals.

**Sediments.** General term applied to sedimentary rocks which may be well consolidated.

**Shaft.** Vertically mined tunnel or hole.

**Siderophore.** An iron chelating substance produced by certain microbes.

**Slime.** Produced mainly by capsule-forming bacteria. Consists of water and polysaccharides.

**Smectite.** A group of montmorillonite-like clay minerals.

**STP.** Conditions of standard temperature (25°C) and pressure (1 atmosphere).
Suphate-reducing bacteria (SRB). Bacteria that reduce sulphate to sulphide (and to elemental S).

Sulphur oxidising bacteria. See Thiobacillus.

Swelling clays. Clay minerals which increase in volume during water uptake. If volume for expansion is constrained, high "swelling pressure" may build up.

Taxis. Locomotory movement of an organism or cell, in response to a directional stimulus, the direction of movement being orientated in relation to the stimulus.

Thermophile. Organism with high optimum growth temperatures (above 40°C).

Thiobacillus (pl. Thiobacilli). Gram negative, rod shaped bacterium. The Thiobacilli are mostly chemolithotrophic and obtain their energy through the oxidation of reduced sulphur compounds.

Transuranic. Element with atomic number greater than that of Uranium (92).

Viable Count. Microbial enumeration by means of culturing of organisms.

Virus. Sub microscopic agent that infects plants, animals, bacteria, usually causing disease, incapable of multiplying outside host tissues. Consists of nucleic acid within a protein-lipid coat. Nucleic acid initiates synthesis of more viruses when in infected cell.

Vitrification. The process of incorporation of radionuclides into a glass matrix.
Weathering. General term for natural, low temperature mineral degradation. Rigidly applicable only to near-surface effects but loosely used for deeper systems.

Yeast. Unicellular fungus.