Microbial Effects on Repository Performance

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Preface

This report has been prepared by Quintessa Limited under contract to the Nuclear Decomissioning Authority (NDA). The report has been reviewed by NDA, but the views expressed and conclusions drawn are those of the authors and do not necessarily represent those of NDA.

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Summary

This report presents a critical review of the international literature on microbial effects in and around a deep geological repository for higher activity wastes. It is aimed at those who are familiar with the nuclear industry and radioactive waste disposal, but who are not experts in microbiology; they may have a limited knowledge of how microbiology may be integrated into and impact upon radioactive waste disposal safety cases and associated performance assessments (PA).

Site selection in the UK will be based on a process of voluntarism, and no particular location has yet been decided upon. Similarly, no repository design or concept has been chosen. Consequently, this report is wide ranging and does not focus on any particular kind of site or repository design. The report draws information from a wide range of sources, including research into near-surface radioactive disposal sites and from the general environmental microbiology literature, as these provide insights into our understanding of microbial impacts on deep geological disposal of higher activity wastes. The report broadly summarises the interactions between microorganisms and the different components of geological disposal facilities (wastes, engineered barriers and natural barriers) and highlights how these interactions influence the behaviour of these components. The tools and techniques available to investigate the microbiology of a geological repository and its associated geology are also reviewed from the perspective of how such data can be integrated into PA and PA models.

The review makes the following conclusions:

Microbiology of relevant geological formations

It is recognised that microbes live in a wide range of geological environments. The reviewed work indicates that all the identified general geological environments in the UK will have an indigenous microbial ecosystem which will be influenced by environmental conditions such as the availability of nutrients and energy for microbial use; groundwater flow; the geological history and characteristics of the site including recent useage; and the site specific geological information.

Microbiology has been investigated in radioactive waste management programmes throughout the world for over 20 years and recognised it is now recognized that microbiology can influence a wide range of safety-relevant processes. Consequently, microbiological investigations are typically included in site investigation studies. The information obtained assists in understanding and predicting the performance of the repository into the long-term future.

Microbiological site characterisation is heavily dependent on obtaining pristine samples from geological materials to eliminate the possibility of contamination. Additionally, any microbiological characterisation programme must be planned at the same time as, for example, the geochemistry programme. Both are sensitive to external changes and both should be undertaken early in any site assessment. Furthermore, some drilling muds / fluids contain materials which will increase the nutrient and energy for microbial useage in the geological environment – probably permanently – thus altering the microbiology of the system in the future. Many characterisation techniques exist which can be utilised when assessing the microbiology of a particular geological environment. These techniques are not all generically applicable with some environments (e.g. clays) posing particular technical difficulties. It will be crucial to focus on microbial activity levels of relevant microbial groups as these will vary with the geological environment.

Microbiology of the near-field

Microbes can tolerate a range of extreme environmental conditions which demonstrates that a repository, even one for High-Level waste (HLW) or Spent Fuel (SF) or one backfilled with cement, cannot be assumed to be sterile for its entire lifetime. Nevertheless, it is very likely that sterile conditions will be found close to the waste although the distribution of sterile zones will be spatially and temporally variable. The existence and viability of life are controlled principally by the availability of water, nutrients and energy sources. The varying materials that may be used in a repository (as waste matrices, canisters, overpacks, buffers, backfills etc) are all potential nutrient and energy sources for microbial use. These materials may also have a microbial load, as will the excavated repository itself.

Internationally, considerable work has been carried out to understand and quantify microbial influences on many of these materials. Much of this work is site- or repository concept-specific but all investigations show the importance of considering microbial impacts in the context of a particular repository concept. Broadly, these studies have shown the following:

1. Microbially-Influenced Corrosion (MIC) has been demonstrated in many repository-relevant studies and all show that MIC must be considered in any repository concept where metals may be included.

2. Canadian studies of a range of backfill / buffer materials have shown that some mixes cannot reduce microbial activity to a point where it is insignificant with respect to the Canadian deep geological concept. As a result, this concept now uses compacted 100% bentonite buffer directly in contact with SF. Thus it is

reasonable to conclude that, for other concepts that employ compacted clay buffer material, direct microbial degradation is unlikely to be a problem.

3. Microbial degradation of organics will be significant in some repository concepts and much work has been undertaken to study biodegradation rates and biogenic gas production. However, the exact microbiological impacts will depend on the nature of the waste and the repository concept.

4. Microbial activity can have a direct influence on the behaviour of some radioelements such as uranium, technetium, neptunium and plutonium and for certain radionuclides such as ^{14}C. The nature and extent of these influences will depend on the prevailing geochemical environment and the chemical form of the radionuclide concerned.

The effect of microbes in the perturbed zone is currently an area of very active research. In particular the microbial impacts on overall solute transport properties and on reduction / oxidation (redox) processes are being investigated, as these are important factors that influence the performance of many geological disposal concepts.

Microbiological influences on Engineered Barrier System (EBS) performance

The overall influences of microbial activity on the performance of an EBS are, again, complex and dependent upon the nature of the wastes and the repository concept under consideration. For example, different concepts place different emphasis on the engineered and natural barriers in order to attain overall performance targets. In those HLW / SF concepts where disposal will occur in fractured igneous rock relatively great emphasis is typically placed on EBS performance and required canister lifetimes are relatively long. In these cases MIC may potentially influence overall barrier performance. In contrast, those HLW / SF concepts where the host rock is plastic clay typically place greater emphasis on the host rock barrier and canister lifetimes are relatively short. In these cases MIC may have a proportionately lower impact on overall barrier performance.

Microbial activity will also influence radionuclide release rates and migration. However, the significance of these impacts is also again dependent on the repository concept itself. For example, biogenic gas production is unlikely to be significant for HLW /SF where organic content will be very low.

Many microbial effects will also be enhanced in a repository concept which has an ‘open period’ during which effectively unlimited air (oxygen) is circulated into the system. Consequently, the effects of an open period on microbial populations and future repository performance would require careful assessment.

Microbiology of the far-field

As in the perturbed zone, microbes can impact on solute transport processes and thus influence radionuclide migration in the far-field. This is an area of active research, but much of the relevant repository performance work has been undertaken in granitic environments using underground laboratories. Little work has been carried out in other geological environments.

Microbial transformation of organic complexing agents has the potential to reduce radionuclide migration in the far-field. Additionally gases such as hydrogen and methane generated within a repository may be subject to further microbial transformations as they move through the far-field.

Integrating microbiology into Performance Assessment

The report focuses on the integration of microbiology into PA, but comments on the implications for safety assessment and safety case development. PA concerns the performance of a system or subsystem and its implications for protection and safety at a disposal facility. Thus, PA has a broader meaning than 'safety assessment' in which the performance measure is radiological impact or some other global measure of impact on safety. Conversely, PA is rather less broad than 'safety case development', a 'safety case' being an integrated collection of arguments and evidence to demonstrate the safety of a facility.

Understanding of microbiological processes is also typically taken into account implicitly when developing numerical models as part of PA. For example, the assessment process normally involves development of scenarios that are then subjected to numerical analysis during PA. These scenarios can be considered collections of Features Events and Processes (FEPs) and expert judgments of those FEPs to include in a scenario are required. Certain FEPs are to a greater or lesser degree influenced by microbial activity and deciding whether or not to include them requires understanding of microbiology. However, usually the application of this understanding is not reported transparently.

There is a wide range of available models that can be used to evaluate various aspects of microbial impacts on repository performance. However, they all have their limitations (e.g significant data requirements) and, for deep environments, there is no single approach or code to quantify microbial influences on solute transport and effects on redox processes. This is particularly the case where some scenarios may be too simplified to allow the impact of microbes to be assessed realistically – especially where microbial processes may support the function of key safety barriers, such as maintenance of reducing conditions.

Nevertheless, significant progress has been made towards incorporating knowledge of microbial processes into overall PA models. Existing models have been shown to make significant contributions to understanding microbial impacts on a particular disposal system. However, these models require site-specific data to be effective. Underground laboratories have a significant role to play in the generation of these data.

Development of a safety case involves using multiple lines of reasoning to support reasoned arguments that safety criteria are met robustly. These lines of reasoning and the arguments developed from them are qualitative and quantitative in nature. Microbiological information must be taken into account in both cases.

The output from a PA is one important input to safety case development. To the extent that microbiology is taken into account in PA, it is also incorporated into the subsequent safety case.

Microbiology is also frequently taken into account implicitly when developing qualitative arguments in favour of safety. For example, reducing conditions in the deep geosphere are generally favourable for safety. It is typically argued that post-closure conditions in a repository environment will become reducing over a short period compared to the overall assessment timeframe. This argument implies knowedge of microbially-mediated processes. However, the precise role of such knowledge in developing these kinds of arguments is typically unclear in literature supporting published safety cases.

The report shows that is not possible to ascertain which microbial effect or effects will predominate in the near-field, the EBS and disturbed zone; microbial effects will be dependent on the selected site, type of waste and the repository concept. Evaluation of these effects will need to be undertaken at a specific site, so the results can be assessed in the overall context of a PA. If the evaluation indicates that all microbial processes would have a significantly negative overall effect on performance then methods to limit these effects may need to be employed. These methods include (but are not limited to) measures to control or limit microbial activity, such as engineering solutions.

Contents

List of Abbreviations

AECL	Atomic Energy Canada Ltd
ANDRA	Agence Nationale Pour la Gestion des Déchets Radioactifs, the French national radioactive waste management agency
AO	Acridine Orange
APHA	American Public Health Association
ATP	Adenosine Tri-Phosphate
CARE	Cavern Retrievable Concept
CES	Cavern Extended Storage Concept
CDC	Concrete Disposal Cask
CDP	Cellulose Degradation Products
CFU	Colony-forming units
CSH	Calcium silicate hydrate (a cement component)
CSTR	Continuously stirred tank reactors
DEFRA	Department for Environment Food and Rural Affairs
DGGE	Denaturing Gradient Gel Electrophoresis
DMRB	Dissimilatory Metal Reducing Bacteria
DNA	Deoxyribonucleic Acid
EBS	Engineered Barrier System
EDZ	Excavation Damaged Zone
Eh	Redox potential
FEPs	Features, Events and Processes
FISH	Fluorescent In Situ Hybridisation
GDF	Geological Disposal Facility
GRA	Guidance on Requirements for Authorisation

GWB	Geochemists Workbench
HLW	High Level Waste
HRL	Hard Rock Laboratory
IAEA	International Atomic Energy Agency
ILW	Intermediate Level Waste
ISA	Iso-saccharinic Acid
JAEA	Japan Atomic Energy Agency
JNC	Japan Nuclear Cycle Development Institute
LLW	Low Level Waste
LLWR	Low Level Waste Repository
MIC	Microbially-Influenced Corrosion
MOX	Mixed oxide fuel
MPN	Most Probable Number
mRNA	Messenger RNA
Nagra	Nationale Genossenschaft für die Lagerung Radioaktiver Abfäller, the Swiss organisation charged with preparing and implementing a sustainable waste management solution for radioactive waste
NDA	Nuclear Decommissioning Authority
NEA	Nuclear Energy Agency (of the OECD)
NRVB	Nirex Reference Vault Backfill
NUMO	Nuclear Waste Management Organisation of Japan
OECD	Organisation for Economic Cooperation and Development
ONDRAF / NIRAS	Organisme National des Déchets Radioactifs et des Matières Fissiles enrichies / De Nationale Instelling voor Radioactief Afval en Veriiikte Splijtstoffen, the Belgian agency for radioactive waste and enriched fissile materials

OPC	Ordinary Portland Cement
PA	Performance Assessment
PCR	Polymerase Chain Reaction
PCRSA	Post-Closure Radiological Safety Assessment
PCSC	Post-Closure Safety Case
PGRC	Phased Geological Repository Concept
PLFA	Phospholipids Fatty Acid
RFLP	Restriction Fragment Length Polymorphism
qPCR	Quantitative PCR
rRNA	Ribosomal RNA
RT-PCR	Reverse Transcription PCR
RNA	Ribonucleic Acid
RWMD	Radioactive Waste Management Directorate (of the NDA)
SKB	Svensk Kärnbränslehantering AB, the Swedish Nuclear Fuel and Waste Management Company
SOCs	Soluble Organic Compounds
SF	Spent Fuel
SRB	Sulphate Reducing Bacteria
SYNROC	“Synthetic rock”, a ceramic waste form material
T-RFLP	Terminal Restriction Fragment Length Polymorphism
TGGE	Temperature Gradient Gel Electrophoresis
TRU	Transuranic Wastes
URL	Underground Research Laboratory
WIPP	Waste Isolation Pilot Plant

1 Introduction

This report presents a critical review of the international literature on microbial effects in and around a deep geological repository for higher activity radioactive wastes. The report is aimed at those who are familiar with the nuclear industry and radioactive waste disposal but who are not experts in microbiology; and who may have limited knowledge of how microbiology may be integrated into and impact upon radioactive waste disposal safety cases and associated performance assessments (PA). The report draws information from a wide range of sources including research into near-surface radioactive waste disposal and general environmental microbiology; these sources are utilised because they inform our understanding of microbial impacts on deep geological disposal of higher active wastes.

The report summarises the interactions between microorganisms and the different components of a geological repository (wastes, engineered barriers and natural barriers) and highlights how these interactions influence the behaviour of these components. The tools and techniques available to investigate the microbiology of a repository and its associated geology are also reviewed from the perspective of how such data can be integrated into a PA and PA models.

The report comprises the following sections:

1. Introduction including the history of radioactive waste microbiology, focussing on the UK, and outlining the context of this report;

2. Review of the microbiology of relevant geological formations including characterisation methodologies;

3. Review of the microbiology of the near-field;

4. Review of microbial influences on repository and Engineered Barrier System (EBS) performance;

5. Review of microbial effects in the far-field;

6. Integrating microbiology into performance assessments;

7. Conclusions.

At the end of sections 1- 6, key summary points are given.

1.1 Introduction to Subsurface Microbiology

To many scientists, the idea of the biosphere extending more than a few metres below the soil is a strange concept. Deeper 'rock' is often perceived as being incapable of supporting life as a consequence of being at high pressure and temperature, often fairly dry or with very slow-moving pore-water and low in nutrients. Consequently 'rock' is thought to be more ideally suited to the preservation and fossilisation of biological materials rather than providing a habitat for life. However, during the last one hundred years, the significance of microbes in element cycling, pH and reduction / oxidation (redox) has been realised (e.g. Baas Becking et al., 1960; references in Ehrlich, 1996). Since the 1980s, interest in the natural microbial activity of indigenous populations deeper in the subsurface has grown, particularly in the context of contaminant biodegradation, secondary oil recovery, and survival and transport of pathogenic organisms (e.g. Chapelle, 1993; West and Chilton, 1997). Considerable work is now undertaken on the 'geomicrobiology' of a diverse range of environments and it is now recognised that microorganisms live in a wide range of deep geological environments (e.g. Lin et al., 2006; Roussel et al., 2008). Indeed, it has been proposed that the mass of subsurface microbes may exceed the mass of biota on the Earth's surface (Whitman et al., 2001). This development of subsurface microbiology as a scientific discipline has been paralleled by the development of the new field of radioactive waste geomicrobiology.

1.2 History of Radioactive Waste Microbiology

With the development of geomicrobiology in the late 20th century and the recognition of the existence of microbial life in deep geological formations, some seminal studies of the potential influence of microorganisms on High-Level (HLW) and Spent Fuel (SF) repositories were carried out (Mayfield and Barker, 1982; West et al., 1982). This early work showed clearly that microbial processes have the potential to affect the performance of a geological repository. These processes can have both direct (e.g. biodegradation and corrosion of some wastes and repository containment materials; gas production; blocking of pores by biofilms) and indirect effects (e.g. alteration of pH and redox resulting in changes to radionuclide mobility). Many radioactive waste management organisations include applied geomicrobiology in their programmes.

1.2.1 History of Radioactive Waste Microbiology Worldwide

Over the past 20 years, radioactive waste geomicrobiology has expanded to be included in analyses of disposal options for varied types of waste and a number of countries now have microbiology programmes. Projects have covered a wide range of areas including:

- fundamental microbiology of deep geological formations;
- microbial tolerances to extreme conditions;
- biodegradation of organic waste components;
- biodegradation of repository materials;
- microbial gas generation;
- direct interactions between microorganisms and their by-products and radionuclides;
- natural analogue studies; and
- microbial influences on the geochemistry and transport properties of the repository environment.

The work has included field sampling, laboratory studies, and the development of mathematical models. Countries in which this research has been undertaken include Canada, Japan, Sweden, Switzerland, UK and USA. A summary of past and present work areas for these programmes is given in Table 1-1. Specific results are discussed in following sections.

Table 1-1: Overview of geomicrobiological research areas in Canada, Japan, Sweden, Switzerland, UK and Japan.

Country	Presence of microbes in relevant formations	Tolerance of microbes to repository conditions	Biodegradation / Biocorrosion	Radionuclide migration	Influences on geochemistry	Model development
Canada	X	X	X	X		X
Japan	X			X	X	X
Sweden	X	X	X	X	X	X
Switzerland	X		X	X	X	X
UK	X	X	X	X	X	X
USA	X	X	X	X		X

1.2.2 History of Radioactive Waste Microbiology in the UK

The United Kingdom programme is one of the longest running, having started in the early 1980s. In this study, the work is reviewed using the following categories: literature surveys; presence of microorganisms in relevant formations; microbial effects on repository construction materials and waste; microbial effects on radionuclide migrations, modelling studies; and natural analogue studies.

Many literature surveys have been performed. These range from the early studies of West et al. (1982) and West and McKinley (1984), where the significance of microbial activity was suggested, to later reviews on gas generation (Norris, 1987), effects on shallow land disposal (McGahan, 1987), effects of humic and fulvic acids on low- and intermediate- level waste (L / ILW) disposal, and reviews of degradation pathways of organic materials (Greenfield et al., 1991). Other reviews in the 1990s again focussed mostly on microbiological effects on L / ILW (e.g. Christofi, 1991; Rosevear, 1991). More recently, reviews have been undertaken on environmental microbiology relevant to radioactive waste disposal (Grant et al., 2000) and on the potential impacts of microorganisms on radionuclide migration (Bass et al., 2002).

Surveys of relevant geological formations have been an important component of the work in the UK and have involved studies on sites in continental Europe and in Britain. Granites (UK, Sweden) have been assessed together with Boom Clay (Belgium), Salt (Asse, Germany) and sedimentary sites (UK and Europe) (for example see summaries in Greenfield et al., 1991 and West, 1995). The microbiology of the UK Low Level Waste Repository (LLWR) near Drigg has also been investigated (Wilkins et al., 2007; Fox et al., 2006; Lockhart et al., 2006; Lockhart 2004; Humphreys et al., 1997a). Work has been undertaken on the microbial degradation of simulated LLW in concrete trenches and the survival of microorganisms in deep cementitious repository environments (e.g. Coutts et al., 1997; Grant et al., 2001). Microbial tolerances to high temperatures and pressures have also been studied (West, 1995).

The performance of the waste itself and repository construction materials was tackled in the early 1990s. The impacts of microorganisms on steel corrosion were studied (Philp et al., 1991) and considerable efforts were made to investigate cellulosic biodegradation and biogenic gas production in the context of I/ LLW (e.g. Coutts et al., 1997). During this period, radionuclide solubility tests were performed to study the effects of leachates from degradation experiments on plutonium solubility (Colasanti et al., 1991). The effects of microorganisms on results from conventional batch sorption experiments were also determined (West et al., 1991b).

Microbiology-related work carried out to support the 2002 Post Closure Safety case (PCSC) for the UK LLWR (BNFL, 2002a) focussed on the microbial characterisation of the site (Lockhart et al., 2006; Lockhart, 2004; Beadle et al., 2001) and the nature and extent of microbial gas generation issues (Humphreys et al., 1997a; Beadle, 2001 and 2002). Research was also commissioned on the impact of microbial activity on redox sensitive radionuclides (Fox et al., 2006) and microbial aspects of the near-field far-field interface (Wilkins et al., 2007; Beadle et al., 2003; Nikolova et al., 2001).

In common with other countries, the UK has sought to produce quantitative results which can then be used in models to evaluate the significance of microbial activity. In the 1990s a computer based mathematical model of microbial metabolism linking all related factors was devised for Nirex (Colasanti et al., 1991). A model for gas production (including biogenic gas) was also written (Agg, 1993). Microbiology is a central component of the DRINK source term model (Humphreys et al., 1997b) employed in the 2002 PCSC for the UK LLWR (BNFL, 2002a) allowing an evolving redox chemistry to be simulated (Small et al., 2000). An alternative modelling approach has been adopted for sites where significant amounts of biodegradable waste streams are not present. These employ nutrient and energy inventories to model microbial effects on repository performance. These models have been developed by the British Geological Survey with NAGRA, Switzerland and are now used for a wide range of applications (e.g. Baker et al., 1998; West et al., 2006).

The UK has also contributed to multi-national studies involving natural analogues where microbiology has played a role. These include studies of alkaline groundwater systems in Oman (Bath et al., 1987) and Jordan (Linklater, 1998), the uranium mine at Poços de Caldas, Brazil (West et al., 1992) and the Needle's Eye secondary uranium deposit in Scotland (Milodowski et al., 1990). Microbiology work has also been undertaken by UK institutions for other national and international programmes. Examples include the REX (Redox in block scale) project at the Äspö Underground Research Laboratory (URL), Sweden (Hama et al., 2001); and studies for NUMO, Japan on the influences of microbes on containment in repositories with extended open periods (West et al., 2006).

1.3 Scope of this Report

The UK will select its site based on a process of voluntarism (DEFRA, 2008) and there are a number of possible geological environments for a Geological Disposal Facility (GDF). Consequently, the review does not focus on any particular kind of site or repository design. Instead, the report is wide-ranging, reviewing work undertaken throughout the world on microbial effects that could occur in and around a geological repository for higher active wastes. The aim is to provide a context for the following sections that review work done in varied environments in different countries, with the aim of highlighting the potential relevance of this work to the UK.

1.3.1 Geological Environments Considered

Watson et al. (2007a) defined a number of generic geological environments in order to assess how the Phased Geological Repository Concept (PGRC) for ILW, developed by Nirex (Nirex, 2003a), might vary if adapted to different geologies (Table 1-2 [1]). However, these general environments might also be appropriate hosts for HLW / SF repositories. Watson et al. (2007b) reviewed international research concerning varied HLW / SF disposal concepts that would employ carbon steel barrier components and that could plausibly be employed in these environments.

The definitions of the generic environments are sufficiently broad that they constitute an adequate basis for discussing the likely microbial populations that may occur or evolve in and around a repository. However, it should be noted that these environments do not cover all those UK geological environments that may in future be considered to host a repository. Some other suitable geological environments may not be exactly the same as any one of these generic environments, but may have properties that are combination of those found in two or more generic environments. For example, plastic clay host rocks at sufficient depth to host a repository lie a short distance offshore from eastern England and potentially may be accessible by tunnels constructed from the onshore area. These plastic clays have mechanical properties that are different to those of the mudrock host rock in Environment 5, but which show some similarities to the mechanical properties of halite in Environment 4. However, in other respects (e.g. their mineralogy, porewater chemistry and transport properties) plastic clays are likely to be similar to the mudrock host rock of Environment 5.

[1] The descriptions differ from those in Watson et al. (2007a), owing to rounding of key parameters (e.g. salinity ranges) and some modifications to indicate ranges of conditions likely to be particularly relevant to microbiological activity.

Table 1-2: Summary characteristics of general geological environments in the UK that might plausibly be considered to host a deep geological repository for radioactive wastes (based on Watson et al., 2007a).

No.	General description	Most Probable Lithologies	Most likely groundwater chemistry	Most likely groundwater flow characteristics
1	Crystalline basement rocks to surface, , most likely in an area with little topographical relief	**Host rock:** 'granitic' (sensu lato) **Surrounding rocks**[2]**:** 'granitic' (sensu lato) and metasediments	**pH:** 6 – 9 **Eh (redox potential):** -0.4 V to -0.1 V (reducing) at depth below a few 10's of meters from the surface **Total Dissolved Solids (TDS):** Fresh[3], up to about 550 mg l^{-1} among HCO_3-dominated waters; brackish or saline, with up to around 10,000 mgl^{-1} in Cl-dominated waters **Range of composition:** dominated by Ca-Na-HCO_3 at shallower levels, possibly dominated by Na-Ca-Cl at deeper levels **Depth-related variations:** No trend in salinity in among HCO_3-dominated waters, but increase in Ca/Na and pH and decrease in pCO_2 with increasing depth. Increase in salinity with depth in Cl-dominated waters **Host rock:** May be either Ca-Na-HCO_3 or Na-Cl dominated, depending on site and repository design	**Driving forces:** Likely low head gradients (little advection) **Host rock + Surrounding rocks:** Diffusion in rock matix, advection through fractures (which overall is the dominant means of groundwater transport)

[2] The term 'surrounding rocks' is used generally to refer to rocks that are lithologically significantly different from the host rock and that may lie above and / or below the host rock and / or occur laterally at some distance from the host rock. The term is more generally applicable than the terms 'overlying rocks' or 'underlying rocks', which imply stratigraphical relationships between the host rock and shallower or deeper rocks respectively. In these cases 'shallower' and 'deeper' could refer either to the present geometrical arrangement of the rocks and / or to their geometrical arrangements at the time when the rocks were formed. Thus, 'overlying rocks' and 'underlying rocks' are usually meaningful only when the rocks being described are sedimentary in nature. Additionally, these terms are inappropriate when the rock being described occurs at the same depth as the host rock owing to faulting.

No.	General description	Most Probable Lithologies	Most likely groundwater chemistry	Most likely groundwater flow characteristics
2	Crystalline basement rocks under relatively permeable sedimentary cover rocks (Basement Under Sedimentary Cover, BUSC, relatively high-permeability cover variant), most probably in an area of high topographical relief near the cost	**Host rock:** 'granitic' (sensu lato) or volcaniclastic **Surrounding rocks:** Mainly sandstones, minor mudrocks, limestones possible	**pH:** 6 – 8.5 **Eh:** -0.3 V to -0.1 V (reducing) at depth below a few 10's of meters from the surface **Total Dissolved Solids (TDS):** Up to about 500 mg l^{-1} among HCO_3-dominated waters; up to around 200,000 mgl^{-1} in Cl-dominated waters **Range of composition:** dominated by Ca-Na-HCO_3 at shallower levels, dominated by Na-Cl at deeper levels; **Depth-related variations:** At any location shallower Ca-Na-HCO_3- dominated water is fresh, deeper Na-Cl dominated water is saline. Salinity of the latter water increases with depth. **Host rock:** Most likely Na-Cl dominated, either saline or brine, depending upon the site.	**Driving forces:** Likely relatively high head gradients **Host rock:** Diffusion in rock matix, advection through fractures (which overall is the dominant means of groundwater transport) **Surrounding rocks:** Variable lithologies, most rocks have advection-dominated flow, some with diffusion-dominated transport, all may support advection through faults / fractures
3	Low-permeability basement rocks under relatively low-permeability sedimentary cover rocks (BUSC, low-permeability cover variant), most likely in an inland area with low topographical relief	**Host rock:** 'granitic' (sensu lato), metasedimentary or volcaniclastic **Surrounding rocks:** Mainly mudrocks, minor sandstones, limestones possible. Evaporites may occur within the surrounding rock sequence. Overlying rocks likely to be thin, possibly only a few tens of metres thick.	**pH:** 6 – 8.5 **Eh:** -0.3 V to -0.1 V (reducing) at depth below a few 10's of meters from the surface **Total Dissolved Solids (TDS):** Up to about 500 mg l^{-1} among HCO_3-dominated waters; up to around 200,000 mgl^{-1} in Cl-dominated waters **Range of composition:** dominated by Ca-Na-HCO_3 at shallower levels, dominated by Na-Cl at deeper levels **Depth-related variations:** At any location shallower Ca-Na-HCO_3- dominated water is fresh, deeper Na-Cl dominated water is saline. Salinity of the latter water increases with depth. **Host rock:** Most likely Na-Cl dominated, either saline or brine, depending upon the site.	**Driving forces:** Likely relatively low head gradients **Host rock:** Diffusion in rock matix, advection through fractures (which overall is the dominant means of groundwater transport) **Surrounding rocks:** Heterogeneous, most rocks have diffusion-dominated flow, some with advection-dominated transport, all may support advection if there are fractures present

[3] Using the classification of Carpenter (1978); fresh waters have TDS <1000 mgl^{-1}; brackish waters have 1000 ≤ TDS < 10,000 mgl^{-1}; saline waters have 10,000 ≤ TDS < 100,000 mgl^{-1}; and brines have TDS ≥ 100,000 $mg1^{-1}$.

No.	General description	Most Probable Lithologies	Most likely groundwater chemistry	Most likely groundwater flow characteristics
4	Bedded evaporite host rock in a sedimentary basin, most likely in an area with little topographical relief	**Host rock:** Bedded halite **Surrounding rocks:** sequence of bedded evaporite and mudstone, overlain by a series of sandstones, marls, and mudstones, with low-permeability mudrocks immediately adjacent to the evaporite.	Likely to be similar to Environment 3, but with Na-Cl dominated brines certain to occur in the host rock. Depending upon the temperature, the brine is likely to have TDS in the range 250,000 mgl^{-1} to 300,000 mgl^{-1}	**Driving forces:** Likely low head gradients (little advection) **Host rock:** Advection in host rock negligible; diffusion-dominated transport **Surrounding rocks:** The lithology adjacent to the host rock has very low permeability and transport is dominated by diffusion. Other lithologies heterogeneous, some with diffusion-dominated transport, others with advection-dominated transport, some may support advection through fractures
5	Mudrock host rock, most likely in an area of low topographical relief	**Host rock:** Mudrock **Surrounding rocks:** low-permeability clays, sandstones and limestones. Sequence may contain evaporites.	**pH:** 7.5 – 8.5 **Eh:** -0.3 V to -0.2 V (reducing) at depth below a few 10's of meters from the surface **Total Dissolved Solids (TDS):** Up to about 500 mg l^{-1} among HCO_3-dominated waters in higher-permeability units; most likely up to around 35,000 mgl^{-1} in Cl-dominated waters in lower-permeability units that are remote from evaporites. If evaporites occur, Na-Cl dominated brines may be present. **Range of composition:** dominated by Ca-HCO_3 at shallower levels and in higher-permeability rocks, dominated by Na-Cl at deeper levels and in lower-permeability rocks **Depth-related variations:** salinity and chemistry variations with respect to depth reflect both the depositional environment of the rock (notably whether marine or non-marine), the spatial distribution of evaporiates and permeability variations **Host rock:** Most likely Na-Cl dominated, saline water	**Driving forces:** Likely low head gradients (little advection) **Host rock:** Advection in host rock negligible; diffusion-dominated transport **Surrounding rocks:** Variable, most with diffusion-dominated transport, but some with advection-dominated transport, some may support advection through fractures
6	Strong, low-permeability sedimentary rock (Chalk) host rock, most likely located near the coast in a sedimentary basin in an	**Host rock:** Low-permeability limestone (Chalk) **Surrounding rocks:** varied low-permeability rocks,	**pH:** 7.5 – 9 **Eh:** -0.3 V (reducing) at depth below a few 10's of meters from the surface **Total Dissolved Solids (TDS):** Fresh, up to about 500 mg l^{-1} among HCO_3-dominated waters; saline, with up	**Driving forces:** Likely low head gradients (little advection) **Host rock:** Advection in host rock negligible; diffusion-dominated transport **Surrounding rocks:** Variable, some with

No.	General description	Most Probable Lithologies	Most likely groundwater chemistry	Most likely groundwater flow characteristics
	area of relatively low topographical relief	mostly mudstones, but may be higher-permeability rocks, such as more permeable limestone and sandstone	to around 35,000 mgl^{-1} in Cl-dominated waters **Range of composition:** dominated by Ca-Na-HCO_3 at shallower levels, in more permeable lithologies, probably dominated by Na-(Ca) -Cl at deeper levels and in less permeable lithologies **Depth-related variations:** Salinity and chemistry variations with respect to depth reflect partly the permeability variations in the host rock and partly depth; fresher water occurs at shallower levels and in more permeable rocks. Salinity increases with depth in the host rock, then decreases downwards towards more permeable deeper horizons. **Host rock:** Na-(Ca)-Cl dominated saline water	diffusion-dominated transport, others with advection-dominated transport, some may support advection through fractures

1.3.2 Disposal Concepts Considered

A wide variety of concepts have been proposed for different kinds of waste and / or different geological environments. Disposal concepts for ILW and HLW / SF that have been proposed by radioactive waste management programmes throughout the world have recently been reviewed for NDA RWMD by Hicks et al. (2008) and Baldwin et al. (2008) respectively. These concepts can be divided broadly into those that are suitable for HLW / SF and those that are suitable for L / ILW or Transuranic Wastes (TRU) wastes. Within each of these two broad groups most of the concepts have been developed with a particular kind of waste and / or geological environment in mind. Some concepts may be less appropriate in some geological environments (e.g. cementitious barriers would probably be ineffective in a salt host rock) or for some wastes (e.g. copper canisters would not be appropriate for ILW because their use would incur excessive costs without resulting in significant benefits relative to other kinds of canister). Furthermore other concepts would probably be considered 'over-engineered' and consequently would not be implemented (e.g. using copper waste canisters for HLW in a plastic clay host rock). However, in practice most concepts could be adaptable for a wide range of specific waste types and geological environments. Furthermore, most of the concepts have not been finalized and many different detailed design options have been considered and / or may be developed in future. Thus, with appropriate adapatation, almost all the concepts that have been proposed by radioactive waste management programmes elsewhere in the world could be employed within the UK for one or more waste types. Of course it is also possible that in future the UK programme may develop concepts that differ substantially from any of those proposed elsewhere.

For the present project, the most important aspects of the concepts that will impact upon microbial activity and influence the techniques that must be employed to investigate this activity are the:

- chemical properties of the host rock and associated groundwater, which will influence the kinds of micro-organisms that might be active (for example, only microbes that are tolerant to highly saline conditions might be present in a brine) and the extent of the activity (for example because concentrations of nutrients and energy sources will differ in chemically distinct groundwaters);

- transport properties of the host rock, which will influence the rate of supply of nutrients and energy sources (notably whether transport is diffusion-controlled or advection-controlled) ;

- repository geometry, which will influence how different emplaced wastes will interact with one another[4];
- geometry of EBS components, which will influence interactions between the components and the accessibility of components to groundwater;
- waste form composition (principally the proportions of organic and inorganic materials), which will influence the chemical environment and its suitability to support microbial activity;
- characteristics of EBS materials, principally the quantities and compositions of:
- metal barriers, which may be composed of iron, steel and non-ferrous metals (most likely copper and / or titanium), and which will influence chemical conditions and prevent water flow between contained materials and other barriers surrounding the metal ones;
- cementitious materials (cement and / or concrete), which will buffer pH at highly alkaline values and react with certain chemical species, thereby preventing their migration (most notably dissolved inorganic carbon species);
- bentonite-bearing materials (bentonite and / or mixtures of bentonite with other materials) are present, which will limit groundwater flux and (chemical buffering will also occur, but will probably be of lesser significance for micro-organisms); and
- organic materials (bitumen employed as an encapsulant or in seals), which could provide a source of organic material that may be metabolized by microbes, but which also will prevent or limit groundwater movement.

1.4 Summary Points

1. It is recognised that microbes live in a wide range of geological environments and their potential influence on the performance of a repository for radioactive waste has been included in national programmes for over 20 years.
2. A number of possible geologies are considered to host a deep repository for radioactive wastes.

[4] The interactions that are most likely to occur will be those between different kinds of ILW and / or LLW emplaced within a particular disposal module. Interactions between co-located HLW and ILW / LLW are much less likely to be significant.

3. All UK geological environments will contain microorganisms.

4. The UK radioactive waste microbiology programme is one the longest running, having started in the early 1980s. Much recent work in the UK has been linked to other national and international programmes, particularly in Japan and Europe.

5. Microbial influences in a wide range of UK-relevant geological environments and repository concepts are considered in this report.

2 Review of Microbiology of Relevant Geological Formations

2.1 Review of Work in National Programmes

Following initial literature searches, an early part of several national geomicrobiology programmes was to establish the presence of microbes in geological formations being considered as host rocks for repositories. The rationale was that if no indigenous populations are present then microbes may not be viable in the environment selected and hence not cause a problem. Many of these studies are relevant to the generic environments identified in Section 1.3.1.

Broadly, characterisation work has been undertaken in granites in Canada (e.g. Stroes-Gascoyne and West, 1996), Finland (e.g. Pedersen, 2008), Japan (e.g. Aoki, 1997; Ishii et al., 2000), Sweden (e.g. Christofi et al., 1985; Pedersen and Ekendahl, 1992; Pedersen, 1993; Pedersen, 2000, Ekendahl et al., 2003; Anderson et al., 2006a; Nielsen et al., 2006; Eydal and Pedersen, 2007; Hallbeck and Pedersen, 2008a and b; Kyle et al., 2008), Switzerland (e.g. Christofi and Milner, 1990) and the UK (e.g. Christofi et al., 1983; 1984). Analyses have also been performed in sedimentary rocks in Belgium, Germany, Hungary and Switzerland (Christofi et al., 1985; Mauclaire et al., 2007; Stroes-Gascoyne et al., 2007a; Poulain et al., 2008, Farkas et al. 2000), the UK (Christofi et al., 1984), Italy and Japan. Evaporites (gypsum, salt) were sampled in Switzerland and Germany (Christofi and Philp, 1997). In the US, work has been undertaken on volcanic tuff (e.g. Pitonzo et al., 1999) and salt formations (Vreeland et al., 1998; Francis et al., 1998).

General reviews of microbial populations in a range of potentially relevant formations have also been presented (Christofi, 1991; Rosevear 1991). Analyses have mostly concentrated on groundwaters, although solid materials have been investigated for microbial content (Christofi et al., 1983; Stroes-Gascoyne, 2007a). In addition, studies on biofilms found on fracture surfaces in granitic environments have also been performed (Stroes-Gascoyne and West, 1997). All these studies have confirmed the presence of microbes in every sampled environment. Numbers vary from 10^2 colony forming units (CFU) per ml to 10^5 CFU per ml in some groundwaters. A single exception is the salt site at Asse in Germany. Here quantification was impossible, even though there were indications of the presence of small populations of extremely oligotrophic organisms adapted to hypersaline conditions. Significant bacterial populations have, however, been found in other salt formations (Vreeland et al., 1998; Francis et al., 1998). A summary of findings at selected sites is given in Table 2-1. Further discussion on specific results is given in the following sections.

Table 2-1: Microbial populations in relevant deep geological environments (from West and McKinley, 2002).

Location	Geology	Depth (mbgl)	Microbial count
Canada	Granite	350 – 400	Total counts 10^3–10^5 cells ml^{-1}
Japan	Granite	Approx 400 - 790	Total counts 10^2–10^7 bacteria ml^{-1}
Stripa, Sweden	Granite	799 – 1240	Total counts $2.0x10^1$–$1.3x10^5$ cells ml^{-1}
Äspö, Sweden	Granite	129 – 1078	Total counts $1.5x10^4$–$1.8x10^6$ cells ml^{-1}
Grimsel, Switzerland	Granite	Approx 350 m	$9.5x10^1$–$9.0x10^4$ CFU ml^{-1}*
Altnabreac, UK	Granite	10-281	$9.4x10^5$ CFU ml^{-1}*
Mol, Belgium	Boom Clay	190 – 223	$1.2x10^3$ CFU ml^{-1}*
Harwell, UK	Oxford Clay	165-331	$8.6x10^3$–$3.5x10^5$ CFU ml^{-1}*
Asse, Germany	Salt	750	ND
Yucca Mountain, USA	Volcanic Tuff	60	10^2–10^3 bacteria g^{-1} dry weight NB Above water table
WIPP, USA	Permian Salt Formation	650	Total Counts 10^3–10^5 bacteria ml^{-1} brine and g^{-1} salt.

Key CFU = Colony forming units, * = aerobic heterotrophs, ND = Not detected, WIPP = Waste Isolation Pilot Plant, an evaporite-hosted repository for TRU waste in New Mexico.

The composition of microbial populations varies with site (West, 1995). It is, however, increasingly recognised that the analysis of ambient microbial populations is part of site characterisation (e.g. JNC, 2000) and may be more critical to understanding the hydrogeochemistry of the site than to assessing the role of microbes on repository performance. For example, the redox conditions of a site are critically important in assessing site suitability. However, many of the redox complexes used to calculate redox potentials (Eh) are multi-electron transfers of sulphur, nitrogen and carbon species, which are kinetically slow at relevant temperatures and will proceed only with microbial catalysis (Hunter et al., 1998). Indeed, in experiments with rock and groundwater from a granitic environment sulphate-reducing bacteria (SRB) and iron-reducing bacteria appeared to catalyse secondary smectite formation (Hama et al., 2001). It is important, therefore, to focus on the activity levels of relevant microbial groups, such as SRB, sulphur oxidisers, methanogens, rather than counting numbers of cells or 'stamp collecting' microbial families or species.

2.2 Review of Methodologies Used to Characterise Geological Environments

2.2.1 Sampling Techniques

Indigenous microbial populations are easily perturbed by contaminant organisms introduced during sampling. Consequently, it is crucial to obtain pristine samples from geological materials so that a microbiological characterisation can be determined with confidence. Avoiding contamination, for example from drilling fluids, during the sampling process is paramount. Methods have been developed to obtain both uncontaminated groundwaters and solid materials, as described in papers listed in the previous section 2.1 (e.g. Christofi et al., 1983 and 1985; Hallbeck and Pedersen, 2008a; Stroes-Gascoyne and West, 1996; Stroes-Gascoyne et al., 2007a). However, it is crucial that any microbiology and biogeochemistry characterisation programme be planned at the same time as, for example, the geochemistry programme. Both are sensitive to external changes and both should be undertaken early in any site assessment. Careful consideration of the use and types of drilling muds / fluids must also be made as these will introduce chemical and microbiological contaminants. Additionally, some drilling muds / fluids contain materials which will increase the nutrient and energy availability in the geological environment, which will also alter the microbiology of the system into the future.

2.2.2 Methodologies

A wide range of approaches is available for the investigation of subsurface microbiology and biogeochemistry (Figure 2-1:). The methodologies employed reflect the aims of the experimental work and the availability of relevant materials and environments. Consequently a wide range of methodologies have been employed to characterise the microbiology of radioactive waste disposal systems (Table 2-2) with the approaches adopted reflecting the development of the waste disposal programme concerned. Microbiology-related work generally splits into two main areas: site characterisation work; and investigations on the impact of microbiology on safety-related functions and processes. The integration of microbiology into site characterisation studies reflects the impact of microbiology on how a geological environment will respond to perturbations resulting from the presence of a disposal facility. In site characterisation, direct isolation, identification and enumeration of microorganisms is generally involved. In work focussed on safety-related functions and processes, microbiology is generally considered in the context of wider processes such as gas generation, corrosion, radionuclide mobility or geochemical evolution. Investigations into these processes usually involve some form of simulation of the expected environment, or the monitoring of real sites / wastes or direct in-situ investigations. In these cases, microbiological investigations run along side chemical and physical investigations and the impact of microbiology is often inferred from the physical and chemical changes; with isolation, identification and enumeration being less important.

2.2.3 Culture Based Techniques

A significant amount of information regarding the potential microbiology of a site can be obtained from an analysis of its geochemical context. An audit of the potential terminal electron accepting processes in conjunction with nutrient availability, which encompasses both the near-field and far-field of a site, will provide a reasonable understanding of the potential microbial impacts (West et al., 1992, 1995, 1998 and 2006; Jolley et al., 2003; Tochigi et al., 2008). If significant microbial activity is likely then a more detailed understanding of the system microbiology will be required to determine how it will evolve in response to the construction of the disposal system.

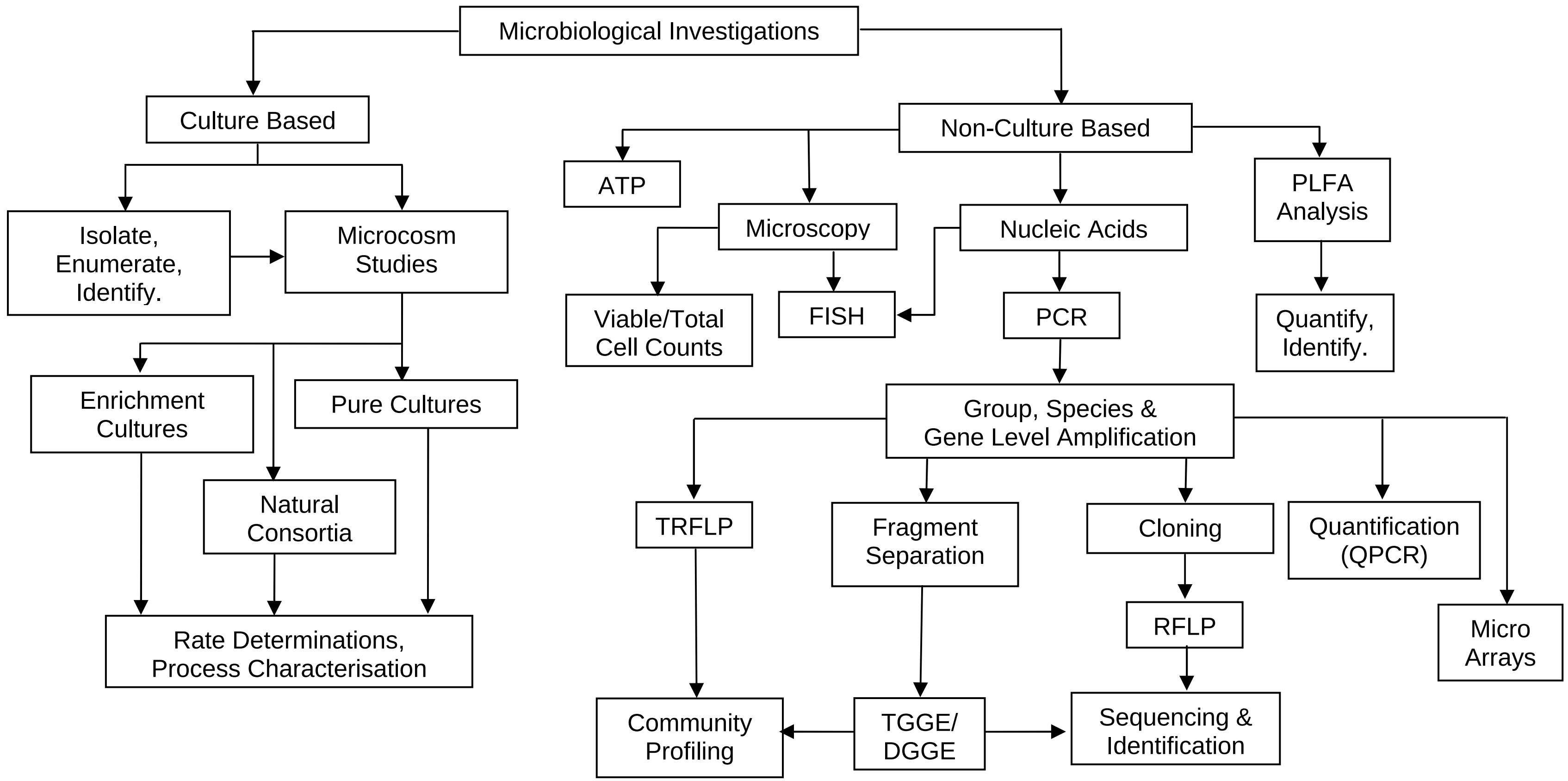

Figure 2-1: Microbiological methods (Modified from Weiss and Cozzerelli, 2008).

Table 2-2: Microbial investigation of radioactive waste disposal systems.

Site	Waste	Near-field (NF), Far-field (FF) or Waste (W) related	Approach – Direct (D), Inoculated Microcosms (IM)	Technique								Objective				Reference
				Culture Based	Direct Measurements	Substrates & Products	PLFA	TGGE/DGGE	RFLP	PCR, Sequencing & Identification	RNA	Site Characterisation	Modelling Support	Data Generation	Conceptual Understanding	
UK LLWR	LLW	NF	D&IM	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓	Humphreys et al. 1997a ; Beadle et al. 2001 ; Lockhart 2004 ; Lockhart et al. 2006 ; Fox et al. 2006.
		FF	D&IM	✓	✓			✓	✓	✓		✓		✓	✓	West et al. 1988 ; Wilkins et al. 2007.
Relevant UK and European sites	I/HLW	FF	D	✓	✓							✓		✓	✓	Christofi et al., 1983 and 1985; Christofi and Milner, 1990; Christofi and Philp, 1997.
Atomic Energy Canada Ltd (AECL) URL, Canada	SF	NF	D	✓		✓	✓							✓	✓	Stroes-Gascoyne et al. 2002 and 2007b.
Yucca Mountain USA	SF	FF	D/IM	✓			✓			✓		✓		✓	✓	Gillow et al. 2000 ; Horn et al. 2004.
WIPP USA	TRU	NF	IM		✓										✓	Leonard et al. 1999.
		FF	D&IM	✓	✓	✓						✓			✓	Vreeland et al. 1998 ; Francis et al. 1998.
GGE Experiment, VLJ Repository Finland	L/ILW	NF/W		✓		✓							✓			Small et al. 2008.
Paks Hungary	L/ILW	W	IM	✓		✓						✓		✓	✓	Molnar et al. 2006 and 2008
Mont Terri, Switzerland	HLW	FF	D&IM	✓	✓		✓			✓		✓			✓	Stroes-Gascoyne et al. 2007a ; Mauclaire et al. 2007; Poulain et al. 2008.
Grimsel Test Site, Sweden					✓											Gillow et al. 2000.
Mol URL, Belgium	HLW	FF	D&IM	✓	✓	✓			✓	✓		✓	✓	✓	✓	Boivin-Jahns et al. 1996 ; Ortiz et al. 2002 ; Aerts et al. 2008.
Äspö , Sweden	SF	FF	D	✓	✓	✓				✓			✓	✓	✓	e.g. Hallbeck and Pedersen 2008a and b; Kyle et al. 2008; Anderson et al. 2006a and b; Pedersen 2000.
		NF	D&IM	✓				✓		✓					✓	e.g. Fru and Athar 2008; Pedersen et al. 2000a and b.

Culture-based microbiological techniques have been used extensively to enumerate and isolate microorganisms from sites and materials associated with radioactive waste disposal (e.g. Francis et al., 1980; West et al., 1988; Vreeland et al., 1998; Farkas et al., 2000; Horn et al., 2004; Fukunaga et al., 2005; Hallbeck and Pedersen 2008a). These techniques involve the growth of organisms present in samples using solid or liquid media with the composition of the media and incubation conditions determining which physiological group of organisms is isolated. These techniques allow an estimate of the numbers of culturable organisms to be determined by either directly counting the organisms on solid media or employing liquid media and statistical approaches such as the Most Probable Number (MPN) technique (APHA, 2005). The advantage of this approach is that it is well established, technically undemanding and allows organisms to be isolated which can then be used for further investigation. However, only a fraction of environmental microorganisms are culturable in this way (<1%, Tanner, 2007), with recovery onto solid media resulting in the lowest recoveries (<0.1%)(Hallbeck and Pedersen, 2008a). It is also important to note that to date some bacterial lineages identified by molecular biology approaches have never been cultured (Rappe and Giovannoni, 2003). This means that culture-based approaches may be not be isolating the most important organisms within an environment, only the most culturable. The use of liquid media specifically designed for the site under consideration, combined with a MPN approach can significantly improve this recovery (Hallbeck and Pedersen, 2008a), however this does involve significant method development. Key to the recovery of environmental microorganisms is the matching of nutrient and culture conditions to the environment under consideration and the use of long incubation times (Tanner, 2007).

Due to the inherent errors associated with culture-based techniques, microbial enumeration from environmental samples may have limited value over and above providing a rough guide to the relative proportions of functional groups. Clearly a large population of culturable organisms will indicate an active population within the environment responsible for the catalysis of associated redox processes. On the other hand, the absence of a large culturable population may not indicate the absence of an active population and their associated redox processes. It should also be noted that the processes in which a group of organisms take part within an environment may not reflect the conditions under which they are isolated and enumerated. For example organisms may be isolated under denitrifying conditions whereas they may be responsible for mainly aerobic metabolism in-situ. It is important to remember when dealing with culture-based techniques that no evidence of microbes is not evidence that microbes do not occur.

The use of culture-based techniques does allow the isolation of specific microbial species, which can then be used in further investigations where a high degree of control is desired. This approach has been used extensively in radioactive waste disposal programmes to investigate, for example, the microbial accumulation of uranium (Nedelkova et al., 2007; Gillow et al., 2000), the survival of bacteria in bentonite (Motamedi et al., 1996; Pedersen et al., 2000a and b), microbial impacts on corrosion (Horn et al., 2002), and microbial biocolloids (Francis et al., 1988).

2.2.4 Microcosm Studies

Microcosms are simulations of the environment of interest and can be operated in a number of different ways depending on the objective of the experiment. They can be run using single species of bacteria, defined communities of microorganisms or undefined communites. With single species and defined community microcosms, the microorganisms employed are either from the environment of interest or possess particular characterisitics of interest. Single species experiments have been used extensively to investigate direct bacterial interactions with radionuclides (e.g. Nedelkova et al., 2007; Boukhalfa et al., 2007; Icopini et al., 2007; Renshaw et al., 2007) and some corrosion studies (Horn et al., 2005). Microcosms can be run as either batch systems which are completely sealed or flow through systems where nutrients are fed into the system. Batch microcosms have been used extensively to investigate gas generation from organic-containing wastes (Caldwell et al., 1988; Francis et al., 1997; McDonald et al., 1997; Beadle, 2001 and 2002). Many of these investigations have used either the microbial load present on the waste or added an inoculum such as soil, which carries a broad microbial load. Flow through systems are generally better suited to investigate microbial impact on dissolved chemical species, for examples they were used to investigate the denitrification (nitrate removal) potential of bacteria present in Yucca Mountain rock samples (Horn et al., 2005). Flow through systems are commonly run as columns and have been used to investigate radionuclide release (Kelly et al., 1998), bioclogging (Nikolova et al., 2001), biofilm / mineral and corrosion interactions (West et al., 1998; Hama et al., 2001; Tuck et al., 2006).

Enrichment cultures (Wolfaardt et al., 2007) are microcosms employed to investigate specific functional groups within an environment. These involve incubating environmental samples under specific conditions which select for and promote the growth of the organisms of interest. Enrichment prevents the enumeration of microbial groups, since a successful enrichment may result in a small number of organisms in the original sample becoming the dominant group in the enrichment. Enrichment cultures suffer from the same problems as all culture based techniques, in that their success is dependent on the ability of culture conditions to match the growth requirements of the organisms of interest. The absence of growth is as likely to be a problem with the culture conditions as evidence of the absence of the microbes concerned. Enrichment cultures have been used in a wide range of investigations, including investigations to confirm the potential for specific terminal electron-accepting processes within sites (Beadle et al., 2001), the impact of iron reduction on uranium behaviour (Fox et al., 2006), the recovery of SRB and acetogens from bentonite (Fru and Athar, 2008), the potential for microbial growth in Yucca mountain geological samples (Kieft et al., 1997), and the extent of cellulose degradation under WIPP conditions (Vreeland et al., 1998).

2.2.5 Physiological Characterisation

At its simplest physiological characterisation involves the determination of functional groups of microorganisms based on the consumption of substrates and the generation of metabolic end products. This approach has been used to support / indicate the presence and activity of organisms at a range of sites and experimental programmes (Tables 2-2). More sophisticated physiological profiling has been employed to investigate the subsurface degradation of organic pollutants and associated bacteria (Garland et al., 2007). Physiological profiling has been used to characterise bacteria from some radioactive waste disposal sites (Vreeland et al., 1998; Nedelkova et al., 2007). Stable isotope probing (Weiss and Cozarelli, 2008) provides a more refined technique for the following element flow through microbial processes. Labelling by ^{13}C- has been extensively employed to investigate the subsurface degradation of organic compounds (Madsen, 2006). Although stable isotope techniques have not been used in radioactive waste disposal situations, radio-labelled carbon compound (^{14}C) and sulphur (^{35}S) have been used to investigate substrate degradation (Kieft et al., 1997) and sulphate reduction (Pedersen et al., 2000b).

2.2.6 Direct Microbiological Techniques

Given the problems inherent in culture-based techniques they are often employed alongside more direct measurement of microbial load. These approaches are most appropriate for liquid samples such as leachates and groundwater and generally involve some combination of microscopic observation and staining. Gillow et al. (2000) determined total microbial counts of WIPP and Grimsel groundwaters using the Deoxyribonucleic Acid (DNA) specific fluorescent stain DAPI and epifluorescence microscopy. The same approach was employed by Francis et al. (1998) for total bacterial counts in WIPP brines. A similar approach was employed by Kieft et al. (1997) when investigating the total microbial content of rock samples from Yucca Mountain and in many European studies (e.g Christofi et al., 1983; 1984 and 1985). In this case acridine orange (AO) stain was employed instead of DAPI. In cases where microbial counts are low, fluorescent microscopic techniques (DAPI or AO) can be combined with filtration allowing the numbers of cells in large volumes of groundwater to be counted. This approach was also employed by Hallbeck and Pedersen (2008a) to determine total cell counts in groundwater from the Fennoscandian shield. Since DAPI and AO stains bind with DNA they provide a total count and are not able to differentiate between viable and non-viable cells. There are however stains which only bind to viable cells, one such stain, CFDA-AM, was developed for soil investigations and have been employed by Fukunaga et al. (2005) to investigate the bacterial content of bentonite deposits. There are also commercial staining kits that allow live and dead bacteria to be visualised. These kits employ combinations of the viable cell stain SYTO 9 and propidium iodide which stains cells with compromised membranes. These stains were employed by Chicote et al. (2005) to investigate bacteria present in SF storage ponds. Electron microscopy has been employed in a number of cases (Poulain et al., 2008; Anderson et al., 2006a) to provide a direct visualisation of cells. These approaches are best applied to surfaces with microbial growth or relatively concentrated suspensions.

A more sophisticated staining technique (Fluorescent In Situ Hybridisation (FISH)) (Moter and Gobel 2000; Bottari et al., 2006) allows the direct visualisation of microbial groups using fluorescent probes specific for ribosomal Ribonucleic Acid (RNA) sequences. These probes can be designed to target microorganisms from the domain to the species level. Different coloured probes allow the direct visualisation, identification and enumeration of multiple species within the same sample / environment. For example Detmers et al. (2004) used FISH to study the community structure of a pristine aquifer by using domain- and species- specific probes. An attempt was made by Stroes-Gascoyne et al. (2007a) to use a variant of the FISH approach, CARD-FISH, in order detect bacteria in Opalinus clay samples from the Mont Terri URL. Unfortunately this approach was not successful, probably due to the very low levels of microorganisms present.

2.2.7 Biochemical Markers of Viable Microorganisms

In addition to direct microscopic investigations biochemical markers have also been employed to provide an insight into the in situ levels of microorganisms in geological environments. Adenosine Tri-Phosphate (ATP) is a coenzyme essential for energy transfer within cells (Knowles, 1980). As such it is a marker for biological activity and consequently its presence can be used as an indicator of active microbial populations.

Eydal and Pedersen (2007) evaluated the use of ATP analysis on shallow and deep groundwaters by comparison with viable and direct microbial counts. The technique was found to be robust and reliable with a detection limit of 2×10^{3} cells ml^{-1}. ATP levels were found to correlate with total cell counts and the ratio of ATP levels to cell counts provided an inication of the metabolic state and viability of the groundwater environment under investigation. The value of ATP analysis in microbial site characterisation is reflected by the fact that it has been integrated into radioactive waste disposal related investigations into Fennoscandian Shield groundwaters (Hallbeck and Pedersen, 2008a and b).

Phospholipids fatty acid (PLFA) analysis (Hedrick et al., 2007) provides an alternative approach for the assessment of viable microbes within an environment. PLFA are microbial membrane components which are rapidly degraded on cell death and consequently represent the living microbial community present within a sample. As well as providing an estimate of the overall biomass levels, PLFA analysis provides an insight into the microbial community structure due to the fact that specific biomarkers exist for specific microbial groups. In contrast the breakdown products of phospholipids, neutral and glyco-lipids can be used as marker for cell debris. PLFA analysis have been used to provide insight in the microbial ecology of a wide range of subsurface habitats (Ringelberg et al., 1997; Green and Scow, 2000; Weiss and Cozzarelli, 2008). The method complements molecular and biochemical profiling techniques (Widmer et al., 2001) and may be more effective at detecting changes in microbial community structures (Ramsay et al., 2006).

PLFA analysis has been employed in both Canadian and US research concerning radioactive waste microbiology. PLFA analysis of Yucca mountain tuff samples (Kieft et al., 1997; Horn et al., 2004; Wang and Francis, 2005) indicated an overall population in between 10^3 and 10^5 cells/g dominated by Gram negative bacteria. It has also been used to assess the overall microbial populations present in long term corrosion test performed under simulated Yucca mountain conditions (Horn et al., 2005). PLFA analysis was used to characterise buffer material removed from in-situ experiments at the URL of Atomic Energy Canada Ltd (AECL) in Canada (Stroes-Gascoyne et al., 2002). This analysis was employed to provide overall levels of biomass and an indication of the overall community structure via the presence of specific PLFA markers. In more recent analysis of Opalinus Clay samples from the Mont Terri Site in Switzerland (Mauclaire et al., 2007; Stroes-Gascoyne et al., 2007a) these markers revealed the dominance of Gram negative anaerobic bacteria, relatively low levels of SRB and potentially the existence of some fungi.

2.2.8 Nucleic Acid Based Methods

Nucleic acid (DNA or RNA) based investigative techniques have revolutionised the study of environmental microbiology by providing culture independent approaches to the identification of the microbes present within a site. The majority of these approaches applied to radioactive waste disposal studies have employed the same overall scheme (Figure 2-2:).

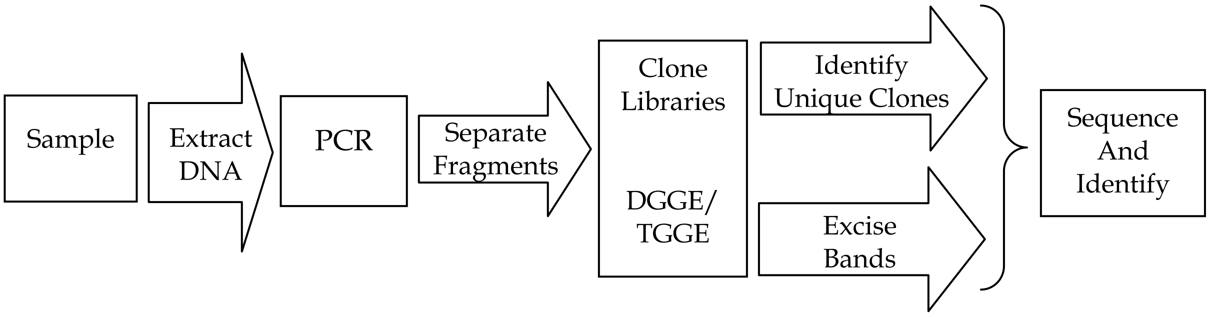

Figure 2-2: Generic application of nucleic acid-based techniques.

All these approaches begin with the extraction of microbial DNA from the environmental samples concerned. Following extraction the polymerase chain reaction (PCR) is used to amplify selected regions of the microbial DNA. Amplification is achieved using PCR primers, which are short sections of DNA that act as starting points for DNA replication. The choice of primers determines the area of DNA to be copied with domain-specific, species-specific and gene-specific primers being available. The commonest regions chosen for amplification are associated with the gene coding for 16s ribosomal RNA (rRNA). The DNA fragments amplified are then separated by either the construction of clone libraries or via electrophoresis. The amplified fragments are different for each species and once they have been sequenced (i.e. the DNA code is read) they can be compared with sequences from known species. This comparison enables the identification of the exact species the sequence originates from or its closest known relative.

Clone libraries are constructed using plasmids to insert individual PCR-amplified fragments into the DNA of *E. coli* cells. These cells are then grown with each colony containing multiple copies of the inserted fragment. The fragments can then be extracted from the cells to yield pure extracts of PCR products. The result is a large number of individual PCR products which may be from the same or different microorganisms present in the original sample. Although in theory all these fragments could be sequenced, in reality only a selection are taken forward for further analysis.

PCR fragments may also be separated via electrophoresis techniques such as Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE). DGGE (Muyzer et al., 1993) is an electrophoresis technique which allows the separation of DNA fragments having different base pair sequences but the same overall size. Separation occurs due to the denaturing of the amplified DNA fragments in response to an increasing gradient of a chemical denaturant. In TGGE a temperature gradient rather than a chemical gradient is used to denature PCR fragments. The result of both approaches is a banding pattern on the gel which reflects the microbial diversity of the sample analysed. DGGE and TGGE offer both a community profiling technique (Osborn and Smith, 2005; Tzeneva et al., 2007) and a way of separating fragments for sequence analysis. There are, however, issues with both DGGE and TGGE when applied to environmental microbiology (Liu and Stahl, 2007), including:

- their inapplicability to complex populations due to their inability to resolve the large number of bands involved (i.e. the band overlap);
- their inability to separate fragments greater than 500bp (base pairs), limiting the phylogenetic information available from the sequencing of excised bands; and
- the fact that some organisms may generate more than one band.

It is important to recognise that these approaches may not be 100% effective when applied to environmental samples. For example, several attempts to extract DNA from Opalinus clay samples from the Mont Terri URL were unsuccessful (Stroes-Gascoyne et al., 2007a), despite the fact that PLFA and enrichment cultures indicated that a small but viable population of organisms was present (Stroes-Gascoyne et al., 2007a; Mauclaire et al., 2007; Poulain et al., 2008). This lack of success was attributed to the potential binding of DNA to clay minerals, the very low levels of microbes present and the fact that the microbes present may be in a viable but non-culturable state (Stroes-Gascoyne et al., 2007a). However, the application of PCR approaches to associated enrichment cultures successfully led to the identification, via sequence analysis, of previously unknown species of the *Sphingomonas* and *Alicyclobacillus* genera (Poulain et al., 2008). Where pure cultures are extracted from environmental samples it is possilbe to fully sequence the genome of the organisms recovered.

Other radioactive waste disposal programmes have been more successful in applying these approaches. Pedersen et al. (1996) amplified, cloned and partially sequenced 155 16s rRNA genes from unattached and attached bacteria present in a range of groundwaters. This analysis identified bacteria of the genera *Acinetobacter*, *Bacillus*, *Desulfovibrio* or *Thiomicrospira*. A number of sulphate-reducing and iron-reducing isolates were also sequenced indicating the presence of *Desulfomicrobiurn*, *Desulfovibrio* and *Shewanella* species. Pedersen (1996) reported over 155 unique sequences taken from 50 independent samples sampled from varied sites, including two natural analogues, the Stripa research mine, the Äspö URL (termed the Hard Rock Laboratory, HRL) and large scale heated waste canister experiments performed in Canada. These sequences were distributed across 11 branches of the eubacterial phylogenetic tree.

Analysis of Yucca Mountain Tuff samples combined PCR, clone library construction and screening via Restriction Fragment Length Polymorphism (RFLP). This approach was used to construct *a more complete roster of the organisms contained within the Yucca Mountain community* (Horn et al., 2002). This project employed Eubacterial primers to generate a clone library which was then screened via RFLP. Two hundred clones were screened resulting in 65 unique sequences, which were used to construct a phylogenetic tree of the Yucca Mountain bacterial community. This allowed the Yucca Mountain sequences to be related to the sequences of known bacteria in the databases. The screening of clones by RFLP allows a percentage composition of the original bacterial population to be determined based on the percentage occurrence of each clone in the clone library. The accuracy of this approach depends on the number of clones screened, assuming that biases in DNA extraction and amplification are not significant. In the Yucca Mountain study statistical analysis indicated that analysis of 200 clones was sufficient. This analysis indicated that close relatives of three species *Arthrobacter nicotianae*, *Microbacterium imperiale* and *Devosia riboflavin* accounted for 43% of the community investigated. The same approach was applied to samples from long-term corrosion tests run to evaluate potential waste package materials. In this case the population was much less diverse, with *Bacillus sp* accounting for 84% of screened clones.

This approach was also employed by Wilkins et al. (2007) to investigate the behaviour of uranium and technetium under iron-reducing conditions within microcosms containing far-field sediments associated with the UK LLWR. This approach allowed shifts in the microbial populations in response to the establishment of iron-reducing conditions and the introduction of radionuclides to be identified. For example under iron-reducing conditions a close relative of the acetate-oxidising, iron-reducing bacteria *Rhodoferax ferrireducens* became a significant component of the population whereas it was not a significant aspect of the population present in un-amended sediments. A similar approach was employed by Fox et al. (2006) to investigate the response of near-field microbial populations from the UK LLWR to uranium amendments. As with Wilkins et al. (2007) this approach allowed Fox et al. (2006) to detect changes in the microbial population when microbial uranium reduction was taking place.

An alternative approach to detecting changes in microbial populations which does not employ cloning is a modification of RFLP known as Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Liu et al., 1997; Osborn and Smith, 2005). This is a community profiling technique where PCR-amplified target genes are digested with one or more restriction enzymes. The size of the subsequent fragments is then detected via a DNA sequencer. In order to do this one or both primers are fluorescently labeled, the output of T-RFLP analysis being a graph of fragment size versus their fluorescence intensity, which is often subject to statistical analysis (e.g. Abdo et al., 2006). T-RFLP is often used to profile and compare communities within environments. Although not applied to radioactive waste disposal environments this approach has been extensively applied to soil environments (e.g. Muckian et al., 2007; Thies, 2007), and some deep geological environments. For example Inagaki et al. (2002) combined T-RFLP with sequencing of 16s rRNA gene sequence analysis to investigate the bacterial and archaeal populations present in deep sea rocks.

Fox et al. (2006) employed DGGE to provide an insight into the evolution of the microbial populations under study. DGGE was also employed by Gillow et al. (2000) in the identification of bacteria present in groundwaters associated with the Grimsel test site. Fru and Athar (2008) employed DGGE to separate PCR product from 16s rRNA gene amplification from a range of samples associated with bentonite experiments carried out at the Äspö HRL. Prominent bands were excised, the associated DNA extracted, re-amplified and sequenced for subsequent phylogenetic analysis. As with Fox et al. (2006), Fru and Athar (2008) combined DGGE analysis with the construction of clone libraries, which indicated that a substantial proportion of the retrieved sequence belonged to *Bacillus sp*. Lockhart (2004) employed TGGE to investigate the diversity of cellulolytic *Clostridium* species and methanogenic bacteria in samples taken from the waste disposal trenches at the UK LLWR. This analysis highlighted differences in the diversity between the known clusters of cellulolytic *Clostridium* sp identified by Collins et al. (1994), with cluster I and IV being of lower diversity than that of cluster (III). This work was also able to determine the spatial diversity of methanogenic bacteria across the site.

Lockhart (2004) employed a range of PCR primers and probes targeted at increasing levels of complexity within the microbial population under study, allowing the analysis to start at the domain level and progress through to genera and species. In the case of cellulolytic Clostridia, specifically designed primers (Van Dyke, 2002) were employed to target the 16S rRNA genes of these groups. SRB were also investigated using a series of PCR primers designed to individually target each subgroup of SRB (Daly et al., 2000). In the case of methanogens a probe hybridisation approach was used to confirm the presence of a range of methanogenic families and genera in the site.

Not all PCR analysis targets the rRNA genes, a number of studies have targeted functional genes associated with specific microbial groups. Fru and Athar (2008) targeted the sub units of the dissimilatory bisulphite reductase gene found in SRB. The presence of this gene was used to confirm the presence of SRB in the groundwater under investigation. Similarly Lockhart (2004) employed the methyl coenzyme M reductase gene, a highly conserved and ubiquitous gene amongst methanogens, to provide insight into methanogenic populations within LLW degradation simulants (Beadle, 2001 and 2002).

A wide range of approaches have been applied to microbial investigations associated with radioactive waste disposal systems. The number of available technologies is extensive and continually expanding (Osborn and Smith, 2005; Liu and Stahl, 2007; Dowd and Pepper, 2007; Weiss and Cozzarelli, 2008). The DNA / PCR-based techniques outlined above do not provide any insight into the size or activity of the microbial populations present. The development of Quantitative PCR (qPCR) (Osborne and Smith, 2005; Dowd and Pepper, 2007) allows the quantification of the starting amount of the nucleic acid templates of interest that are present in a sample. Quantification is achieved by comparison with a standard curve, the generation of the PCR product being detected using a fluorescent dye, which binds to the PCR product as it is generated. This approach has been used to analyse microbial communities via ribosomal RNA gene analysis in sediments (Stults et al., 2001), soils (Hermansson and Lindgren 2001; Fierer et al. 2005) and groundwater (Miller et al., 2007). The levels of functional genes within environments can also be determined via qPCR with denitrification (Kandeler et al., 2006), ammonification (Okano et al., 2004), sulphate reduction (Foti et al., 2007) and methanogenesis (Nunoura et al., 2006).

It is possible to gain insight into the activity of specific genes within a microbial population by the analysis of messenger RNA (mRNA) rather than DNA. Due to the rapid turnover of mRNA within bacteria (Ross, 2001) the detection of mRNA within a microbial population is significant evidence for the activity of the associated process within that population (Osborn and Smith, 2005). In order to study gene expression via mRNA, reverse transcription PCR (RT-PCR) is employed (Osborn and Smith, 2005). This approach has been used to investigate the biodegradation of environmental pollutants (e.g. Röling, 2004) and microbial processes including denitrification (Nogales, 2002), iron reduction (Chin et al., 2004) and nitrogen fixation (Holmes et al., 2004). RT-PCR approaches have been combined with qPCR approaches allowing mRNA levels within an environment to be determined (Sharkey et al., 2004). RT-PCR has also been combined with DGGE techniques to provide community profiles of active microbial population (Nakatsu, 2007).

A further development in the analysis of RNA is the use of stable isotope probing. Here ^{13}C-labelled organic compounds are degraded by microbial populations and the subsequently labelled RNA is extracted and analysed via RT-PCR, to allow the organisms responsible or the degradation process to be identified. This approach was combined with DGGE to investigate phenol degrading populations by Manefield et al. (2002) and has been applied to marine sediments (MacGregor et al., 2006), contaminated groundwaters (Kasai et al., 2006) and soils (Pumphrey and Madsen, 2008).

The screening of microbial populations for the presence of larger numbers of target genes has been simplified by the application of microarrays (Dowd et al., 2007). These arrays are constructed by depositing an array of DNA probes corresponding to specific genes or species, onto glass or silicon beads or surfaces. These arrays are then challenged with fluorescently labelled nucleic acid extracts, with the presence of the DNA of interest being detected by the retention of the fluorescent label to the array. This approach has been used in a number of environmental applications including to screen for the presence of all lineages of SRB (Loy et al., 2002), active methanotrophic populations (Bodrossy et al., 2006), and microbial uranium oxidation and reduction (Brodie et al., 2006).

This section has overviewed the nucleic acid-based techniques that have been applied within radioactive waste disposal programmes and related environmental research, focussing on those techniques that involve a PCR amplification stage. It is not within the scope of this section to provide an in-depth discussion of the limitations and biases inherent within these techniques.

2.3 Summary of Organisms Potentially Occurring in UK Generic Geological Environments

Section 1.3.1 describes general geological environments in the UK that might plausibly be considered to host a deep geological repository for radioactive wastes. Although microbial populations in any geological environment will be site-specific, as previously discussed, it is likely that broad 'functional groups' in these general UK environments may be similar to other analogous environments. Some specific studies of the microbiology of some relevant UK formations are available, mostly dating from the 1980 and early 1990s (see Section 1.2.2). However, Table 2-3 summarises some of the recent work, mostly performed for other radioactive waste programmes, in the context of potential UK host rocks. It must be emphasised that reference to this other work does not suggest that microbiological characterisation of UK geological environments is unnecessary. It merely indicates the potential range of organisms likely to be detected in a particular geological environment. It should also be noted that much of the work undertaken in UK sandstone, limestone and chalk has targeted shallow contaminated aquifers and is thus of limited relevance to deeper geological environments, especially those in which lithologies have low permeabilities. Nevertheless, it is possible to summarise, broadly, the main environmental controls on the microbiology of any UK generic geological environment. These will be:

- the availability of nutrient and energy sources for microbial useage;
- groundwater flux;

- the geological history of the site including recent useage such as water abstraction, proximity to contaminated sites, landfills, industrial features which will impact on the subsurface ecosystem; and
- the specific geological environment, for example geochemistry.

2.4 The Impacts of UK Generic Geological Environments on Microbial Populations and Activity

An examination of some generic UK geological environments (Section 1.3.1) highlights certain characteristics of each environment that could potentially impact on microbiological populations and activity:

- *Crystalline basement rocks*: growth along fracture surfaces; heterogeneous nutrient supply;
- *Bedded evaporite*: high porewater / groundwater salinity; potentially high sulphate; diffusion control of nutrient supply;
- *Mudrock host rock*: diffusion control of nutrient supply; potentially high organic and sulphide content in rock;
- *Strong low-permeability sedimentary rock*: dual porosity characteristics likely; possible high organic and Fe content in rock.

Additionally, thoe characteristics of these different environments that may impact upon microbiology would have different susceptibilities to being affected by climate change. For example, a fractured host rock that extends to the surface would be more likely to be affected by inputs of glacial water than an evaporite host rock beneath low permeability sedimentary cover rocks.

By analogy with experience internationally, site-specific data will be needed to predict the actual characteristics of microbiology in these different generic UK geological environments. Studies in other countries show that modelling of microbiological processes can make a significant contribution to understanding microbial impacts on a particular disposal system. However, the models require site-specific data to be effective. This international experience also highlights that underground laboratories have a significant role to play in the generation of these data.

It can also be concluded from reviewing international experience that the geological characteristics of a site will also influence how readily its microbiology can be characterised. In fractured hard rock environments, experience suggests that it is possible to gain significant insight into the role of microbiology in maintaining *in-situ* conditions. However, characterisation of mudrock environments appears to be more technically challenging, although it is being actively investigated. The characterisation of other sedimentary rocks has been investigated but mainly in the context of near-surface non-radioactive contaminants.

Information from other programmes suggests that in the generic UK environments, levels of indigenous microbial activity will be dependent on the characteristics of the undisturbed geological environment (e.g. the Boom Clay and Swedish granite have contrasting microbial activities). There is also a relationship between the characteristics of the geological environment and the microbiology of the system and, consequently, in the techniques used to investigate them. Thus any geochemical and microbiological investigations need to be fully integrated and included in geological / hydrogeological site investigations; optimisation of an overall site characterisation programme needs to include consideration of microbiological characterisation efforts. For example, if there is a requirement to obtain good quality rock core then it may be necessary to use drilling muds. However, to do so would detract from any microbiological investigation as muds can be a source of organic carbon. Therefore, rock sampling and microbiological sampling need to be undertaken with consideration of their mutual impacts.

In each of the generic UK environments, microbiological changes in response to the presence of a repository would be determined by the environmental characteristics and the repository concept. For example, where fluid and solute transport occur dominantly by diffusion, the disturbance associated with facility construction of the facility would be less spatially extended than would be expected in environments where transport is advection-dominated.

Table 2-3: Examples of potential organisms in generic UK geological environments based on recent examples from other relevant sites.

Host rock	**Source of information**	**Summary of detected microbial types**	**Example References**
Granitic	Olkiluoto, Finland; Kamaishi Mine, Japan; Altnabreac, Scotland; Äspö HRL; Forsmark; Laxemar, Sweden (NB Groundwater analyses only).	Prokaryotes: Nitrate-reducers; Iron-reducers; Manganese-reducers; Sulphate-reducers; Acetogens; Methanogens. Viruses: 4 bacteriophage groups. Eukaryotes: 5 yeast, 3 yeastlike and 17 mold strains.	Finland: Pedersen, 2008; Japan: Ishii et al., 2000; Scotland: Christofi et al., 1983; Sweden: Ekendahl et al., 2003; Kyle et al., 2008; Hallbeck and Pedersen, 2008b.
Halite	Asse, Germany (Groundwater analyses only) WIPP, USA.	Non detected although indication of presence of very oligotrophic organisms. Halophilic and halotolerant bacteria and archaea including Aerobes, denitrifyers, fermenters, sulphate reducers and methanogens.	Germany: Christofi et al., 1985. USA : Vreeland et al. 1998 ; Francis et al. 1998 ; Gillow et al. 2000.
Low-permeability limestone (Chalk)	No studies that are directly relevant; although there is much information on microbiology and water quality eg denitrification; pathogen survival and transport.	Prokaryotes: Denitrifiers, Survival of viruses and prokaryotic *Cryptosporidium* spp oocysts demonstrated	UK: e.g. West and Chilton, 1997; Pedley et al., 2006
Low-permeability rocks e.g. mudstones, clays, marls, etc	Harwell, England; Mont Terri Underground Research Laboratory, Switzerland (Opalinus Clay)(NB Rock analyses).	Prokaryotes: Organotrophic aerobes and anaerobes; Anaerobic lithotrophs; Sulphate-reducers. NB Small viable population indicated which is mostly dormant.	England : Christofi et al., 1983; Switzerland : Mauclaire et al., 2007; Stroes-Gascoyne et al., 2007a; Poulet et al., 2008.
Higher-permeability rocks e.g. certain kinds of sandstones, limestone	No studies that are directly relevant; although there is much information on microbiology and water quality eg pathogen survival and transport. Much of the more radioactive waste-relevant work on these rock types has focussed on shallow contaminated aquifers.	Prokaryotes: Survival of a range of Thermotolerant coliforms. Viruses: Survival of Coliphage and enteric groups.	UK: e.g. Powell et al., 2003.

2.5 Summary Points

1. Microbes have been shown to be present in many relevant geological formations. However, the particular characteristics of the active microbial population will be specific to the particular site / environment within which it occurs.

2. Identified general geological environments in the UK will have an indigenous microbial ecosystem. This ecosystem will be influenced by environmental conditions such as the availability of nutrients and energy for microbial use; groundwater flow; the geological history of the site, including recent useage; and the site-specific geological environment.

3. It is crucial to obtain pristine samples from geological materials so that a microbiological characterisation can be determined with confidence. Additionally, any microbiology characterisation programme must be planned at the same time as other aspects of site characterisation, for example the geochemistry programme. Both microbiology and geochemistry are sensitive to external changes and both should be undertaken early in any site assessment. Furthermore, some drilling muds / fluids contain materials which will increase the nutrient and energy for microbial useage in the geological environment, thus altering the microbiology of the system into the future.

4. Many characterisation techniques exist which can be utilised when assessing the microbiology of a geological environment. Many of these have been developed within the general field of environmental microbiology of which radioactive waste microbiology is a subset. These techniques are not all generically applicable with some environments. For example clays, pose particular dificulties. However, it is crucial to focus on microbial activity levels of relevant microbial groups as these will vary with the geological environment.

5. Microbiology is now included in radioactive waste disposal programmes throughout the world and is included in site investigations. It is recognised that microbiology can influence a wide range of safety-relevant processes and microbiological knowledge can assist in understanding and predicting the performance of a repository in the long-term future.

3 Review of Microbiology of the Near-field

3.1 Microbial Tolerances to Repository Conditions

The varied disposal concepts that have been proposed for the different kinds of higher-activity wastes will contain a wide range of physical and chemical environments (Section 1.3.2). These varied environments will impact to differing degrees on the characteristics of micro-organisms that can survive and upon the activity of these organisms.

The near-field environment of an HLW / SF repository is often perceived as being too extreme for life because it is hot and dry, has few nutrients to sustain life and, in some designs, is highly radioactive.

Conditions in a repository will, however, vary with:

- waste type;
- the varied materials used in repository construction;
- repository geometry (e.g. spacing of waste canisters will influence temperature); and
- the characteristics of the host geological environment.

For HLW / SF, conditions will be very radioactive (hundreds to thousands of Sieverts at the surface of the waste) and hot (in most cases ~50 °C to ~100 °C). Indeed, in the early phases of some concepts for the proposed Yucca Mountain site in Nevada, USA, the temperatures could be up to 300 °C – 400 °C. There will also be considerable pressure generated from the overlying water[5] and rock burden (up to ~10 MPa and ~25 MPa, respectively) and, for some concepts, high salinity with concentrated brines occurring in some environments.

Heat and radiation will be much less in a LLW / ILW repository. However, in most proposed deep disposal concepts for LLW / ILW cementitious materials will be used to encapsulate the waste and / or as a backfill material. These cementitious materials

[5] In all presently proposed concepts worldwide except for the one proposed for Yucca Mountain where the repository will be within the unsaturated zone.

will generate a hyperalkaline environment in the vicinity of the waste (with pH decreasing towards the far-field with increasing distance from the concrete itself).

Such extreme conditions were thought to preclude life and hence most early analyses considered that a repository would be a completely sterile environment. However, many studies have shown that there are many examples of microorganisms tolerating extreme environments. Some of those relevant to a repository are given in Table 3-1. However, it is difficult to find reported work on natural extreme environments where all the conditions that will occir within a repository will be produced (West and McKinley, 2002).

Table 3-1: Tolerance of microbes to extreme environments (from West and McKinley, 2002).

Condition	Example of organism	Limit of growth
High temperature	'Black smoker' bacteria	Reported to 113 °C
Low temperature	*Sporotrichum carnis*	-20 °C
High pH	Nitrifying bacteria	12
Low pH	*Thiobacillus ferrooxidans*	0
High salinity	*Halobacterium halobium*	50% salt by weight
Low salinity	*Salmonella oranienburg*	70ppb dissolved salts
Radiation	*Deinococcus radiodurans*	Single dose 5000 Gy
Chemical toxins e.g. $PbCl_2$	*Aspergillus niger*	67 mg ml^{-1}
High pressure	*Desulfovibrio desulfuricans*	180 MPa

The ability of microbes to simultaneously tolerate high radiation doses and high temperatures is of particular interest to HLW / SF programmes. Studies in Canada suggest that radiation and desiccation effects could create a zone of depleted or reduced microbial activity extending a few tens of cm into a buffer material (Stroes-Gascoyne and West, 1997). Work on indigenous microbes from the Yucca Mountain, Nevada Test Site demonstrate that they are capable of surviving gamma radiation up to 10^4 Gy (at 1.63 Gy min^{-1}) in a viable but non-culturable form and can be resuscitated to a culturable form (Pitonzo et al., 1999). A study by Billi et al. (2000) on ionising radiation (X-ray) resistance in dessication tolerant cyanobacteria showed recovery of viable cells after exposure to 15 kGy but not after exposure to 20 kGy. The bacteria studied were isolated from desert and hypersaline environments and the radiation resistance observed was thought to reflect the ability of these extreme-environment bacteria to survive prolonged dessication through efficient DNA damage repair.

The photosynthetic nature of cyanobacteria means that their impact on radioactive waste disposal will be confined to the operational phase where artificial lighting will be present. Other radiation work with SRB found a joint tolerance up to 80 °C and 310 bars and a separate tolerance of up to 10^3 Gy over 40 hours (West, 1995). Observations of the reactor core at Three Mile Island showed that microbes were present and were receiving doses of 10 Gy hr^{-1} despite the hydrogen peroxide used regularly as a biocide (Booth, 1987). All these figures are compatible with dose estimates of HLW / SF disposal.

The effects of moisture content on microbial presence were investigated further in an *in-situ* experiment with a full scale nuclear fuel waste disposal container. In this experiment a heater (simulating a nuclear fuel waste container) was surrounded by sand and bentonite backfill material (Stroes-Gascoyne et al., 1997). Viable microbes could be isolated from the backfill material only where the moisture content was above 15%. This finding suggests that buffer material will be populated by viable microorganisms only where the moisture content is above a value where free water is available for active life. The effects of temperature on microbe survival and migration were studied for the Yucca Mountain project. Here a block of tuff was heated to a maximum temperature of 142 °C. Two test isolates were found to tolerate the conditions and to migrate through the tuff itself to a distance of 1.5 m from their injection point (Chen et al., 1999). Additional work demonstrated that some isolates from Yucca Mountain were able to withstand repeated exposure to 120 °C, probably through the generation of spores (Horn et al., 1998).

Within ILW repositories, where radiation fields and temperatures will be lower, moisture content, nutrient availability and, where cementitious materials occur, high pH, are likely to be the controlling environmental factors for microbial activity. In the cementitious geological disposal concept developed by Nirex (2003a and b, 2005) alkaline conditions would be established post closure through the use of a cementitious backfill material (Nirex Reference Vault Backfill, NRVB). Under these conditions equilibration with $Ca(OH)_2$ is expected to maintain a pH around pH 12 for tens of thousands of years, until evolved cement phases develop and the system pH falls to pH 11.0 (Evans and Heath, 2004). Estimates of post-closure repository temperatures within a UK ILW repository indicate that 60 °C is a more likely peak value, with temperatures falling to below 35 °C after a few decades (Chambers et al., 2003). These repository temperatures are within the temperature range over which a large variety of micro-organisms may be active.

Tolerance to alkaline conditions has been shown in a study of alkaline groundwaters in Jordan, where a range of microbes tolerated pH 12 and above (Linklater, 1998). Work in France has shown also that fungi, likely to be important in LLW, will grow when in contact with cement and will reduce the water's pH (Perfettini et al., 1991). In experiments where SRB have been grown over a range of pH and Eh, activity at pH 8 to 10 was found to be enhanced by decreasing Eh (Fukunaga, 1995). Studies under simulated UK repository conditions (Gardiner et al., 1997) have demonstrated the survival of some bacteria at elevated temperatures and a wide range of pH values, with survival at temperatures as high as 90 °C being enhanced through attachment to surfaces.

These examples of microbial tolerances to a range of extreme environmental conditions demonstrate that even a HLW / SF repository or an ILW repository backfilled with cement, cannot be assumed to be sterile for its entire lifetime. Given this fact, it is clear that the availability of water, nutrients and energy sources are the only certain controls on the viability of life. Thus an assessment must be made of the likely impacts of microbial activity on the waste itself, on the containment materials and on subsequent radionuclide transport, which are discussed in Section 3.2.

During repository excavation, construction and waste emplacement a range of microbes, potential energy and nutrient sources will be added to the subsurface environment. These changes may convert the environment from a low nutrient system to one that, at least temporarily, could support substantial microbial growth and metabolic activity. Work on disposal of spent fuel in granitic environments, for example, has shown that a significant amount of nutrients may be introduced from explosive residues associated with excavated rock, which is reused in backfill material. Also, organic matter in backfill clays has been shown to increase microbial viability when treated with heat and radiation (Stroes-Gascoyne and West, 1997). For assessing microbial effects on the EBS, measurements on microbial populations in emplaced materials are probably more relevant than studies of the host rock. Alternatively, studies of natural alkaline environments could be relevant where cement and concrete are of interest in the waste disposal concept (Linklater, 1998).

3.2 Biodegradation of Repository Materials

Many different combinations of engineered and geological barriers have been designed to provide safe repository concepts for particular combinations of waste type(s) and geographical / geological setting. Repositories may contain a great diversity of materials, which can be sub-divided in terms of their function as waste matrices, canisters and overpacks, buffers and backfills and assorted structural elements (Section 1.3.2; Baldwin et al., 2008; Hicks et al., 2008). The main materials used or proposed are summarised in Table 3-2. Their performance over very long time scales must be assessed and consequently, it is important to consider the many potential effects of microbial activity on these materials.

The various groups of materials listed in Table 3-2 may have various roles within in a repository, depending upon the concept and the time following closure. For example, in some concepts an iron canister may be required to provide containment for a substantial time following closure, whereas in other concepts the primary purpose of the container is to provide containment during the operational phase. Thus, the impact of microbes on a particular material and hence on repository performance, will depend upon the particular concept and time period of interest. It should be noted that the specific examples discussed below concentrate on the main materials used in a repository.

3.2.1 Stable HLW Waste Matrices

Little, if any, work has been reported on the biodegradation of HLW glass, synthetic rock (SYNROC) or SF. Experimental studies are, however, carried out in conditions which are certainly not sterile and hence empirical degradation rates may contain a contribution from microbially catalysed processes. Direct Microbially-Influenced Corrosion (MIC) of materials such as borosilicate glass, uranium dioxide or SYNROC is not to be expected, but microbial by-products could be of significance. For example, etching of glass by fungal by-products such as organic acids is well known (West et al., 1982) but is related to contamination of glass surfaces by organic material.

3.2.2 Metals

MIC may affect the integrity of metals and alloys, which may be used as encapsulation (container) materials, tunnel liners or may even be included as waste. For metals acting as containers / overpacks, an important factor is their lifetime before physical failure so that localised corrosion may be particularly important. However, for metals which are waste forms (e.g. activated steel or zircalloy) only the total corrosion rate, which is used to derive the rate of release of contained radionuclides, is important.

In addition to corrosion rate, the products of microbially catalysed corrosion can be important if they differ from those resulting from 'inorganic' corrosion. Of particular relevance are the radionuclide sorption and redox-buffering properties of solid phase products. Gas may be also generated by such corrosion and this could exert pressure on containment materials, again potentially affecting integrity.

Table 3-2: Major repository materials.

	Waste Matrix	Canister / Overpack	Backfill / Buffer	Others
HLW / SF	Glass Spent Fuel (UO_2/MOX/U metal) SYNROC	**Iron / Steel** **Copper** **Titanium**	*Clay* *Crushed rock* *Cement*	Liners – *concrete*, **steel** Plugs /seals – *concrete*, *clay* Grouts – *cement*, *clay*
TRU/L/ILW	**Metals** *Cement / concrete* Resins Bitumen Organic waste components e.g. cellulosic materials	**Steel** *Concrete*	*Clay* *Crushed rock* *Cement* *MgO (WIPP)*	Plugs / seals – *concrete*, *clay* bitumen Grouts – *cement*, *clay*
Key: Groupings: Stable waste matrices, **Metals**, *Cement and concrete*, *Clays and crushed rock*, Organics and miscellaneous materials. HLW – High level waste; SF – Spent Fuel; TRU – wastes containing long lived Transuranic radionuclides; ILW – Intermediate level waste; LLW – Low level waste; WIPP – Waste Isolation Pilot Plant; MOX – Mixed oxide fuel; SYNROC – Waste isolation material. 'Clay' is most commonly a bentonite-dominated, bentonite-sand mixture, but other compositions are proposed for different purposes, for example pure bentonite or sand- or crushed rock-dominated mixture. 'Cement' covers the variety of cementitious materials that may be used for different purposes, such as concrete, Ordinary Portland Cement (OPC) and low-pH cement.				

MIC can be either direct, with microbes interacting directly with the metal surface, or indirect where microbes change the local conditions to allow chemical corrosion to take place. MIC of metallic materials is an electrochemical process and the physical presence of microbial cells, in addition to their metabolic activities, modifies these electrochemical processes (Videla and Herrara 2005, Little et al., 2007). Adsorbed cells grow, reproduce and form colonies that develop into unevenly distributed biofilms, occluding some areas and leaving others exposed to the bulk environment. This process results in the formation of differential aeration cells leading to enhanced, uneven corrosion (Little and Wagner 1996; Geesey, 1993). In all cases such effects will be controlled by the amounts of nutrients and energy sources available to the organisms. In laboratory experiments simulating an LLW / ILW disposal regime, SRB enhanced carbon steel corrosion by three times the rate of a control (Philp et al., 1991). Other experiments in realistic conditions have also shown localised and deep pitting of steels, which was directly attributable to microbial action (West et al., 1998). In Canada, Ogundele and Jain (1999) determined the effect of a host of microbial metabolites on the corrosion of copper in saline solution. They found that, within the concentration ranges examined, the corrosion rate of copper was slightly enhanced by microbial metabolites compared to the average abiological rate.

Titanium and / or copper alloys are candidate container materials in several HLW / SF disposal concepts. The use of such expensive material is justified by their corrosion resistance over very long periods of time. Thus the possible role of microbes affecting these materials has been a significant area of study.

Within a HLW repository, the environmental conditions will change with time. Upon emplacement of the waste, the conditions will be warm and oxidising. As the radionuclides contained in the waste decay and the initially trapped oxygen is consumed (by corrosion of the container, by reaction with reduced components in the rocks and backfill, and by microbial activity), conditions will change to be cool and anoxic. Consequently the nature of the chemical corrosion reactions will also change with time. In general, localised corrosion and fast uniform corrosion are only expected to occur in the oxidising phase when sufficient oxidant is present (Stroes-Gascoyne and West, 1996). For HLW such a timescale would correspond to when conditions are most hostile and where lack of moisture might inhibit microbial activity near containers (Stroes-Gascoyne et al., 1997) and, therefore, MIC effects. Repopulation of the area near the containers by microbes later in the life of a repository will depend on whether organisms can move through the pore space in any surrounding compacted buffer material. Experiments to investigate buffer material repopulation with viable *Pseudomonas stutzeri* after a 'sterilisation' period suggested that movement could not take place within a compacted bentonite matrix (Stroes-Gascoyne and West, 1997). Swedish studies (Motomedi et al., 1996; Pusch, 1999; Pedersen, 2002) have also demonstrated the lack of movement (and even survival) in compacted backfills consisting mostly of bentonite. However, Stroes-Gascoyne et al. (1997) showed that movement could occur at interfaces between the backfill and other experimental materials. This finding suggests that fractures or discontinuities, for example cracks in the buffer resulting from initial desiccation, may be a preferred pathway for microbial migration. Resealing will occur as groundwater resaturates the buffer, but resealing may be slower than microbial movement. If a zone of depleted microbial activity is created around the waste containers during the high heat and desiccation period, and repopulation is limited by the pore size of the buffer material, then microbial activity would be limited to regions outside this depleted zone. Only anaerobic corrosion could then occur, probably involving SRB as the repository would no longer be in the oxidising phase. In this case, the only microbial impact on the container materials will result from the diffusion of microbially reduced sulphur species to the container surface. Modelling studies have predicted the extent of sulphate reduction in such a situation and have shown that the consequent effects on copper corrosion are likely to be minimal (King et al., 1999).

MIC-related work at the Yucca Mountain site initially focussed on the isolation of bacteria with corrosion-related capabilities (Horn et al., 2005), for example the ability to generate acids, polysaccharides or sulphides. Horn et al. (1998) isolated from disturbed and undisturbed geological materials associated with the Yucca Mountain site, a number of heterotrophic bacteria that demonstrated these properties. In addition iron-oxidising bacteria capable of generating acidic conditions from the oxidation of reduced iron and sulphur compounds were also isolated (Horn et al., 2005). These bacteria are a potential concern for Yucca Mountain since they can use groundwater nitrates present at the site in the absence of oxygen. Microbial analysis of long-term corrosion tests designed to simulate extreme repository conditions indicated that the tested materials had been colonised by bacteria even though they had not been innoculated (Horn et al., 2005). Denitrification (the reduction of nitrate to nitrogen gas) has received significant attention at Yucca Mountain since groundwater nitrate is expected to counter the corrosive effects of chloride ions (Horn et al., 2005). Consequently, its removal may enhance corrosion rates. Oxidising and reducing experiments on Alloy 22 (a candidate material for HLW containers) (Ahn et al., 2008) have shown enhanced corrosion in the presence of a carbon source. Biofilm formation enhanced corrosion through either organic ligand formation or pH reduction. Corrosion was confirmed by the accumulation of alloy components such as Cr within the biofilm (Horn et al., 2002). Extended corrosion experiments (up to 5 yrs) with Alloy 22 coupons showed pinhole formation in the presence of Yucca Mountain tuff that had been amended with glucose. No corrosion was observed in sterile controls. The distribution and frequency of corrosion pinholes differed depending on incubation temperature (room temperature or 30 °C), as did the microbial populations present at the different temperatures (Martin et al., 2004).

There are no reports on MIC of titanium (Little and Wagner, 1996). Furthermore no literature has been found on direct studies of biodegradation of other possible metallic waste components (e.g. zircalloy, control rod materials) under repository conditions. However, the extensive studies of MIC in reactor systems may contain relevant information. Generalised corrosion of titanium coupons has been observed in the presence of actively growing, thiosulphate-fed *T. ferrooxidans* cultures (Horn et al., 2001). These experiments represented an extreme case potentially relevant to the Yucca Mountain facility (Horn et al., 2005).

3.2.3 Cement and Concrete

Cement and concrete may be present in tunnel liners, plus seals and grouts used in HLW / SF repositories. In the so-called Belgian supercontainer concept for HLW, cement is also proposed as a buffer and a backfill (Baldwin et al. 2008 and references therein). A variant of the Japanese Cavern Retrievable Concept (CARE) for HLW / SF envisages concrete disposal casks (CDCs) and possibly cement backfill (Baldwin et al. 2008)). However, in many cases it is planned to keep cementitious material away from any clay-based backfills, because of the potentially detrimental interaction of cement leachates with the clays. In such roles, the main features of interest are one or more of: the structural integrity of the cementitious material (mechanical strength); its hydraulic properties (sealing groundwater movement); and its chemical buffering capability (in the supercontainer concept).

Cementitious materials are generally proposed much more widely in repositories for other waste types. In these cases the cementitious materials will similarly perform structural roles and / or act as backfills, buffer or containers. Radionuclide sorption, chemical buffering and colloid filtration may all be important. Activated concrete or concrete / cement solidification matrices are often major waste components and here a further property of importance is the leaching rate of radionuclides from the waste matrix.

Microbial degradation of cements and concretes is commonly observed under aerobic conditions (Philp et al., 1991) and could occur in the early phase of a repository. Sulphur oxidising bacteria such as *Thiobacillus* sp. oxidise sulphur, sulphides and thiosulphates, producing sulphuric acid under aerobic conditions. Nitrifying organisms use ammonia and produce nitric acid in the same conditions. These acids can then attack the concrete matrix by dissolving calcium silicate hydrate (CSH) gel and $Ca(OH_2)$. Direct anaerobic corrosion of concretes is not known, although organic acids produced by microbial action on organic materials could be a significant factor (Perfettini et al., 1991). Biofilms can also develop on surfaces (Colasanti et al., 1991; Rogers, 1995) although the organisms may be utilising the organic plasticisers added to concrete to increase their workability (Haveman et al., 1996). The local alkaline conditions produced by the concretes seem unlikely to prevent any potential microbial growth.

3.2.4 Clays

Clay-based backfills / buffers, often involving compacted bentonite or bentonite / sand mixtures, are included in the EBS designs for most HLW / SF concepts and some designs for other waste types, particularly if a barrier to water advection is required.

The very low permeability of such clays also leads to their use in plugs, seals and grouts. As listed previously, the compacted clays have a wider range of favourable properties which are due to their microporous nature and the presence of large areas of reactive surfaces. As a result, work has been undertaken to investigate the occurrence of microbes in clay (particularly in bentonites) from various geological deposits (e.g. Philp et al., 1984; Fukunaga et al., 2005). These studies showed the presence of organisms although reported microbial numbers are lower than in soils and aquatic sediments (Fukunaga et al., 2005). However, detailed microbial studies of several bentonite-based buffer, backfill and sealing materials[6],used in large-scale experiments at AECL's URL, have demonstrated the presence of culturable populations in all bulk materials and, in particular, in interface environments including that of the concrete buffer (Stroes-Gascoyne et al., 2002, 2007b and 2008; Stroes-Gascoyne, 2008). Compaction of the materials upon emplacement reduced the numbers of culturable aerobic organisms but had limited effect on anaerobic populations. Additionally, the viable microbial population was considerably larger than the culturable population suggesting that there was a potential for future activity should conditions become favourable. Indeed, these experiments appeared to show that these buffer materials are not able to reduce microbial activity to the point where it is insignificant with respect to the Canadian deep geological repository concept. Consequently, the Canadian concept now uses compacted 100% bentonite buffer directly in contact with used fuel containers.

The Canadian position mirrors that developed for the Swedish HLW / SF disposal concept, where in-situ (Pedersen et al., 2000a) and laboratory experiments (Pedersen et al., 2000b) demonstrated that the number of viable cells decreased rapidly during bentonite swelling, with only spore-forming bacteria (SRB and *Bacillus sp*) demonstrating significant survival times. Pedersen et al. (2000b) outlined a conceptual model of bentonite bacterial interactions in a HLW / SF repository in which very few if any vegetative cells survived.

There is also an increasing awareness that microbial activity can be involved in mineral alteration. A recent paper by Mulligan et al. (2008) has discussed these effects broadly and suggested that the kinetics of montmorillonite conversion to illite, which could alter hydraulic conductivity, may be enhanced by microbial activity. However, this suggestion remains to be investigated in detail and under relevant repository conditions. Additionally, as microbial activity is low in compacted bentonite (Stroes-Gascoyne et al., 2002, 2007b and 2008; Stroes-Gascoyne, 2008), this enhanced mineral

[6] Buffer/sealing materials, 50-70% bentonite/30% silica sand; backfill materials, 25% clay/75% crushed graded rock or 10% bentonite and 90% sand

conversion would seem unlikely. Furthermore, since the long-term evolution of bulk backfill material at temperatures over 100 °C dominates the near-field (Arcos et al., 2008). It is reasonable to conclude that direct microbial degradation would not be significant for bulk compacted clay materials.

3.2.5 Organics

Organic materials such as cellulose, plastics / rubbers and ion exchange resins are key components of wastes such as LLW (NDA, 2008) and TRU (Cohen, 2006). The potential nature and extent of their microbial degradation may have a significant impact upon repository performance and radionuclide release.

Cellulose Degradation

The degradation of cellulosic materials is a well-defined process. Under anaerobic conditions it involves a wide variety of microbial groups generating a range of gases such as H_2, CO_2, CH_4 and H_2S and soluble organic compounds (SOCs) such as volatile fatty acids (Greenfield et al., 1991; Humphreys et al., 1997a; Askarieh et al., 2000).

However, in repository concepts with a cementitious backfill (e.g. Nirex, 2005) the primary mechanism of cellulose degradation is likely to be chemical rather than microbiological. This alkaline degradation of cellulosic materials has been extensively documented (Greenfield et al., 1991; Greenfield et al., 1993; Van Loon and Glaus, 1997; Askarieh et al., 2000; Pavasars et al., 2003), with the degradation products being a range of soluble organic molecules (Knill and Kenedy, 2003). The dominant products under simulated repository conditions are α- and β- iso-saccharinic acid (ISA) (Greenfield et al., 1995).

Within the PGRC (Nirex, 2005) the high pH of the backfill- modified pore water (Evans and Heath, 2004) will prevent the general establishment of microbial cellulose degradation. However, it has been recognised that the chemical environment will not be uniform and it is likely that niches will exist where micro-organisms may be active (Gardiner et al., 1997; Grant et al., 2000; Askarieh et al., 2000). Anaerobic cellulose-degrading bacteria able to live at high pH have been described (Sonne-hasen, 1997; Grant, 2007) and appear to operate at a pH around pH 10 (Zhilina and Zavarzin, 1994; Zvereva et al., 2004). Consequently it is possible that chemical and microbial cellulose degradation will take place simultaneously, but with chemical degradation being the dominant process. Small-scale LLW degradation studies (Kidby and Billington, 1992) carried out between pH 10 and 13, showed microbial activity at all pH, with the exception of pH 13. Microbial activity was able to reduce the pH of these experiments to below pH 7.0 through the generation of volatile fatty acids.

Near-field microbial activity may also impact on the concentration of alkaline cellulose degradation products (CDP). Compounds generated alongside α- and β- ISA include organic acids such as formic, lactic, acetic and propanoic acid (Hurdus and Pilkington, 2000). Many of these acids are common products of microbial fermentation reactions (Grant et al., 2002) and can be found in a range of anaerobic environments such as landfill sites, anaerobic digesters and sediments. The degradation of these products within anaerobic environments depends on the activity of methanogenic and acetogenic bacteria (Pedersen, 2000; Grant et al., 2002), or bacteria able to oxidise these compounds via the reduction of terminal electron acceptors such as ferric iron or sulphate (Grant et al., 2000; Pedersen, 2000; Grant et al., 2002). The degradation of these small CDPs is likely to be controlled by the ambient pH, with degradation being confined to low-pH niches and biofilms (<pH 10.0). The removal of volatile fatty acids in alkaline environments such as soda lakes has been described (Grant, 2007).

Bacteria able to degrade ISA under aerobic and anoxic (denitrifying) conditions have been found in a range of environments (Strand et al., 1984; Grant et al., 2002; Francis and Dodge, 2006). Grant et al. (2002) isolated ISA degraders from a number of naturally occurring alkaline sites at 25 °C, pH 10.5 and under anoxic conditions (Table 3-3). ISA degraders were also isolated from sites contaminated with industrial effluents containing ISA, for example the Sodra Site in Sweden (Table 3-3). These sites were chosen since they were likely sources of ISA degrading bacteria. The Sodra site demonstrated that ISA-degraders could evolve relatively quickly once ISA becomes available. Sodra soil samples were taken from areas that had been regularly contaminated with Black Liquor over a period of 10 - 15 years. Only those areas directly associated with contamination yielded ISA-degrading organisms. Samples of soil taken outside the contaminated zone did not contain any culturable ISA degraders. These results suggest that the normal soil population had evolved to degrade ISA in areas where ISA represented a significant proportion of the available organic carbon.

Attempts to isolate ISA degraders from uncontaminated soils, compost and anaerobic digester sludge under alkaline conditions were unsuccessful (Grant et al., 2002). Since the microbial populations of these sites are likely to more closely resemble those of the repository at closure, it is therefore likely that the repository will start with a largely non-specialised microbial community where the ability to degrade ISA is either absent or very limited. However, results from the Sodra site suggest that these initial microbial communities may evolve the ability to degrade ISA relatively quickly.

Grant et al. (2002) carried out more detailed studies on ISA-degrading isolates and demonstrated degradation between pH 7.0 and pH 11.5, with a maximum between pH 9.0 and pH 10.0. Further studies using NRVB indicated a maximum pH of 12.5 for ISA degradation. The most pH-tolerant bacteria were present from industrially contaminated sites that are calcium dominated, rather than the sodium dominated soda lakes. These calcium dominated environments more closely resemble repository conditions than natural alkaline environments, which tend to be sodium dominated. These results indicate that ISA degraders able to withstand the pH of a backfilled repository are possible and may evolve within the repository. ISA-degrading biofilms were also investigated by Grant et al. (2002) who were able to establish viable biofilms on both NRVB and concrete surfaces. ISA degradation rates for bacterial suspensions ranged from 0.2 to 5.6 mol ISA yr^{-1} g^{-1}, whereas biofilms removed ISA at between 40 and 350 mol ISA yr^{-1} m^{-2} of surface area. These microbial cultures were also able to reduce ISA concentrations from 10^{-2} M to below 10^{-4} M.

The ISA-degrading isolates described by Grant et al. (2002) coupled ISA degradation to nitrate reduction (denitrification), with the concurrent oxidation of ISA to carbon dioxide. The coupling of ISA oxidation to more reduced terminal electron acceptors such as sulphate remains to be demonstrated, as does the degradation of ISA by methanogenic microbial communities. However the successful treatment of Kraft pulp mill effluents by anaerobic treatment systems (Buzzini et al., 2006) and observations in anaerobic soils (Maset et al., 2006) suggests that ISA degradation under methanogenic conditions is likely.

Table 3-3: Isolation of ISA-degrading bacteria (Grant et al., 2002).

Source	Growth on ISA/Yeast Extract	ISA Removal Demonstrated	Age of Site
Crater Lake consortium	Strong Growth	✓	$>10^3$ Yrs
Elmenteita consortium	Strong Growth	✓	$>10^3$ Yrs
CS3 isolate from Crater Lake	Strong Growth	✓	$>10^3$ Yrs
Wood from Crater Lake	Strong Growth	✓	Unknown
Mono Lake wood	Strong Growth	X	Unknown
Alkaline effluent lagoon. New Zealand paper pulping site.	Some Growth	✓	8 Yrs
Soil from Sodra site (Sweden)	Strong Growth	✓	10-15 yrs
Tank of Black Liquor from Modo	Strong Growth	✓	4 yrs
Alkaline soils	No Growth	X	Wide Range
Pulverised Fuel Ash Soil Mix	Strong Growth	✓	<20 yrs

Gas Generation

Gas generation originating from the microbial degradation of organic waste components has received considerable attention worldwide (Table 3-4). This focus originates from issues related to repository pressurisation and associated impacts on engineered structures and water flow, and the generation and release of radioactive gases (e.g. ^{14}C- and ^{3}H- labelled methane). A range of approaches have been used to investigate gas generation, including inactive simulants, instrumentation of stored radioactive wastes and investigations of existing repositories (Table 3-4). The aims of these investigations include improved conceptual understanding, data generation, model testing and site characterisation (Table 3-4).

Under the aerobic conditions encountered in a repository soon after waste emplacement, carbon dioxide is likely to be the main gas produced by direct microbial action. As the redox conditions change, a range of other gases may be produced depending on the nature of the environment and the wastes involved. Systems with significant amounts of degradable organic materials generally result in the generation of carbon dioxide and methane (Kidby and Billington, 1992; Beadle 2001 and 2002; Molnar et al., 2008; Small et al., 2008). When such gas generation does not occur, the reason is likely to be either acidification or other inhibitory factors (Kidby and Billington, 1992; Agg et al., 2002). Generally the production of hydrogen and the production of methane are mutually exclusive (Kidby and Billington, 1992; Molnar et al., 2001; Agg et al., 2002; Small et al., 2008; Small and Dutton, 2009), which can be attributed to the consumption of hydrogen via hydrogen-oxidising methanogenic bacteria. If these bacteria are absent or inhibited, hydrogen generated via corrosion or fermentation reactions may accumulate. It should be noted that other hydrogen-consuming microbial pathways do exist, where hydrogen oxidation is coupled with the reduction of oxygen, nitrate, ferric iron and sulphate (Schwartz and Friedriich, 2006).

Gas generation experiments on simulated TRU wastes (Caldwell et al., 1988; Francis et al., 1997; Felicione et al., 2003) carried out in the presence of WIPP brines demonstrated significant levels of carbon dioxide, but methane was either absent or produced at much lower levels. The work carried out by Francis et al. (1997) generated low volumes of gas, which may have been due to the acidic conditions (which typically inhibit methane production) that developed in most of these experiments. In general, significant gas generation was only observed in the presence of nitrate and / or bentonite. Felicione et al. (2003) reported on a 6.5 year experiment investigating gas generation from contact-handled transuranic wastes. The majority of these experiments did not produce significant amounts of methane, suggesting that the carbon dioxide measured reflects incomplete cellulose degradation.

In addition to cellulose, TRU, and some ILW contains significant amounts of plastics and rubber. Some authors have suggested that these components are recalcitrant under repository conditions (Grant et al., 2000; BNFL 2002b). This position was supported by Francis et al. (1997) who found no evidence of biodegradation of plastic and rubber that had been irradiated by electron beam irradiated. However, Cohen (2006) concluded that some degradation of plastics and rubbers may be possible over timescales relevant to the WIPP site. This conclusion is founded on the possibility that oxidation and radiation damage may enhance plastic biodegradation or generate soluble intermediates amenable to microbial attack.

Ion exchange resins may also be a significant portion of the non-cellulose organic waste inventory. There is disagreement as to the biodegradability of these materials. Bracke and Muller (2003) reported gas generation from resin wastes in interim storage and Bowerman et al. (1988) reported the aerobic biodegradation of ion exchange media. More degradation was seen when the resins contained organic anions such as citrate or where they were subject to radiation damage. Some growth was also seen in cultures where the resins were equilibrated with inorganic salts. The biological degradation of resins was exploited by Finland's IVO company as a biological treatment process (Tusa et al., 1989; Tusa, 1992) however, few details of the process are available.

Other Organics

Bitumen is employed in some radioactive waste disposal concepts as either an engineered barrier material (Luey and Li, 1993) or as a matrix for the immobilisation of waste (Jacquot et al., 1997; Springael et al., 1997). The microbial degradation of bitumenised waste has been demonstrated (Jacquot et al., 1997; Springael et al., 1997). Further insight into the anaerobic degradation of asphalt has recently emerged from research on natural asphalts from tar pits, which has indicated that these materials are able to support complex microbial communities (Kim and Crowley, 2007).

3.3 Direct Interactions with Radioelements

Microorganisms have the ability to directly interact with radioelements present within radioactive wastes. These direct interactions occur when microorganisms integrate radioelements into their metabolism. The ability of microrganisms to catalyse these reactions is highly dependent on the physical and chemical nature of the radioelements concerned and the prevailing environmental constraints on microbial activity. These interactions may potentially enhance or retard the migration of radioelements out of a repository.

3.3.1 Oxidation / Reduction (Redox) Reactions

Certain microorganisms[7] have the ability to oxidise and / or reduce a range of radioelements, in some cases using them as electron donors or acceptors in microbial energy generation reactions (See Appendix 1). These processes have been observed for uranium, plutonium, technetium and neptunium. However, the vast majority of work on this topic has been focussed on microbial transformations in near-surface sediments and may be more relevant to environmental emissions and biosphere assessments, rather than the deep geological disposal of radioactive wastes. A considerable amount of research has focused on microbial interactions with uranium (Wilkins et al., 2006; Davis et al., 2006; Merroun and Selenska-Pobell, 2008). A wide variety of microorganisms, including iron-reducing bacteria and SRB, have been shown to reduce U(VI) to U(IV) by employing both organic compounds and hydrogen as electron donors (Merroun and Selenska-Pobell, 2008). The prevalance of this process is dependent on the presence of oxidised U(VI) species within a reducing environment where appropriate electron donors are available. If these donors were present then the impact of uranium reduction would be to transform highly moble U(VI) species to low-solubility U(IV) minerals (Davis et al., 2006). Reduced uranium phases may also be subjected to microbial oxidation on exposure to oxidising environments (Merroun and Selenska-Pobell, 2008). However, these microbially driven processes compete with chemical oxidation at the expense of oxygen, ferric iron and nitrite (Merroun and Selenska-Pobell, 2008).

Whereas the microbial reduction of uranium results in the generation of immobile phases (Wilkins et al., 2006; Davis et al., 2006), plutonium reduction potentially results in enhanced mobilisation (Renshaw et al., 2007). The biogeochemistry of plutonium is complex due to the complex redox chemistry and toxicity of this element (Simonoff et al., 2007). Boukhalfa et al. (2007) demonstrated the microbial reduction of plutonium by metal-reducing bacteria. However, these bacteria were unable to sustain growth with Pu(IV) as the sole terminal electron acceptor.

Microbial transformations of technetium have received considerable research attention (Renshaw et al., 2007; Simonoff et al., 2007; Lloyd, 2003). The reduction of highly mobile Tc(VII) species such as pertechnetate ions by a wide range of microorganisms has been demonstrated. These organisms employ organic carbon compounds and hydrogen as electron donors and the final technetium phases are insoluble Tc(IV) oxides (Renshaw et al., 2007; Simonoff et al., 2007; Lloyd, 2003). Indirect microbial

[7] These microorganisms are sometimes referred to as Dissimilatory Metal Reducing Bacteria (DMRB).

technetium reduction is also possible, with biogenic Fe (II) and sulphides catalysing the reduction process (Lloyd, 2003). The microbial reduction of neptunium mirrors that seen with technetium, but significantly less research has been reported than for technetium. The microbial reduction of Np(V) to Np(IV) at the expense of hydrogen and organic electron donors has been demonstrated (Rittmann et al., 2002; Icopini et al., 2007).

The impact of these processes on radioactive waste disposal is dependent on the speciation of the disposed radionuclides and the response of these radionuclides to the prevailing redox conditions. It is likely that radio-elements such as technetium will only be accepted for disposal if they are in their less mobile reduced forms. Any radionuclides present in oxidised forms may also be subject to chemical reduction through the prevailing reducing conditions established in the near-field by corrosion processes.

3.3.2 Carbon-14

The release of ^{14}C via biogas has featured in a range of safety cases and risk assessments (BNFL, 2002a; Hock and Rodwell, 2003; Bracke and Muller, 2007 and 2008). However, in reality it is likely that ^{14}C may not all be metabolised by microbial activity due to the process of isotope fractionation, or to its chemical form within the waste inventory (e.g. if it is located within steel or irradiated graphite).

The LLWR Post-Closure Radiological Safety Assessment (PCRSA) (BNFL, 2002c) commented that:

'As a consequence (of isotope fractionation) lighter isotopes are used preferentially at a faster rate. Consequently, biologically mediated products are enriched in lighter isotopes. DTP/105 has reviewed the effects and significance of isotope fractionation in the Drigg near-field and concludes that the effects of isotope fractionation would in general enrich ^{14}C in solid immobile phases.'

The nature of the ^{14}C inventory will have a significant affect on the fate of ^{14}C in the repository. If the ^{14}C is present as carbonates then it is unlikely that it will be involved in microbial metabolism. This is because bacteria will favour ^{12}C carbonates over ^{14}C carbonates. The impact of methanogenesis on dissolved inorganic carbon is to generally enrich it in heavier isotopes. As Grossman (1997) points out:

'Isotopic effects associated with methanogenesis are amongst the largest in nature and microbial methane represents the most ^{13}C-depleted substance on Earth.'

However if ^{14}C is incorporated into an organic compound, for example as part of the cellulose, then it could become incorporated into biogas since the microbes would be unable to distinguish the ^{14}C from the ^{12}C. This may be what is happening in experiments reported by Molnar et al. (2006), where ^{14}C enrichment of the methane fraction in the headspace of waste drums was detected.

3.3.3 Biomethylation

Biomethylation is the microbial generation of volatile compounds through the transfer of methy groups (Thayer, 2002). From a radioactive waste disposal perspective, biomethylation is important because it may modify the transport of radioactive isotopes of elements such as chlorine, iodine, selenium, polonium, nickel, lead and tin. Generally, metals are primarily methylated by bacteria, whereas fungi and algae dominate the methylation of non metals and metaloids (Thayer, 2002).

The generation of methylated forms of selenium appears to be a detoxification process of a type employed by a wide range of microorganisms to deal with the toxic oxianions selenate and selenite (Stolz et al., 2006). Although the generation of methylated selenium compounds has been attributed to both aerobic and anaerobic environments, generation under anaerobic conditions can be considerably reduced and often undetectable (Hapuarachchi et al., 2004; Haudin et al., 2007; Zannoni et al., 2008) even though bacteria able to generate methylselenium can be cultured from these or similar environments (Chasteen and Bentley, 2003; Meyer et al., 2008; Dungan and Frankenberger, 2000).

Under anaerobic conditions the fate of selenium is determined by competing reduction and methylation reactions. The dominant form of selenium is elemental selenium (Se^0) generated by the microbial reduction of oxidised forms such as selenate and selenite (Stolz and Oremland, 1999; Zannoni et al., 2008; Stolz et al., 2006). Stolz and Ormland (1999) suggest that bacteria capable of selenium reduction are abundant in nature. In anaerobic experiments containing selenite undertaken by Hapuarachchi et al. (2004) <0.1% of added selenium was lost in a volatile organic form. Anaerobic soil experiments reported by Haudin et al. (2007) demonstrated that added selenium was strongly immobilised within the system. In this study the reduction and immobilisation of selenium was enhanced by the addition of a cellulose source (straw), which has some similarities to the cellulose contained in certain radioactive waste. Pure culture experiments with selenite (Dungan and Frankenberger, 2000) demonstrated that the partitioning between methylation and reduction is inversely dependent on the starting concentration, with methylated forms being enhanced at low selenite concentrations (10 μM) and reduction more dominant at higher concentrations (1.0 mM).

Not only is selenium reduction the dominant process in anaerobic environments, it is also likely that selenium methylation and reduction are mutually exclusive processes

(Zannoni et al., 2008). The highly insoluble selenium metal is a stable sink for selenium within anaerobic systems (Knotek-Smith et al., 2006). The stability of reduced selenium appears to be further enhanced by reactions with iron corrosion products (Knotek-Smith et al., 2006). Similar reactions may potentially occur in the environment present in a radioactive waste disposal site. The published data on selenium suggests that although the formation of methylated selenium compounds cannot be ruled out the dominant process under anaerobic conditions is more likely to be selenium reduction. In this case enhanced immobilisation occurs through interactions with anaerobic corrosion products.

Investigations into the methylation of tin have focussed on marine systems (Thayer, 2002) where organic tin compounds have been used in antifouling preparations. Consequently, the applicability of this work to radioactive waste disposal may be limited. There are, however, published data on the generation of tetramethyltin from inorganic tin in anaerobic soils (Meyer et al., 2007) and from anaerobic digesters (Michalke et al., 2000). However, in the latter case the generation of tetramethyltin was not confirmed in pure culture experiments.

Meyer et al. (2007) also demonstrated that methylated lead compounds form in anaerobic soils supplemented with inorganic lead salts. Methylated lead has also been detected in a range of anaerobic environments (Thayer, 2002).

The biomethylation of nickel appears to be confined to methanogenic bacteria (Thayer, 2002) and consequently there is the potential for the volatilisation of nickel in anaerobic repositories.

The volatilisation of polonium has been observed from microbial systems, but the generation of methylated forms has not been confirmed (Thayer, 2002). Several of these investigations have been under aerobic conditions (Momoshima et al., 2001 and 2007), conditions not relevant to the deep disposal of radioactive wastes following repository closure.

The methylation of chlorine, bromine and iodine has been described. The methylation of chlorine and bromine is primarily associated with fungi and algae (Thayer, 2002). In contrast, iodine methylation is associated with bacterial metabolism, as demonstrated by Amachi et al. (2001), who screened a range of bacteria for iodine methylation potential. Of the bacteria screened, strict anaerobes, including methanogens, produced no methylated iodine. This observation prompted the authors to state that "methylation would not occur in strictly anoxic sediments or soil subsurfaces". Subsequent research by Muramatsu et al. (2004) did measure low levels of methyl iodide production from soils incubated under anaerobic conditions, but the rates of generation were much lower than those observed under aerobic conditions. At iodine concentrations below 10 mM, methyl iodide levels were below detection limits. However, at 10 mM iodine concentrations, 0.1 nmol l^{-1} of headspace methyl iodide was generated over a 20 day incubation period, equating to a methyl iodide production rate of approximately 2.5×10^{-5} nM g^{-1} soil/day. The authors suggest that these results indicate that iodine methylation is primarily an aerobic process.

3.4 Summary Points

1. Microbes can tolerate a range of extreme environmental conditions which demonstrates that a repository, even one for HLW / SF and / or one backfilled with cement, cannot be assumed to be sterile for its entire lifetime. Nevertheless, it is very likely that sterile conditions will be found close to the waste, although sterile zones will be distributed variably, both spatially and temporally. The only certain controls on life are the availability of water, nutrients and energy sources.

2. The varying materials that may be used in a repository (as waste matrices, canisters, overpacks, buffers and backfills etc) are all potential nutrient and energy sources for microbial use.

3. Considerable work has been undertaken to understand and quantify microbial influences on many of these materials and this work is overviewed in this report. Much of the work is site- or repository concept- specific, but all studies show the importance of considering microbial impacts in the context of a particular repository concept.

4. MIC has been demonstrated in many respository-relevant studies and these have shown that MIC must be considered in any repository concept where metals may be included. However, the extent of MIC will depend on the repository concept and may be quite limited in impact.

5. Canadian studies of a range of backfill / buffer materials have shown that that some mixes cannot reduce microbial activity sufficiently to a point where it is insignificant with respect to the Canadian deep geological concept. Consequently, the Canadian concept now uses a buffer composed of compacted pure bentonite directly in contact with used spent fuel. Thus, it may be reasonable to conclude that, for other similar disposal concepts, direct microbial degradation would not be expected to be a problem for bulk compacted clay materials.

6. Microbial degradation of organics will be significant in some repository concepts and much work has been undertaken to study biodegradation

products and biogenic gas production. However, once again, the exact impacts of microbes will be dependent on the nature of the waste, the repository environment and physical structure.

Microbes can have direct impacts on the speciation and mobilisation of radioelements through processes such as redox reactions and methylation.

Table 3-4: Biogenic gas generation studies.

	Waste Type	Waste State		Analysis					Objective					
	LLW, LLW+Cement (LLWC), ILW, Resins&Plastics (RP), TRU	Saturated (S) /Unsaturated (US)	Compacted (C)/ Uncompacted (UC)	Gas Composition	Gas Generation Rate	Solution Chemistry	Microbial Analysis	Corrosion	Conceptual Understanding	Data Generation	Model Testing	Site Characterisation	Nationality	Reference
Simulant Studies	LLW	S	UC	✓	✓	✓	✓	✓	✕	✓	✕	✕	UK	Beadle, 2001 and 2002.
	LLW	US	UC	✓	✕	✓	✕	✓	✓	✕	✕	✕	UK	Kidby and Billington, 1992.
	LLW LLWC	US&S	UC	✓	✓	✓	✕	✕	✕	✕	✓	✕	UK	McDonald et al., 1997.
	LLW, LLWC	US&S	UC	✓	✓	✓	✕	✕	✕	✕	✓	✕	UK	Agg et al., 2002.
	TRU	US&S	UC	✓	✓	✕	✕	✕	✓	✓	✕	✕	USA	Caldwell et al., 1988.
	RP, TRU	US&S	UC	✓	✓	✓	✓	✕	✓	✓	✕	✕	USA	Francis et al., 1997.
Radioactive Waste Studies	ILW	US	C&UC	✓	✓	✕	✕	✕	✓	✕	✕	✕	Hungary	Molnar et al., 2006 and 2008.
	LLW	S	C	✓	✓	✓	✓	✓	✓	✕	✓	✕	Finland	Small et al., 2008.
	LLW, RP	US	C&UC	✓	✓	✕	✕	✕	✕	✓	✕	✕	Germany	Kannen and Muller, 1999.
	TRU	S	UC	✓	✓	✕	✕	✕	✓	✕	✓	✕	USA	Felicione et al., 2003.
	TRU	S	UC	✓	✓	✕	✕	✕	✓	✕	✓	✕	USA	Hollis et al., 1998.
	RP	S	UC	✓	✓	✕	✓	✕	✓	✕	✓	✕	USA	Bowerman et al., 1988.
	TRU	S	UC	✓	✕	✕	✓	✕	✓	✕	✓	✕	USA	Leonard et al., 1999.
Site Investigations	LLW	S	UC	✓	✕	✓	✓	✓	✓	✓	✕	✓	UK	Humphreys et al., 1997a, Beadle et al., 2001, Lockhart, 2004.
	LLW, LLWC	US	UC	✓	✕	✕	✕	✕	✓	✕	✕	✓	Hungary	Molnar et al., 2002.

4 Review of Microbial Influences on Repository and EBS Performance

4.1 Effects of Microbial Influences on EBS Components

4.1.1 Influencing Processes

In multi-barrier concepts, the various EBS components provide 'defence in depth', thereby ensuring that no significant release of radionuclides occurs. As discussed in the previous section, the presence of microbes could potentially degrade the functioning of some of these barriers and the challenge is thus to assess if such degradation is significant compared to the considerable safety margins included.

Focusing first on the relatively simple (from a microbiological perspective) HLW case, it was concluded above that microbial activity is probably unlikely to affect the performance of backfill with a high proportion of expandable clay minerals. The corrosion rate and failure time of the canister could potentially be influenced, but for vitrified HLW this is not usually seen to be a factor that critically affects the safety case (e.g. NAGRA, 1994). For spent fuel, however, safety cases often assume very long canister lifetimes. These safety cases need to take full account of microbial effects. The importance of considering these effects has been highlighted by investigations of the Canadian concept.

When the canister fails, corrosion of the waste form will effectively commence. As indicated above, the corrosion rate is not expected to be directly influenced by microbial activity. The corrosion of spent fuel is, however, very dependent on redox conditions and the potential exists for radiolysis of water to lead to locally oxidising conditions which accelerate dissolution. Although the significance of this mechanism is actively debated at present, it is possible that microbial activity could have a positive effect by consuming oxidants and extracting energy from their reaction with kinetically-hindered reductants present in the system (e.g. hydrogen, metallic iron). This microbial impact is dependent on the prevailing environmental conditions (temperature, radiation, water availability) and its significance is yet to be established.

The release of many key radionuclides is constrained not by the corrosion rate of the matrix but by their very low solubility under reducing, neutral-alkaline conditions. Microbial activity could play an important rôle by either enhancing radionuclide solubility via by-products (organic complexants, carbonate, acids), or reducing radionuclide solubility by catalysis of redox reactions (especially for radionuclides which have relatively soluble oxidised forms (e.g. UO_2^{2+}, TcO_4^-)) (Wilkins et al., 2006; Renshaw et al., 2007).

It is known that common groups of microbes often influence the behaviour of such trace nuclides in their catalysis of reactions of major elements (e.g. SRB-producing selleno-sulphides (West et al., 1995). Another example is iron-reducing bacteria, which have been shown to reduce U(VI) to U(IV), hence making uranium less soluble and mobile (Gorby and Lovley, 1992; Wilkins et al., 2006). Microbial reduction of technetium and neptunium may also result in the immobilisation of these radionuclides (Rittmann et al., 2002; Icopini et al., 2007; Renshaw et al., 2007). In the case of plutonium, microbial reduction may under certain circumstances result in enhanced mobilisation (Renshaw et al., 2007; Boukhalfa et al., 2007).

The production of mobile organic by-products, which can complex and therefore mobilise trace elements, is well known for a wide range of relevant organisms. The most extreme examples are organic molecules which are directly utilised for this purpose (e.g. siderophores). These molecules can complex extremely strongly (effectively irreversibly) with a range of relevant elements and notably with actinides. Nevertheless, a very wide range of by-products ranging from simple organics (e.g. formate, acetate, oxalate), through larger biodegradation products (e.g. ISA from breakdown of cellulose) to large macromolecules (fulvic and humic acids) can be important. The influence of these molecules has been reviewed in detail elsewhere (Birch and Bachofen, 1990).

The final key factor constraining the release rates of nuclides from the EBS is retardation during slow nuclide transport through the different EBS components. Microbes could potentially decrease sorption by covering active mineral surfaces, or increase sorption if immobile organisms (i.e., in biofilms) actively take up radionuclides.

The net effect of such sorption depends on the extent of its reversibility and the mobility of the organisms involved. A significant amount of research has been undertaken investigating reversable metal adsorption to microbial cells (e.g. Haas et al., 2001; Ngwenya et al., 2003). However, irreversible uptake is generally of most significance but its net effect is negative only if the organisms are mobile. Biofilms, which are expected to form extensively in oligotrophic rock matrices, play a vital role in controlling both the mobility of organisms and the sorption of radionuclides in and around organisms in the biofilm. Uptake into micro-organisms may, superficially, be difficult to distinguish from sorption onto outer membranes. The difference becomes particularly significant for the cases in which internal mineralisation occurs, which is well known for many microbial groups (Fortin et al., 1997). In the most extreme case, this process can result in the immobilisation of radionuclides in mineral forms, as can occur in the formation of ores of elements such as uranium. This immobilisation has been shown by observations of secondary uranium precipitation at the Needles Eye natural analogue site in Scotland (Milodowski et al., 1990). The same process can be detrimental, however, if the microbes containing a concentrated radionuclide can migrate through the engineered or natural barriers and then release the radionuclide when they die, or if the mineralised form is released as a mobile colloid. Both these processes are most unlikely in a compacted bentonite buffer.

The same basic appraisal can be carried out for the other waste streams, but it is very much more difficult to scope the likely magnitude of possible effects due to greater system heterogeneity. Biodegradation may thus have little direct influence on the performance of cement or zircalloy waste forms, but could be significant for steels and bitumen, and very important for cellulosic materials. Similarly, the effect of microbes on radionuclide solubility or mobility is much harder to assess in the hyperalkaline environment that will occur in those repositories containing large quantities of cementitious materials. Concrete may be a very effective buffer when intact, but fracturing, due either to non-biological processes or microbial by-products (e.g. gas), could greatly reduce performance, allowing advective flow of fluids, transport of colloids etc. to occur.

Fracturing of the backfill is an example of a perturbation that could potentially short-circuit a key barrier. Microbial processes that could cause such a short-circuit (such as biogenic gas production), or which could increase the consequences of such a short-circuit (movement of microbes as 'organic colloids') are of particular concern.

4.1.2 Effect of Open Period

A number of approaches and options have been proposed for closing a repository. Some of these approaches include a period of potentially prolonged institutional control following operations. In some cases staged closure is also proposed, again resulting in a post-operational period when all or part of the repository remains open. Many of the biodegradation processes described in Section 3 would be greatly enhanced during such an open period, where the availability of oxygen for use by microbes is effectively unlimited. In particular, the biodegradation of organic materials could be significant under these circumstances, although grouted waste would be less affected by these processes. However, microbially-mediated oxidation of pyrite, which is present in some rocks, could result in the development of acidic conditions (pH as low as 2.4). These conditions could have an adverse impact on cementitious grouts (Jackson et al., 2002).

There would also be microbial growth on rock surfaces in some rock types, which would accompany the development of a redox front. Additionally, nutrients and energy sources (and microbes) would be introduced from the surface during operational and monitoring procedures, which will also change the microbiological environment. In the long-term, the generation of biomass in the open period could also impact on the long-term performance of the repository by generating organic material (biomass). West et al. (2006) have made some simple quantitative calculations concerning the microbiological effects on the Japanese Cavern Extended Storage (CES) repository concept. These calculations have shown that generation of biomass is a key feature of concepts with an extended open period. However, the exact significance would be dependent on the specific repository concept and geological environment.

4.2 Effects of Microbes in the EBS and Damaged Zone

Microbial influences on radionuclide migration through a perturbed EBS need to be assessed. The radionuclide transport properties of any medium depend on both the processes that move radionuclides in a mobile phase (usually gas or water) and those that transfer radionuclides from this mobile phase to a solid phase. Radionuclide movement depends principally upon advection, diffusion and dispersion, whereas radionuclide partitioning between the mobile phase and the solid phase depends mainly upon sorption and precipitation (noting that immobilisation of a gaseous species first requires dissolution in an aqueous phase before precipitation of a solid product can occur).

The physical processes of advection, diffusion and dispersion can be influenced by microbes only if the microbes can increase fluid-filled pore space due to dissolution of solid phases, or reduce the pore space due to clogging by the formation of biofilms. If very intense microbial activity occurred in an EBS in which porosity is very small and/or pore throats are very narrow, an influence might well be expected. However, significant effects for EBS systems with large pores or for the wider geosphere would require either very high concentrations of biomass (or biofilms) or large amounts of secondary alteration products.

Laboratory experiments have, rather unexpectedly, observed blocking of flow cells within 2 days due to intense microbial growth / alteration of crushed rock (Hama et al., 2001). The extent to which this is significant is presently being studied in controlled flow systems (e.g. Tuck et al., 2006; Coombs et al., 2008). More details of this work are given in the following section. However, Lucht et al. (1997) investigated the occurrence of pore clogging in backfill (a mixture of 25% bentonite and 75% crushed granite rock) by bacterial activity, but found no evidence for it over a 180 day period.

Flow could also be slowed in cases where advection occurs in fractures, but where there is also diffusion into the non-flowing porosity of the surrounding rock. Biofilms on fracture surfaces could certainly limit access to the matrix and thus greatly reduce the retardation of many key radionuclides (Anderson et al., 2007). To complicate the situation, radionuclides could also be directly immobilised by the biofilms themselves, either by adsorption onto cell surfaces, intracellular uptake, or via micorbially mediated precipitation.

Sorption is a bulk term for a range of processes causing uptake of solutes onto surfaces and is generally assessed in laboratory uptake experiments. In cases where microbiology is considered at all, it is often assumed that the experimental systems already include microbial effects as no attempts are made to limit microbial growth. In some cases, an agreement between the predicted retardation rate based on laboratory sorption measurements and that observed in in-situ tracer tests argues that this assumption is justifiable. Experiments suggesting that microbial activity plays a role (West et al., 1991a; Johnsson et al., 2006) do, however, suggest that care must be taken when attempting to develop mechanistic sorption models based entirely on physico-chemical mechanisms.

The direct effects of microbes on radionuclide migration can be positive if radionuclides sorb onto attached organisms or onto biofilms. Alternatively the effects can be negative if radionuclides sorb onto mobile or motile organisms. Biofilms could potentially form on any wet surface in the near- and far-fields and have been observed forming on surfaces in deep subsurface environments (Brown and Hamon, 1994). Some biofilms have included *Gallionella* spp, which produces iron oxyhydroxide. This solid can sorb radionuclides during the oxidation of ferrous iron present in groundwater. Although such biofilms are not necessarily representative of those expected in the host rock or in the EBS, these studies demonstrate the potential redox regimes possible in biofilms and the potential role that they may play in radionuclide retardation.

Extensive biofilms are unlikely to form in a compacted buffer, because of the small pores present, but they are more likely in the backfill and host rock. Laboratory experiments investigating ^{75}Se, ^{113}Sn and ^{137}Cs migration through artificial granite fractures containing biofilms indicated that the presence of biofilms had no effect on retardation (Vandergraaf et al., 1997). However, some studies of similar low-nutrient environments also suggest that some microbes become starved. As a result the microbes become small and mobile and capable of penetrating deeply into geological formations (Lappin-Scott and Costerton, 1990). This effect also has implications for radionuclide retardation. Experiments where microbes are in solution suggest that they have varying effects in conventional radionuclide sorption experiments. For example, in experiments with ^{137}Cs, microbes appear to compete for sites on rock materials with the radionuclide (West et al., 1991b).

Finally, the influences of perturbations in the geological barrier that are caused by a repository need to be assessed. To date, one hydrological and three main chemical perturbations of this type have been identified. Due to excavation practices in hard rock, a so-called excavation damage zone (EDZ) may extend some distance into the rock (typically up to about one meter, but dependent upon rock type, stress regime and excavation method). This zone may have higher permeability than the bulk rock potentially resulting in enhanced microbial transport.

The main chemical perturbations are:

- the oxidising redox front resulting from diffusion of air and / or water into porous rock or along fractures during the operational phase;
- the oxidising redox front resulting from radiolysis due to HLW (especially important for directly-disposed spent fuel); and
- the high-pH plume resulting from hyperalkaline leachates from certain kinds of repository.

The extent to which these reaction fronts can be locations of enhanced microbial activity and the consequences of such activity have been reviewed (McKinley et al., 1997). It was concluded that microbial processes probably play a critical role in the development and movement of redox fronts as commonly observed in nature (e.g. roll-front ore deposits). This conclusion has been supported by microbial observations carried out within the Poços de Caldas natural analogue study in Brazil (West et al., 1992), which showed the critical role of microbes in influencing movement of chemical species across this roll-front deposit.

Consequently, as prediction of the future development of redox is critical to determine the efficiency of many geological disposal concepts, consideration of microbial influences is now included in many redox studies. For example, there is controversy over the interpretation of oxidised regions around fractures in otherwise reduced deep rocks, in environments where the conventional explanation of flow of 'oxidising' groundwaters is difficult to justify using hydrogeological principles. One school of thought favours perturbation scenarios that could drive oxidising water to depth (e.g. Akagawa et al., 2006; Lin et al., 2003). Alternatively, such features could represent microbial catalysis of the oxidation of reduced minerals coupled to complex oxyanion reduction. This process is illustrated by recent work of Yoshida et al. (2008a and b), who examined past redox front formation in sedimentary rocks. The observations suggest that microbes play a key role in the concentration of Fe-oxyhydroxides across the redox front. Further work by West et al. (2008) and McKinley et al. (2009) has used data obtained from across ancient palaeo-redox front in fractured crystalline rock (Akagawa et al., 2006) as evidence of microbial involvement in deep redox reactions.

Addtionally, the role microbes can play in the oxidation and reduction of Fe(II) and Fe(III) respectively, could be of particular relevance to repository concepts where C-steel is included (King and Stroes-Gascoyne, 2000).

A microbial role in the development of the high-pH front was shown to be theoretically possible, but was identified as an area in which relevant experimental data and field observations were lacking.

4.3 Summary Points

1. The effects of microbial activity in the near-field are, again, complex and depend on the repository concept under consideration. For example, for HLW / SF concepts where long canister lifetimes are required, there must be careful consideration of the effects of MIC. However, for concepts for vitrified HLW where shorter-lived canisters are proposed, canister corrosion may be of lesser importance in the safety case. Evaluations of other waste streams can be made, but these will be more complicated due to greater system heterogeneity.

2. Microbial activity will influence radionuclide release rates and migration either directly (by uptake onto mobile organisms or gas production) or indirectly (e.g. by altering redox conditions or producing organic by-products including gases). The significance of these impacts is again dependent on the repository concept itself. For example biogenic gas production is unlikely to be significant for HLW / SF where the organic content will be very low.

3. The effects of microbes in the perturbed zone is currently an area of very active research. Experimental work examining the effects of biofilms on radionuclide migration and overall transport properties are being undertaken in several programmes and show that microbial activity can have a positive effect by reducing fluid flow. Studies of natural systems are also examining the role of microbes in chemical perturbations of the geological barrier. In particular the influence of microbes on redox processes is receiving attention, as these processes may impact upon the efficiency of many geological disposal concepts.

4. Many of these microbial effects will be enhanced in a repository concept which has an ‘open period’ during which effectively unlimited air (oxygen) is circulated into the system. Consequently, the effects of an open period on microbial populations and future repository performance would require careful assessment.

5. It is not possible to ascertain which microbial effect or effects will predominate in the near-field, the EBS and damaged zone as this will depend on the selected site, type of waste and the repository concept. The microbial effects will need to be assessed using site-specific data so the results can be taken into account in the context of PA. If the evaluation indicates that all microbial processes would have a significantly negative overall effect on performance then methods to limit these effects will need to be employed. These methods include (but are not limited to) measures to control or limit microbial activity, such as engineering solutions.

5 Review of Microbial Effects in the Far-field

The function of the repository is to contain radionuclides and thus any process that may cause radionuclide transport needs to be evaluated. In deep geological systems groundwater advection, where dissolved or colloidal species are transported by the bulk motion of groundwater, is usually the main process of concern. Diffusion-controlled transport will be orders of magnitude slower. Therefore this mechanism will not permit significant transport of radionuclides from a repository unless the medium through which diffusion occurs is of only very limited thickness. The relative importance of advective and diffusive transport will depend on the rock types that comprise the geosphere around the repository. As discussed above, some engineered barriers (e.g. clays) have such low hydraulic conductivities that advective transport is minimal and diffusion will dominate solute transport.

5.1 Radionuclide Migration in Undisturbed Systems

5.1.1 Influence of Biofilms on Transport Processes

It is now generally recognised that microbes living in deep geological environments can impact upon solute transport processes (Chapelle, 2000; Cunningham et al., 1997; Fredrickson et al., 1989; Keith-Roach and Livens, 2002; West and Chilton, 1997, Section 4). Microbial activity in any environment is generally located on chemical or physical interfaces, usually within biofilms. The impacts can be both physical (e.g. altering porosity) and / or chemical (e.g. changing pH, redox conditions). These impacts may result in intracellular or extracellular mineral formation or degradation (Coombs et al., 2009; Beveridge et al., 1997; Ehrlich, 1999; Konhauser et al., 1998; Milodowski et al., 1990; Tuck et al., 2006). Biofilms can also immobilise radionuclides via cell surface metal adsorption (Haas et al., 2001) All these processes potentially could influence transport of radionuclides in the far-field of a repository. To date, most work of direct relevance to repository performance has focussed mostly on granitic environments in URLs.

Considerable work on the effects of biofilms on overall transport processes has been undertaken at the Äspö HRL in Sweden, which has been used to evaluate aspects of the geological disposal of radioactive waste in hard rock (granodiorite) environments. An in-situ study at the HRL examined the redox buffering of groundwater in vertical fracture zones penetrated by recently recharged, meteoric water. Such processes might occur during repository construction, and could alter the geochemical environment of the repository. The results showed that indigenous bacteria were capable of maintaining reducing conditions in the deep groundwaters (Banwart, 1995).

As a result, a further laboratory experimental study was undertaken to simulate the interactions of microbes with mineralogical surfaces associated with groundwater flow systems at Äspö (Hama et al., 2001). Indigenous iron-reducing bacteria and SRB were introduced in Äspö groundwater flowing through either columns or continuously stirred tank reactors (CSTR) packed with crushed Äspö granodiorite rock. The columns containing the organisms became blocked within a few days and no flow was possible. Petrographic analyses of the column residues that had been inoculated with the bacteria indicated that the permeability reduction in the columns was associated with: (1) mobilisation of 'fines' from grain surfaces and their accumulation in intergranular pore throats; (2) development of filamentous organic biofilaments; and (3) formation of secondary mixed-layer chlorite-smectite. The observed change in permeability in the biotic experiments was surprising because of the low concentration of available nutrients and because any mineralogical changes involved (and hence changes in porosity) were volumetrically small. The same formation of secondary clay was also observed in the 3 month long CSTR experiments, although smectite, rather than the mixed-layer chlorite-smectite, was detected. Once again, the amount of smectite formed was greater in the biotic CSTR experiments demonstrating that the bacteria were having a significant influence on clay mineral formation in the experiments.

Further experiments by Tuck et al. (2006) used packed columns and stirred batch reactors to simulate microbial-geochemical interactions in deep subsurface low-nutrient granitic environments. The columns, containing crushed Äspö granodiorite and a single or mixed culture of microbes, became impermeable to synthetic Äspö groundwater after 5 days. Analysis revealed copious filamentous biofilm and suggested that the indigenous bacteria were capable of surviving in relatively low-nutrient conditions. The results are consistent with the hypothesis that bacteria can either concentrate relevant chemical species for mineral formation in localised microenvironments, or may accelerate clay formation. This study implies that the localised hydrological regime of a granitic environment can be changed, particularly if new nutrient sources are introduced (e.g. via links to surface water; or via links to the repository components). Biogenic mineral precipitates and trapped mineral matter are much more chemically and physically stable than the biofilm, and can persist in the pore system long after the biofilm has decayed or been removed (Brydie et al., 2005).

These results illustrate the complex interactions between microbes, geochemistry and mineralogy at this site, which potentially may influence the overall transport properties. More details of these studies are described elsewhere (Hama et al., 2001; Tuck et al., 2006). Work is now progressing on developing quantitative flow systems to evaluate microbial effects under repository conditions in different host rock environments (Coombs et al., 2008).

Other studies at the Äspö HRL examined radionuclide sorption processes. The adsorption capacity of granitic rock was compared to the adsorption capacities of biofilms grown in situ on glass and rock surfaces (Anderson et al., 2006b). After immersing the surfaces for 42 days in anaerobic synthetic groundwater containing a number of radioactive tracers, the adsorption and distribution of the radionuclides was investigated using 2D autoradiography. Results showed that the rock adsorbed more ^{60}Co, ^{99}Mo, ^{241}Am, ^{237}Np and ^{234}Th per unit area when compared to the biofilm grown on the glass slides, whilst the biofilm adsorbed more ^{147}Pm than the rock. Biofilms can form a barrier between the rock and groundwater and may slow down radionuclide diffusion to the rock. These results suggested that differences in adsorption were dependant upon the chemical properties of the individual radionuclides and the availability of different surface functional groups modified by the presence or absence of biofilms.

Studies using iron oxides and groundwater samples collected from the Äspö site (Ferris et al. 1999) and other similar sites (Ferris et al., 2000) investigated the sorption of dissolved metals onto bacteriogenic iron oxides in deep groundwater from hard rocks. This work suggested that bacteriogenic iron oxides are an important sink for dissolved metals. However, measured metal distribution coefficients (Kd values) decreased with increasing iron oxide content, apparently due to the influence of bacterial organic matter in the solids. This finding has implications for the transport and fate of dissolved metals in groundwater systems where there is contact between iron oxides and bacterial organic matter.

5.1.2 Other Relevant Studies

Experiments to better understand the physicochemical processes occurring at the biofilm-mineral interface were designed by Vaughan et al. (2001). These experiments used miniature flow cells to grow single-species biofilms within a simulated rock fracture environment. The structure of the resulting biofilms and the bacterial distribution suggested that biofilms might have the effect of producing localised flow gradients and may have an important impact on hydraulic properties when modelling a flowing system.

Other experiments using porous media have also shown that, where conditions are suitable, there is potential for biofilaments to establish (in a matter of days), causing pore blockages resulting in a decrease in permeability and a change in the local fluid flow patterns. Extensive formation of biofilm reduces pore space, will then lead to eventual blocking of the pore system (Taylor and Jaffé, 1990a and b; Taylor et al., 1990) and is referred to as 'bioclogging' (Brydie et al., 2005). Brydie et al. (2005) observed a 70% reduction in the permeability of sand due to bioclogging. Even greater permeability reductions (three orders of magnitude) were observed in earlier studies by Taylor and Jaffé (1990a). Similar work has also been described in the context of oil exploration, where well productivity has been damaged by microbial biofilm formation.

In summary, the effects of microbial activity (usually as biofilms) on transport of radionuclides in the EDZ and far-field appear to be potentially significant, with flow characteristics possibly changed in both fractured and porous media. Additionally, biofilms appear to influence radionuclide sorption onto rock surfaces although this affect appears to be dependent on the geochemistry (particularly redox conditions) and mineralogy of the host rock. Microbial activity may also influence radionuclide transport via cell surface adsorption, intracellular uptake, mineral precipitation and the formation of bacteriogenic iron oxides Considerable efforts are now focussed on understanding these microbiological processes, some of which are included within the new European Community ReCosy (Redox phenomena controlling systems) consortium project (http://www.recosy.eu/index.php - insertion date 12 January 2009) which started in 2008.

5.2 Microbial Influences on the Fate of Soluble Organic Compounds (SOCs)

In the case of repositories receiving organic-containing wastes such as certain ILW (NDA, 2008) and TRU (US DoE, 1995), it is possible that SOCs may leave the near-field in a plume of water that has been chemically conditions by processes in the repository. Thereafter, the SOCs may impact upon the far-field. These organic materials may be either fermentation products such as volatile fatty acids, alkaline CDP or soluble organic material present in the waste or repository structure. The fate in the geosphere of these SOCs, including ISA and associated CDPs, is determined by a range of inter-related processes such as microbial and chemical degradation, precipitation and sorption. Processes that influence the transport of SOCs may be important since many SOCs such as ISA are able to complex radionuclides and therefore enhance their transport. The nature and extent of these processes will depend on the relative geochemical characteristics of the geosphere and the plume. As the plume moves out from the repository, dilution and dispersion will have a significant impact on the concentration of these SOCs in the geosphere. These processes will also reduce the chemical impact of the plume, with the plume pH and Eh being modified until they eventually approach those of porewater in the host rock.

In the geosphere, microbial degradation processes will be determined by the prevailing geochemistry and mineralogy since they will be dependent on the availability of terminal electron acceptors. ISA degradation under geosphere conditions has not received significant study. Instead research has focussed on degradation under aerobic (Strand et al., 1984) and near-field conditions (Grant et al., 2002; Francis and Dodge, 2006), and has demonstrated ISA degradation in both cases. Although not demonstrated, the degradation of ISA under more reduced environmental conditions seems likely (Grant et al., 2002). In the deep subsurface surrounding a repository, oxygen and nitrate are unlikely to be present in significant quantities, unless waste-derived nitrate is a component of the near-field plume. Manganese (IV), ferric iron and sulphate are microbial terminal electron acceptors that are more common in the geosphere are; in addition, fermentation processes and methanogenesis are also possible. ISA oxidation coupled to manganese, iron and sulphate reduction will result in the complete oxidation of ISA to carbon dioxide, with the generation of associated reduced manganese, iron and sulphur species. In the absence of these terminal electron acceptors, anaerobic degradation of ISA with the associated generation of methane may occur. Data from environments contaminated with ISA suggest that microbial communities are able to adapt to ISA degradation relatively quickly in comparison to the lifetime of a repository (Grant et al., 2002). Under neutral conditions microcosm studies (Maset et al., 2006) suggest that soil microbial communities can degrade ISA without the need for an adaption period. The most non-ISA CDPs are common microbial substrates such as volatile fatty acids, and these, along with fermentation end products that may enter the geosphere, are likely to be degradable under geosphere conditions.

5.3 Microbial Transformation of Gases

Microbial- and corrosion- related gas generation may result in a mixture of gases migrating into the far-field of a geological disposal site. A range of gases may be produced, with the most likely being carbon dioxide, methane and hydrogen. The most likely to perturb the far-field microbiology are methane and hydrogen since these are potential energy sources for microbial metabolism. However, carbon dioxide should not be overlooked since it is a potential carrier of ^{14}C, although in many disposal systems it is likely to be immobilised by precipitation processes.

A wide variety of microorganisms are able to facilitate the oxidation of hydrogen (Schwartz and Friedrich, 2006) using a range of terminal electron acceptors including nitrate (Smith et al., 1994; Vasiliadou et al., 2006; Harris et al., 2007) ferric iron (Lovely, 1991; Harris et al., 2007), sulphate (Matias et al., 2005; Moser et al., 2005; Harris et al., 2007), and carbon dioxide (Kotelnikova and Pedersen, 1998; Moser et al., 2005; Harris et al., 2007). The reaction schemes for these processes are outlined below.

Hydrogen-consuming microbial communities have been described or inferred in a range of geological environments (Stevens, 1997) including Swedish granite aquifers (Pedersen 2000; Hallbeck and Pedersen, 2008a and b), Belgian Boom Clay (Ortiz et al., 2002), the Columbia River Basalt Group (Stevens and McKinley, 1995), the Lidy Hot Springs (Chapelle et al., 2002) and the South African Witwatersrand basin (Ward et al., 2004). First order rate constants for hydrogen-consuming denitrifying (0.05 h^{-1}), iron-reducing (0.18 h^{-1}) and sulphate-reducing (1.2 h^{-1}) communities were published by Harris et al. (2007) for uncontaminated aquifers (where there is denitrification) and contaminated aquifers (where there is iron and sulphate reduction).

When carbon dioxide is used as a terminal electron acceptor for hydrogen oxidation, there are two potentially competing reactions, namely acetogenesis and methanogenesis (Pedersen, 2000; Hallbeck and Pedersen, 2008a). Hydrogen-driven acetogenic microbial ecosystems have been suggested by Pedersen (2000) for the Swedish deep granitic host rocks that are proposed for deep radioactive waste disposal. In this environment, rates of methanogenic hydrogen consumption ranged up to 5.6 $\mu M\ h^{-1}$ where as acetogenic hydrogen consumption was much higher, ranging up to 0.13 $mM\ h^{-1}$ (Kotelnikova and Pedersen, 1998). Not all hydrogen-driven subsurface environments are dominated by acetogens. The Lidy Hot Springs site described by Chapelle et al. (2002) is dominated by a methanogenic hydrogen-consuming community. In addition, hydrogen metabolism in the Boom Clay environment is coupled to sulphate and thiosulphate reducers as well as methanogens (Ortiz et al., 2002). The reaction schemes for these processes are outlined below:

$5H_2 + 2NO_3^- + 2H^+ \rightarrow N_2 + 6H_2O$ Hydrogen oxidation via nitrate reduction;

$H_2 + 2Fe(III) \rightarrow 2Fe(II) + 2H^+$ Hydrogen oxidation via iron reduction;

$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O$ Hydrogen oxidation via sulphate reduction;

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ Methanogenesis;

$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$ Acetogenesis.

Given that hydrogen-oxidising microbes are common in a wide range of subsurface environments, it is likely that hydrogen oxidation will be a feature of the far-field of any future UK geological disposal site. The hydrogen-degrading processes dominant within a system will depend on the geology and associated geochemistry. Based on the kinds of geological environments that might potentially be used for deep disposal in the UK (Watson et al., 2007a), the most likely pathways for hydrogen metabolism are iron reduction, sulphate reduction, methanogenesis or acetogenesis. It is not possible to provide rates for these processes as these will be site specific and dependent on the specific nutrient and energy supply but, based on the first order rate constants from Harris et al. (2007), 0.05 h^{-1} seems a conservative estimate. Ferric iron may be present as iron minerals and sulphate and carbon dioxide / carbonate being present as dissolved groundwater species.

Methane may also be subject to microbial oxidation provided the appropriate terminal electron acceptors are available within the environment. Methylotrophy, the ability to utilise single carbon compounds as carbon and energy sources in aerobic environments is quite widespread in nature (Lidstrom, 2006). However, it is only likely to be important in geological disposal contexts once gas has migrated close to the surface. However, methane oxidation may also be coupled to the oxidation of more reduced terminal electron acceptors such as nitrate and sulphate (Valentine and Reeburgh, 2000; Thauer and Shima, 2006). Although energetically possible methane oxidation coupled to iron reduction has yet to be confirmed. One important implication of anaerobic methane oxidation is its impact on ^{14}C migration and ultimate release from a disposal site.

Studies of gas production from laboratory systems containing backfill and groundwater from granite have shown that the backfill may have a suppressing effect on methane production, which may be attributable to competing sulphate reduction processes (Stroes-Gascoyne and West, 1997). This hypothesis appears to be supported by a study by Stroes-Gascoyne et al. (2002). Here, changes in gas composition in compacted 50% bentonite buffer, which had been sealed in a borehole for 6.5 years at *in situ* temperatures (13 °C - 17 °C), were examined and compared to the gas composition at the time of buffer emplacement. There was no significant difference between the gas compositions despite the presence of a large population of viable microbes. This finding suggests that the population was inactive and that the organics associated with the bentonite were not easily degradable. The results suggest that the presence of oxygen suppressed methanogenesis since methanogens are strict anaerobes. Some SRB activity was indicated, not by the presence of hydrogen sulphide, but by a small increase in sulphide in the buffer.

5.4 Summary Points

1. Microbes can impact on solute transport processes thus influencing radionuclide migration in the far-field.

2. Microbial transformation of organic complexing agents has the potential to reduce radionuclide migration in the far-field.

3. Biofilms can alter transport properties. To date, most of the work directly relevant to repository performance has been undertaken in granitic environments using URLs. These studies have shown the complex interactions between microbes, geochemistry and mineralogy. This is an active research area but demonstration of impacts of biofilms in other geological environments has not yet been achieved.

4. Hydrogen and methane generated within radiaoctive waste disposal sites may be subject to further microbial transformation as they travel through the geosphere to the biosphere.

6 Integrating Microbiology in Performance Assessments

There are several different definitions of the term 'Performance Assessment' (PA), but IAEA (2003) defines it to mean:

'An assessment of the performance of a system or subsystem and its implications for protection and safety at a planned or an authorized facility. This differs from safety assessment in that it can be applied to parts of a facility, and does not necessarily require assessment of radiological impacts.'

Thus, PA has a broader meaning than 'safety assessment' in which the performance measure is radiological impact or some other global measure of impact on safety.

Conversely, it should be noted that PA usually focuses on the development of numerical models and expresses performance measures in numerical terms. Consequently, the term is rather less broad than 'safety case development', a 'safety case' being defined by IAEA (2003) as:

'An integrated collection of arguments and evidence to demonstrate the safety of a facility. This will normally include a safety assessment, but could also typically include information (including supporting evidence and reasoning) on the robustness and reliability of the safety assessment and the assumptions made therein.'

The following text focuses on the integration of microbiology into PA, but comments on the implications for safety assessment and safety case development.

PA's contribute to the rigorous and comprehensive appraisal of any site. They provide a focus for the underlying science and its implications for confidence in long-term containment and isolation, and are an important contributor to the long-term 'safety case'. Until the early 1980s, microbial processes were not even mentioned in PA of potential deep disposal facilities (e.g. SKB, 1983). Even in the mid 1980s when the potential for microbial perturbations was recognised, microbes (together with organics and colloids) were usually considered as 'problem areas' or 'open questions' (e.g. Nagra, 1985). More explicit acknowledgement is, however, made in later assessments (e.g. JNC, 2000) with assessments where high-organic wastes are important making microbial processes an integral part of the PA calculations (BNFL, 2002a).

The IAEA ISAM programme (IAEA, 2004) outlined a generic safety assessment methodology (Figure 6-1), which provides a useful framework against which to discuss safety assessment, which can be considered a kind of PA.

6.1 The Assessment Context

The assessment context provides the framework within which a safety assessment is carried out, and covers the purpose, regulatory framework, assessment end-points and philosophy, the disposal system characteristics, and assessment timeframes (IAEA, 2004). These key aspects of the context are outlined within the UK's current *Guidance on Requirements for Authorisation* (GRA) for deep geological disposal systems (Environment Agency et al., 2009). A future safety assessment that is carried out as a contribution to safety case development by the NDA RWMD must be demonstrated to conform to the principles of the GRA. The highest level of this guidance details several GRA 'Requirements' and the relationship between these GRA requirements and microbiology are outlined in Table 6-1. The progress seen internationally in radioactive waste microbiology in recent years, means that microbiology can be considered in relation to the GRA requirements. That is, the subject has certainly moved into the realm of quantifying uncertainties, in that the impacts of microbiology on a disposal system can be identified, evaluated and hence used to develop qualitative arguments to support a safety assessment and hence safety case development.

6.2 Systems Description

The ISAM methodology (IAEA, 2004) breaks a disposal system down into internal and external components. The internal components are those that occur within the spatial and temporal boundaries of the disposal system, while the external components occur outside these boundaries. While the distinction between the internal and external components depends upon the context, the internal components typically comprise the near-field, far-field (geosphere beyond the near-field) and biosphere. Descriptions of these components are in turn informed by the characterisation of the site (geosphere and biosphere), the waste and waste form and the EBS.

As the study of radioactive waste microbiology has developed internationally the nature and focus of the work has matured in line with the specific characteristics of the disposal system under consideration. Broadly this work can be split into two areas; firstly developing a comprehensive understanding of the disposal system; and secondly developing understanding of those processes related to the safety functions of disposal system components. Safety function-related work has generally focussed on microbial impacts on the evolution of the waste, waste form, EBS and associated geological materials. Work related to understanding the disposal system has focussed on characterising the nature and extent of the microbial communities present in the geosphere, associated with system components (e.g. bentonite) and in the wastes. This work extends to an understanding of the activity of the ambient microbial communities and associated geochemistry and the response of these communites to perturbations associated with the presence of the disposal system.

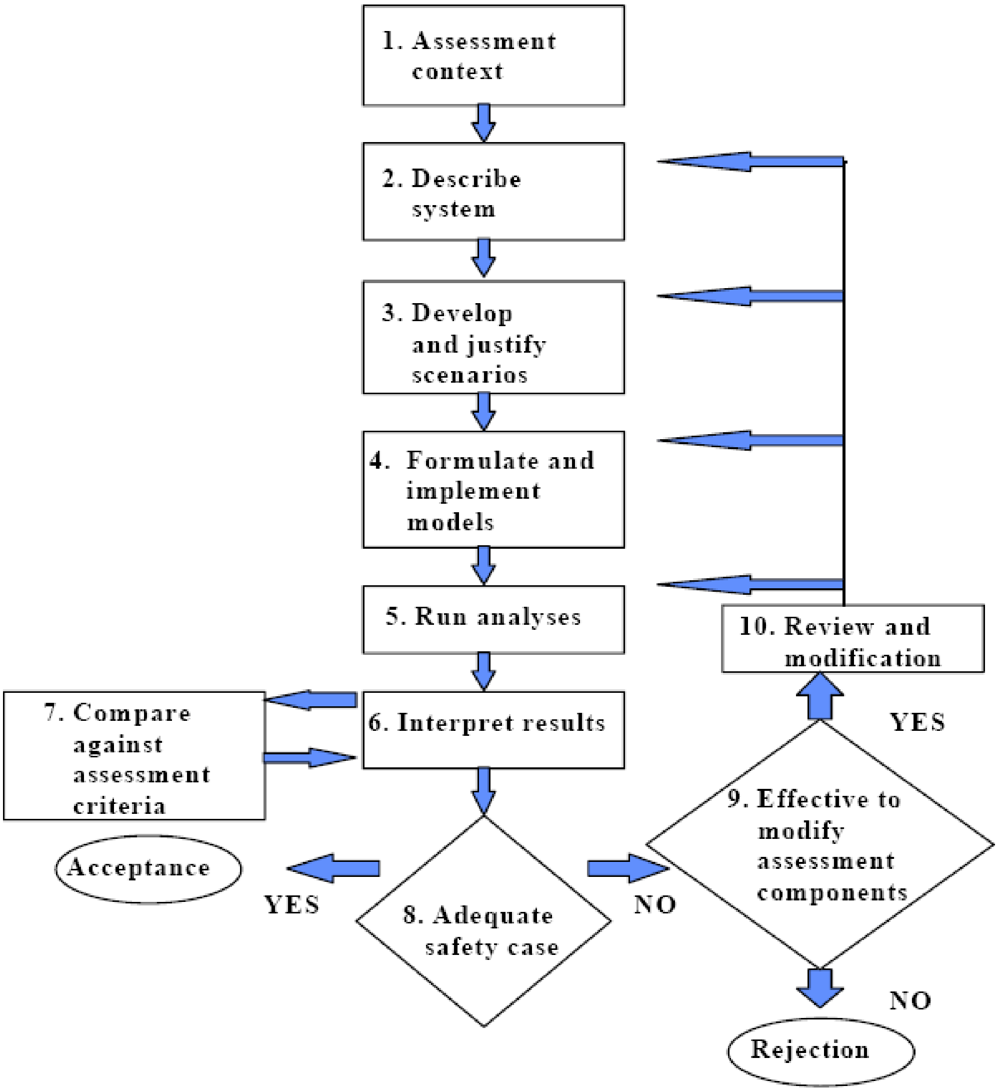

Figure 6-1: IAEA ISAM methodology (after IAEA, 2004).

Internationally, a microbial component to geological site characterisation is well established (e.g. Jain et al., 1997; Vreeland et al., 1998; Farkas et al., 2000; Pedersen, 2000, Horn et al., 2004; Stroes-Gascoyne et al., 2007) and it should be noted that the depth and extent of this work has been significantly enhanced by the use of URLs (IAEA, 2001). The value of integrating microbiology into these site investigations goes beyond developing a simple description of the system components; it allows an insight into one aspect of the nature of the geochemical environment and how that environment will evolve in response to perturbations originating from the installation of the disposal system.

Table 6-1: Alignment of microbiology with aspects of the GRA.

Requirement	Details	Status of Microbiological Investigation
R4	*…application of sound science and good engineering practice…*	The microbiology of corrosion, organics degradation, biogeochemistry and subsurface survival is a mature science. Consequently the integration of microbiology within a PA constitutes the application of sound science.
R6	*..the guidance requires the consideration of quantifiable and unquantifiable uncertainties within a safety case.*	The current status of microbiology as a science and the tools and techniques available for the analysis of microbial activity means that the uncertainties associated with microbiology are quantifiable. That is the impacts of microbiology on a disposal system can be identified, evaluated and integrated into a safety case.
R11	Requires *that the geological environment is characterised, understood and can be analysed to the extent necessary to support the environmental safety case.*	The intimate relationship between microbiology and the ambient geochemistry and the role played by microbiology in mediating perturbations to the geological environment means that a geological environment cannot be fully characterised or understood without a microbiological component to investigations.
	, the environmental safety case should demonstrate a clear understanding of the disposal facility in its geological environment ('the disposal system') as it evolves	Microbial activity may have a significant impact on the evolution of the disposal system through processes such as MIC, waste degradation, barrier degradation and gas generation. A full understanding of the disposal system is not complete without a proper consideration of these processes.
	an explanation of, and substantiation for, the environmental safety functions provided by each part of the system	The safety functions of a number of system components e.g. EBS, waste containers, sorption in the far-field may be influenced and compromised by microbial activity. The substantiation of these functions requires an accurate assessment of microbial activity.

An example of a highly characterised system is that of the Äspö site in Sweden (Pedersen, 2000; Hallbeck and Pedersen, 2008a and b) where the characterisation of the microbiology of the system has allowed the identification of the processes controlling the ambient redox chemistry and carbon speciation (Pedersen, 2000; Samper et al., 2003; Hallbeck and Pedersen, 2008a). Some of the microbial processes of this system (i.e. homoacetogens) are unlikely to have been identified from literature studies, emphasising the need for site-specific research. This understanding has been conceptualised and integrated into models that then allow the evolution of the site to be investigated (Samper et al., 2003). This theoretical and modelling platform has then been used to investigate the impact of geological disposal facility construction on the re-oxidation of the host geology (Yang et al., 2007). The role played by microbiology in catalysing the far-field geochemical response to near-field perturbations is a theme picked up in Boom Clay investigations; where the re-oxidation of sulphides and the impact of waste related nitrates are thought to be microbially driven (Van Geet et al., 2006; Aerts et al., 2008).

Microbiological studies on the safety functions of disposal system components have focussed on a number of key areas with barrier performance being a common theme. Microbiology research in this area has looked at the impact of microbiology on radionuclide sorption to minerals (Anderson et al., 2006b and 2007), canister corrosion (Ogundele and Jain, 1999; King et al., 1999; Horn et al., 2005), and bentonite performance (Pedersen 2000a and b; Stroes-Gascoyne, 2008; Mulligan et al., 2008). The Canadian decision to move to 100% bentonite backfill in response to microbiological studies on bentonite performance can be considered to be an example of an iterative approach to repository design indicative of optimisation (Requirement R8, Environment Agency et al., 2009).

From a microbiology perspective the introduction of a disposal system into a geological environment generally supplies additional sources of electron donors (e.g. polymeric and soluble organic carbon, molecular hydrogen), whereas the geological environment provides the electron acceptors (e.g. groundwater species such as nitrate, sulphate or carbonate and iron and manganese minerals). The exact nature and extent of these impacts on disposal system performance is determined by the waste it accepts; the disposal system design and containment materials; and the geological environment and how the system evolves with time. Complex wastes with significant organic components such as ILW (NDA, 2008) and TRU (US DoE, 1995) contribute significant amounts of nutrients and energy to the disposal system. Microbiology work on these types of waste generally include the consideration of biogenic gas generation (e.g. methane) (Francis et al., 1997; Askarieh et al., 2000) and the impact of SOCs generated by waste degradation, particularly if cementitious backfills are employed (Greenfield et al., 1994; Askarieh et al., 2000). Modelling studies for organic-rich wastes are often kinetically based (Small et al., 2000, and 2008; Bracke and Muller, 2003; Kidby and Rosevear, 1997). Terminal electron acceptors for microbial metabolism are generally contributed by either the waste or the groundwater, consequently microbial activity is focussed for these wastes on the near-field and engineered disturbed zones of the system.

Those disposal systems housing spent fuel or HLW that employ bentonite buffers are organic-poor systems with low microbially-available water contents. In these systems near-field sources of electron donors are confined to corrosion hydrogen and any organic material present in the bentonite. The harsh physical environment of these systems means that microbial activity is likely to be excluded from the near-field for considerable lengths of time (Stroes-Gascoyne, 2008; Pedersen et al., 2000a and b). In other, bentonite free spent fuel / HLW disposal concepts (e.g. concepts with a halite host rock) the environmental conditions may be equally harsh. Microbial impacts are more likely in the engineered disturbed zone and the geosphere with the intrinsic geosphere microbiology being an important consideration. This intrinsic microbiology may be driven by external sources of energy (e.g. Pedersen, 2000) and site construction may have a significant impact on the oxidation state of the system (Van Geet et al., 2006; West et al., 2006; Yang et al., 2007; Aerts et al., 2008). These systems are generally modelled in a manner that takes into account the overall levels of energy and nutrients available (Baker et al., 1998; Jolley et al., 2003; West et al., 2006) for microbial growth. This contrast between these two broad types of disposal system is highlighted by the comparison made by Wang and Francis (2005) of the near-field chemistry of the WIPP and Yucca Mountain repositories. They concluded that microbiology would have a significant impact in the WIPP system due to the significant organic waste component, whereas it was unlikely to make a significant impact at Yucca Mountain due to the limited nutrient supplies (which reflect in part the low organic component of the waste) and harsh environmental conditions.

Consideration must also be made of the effects of microbes in concepts where there is a period of extended waste storage, whether at the surface or at depth. This would ensure a prolonged exposure of the waste to both aerobic conditions and to microbial contamination. As a result, rates of biodegradation of materials, gas production etc will increase, with consequences that are only starting to be assessed (e.g. West et al., 2006).

6.3 Scenario Development

The ISAM process (IAEA, 2004) defined scenarios as:

'…descriptions of alternative, but internally consistent, future evolutions and conditions of the waste disposal system. They handle future uncertainty directly by describing alternative futures and allow for a mixture of quantitative analysis and qualitative judgements. Essentially, the main purpose of scenario generation in the post-closure safety assessment of a radioactive waste disposal system is therefore to use scientifically-informed expert judgement to guide the development of descriptions of the disposal system and its future behaviour.'

A central element in the development of scenarios is often the compilation of a list of Features, Events and Processes (FEPs) that could directly or indirectly impact the disposal system and associated radionuclide migration (IAEA, 2004).

The ISAM programme (IAEA, 2004) defined FEPs as:

- Features – prominent or distinctive parts or characteristics (of the repository or its environment);
- Events – a qualitative or quantitative change or complex of changes located in a restricted portion of time and space;
- Process – a phenomena marked by gradual changes that lead towards a particular result.

The NEA (2000) provided a more descriptive description of a FEP as:

'..a feature, event, process or other factor, that may be necessary to consider in a repository safety assessment. This includes physical features, events and processes that could directly or indirectly influence the release and transport of radionuclides from the repository or subsequent radiation exposures to humans, plus other factors, e.g. regulatory requirements or modelling issues, that constrain or focus the analysis.'

As far as possible these FEPs are identified from the disposal system description, but are also often compiled from the system description and generic information available in the scientific literature. There are also published generic FEP lists (e.g. NEA, 2000; IAEA 2004) against which project FEP lists can be checked. A summary of the microbiology-related FEPs taken from the NEA database can be found in Table 6-2, the NEA database employs a layering system to organise these FEPs, with microbiology-related FEPs mainly residing within layers 2 and 3 (Figure 6-2). The FEPs abstracted from the NEA database are those where microbiology is explicitly mentioned along with those where microbiology is likley to be involved, but is not explicitly mentioned.

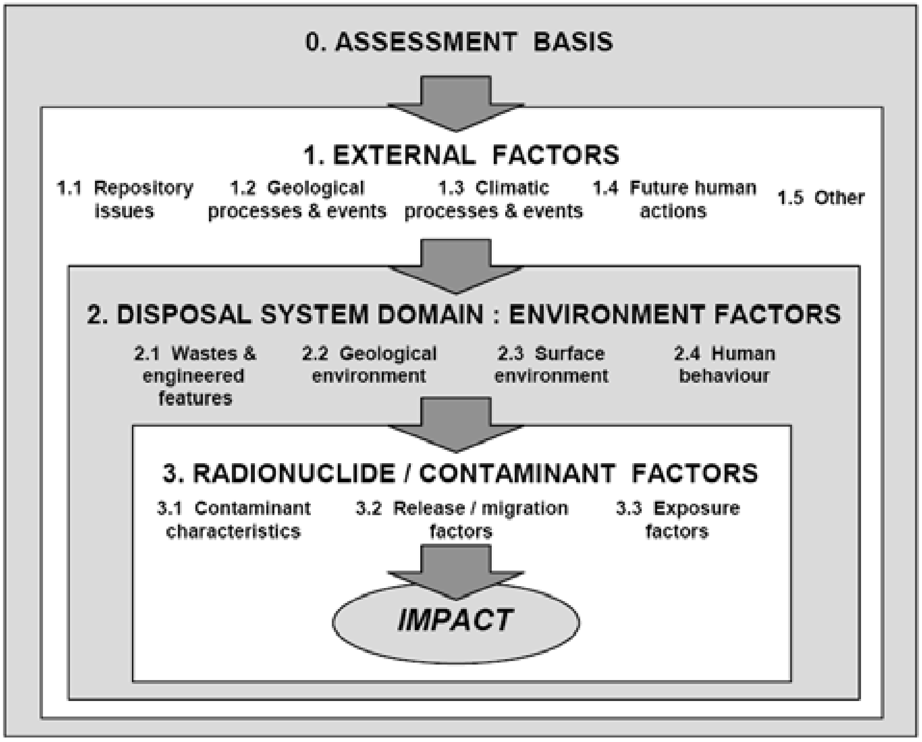

Figure 6-2: The NEA FEPs layering system (after NEA, 2000).

Table 6-2 Microbiology- related FEPs listed in the International FEP Data Base (NEA, 2000).

FEP & Number	Details	Microbes Explicit?	Comments
Species evolution 1.5.02	FEPs related to the biological evolution of humans, other animal or plant species, by both natural selection and selective breeding/culturing.	✓	
Waste form materials and characteristics 2.1.02	FEPs related to the physical, chemical, biological characteristics of the waste form at the time of disposal and also as they may evolve in the repository, including FEPs which are relevant specifically as waste degradation processes.	✓	
Container materials and characteristics 2.1.03	FEPs related to the physical, chemical, biological characteristics of the container at the time of disposal and also as they may evolve in the repository, including FEPs which are relevant specifically as container degradation/failure processes.	✓	
Buffer/backfill materials and characteristics 2.1.04	FEPs related to the physical, chemical, biological characteristics of the buffer and/or backfill at the time of disposal and also as they may evolve in the repository, including FEPs which are relevant specifically as buffer/backfill degradation processes.	✓	
Seals, cavern/tunnel/shaft 2.1.05	FEPs related to the design, physical, chemical, hydraulic etc. characteristics of the cavern/tunnel/shaft seals at the time of sealing and also as they may evolve in the repository, including FEPs which are relevant specifically as cavern/tunnel/shaft seal degradation processes.	**X**	Microbial activity may also influence the chemical and physical characteristics of these features as it can with other EBS/Waste form components.
Other engineered features materials and characteristics 2.1.06	FEPs related to the physical, chemical, biological characteristics of the engineered features (other than containers, buffer/backfill, and seals) at the time of disposal and also as they may evolve in the repository, including FEPs which are relevant specifically as degradation processes acting on the engineered features.	✓	
Chemical/geochemical processes and conditions (in wastes and EBS) 2.1.09	FEPs related to the chemical/geochemical processes that affect the wastes, containers, seals and other engineered features, and the overall chemical/geochemical evolution of near-field with time. This includes the effects of chemical/geochemical influences on wastes, containers and repository components by the surrounding geology.	**X**	It is not possible to separate geochemical impact from microbiological impact since the two are intimately linked.
Biological/biochemical processes and conditions (in wastes and EBS) 2.1.10	FEPs related to the biological/biochemical processes that affect the wastes, containers, seals and other engineered features, and the overall biological/biochemical evolution of near-field with time. This includes the effects of biological/biochemical influences on wastes, containers and repository components by the surrounding geology.	✓	
Gas sources and effects (in wastes and EBS) 2.1.12	FEPs within and around the wastes, containers and engineered features resulting in the generation of gases and their subsequent effects on the repository system.	**X**	Gas generation may be a microbial process.

FEP & Number	Details	Microbes Explicit?	Comments
Contaminant transport path characteristics (in geosphere) 2.2.05	FEPs related to the properties and characteristics of smaller discontinuities and features within the host rock and other geological units that are expected to be the main paths for contaminant transport through the geosphere, as they may evolve both before and after repository closure.	X	Microbial growth may influence the hydraulic and chemical characteristics of these paths.
Chemical/geochemical processes and conditions (in geosphere) 2.2.08	FEPs related to the chemical and geochemical processes that affect the host rock and other rock units, and the overall evolution of conditions with time. This includes the effects of changes in condition, e.g. Eh, pH, due to the excavation, construction and long-term presence of the repository.	X	It is not possible to separate geochemical impact from microbiological impact since the two are intimately linked.
Biological/biochemical processes and conditions (in geosphere) 2.2.09	FEPs related to the biological and biochemical processes that affect the host rock and other rock units, and the overall evolution of conditions with time. This includes the effects of changes in condition, e.g. microbe populations, due to the excavation, construction and long-term presence of the repository.	✓	
Inorganic solids/solutes 3.1.03	FEPs related to the characteristics of inorganic solids/solutes that may be considered.	X	Microbial activity may directly or indirectly influence solubility/precipitation of inorganic compounds.
Volatiles and potential for volatility 3.1.04	FEPs related to the characteristics of radiotoxic and chemotoxic species that are volatile or have the potential for volatility in repository or environmental conditions.	X	Microbial activity may directly or indirectly influence the volatility radiotoxic and chemotoxic species.
Organics and potential for organic forms 3.1.05	FEPs related to the characteristics of radiotoxic and chemotoxic species that are organic or have the potential to form organics in repository or environmental conditions.	X	Microbial activity may directly or indirectly influence the characteristics of organic radiotoxic and chemotoxic species.
Dissolution, precipitation and crystallisation, contaminant 3.2.01	FEPs related to the dissolution, precipitation and crystallisation of radiotoxic and chemotoxic species under repository or environmental conditions.	X	Microbial activity may directly or indirectly influence the dissolution, precipitation and crystallisation of radiotoxic and chemotoxic species.
Speciation and solubility, contaminant 3.2.02	FEPs related to the chemical speciation and solubility of radiotoxic and chemotoxic species in repository or environmental conditions.	X	Microbial activity may directly or indirectly influence the speciation and solubility of radiotoxic and chemotoxic species.
Sorption/desorption processes, contaminant 3.2.03	FEPs related to sorption/desorption of radiotoxic and chemotoxic species in repository or environmental conditions.	X	Microbial activity may directly or indirectly influence the sorption/desorption of radiotoxic and chemotoxic species.
Colloids, contaminant interactions and transport with 3.2.04	FEPs related to the transport of colloids and interaction of radiotoxic and chemotoxic species with colloids in repository or environmental conditions.	X	Microbial cells may contribute to the colloidal transport of radiotoxic and chemotoxic species.
Chemical/complexing agents, effects on contaminant speciation/transport 3.2.05	FEPs related to the modification of speciation or transport of radiotoxic and chemotoxic species in repository or environmental conditions due to association with chemical and complexing agents.	X	Microbial activity may directly or indirectly influence speciation or transport of radiotoxic and chemotoxic species due to association with chemical and complexing agents.
Microbial/biological/plant-mediated processes, contaminant 3.2.06	FEPs related to the modification of speciation or phase change due to microbial/biological/plant activity.	✓	
Gas-mediated transport of contaminants 3.2.09	FEPs related to transport of radiotoxic and chemotoxic species in gas or vapour phase or as fine particulate or aerosol in gas or vapour.	X	Microbial activity may directly or indirectly influence the transport of radiotoxic and chemotoxic species in gas or vapour phase or as fine particulate or aerosol in gas or vapour.

FEP & Number	Details	Microbes Explicit?	Comments
Animal, plant and microbe mediated transport of contaminants 3.2.11	FEPs related to transport of radiotoxic and chemotoxic species as a result of animal, plant and microbial activity.	✓	

In order to construct a comprehensive FEP list a number of methodologies have been developed (IAEA, 2004), one of which is the use of interaction matrices. In interaction matrices the features of the systems are included as leading diagonal elements and processes involving individual features are noted in the associated off diagonal elements. One example of the use of interaction matrices, which included a focus on microbial activity, was the FEP analysis that supported the 2002 safety assessement of the UK LLWR (BNFL, 2002a; Beadle et al., 2001). Here, in order to incorporate microbial processes, microbiota was included as a feature within the system and as such was included as a leading diagonal element (Figure 6-3). A generic interaction matrix focussed on microbiology-related FEPs can be found in Figure 6-4.

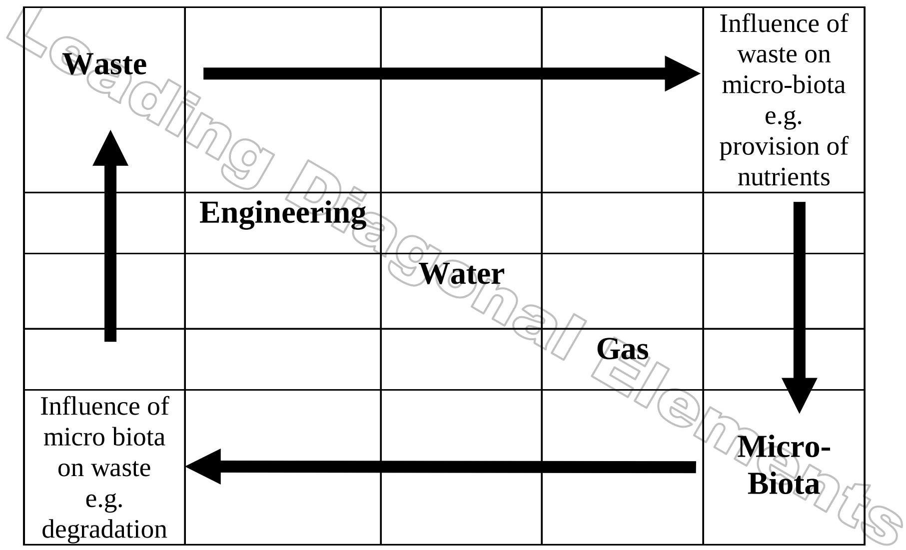

Figure 6-3: Microbiology-related interaction matrices (Beadle et al., 2001).

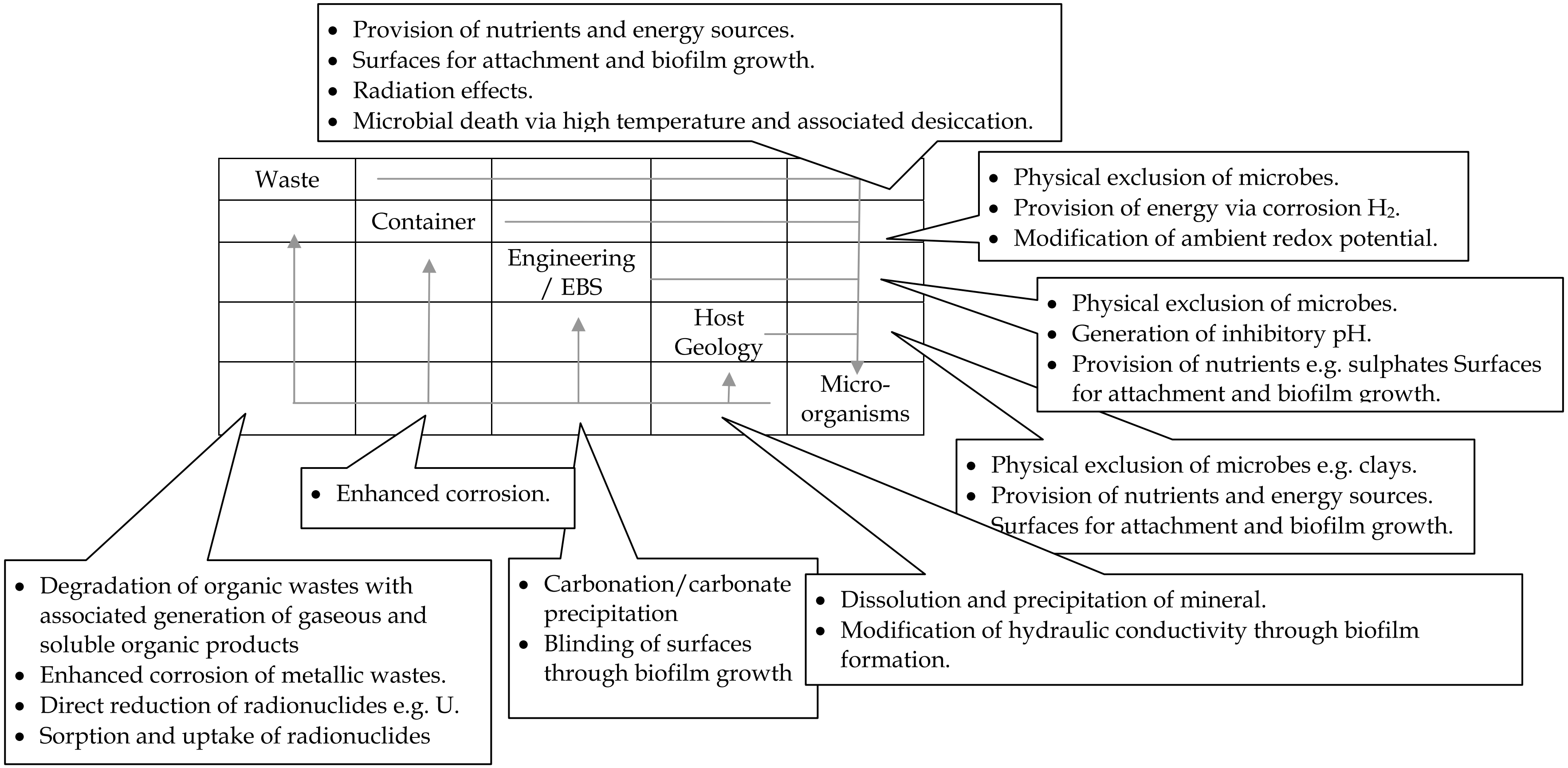

Figure 6-4: Microbiology-related interactions.

6.4 Models for Evaluating Microbial Effects on Repository Performance

This review has shown that the potential effects of microbiological activity on the containment of radioactive waste are complex. However, it is important that these effects are quantified in order to provide information to demonstrate the safety of disposal facilities over the period for which the radionuclides remain harmful; up to hundreds of thousands or millions of years. In view of these potential impacts, several national radioactive waste disposal programmes have developed numerical approaches for examining the effects of microbiological processes. The outputs from these numerical approaches contribute to understanding disposal system performance.

Although these modelling studies and associated codes are diverse and depend on the waste, disposal system characteristics / geological environment and exposure pathway of interest, their development does follow a generic pattern (Figure 6-5). These modelling approaches will be discussed, broadly, in the context of two waste content settings: environments containing high-organic content wastes; and environments containing low-organic content waste.

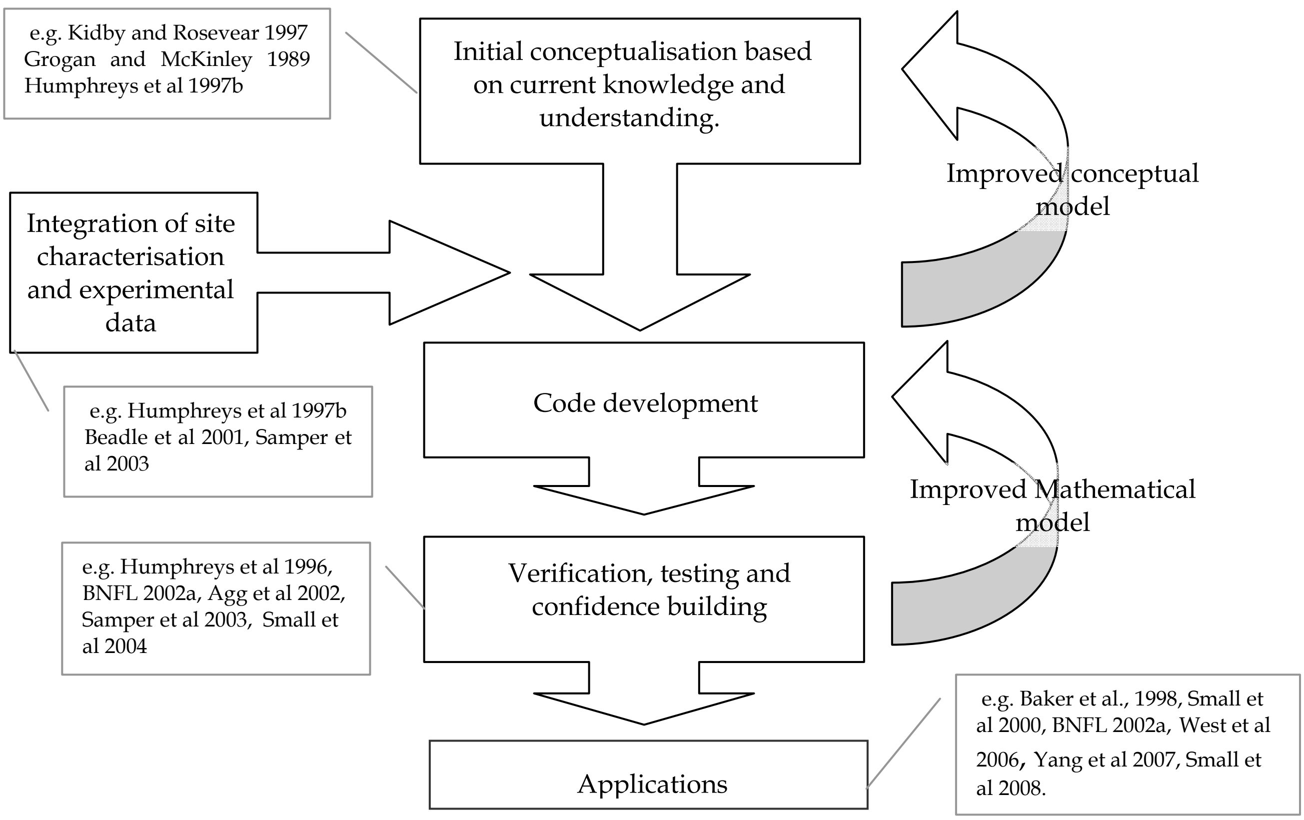

Figure 6-5: Development of microbiological models.

6.4.1 Environments Containing High-Organic Content Waste

Some of the first radioactive waste disposal models to incorporate microbial processes were those specifically investigating the generation and release of gas from wastes with significant organic components. Models developed for gas generation assessment have been reviewed by Rodwell et al. (1999 and 2002) who point out two distinct approaches; firstly the use of empirical models and secondly a more mechanistic approach. In both cases models often include both corrosion and microbiological processes, which may or may not interact. Examples of empirically based models include DEGAS (BNFL, 2000a) and the recently developed SMOGG (Rodwell, 2004; Small and Dutton, 2009). More mechanistic models include the GRM / DRINK code (Humphreys et al., 1997a; BNFL, 2000; Small et al., 2008) and GAMMON (Kidby and Rosevear, 1997).

The adoption of more mechanistic based models allows the integration of a range of processes and a more complete representation of the system under investigation. For example, the GAMMON model (which in RWMD's assessments has been superceded by SMOGG) incorporates a representation of alkaline cellulose degradation alongside microbial processes and corrosion. This mechanistic approach also allows a more complete integration of microbiology and geochemistry allowing an evolving redox potential and environmental pH to be modelled. These outputs may in turn provide an insight into radionuclide transport. The approach has been used in a broad context in BIORXNTRN (Hunter et al., 1998) and has also led to the development of codes able to simulate the biogeochemical evolution of both the near-field (Small et al., 2000 and 2004; Yang et al., 2007) and the far-field (BIO-CORE, Zhang, 2001; Samper et al., 2003) of radioactive waste disposal sites. A summary of these biogeochemical modelling codes is given in Table 6-3.

However, this mechanistic approach does have negative aspects such as significantly greater data input requirements, greater computational requirements, longer run times and more complex verification and testing. These issues limit the application of these models in PA's and prompted the production of the SMOGG code (Rodwell, 2004 and 2005) as a more empirical alternative to the GAMMON code. The capabilities of GAMMON and SMOGG are related in a similar way to those of GRM / DRINK and DEGAS as applied to the 2002 PCSC of the UK LLWR (BNFL, 2002a).

Table 6-3: Codes used to model radioactive wastes with high organic carbon contents.

NAME	BIO-CORE	GRM/DRINK	GAMMON	BIORXNTRN
Reference	Zhang, 2001; Samper et al., 2003	Humphreys et al. 1997b ; BNFL, 2002a.	Kidby and Rosevear, 1997.	Hunter et al., 1998.
Developer	University of Coruna	BNFL/UK National Nuclear Laboratory.	AEA-T/NIREX.	Sandia National Laboratory
Microbiological context	Geochemical code with additional microbiology featured (some optional components)	Biogeochemical reaction-transport code. Microbial gas generation via cellulose degradation and fermentation processes. Corrosion processes modelled.	Microbial gas generation via cellulose degradation and fermentation processes. Alkaline cellulose degradation and corrosion modelled	Biogeochemical reaction-transport code.
Brief description	Coupled 2-D	Modular structure with sequential 2 step approach to coupling of transport with other processes. Calculates an evolving redox potential and uses a modified PHREEQE.	1D	1-D transport model coupled to kinetic reaction network for biogeochemistry of groundwater systems
Biomass predictions	Yes	Partially	Partially	No
Geochemical links	Yes	Yes	Limited	Yes
Redox processes	As function of microbiological processes	Yes	As function of microbiological processes	Yes
Effects on radionuclide migration	No	Yes	Only tritiated and 14-C labelled gases.	Yes
Gas production	No	Yes	Yes	No (but probably possible)
Required data	Site data from in-situ investigations	Site data from in-situ investigations. Literature information	Site data from in-situ investigations. Literature information	Site data from in-situ investigations. Site data from in-situ investigations
TDB* used	EQ3/6	PHREEQE database (Chandratillake et al., 1998).	No	Own calculations
Availability	Through SKB and University of Coruna	Through the UK National Nuclear Laboratory.	Through Serco Assurance/NDA	Through Sandia (with amended code from JAEA)
Example of use	SKB Äspö Facility – Redox zone Experiment	Drigg 2002 Post Closure Safety Case, GGE Experiment, VLJ Repository Finland.	Gas generation from UK ILW disposal scenarios.	Aquifer evolution, radionuclide transport

* Thermodynamic Database

6.4.2 Environments Containing Low-Organic Content Waste

In these environments, two general types of model have been used to assess microbial influences, particularly on the geochemistry of the system. BIO-CORE (Zhang, 2001; Samper et al., 2003) and MINT (Tochigi et al., 2008) are examples of geochemical / biogeochemical codes which are linked to biodegradation processes. MGSE / BGSE and MING are examples of models which assess maximum growth rates in environments, based on the availability of nutrients and energy, calculated from solid and liquid phase analyses. Essentially, mass balance and thermodynamics are used for the calculations. This second approach is particularly useful in deep environments containing wastes with a low organic content as it can demonstrate sensitivities to the availability of a particular nutrient or energy source. It is also useful for making rapid scoping calculations for a particular environment or disposal concept. As a result, this second approach has been developed and adopted by several national radioactive waste disposal programmes (Stroes-Gascoyne, 1989; Coombs et al., 1998; Grogan and McKinley, 1989; Baker et al., 1998; West et al., 1992, 1995, 1998 and 2006; Jolley et al., 2003, Tochigi et al., 2008). It has been used to study microbial effects at the Tono research site in Japan (Baker et al., 1998) and Yucca Mountain, USA (Jolley et al., 2003). Additionally, the approach has been used to assess microbial effects on particular disposal concepts, for example: the Canadian HLW concept (Stroes-Gascoyne, 1989); the Japanese CES concept (West et al., 2006) and the Swiss L / ILW repository (Grogan and McKinley, 1989). It has also been used to indicate microbial growth limitations in experimental situations (Hama et al., 2001) and to assess microbial populations during the Maqarin natural analogue study (West et al., 1995; Coombs et al., 1998).

However, for deep environments, no single approach or code has been developed which can realistically quantify microbial influenced redox processes, particularly under different pH conditions. Accordingly, the consequences of these processes cannot be simulated, nor can the significance of microbial processes on the transport of solutes be determined. A summary of these modelling codes is given in Table 6-4.

Table 6-4: Codes used to model model radioactive wastes with low organic carbon contents.

NAME	MING	MGSE/BGSE	MINT
Reference	Jolley et al.., 2003	Approach first adopted by Stroes-Gascoyne, 1989; Coombs et al., 1998; Grogan and McKinley, 1989; Baker et al.., 1998; West et al., 1992, 1995, 1998 and 2006.	Tochigi et al., 2008; Miyasaka, pers. comm..
Developer	Framatome and Lawrence Livermore Lab	NAGRA/British Geological Survey	JAEA
Microbiological context	Generic microbiological code coupled to energy from redox processes. Requires inventories of nutrients and energy	Generic microbiological code coupled to energy from redox processes. Requires inventories of nutrients and energy	Currently, effects of microbes on groundwater chemistry
Brief description	Mass balance and thermodynamics. Plus kinetic rates	Mass balance and thermodynamics	Models biochemistry and geochemical equilibrium with consideration of solute transport. Focus is on controls of specific microbial group activity
Biomass predictions	Yes	Yes	Yes
Geochemical links	Yes	Yes	Yes
Redox processes	Yes	Requires thermodynamic database	Yes
Effects on radionuclide migration	Yes	No	Yes
Gas production	Yes	Can be extrapolated	
Required data	Literature sufficient for scoping calculations. Site and experimental data for focussed work	Literature sufficient for scoping calculations. Site and experimental data for focussed work	Site data from in-situ investigations
TDB* used	Own database but could be linked to others	Own database but could be linked to others	Own. Comparisons also with PHREEQC
Availability	Developed for Yucca Mtn. Project – likely to be available	Through Nagra and BGS – source code likely to be unavailable	Limited, detailed reports in Japanese
Example of use	Yucca Mtn project	Swiss LLW/ILW concepts. Extended storage concepts, Laboratory Experiments,Natural analogues	Horonobe URL

* Thermodynamic Database

6.4.3 Other Modelling Approaches

A purely geochemical approach to evaluating microbiological impacts is possible. PHREEQC Version 2 and its predecessors (Parkhurst and Appelo, 1999) is a freely available code developed by the US Geological Survey. PHREEQC Version 2 does not contain specific microbiological input or output and is designed to perform a wide variety of low-temperature aqueous geochemical calculations so it has limited applicability. However, it has been used for evaluating biodegradation of organics using a modified Monod rate equation and experimental data. It has also been modified for use as part of the DRINK code (see above). The PHREEQC Version 2 thermodynamic database could also be used in MGSE / BGSE and MINT.

The geochemical effects of microbiolgical activity can also be simulated using the geochemical modelling code Geochemist's Workbench, developed by the University of Illinois (Bethke, 2008). Version 7 contains an explicit microbiological kinetics feature. However, to date, this code does not appear to have been used in the context of microbiological impacts on a radioactive waste disposal facility.

6.4.4 Examples of Useage

All the above microbiological codes are capable, potentially, of evaluating microbial effects in the deep subsurface. They can be divided into those best suited for evaluating sites where gas generation and kinetic processes are important and those where microbial processes are energy- and nutrient- limited. However, the approach adopted by MGSE / BGSE, MING or MINT does appear to be the most adaptable to different geological environments, particularly those in which activity levels are constrained by low nutrient fluxes. Importantly, this approach is also significantly less reliant on microbial kinetic data; obtaining rate data can be a significant overhead for kinetic microbial models. However, codes such as MGSE / BGSE, MING or MINT are very limited in their ability to deal with a range of redox processes particularly when these are coupled to the pH evolution of a system.

It is possible that both types of codes could be employed within a deep disposal programme.Mass balance- and equilibrium- based models and empirical gas generation- based models could be used in early stages of a site's development. During this period the lack of direct site investigation data, microbial conceptual models and site-specific microbial kinetic data makes the application of biogeochemical models difficult and inappropriate. If early geochemical modelling, waste characterisation and site investigation suggests that a microbially driven evolving geochemistry is important then biogeochemical models can be developed. On the other hand if these investigations suggest that microbial activity is not important then biogeochemical modelling is not required. It is worth noting that even in HLW scenarios, where carbon- based wastes are absent and energy inputs may be limited to corrosion hydrogen, far-field microbiology may still play an important role in maintaining the geochemical environment. Such a role can be seen in the hydrogen transformations in Boom Clay (Ortiz et al., 2002), near-field oxygen transformations (Yang et al., 2007) or when far-field biogeochemistry is mediated by environmental carbon inputs (Samper et al., 2003).

The mass balance and equilibrium based codes such as MGSE / BGSE, MING and MINT (Table 6-5) have been more extensively applied to existing and potential radioactive waste disposal scenarios than the kinetic-based biogeochemical codes. The latter have been used in near-surface LLW disposal situations (BNFL, 2002a). However, the nature of the waste and the processes modelled are closely comparable to ILW, since both waste types contain a significant organic component. The successful integration of these modelling approaches into the PA of an operational disposal site (BNFL, 2002a), indicates that models incorporating microbial processes can play an important role. However, the past experience of Nirex also highlights the need for both empirical and mechanistic codes (e.g. Rodwell, 2004 and 2005). Nirex discovered that a limitation of GAMMON was the large number of parameters needed to simulate biogeochemical mechanisms modelled.

6.5 Integration of Microbiology into Radioactive Waste Disposal Programmes

As radioactive waste disposal programmes have matured worldwide, the consideration of microbiology and related impacts has evolved into a number of key steps that reflect the development of the waste disposal programme concerned (Figure 6-6). In countries such as those of the UK, where a repository site has yet to be identified, and consequently a disposal concept has not been specified, the disposal system has unknown characteristics. At this stage, investigations are necessarily generic and can only consider microbial processes that potentially could be important in a range of plausible host geological environments and possible repository concepts. A focus of microbiological assessments is therefore to understand the differences between the different potential disposal systems, which can be used to inform initial specifications of the kinds of study that might be undertaken once a site is specified.

After a site has been identified, initial investigations generally focus on the considerable amount of relevant data available in the scientific literature and reference to existing lists of FEPs relevant to radioactive waste disposal (e.g. NEA, 2000). This auditing of potentially relevant processes gives way to a stage focussed on verifying which processes are relevant to the disposal system under consideration. The nature of the work carried out during this stage depends on the nature of the candidate geological environment being evaluated and the disposal system that is planned. The maturity of international radioactive waste disposal programmes is reflected in the fact that significant microbiology-related investigations have been integrated into the programmes of established sites. Such investigations include those at the WIPP, Yucca Mountain and investigations into candidate geologies in Switzerland, Belgium, Canada, Sweden. Several of these investigations have been enhanced by the availability of URLs (IAEA, 2001). Once the importance of microbiology-related processes has been verified, focus moves onto parameterising the impact of these processes on the disposal system, which in turn allows the integration of these processes into PA.

6.6 Summary Points

1. There is a wide range of available models that can be used to evaluate various aspects of microbial impacts on repository performance. However, they all have limitations (e.g. onerous data requirements; over-simplistic approaches). It may also be possible to use geochemical codes for some modelling of kinetic processes although there are few relevant examples in the radioactive waste disposal field. As a result, there is no consensus or consistency in how microbiological effects are tackled in different national programmes and thus how they can be integrated into existing PAs.

2. For deep environments, there is no single approach or code that can realistically quantify microbial influences on redox processes and their consequences, nor evaluate the transport of solutes involved in microbial processes. Consequently, there is a need to develop microbiological modelling (and the resulting codes) in order to ensure that microbial activity is adequately analysed when considering scenarios for post-closure PA. In particular, some scenarios may be too simplistic to allow the impact of microbes to be assessed more realistically – particularly where microbial processes may support the function of some key safety barriers, such as maintenance of reducing conditions.

3. Detailed mechanistic codes and simpler, empirical codes, used together, could improve confidence in the results of an assessment. The former codes can be used to gain understanding of biogeochemical mechanisms, in particular by allowing a user to explore sensitivities among processes. This understanding can be used to develop more robust and easily parameterized empirical models.

4. In recent years significant progess has been made towards incorporating knowledge of microbial processes into PA. However, understanding of microbial processes has been employed less explicitly when developing qualitative arguments to support broader safety case development.

5. Knowledge of microbiology is implicit in many of the quantitative and qualitative arguments that have underpinned recent PA and wider safety case development. However, this knowledge is not always reported transparently.

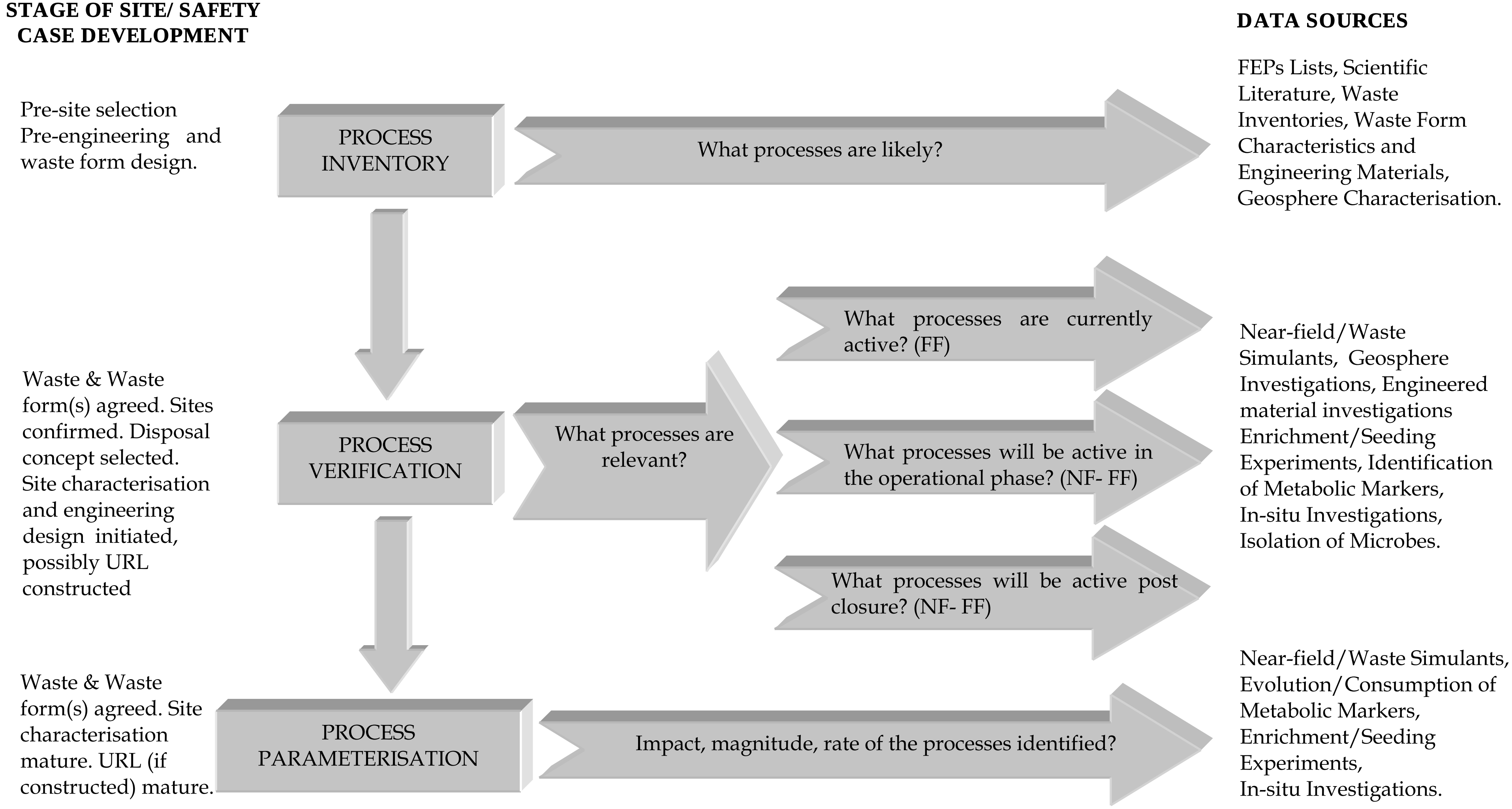

Figure 6-6: Integration of microbiology into radioactive waste disposal programmes.

7 Conclusions

This report has reviewed and evaluated international literature dating back to the early 1980s, concerning microbial effects in and around a deep geological repository for higher activity radioactive wastes. As the UK will select its site based on a process of voluntarism, no particular kind of site has yet been chosen and there are a number of possible geological environments in the UK that might potentially host a GDF. Moreover, no facility design or concept has been decided. As a result, this review does not focus on any particular kind of site or GDF, but broadly examines the literature to provide insights into microbial effects.

7.1 Microbiology of Relevant Geological Formations

It is recognised that microbes live in a wide range of geological environments and their potential influence on the performance of a repository for radioactive waste has been included in national programmes for over 20 years. This work indicates that all the identified general geological environments in the UK will have an indigenous microbial ecosystem, which will be influenced by inter-related environmental conditions, notably:

- the availability of nutrients and energy for microbial use;
- groundwater flow directions and fluxes;
- lithological characteristics, including hydraulic conductivity, porosity and mineralogy;
- the geological and climatic history; and
- anthropogenic impacts, including recent land useage and resource exploitation.

Site-specific information is required to evaluate the impacts of these conditions on microbiology.

Microbiology is now included in radioactive waste disposal programmes throughout the world. It is included in site investigations as it is now recognised that microbiology can influence a wide range of safety-relevant processes. Knowledge of microbiological processes can assist in understanding and predicting the performance of a repository into the long-term future.

Microbiological site characterisation will be heavily dependent on obtaining pristine samples from geological materials to eliminate the possibility of contamination.

Additionally, any microbiological characterisation programme must be planned at the same time as other aspects of site characterisation, for example the geochemistry programme. Both the microbiology and geochemistry of an investigated rock body are likely to be perturbed by factors such as borehole drilling. Consequently, both should be investigated early in any site assessment.

Invasive investigations in boreholes and URLs will cause perturbations to the nutrients and energy sources available for microbial useage, principally by changing groundwater flow patterns and fluxes, and by introducing extraneous materials to the subsurface. These changes are likely to be effectively permanent from the perspective of PA. For example, some drilling muds / fluids contain nutrients and energy sources and their introduction to the subsurface will alter the microbiology of the system in the future.

The nature of the perturbations will be site-specific and investigation-specific. For example the characteristics of the drilling fluids that must be used will depend partly upon the mechanical characteristics of the lithologies being investigated. The nature of the drilling fluids will also be partly dependent upon the nature of the investigations to be carried out in the boreholes. For instance, retrieval of high-quality rock core typically requires drilling muds to be used.

Many characterisation techniques exist that can be utilised when assessing the microbiology of a geological environment. These techniques are not all generically applicable with some environments (e.g. those with clays) posing particular technical difficulties. It will be crucial to focus on microbial activity levels of relevant microbial groups as these will vary with the geological environment.

7.2 Microbiology of the Near-Field

Microbes can tolerate a range of extreme environmental conditions which demonstrates that a repository, even one for HLW / SF or one backfilled with cement, cannot be assumed to be sterile for its entire lifetime. Nevertheless, it is very likely that sterile conditions will be found close to the waste although the distribution of sterile zones will be spatially and temporally variable. The only certain controls on life are the availability of water, nutrients and energy sources. The varying materials that may be used in a repository (as waste matrices, canisters, overpacks, buffers, backfills etc) are all potential nutrient and energy sources for microbial use. They will also have a microbial load associated with them, as will the repository excavations. Internationally, considerable work has been carried out to understand and quantify microbial influences on many of these materials. Much of this work is site- or repository concept-specific but all investigations have shown the importance of considering microbial impacts in the context of a particular repository concept. Broadly:

1. MIC has been demonstrated in many repository-relevant studies and all show that MIC must be considered in any repository concept where metals may be included.

2. Canadian studies of a range of backfill / buffer materials have shown that some mixes cannot reduce microbial activity to a point where it is insignificant with respect to the Canadian deep geological concept. As a result, this concept now uses compacted 100% bentonite buffer directly in contact with SF. Thus it is reasonable to conclude that, for other similar concepts, direct microbial degradation would not be expected to be a problem for bulk compacted clay materials.

3. Microbial degradation of organics will be significant in some repository concepts and much work has been undertaken to study biodegradation rates and biogenic gas production. However, the exact microbiological impacts will depend on the nature of the waste and the repository concept.

4. Microbial activity can have a direct influence on the behaviour of some radioelements such as uranium, technetium, neptunium and plutonium, and nuclides such as ^{14}C. The nature and extent of these influences will depend on the prevailing geochemical environment and the chemical form of the radionuclide concerned.

The effects of microbes in the perturbed zone is currently an area of very active research, particularly their impacts on overall solute transport properties and on redox processes, as these are key to understanding the efficiency of many geological disposal concepts.

7.3 Microbiological Influences and EBS Performance

The overall influences of microbial activity on the performance of an EBS are, again, complex and dependent upon the nature of the wastes and the repository concept under consideration. For example, different concepts place different emphasis on the engineered and natural barriers in order to attain overall performance targets. In those HLW / SF concepts where disposal will occur in fractured igneous rock relatively great emphasis is typically placed on EBS performance and required canister lifetimes are relatively long. In these cases MIC may potentially influence overall barrier performance. In contrast, those HLW / SF concepts where the host rock is plastic clay typically place greater emphasis on the host rock barrier and canister lifetimes are relatively short. In these cases MIC may have a proportionately lower impact on overall barrier performance.

Many microbial effects will also be enhanced in a repository concept which has an extended 'open period' during which effectively unlimited air (oxygen) is circulated into the system. Consequently, the effects of such an open period on microbial populations and future repository performance would require careful assessment.

7.4 Microbiology of the Far-Field

As in the perturbed zone, microbes can impact on solute transport processes and thus influence radionuclide migration in the far-field. This is an area of active research, but much of the relevant repository performance work has been undertaken in granitic environments using underground laboratories. Little work has been carried out in other geological environments.

Microbial transformation of organic complexing agents has the potential to reduce radionuclide migration in the far-field. Additionally gases such as hydrogen and methane generated within a site may be subject to further microbial transformations as they move through the far-field.

7.5 Integrating Microbiology into Performance Assessments

Understanding of microbiological processes is typically taken into account implicitly when developing numerical models as part of PA. For example, the assessment process normally involves development of scenarios that are then subjected to numerical analysis during PA. These scenarios can be considered collections of Features Events and Processes (FEPs) and expert judgments of those FEPs to include in a scenario are required. Certain FEPs are to a greater or lesser degree influenced by microbial activity and deciding whether or not to include them requires understanding of microbiology. However, usually the application of this understanding is not reported transparently.

There is a wide range of available models that can be used to evaluate various aspects of microbial impacts on repository performance. However, they all have their limitations (e.g significant data requirements) and, for deep environments, there is no single approach or code to quantify microbial influences on solute transport and effects on redox processes. This is particularly the case where some scenarios may be too simplified to allow the impact of microbes to be assessed realistically – especially where microbial processes may support the function of key safety barriers, such as maintenance of reducing conditions. Nevertheless, significant progress has been made towards incorporating knowledge of microbial processes into overall PA models. Existing models have been shown to make significant contributions to understanding microbial impacts on a particular disposal system. However, these models require site-specific data to be effective. Underground laboratories have a significant role to play in the generation of these data.

Development of a safety case involves using multiple lines of reasoning to develop reasoned arguments that safety criteria are met robustly. These lines of reasoning and the arguments developed from them are qualitative and quantitative in nature. Microbiological information must be taken into account in both cases.

The output from a PA is one important input to safety case development. To the extent that microbiology is taken into account in PA, it is also incorporated into the subsequent safety case. Microbiology is also frequently taken into account implicitly when developing qualitative arguments in favour of safety. For example, reducing conditions in the deep geosphere are generally favourable for safety. It is typically argued that post-closure conditions in a repository environment will become reducing over a short period compared to the overall assessment timeframe. This argument implies knowedge of microbially-mediated processes. However, the precise role of such knowledge in developing these kinds of arguments is typically unclear in literature supporting published safety cases.

The report shows that is not possible to ascertain which microbial effect or effects will predominate in the near-field, the EBS and disturbed zone; microbial effects will be dependent on the selected site, type of waste and the repository concept. Evaluation of these effects will need to be undertaken at a specific site, so the results can be assessed in the overall context of a PA. If the evaluation indicates that all microbial processes would have a significantly negative overall effect on performance then methods to limit these effects would need to be employed. These methods include (but are not limited to) measures to control or limit microbial activity, such as engineering solutions.

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Appendix A Introduction to Microbiology

A-1 Classification of Microorganisms

The term microorganism is used to describe those organisms that are, individually, not visible to the naked eye. The none-specific nature of this definition means that microorganisms are a very diverse group of organisms. Modern molecular techniques have used DNA sequences to provide a much more specific classification of organisms based on their genetic relatedness. The application of this phylogenetic approach has generated a tree of life consisting of three domains (Woese et al, 1990) namely the Eukarya, the Bacteria and the Archaea. A further classification system that is based on the structure of individual cells is also commonly used and splits life into the prokaryotes and the eukaryotes (Figure A-1). Prokaryotes are generally single celled organisms which do not house their DNA within a nucleus, whereas the eukaryotes do house their DNA in a nucleus and are commonly either single-celled or multi-cellular. The Bacteria and Archaea are all prokaryotes whereas the Eukarya are eukaryotes. Microorganisms are found in all three of these domains, however the Bacteria and Archaea are exclusively microorganisms.

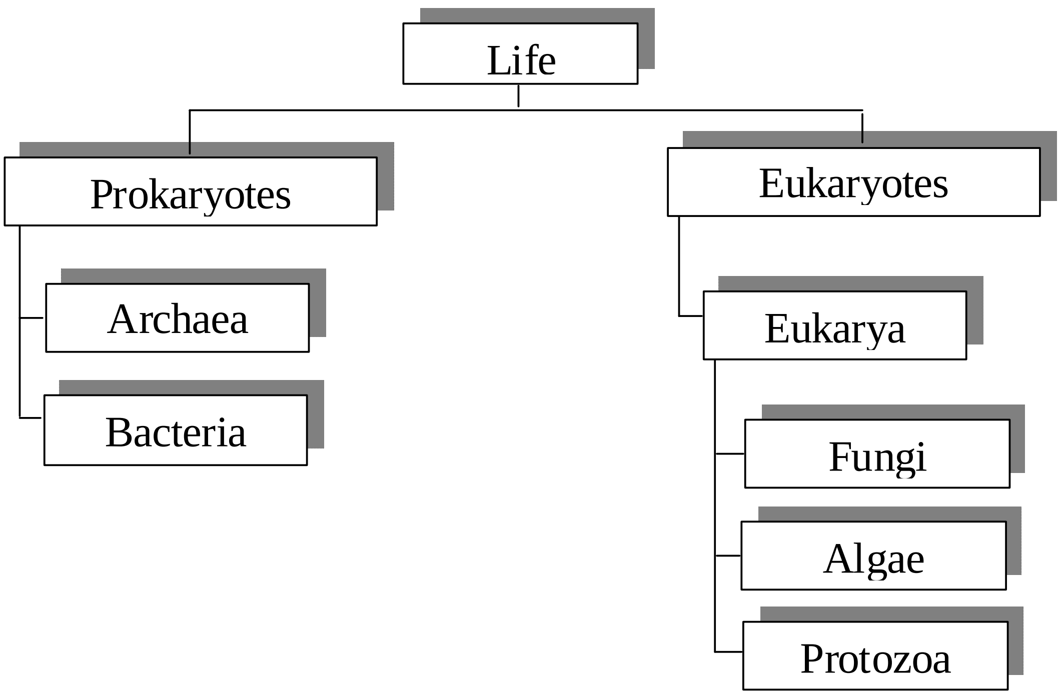

Figure A-1: Classification of microorganisms.

Fungi are either multi-cellular or unicellular (yeasts) eukaryotes which posses rigid cell walls and feed through the excretion of enzymes and absorption of dissolved nutrients.

Multi-cellular fungi grow as filaments know as hyphae, but may also have unicellular aspects to their life cycle. The majority of microscopic algae are either unicellular or colonial and are distinguished from other eukaryotic microbes by their ability to photosynthesise. Protozoa are generally unicellular eukaryotes which predate other microorganisms such as bacteria, fungi and algae. Some protozoa also consume particulate and / or dissolved organic matter.

A-2 Viruses

Viruses are a complex group of intracellular parasites which are unable to grow or reproduce outside of living cells. All cellular life has viruses associated with it and the viruses which infect bacteria are known as bacteriophages. Significant numbers of bacteriophages have been isolated from deep granitic groundwater from the Äspö HRL in Sweden (Kyle et al, 2008) and it is likely that any environment with a microbial population will also contain the associated viral population. The impact of viruses which infect microorganisms within potential radioactive waste disposal sites is difficult to determine. However, it should be noted that bacteriophages are implicated in the transfer of genetic materials and associated characteristics between bacterial species and are therefore agents of bacterial evolution. Hallbeck and Pedersen (2008a and b) suggested that these bacteriophage particles may provide a biological origin for colloidal particles.

A-3 Microbial Energetics and Nutrition

From the perspective of radioactive waste disposal a more useful classification of microorganisms is one based on nutritional requirements and sources of energy rather than those based on evolutionary relationships. This is because it more closely aligns with the how microorganisms impact upon a disposal system. From an energetic perspective life has two potential sources of energy i.e. chemical oxidation-reduction (redox) reactions or light. Microorganisms able to harvest light in order to generate energy are known as phototrophs and include some Bacteria, some Archaea and the algal eukaryotes. The impact of phototrophs on the evolution of a deep disposal site will obviously be controlled by the absence of light. It should be noted however, that the growth of phototrophic organisms during the construction and operational phase of a repository is likely due to the use of artificial lighting. Consequenlty these organisms may contribute organic carbon that may be used in further microbial growth following closure.

Those microorganisms harvesting energy from redox reactions can be split into lithotrophs and organotrophs. Lithotrophs are organisms which are able to generate energy from the oxidation of inorganic compounds (Table A-1) and organotrophs are those which generate energy from the oxidation of organic compounds. Lithotrophy is the domain of the Bacteria and Archaea and is not a process associated with Eukaryotes. When a radioactive waste repository is introduced into a geological environment, sources of electron donors and electron acceptors are contributed by both the facility and its associated waste, and the geological environment. The potential electron donors and acceptors present within a repository are outlined in Table A-2. This is not a comprehensive list and the actual inventory of donors and acceptors will be site- and waste- specific.

Table A-1: A selection of microbial lithotrophic processes.

Electron Donor	Electron Acceptor					
	O_2	$N0_3^-$	Mn (IV)	Fe (III)	$S0_4^{2-}$	CO_2
Sulphides (Mineral/Dissolved)	✓	✓				
Elemental Sulphur	✓	✓				
Ferrous Iron	✓	✓				
Uranium	✓	✓				
Ammonia	✓	✓				
Methane	✓	✓	?	?	✓	
Hydrogen	✓	✓	✓	✓	✓	✓
? = Theoretically possible but not experimentally confirmed.						

Organotrophic organisms may gain energy from the complete oxidation of carbon compounds by coupling this oxidation to the reduction of terminal electron acceptors such as oxygen, nitrate etc. Alternatively they may gain energy via fermentation, where energy is generated in the absence of external electron acceptors. In fermentation the electron donor and acceptor are components of the original substrate, which results in the partial oxidation of the substrate and the generation of both oxidised and reduced carbon compounds. Typical fermentation end products are alcohols, organic acids, carbon dioxide and molecular hydrogen. Fermentation end products are often subject to further oxidation via other microbes employing terminal electron accepting processes (Figure A-2).

Table A- 2: Potential electron donors and acceptors.

	Near-field				Far-field	
	Waste & Waste Form (Present or Derived From)			Backfill and Buffer	Groundwater	Geology
Electron Donors	ILW	HLW	SF			
Organic Carbon	✓			✓	✓	✓
Ferrous Iron					✓	✓
Sulphide				✓	✓	✓
Hydrogen	✓	✓	✓			
Electron Acceptors						
Oxygen	✓	✓	✓	✓		
Nitrate	✓				✓	
Ferric Iron	✓					✓
Manganese	✓					✓
Sulphate				✓	✓	✓
Carbon Dioxide	✓			✓		

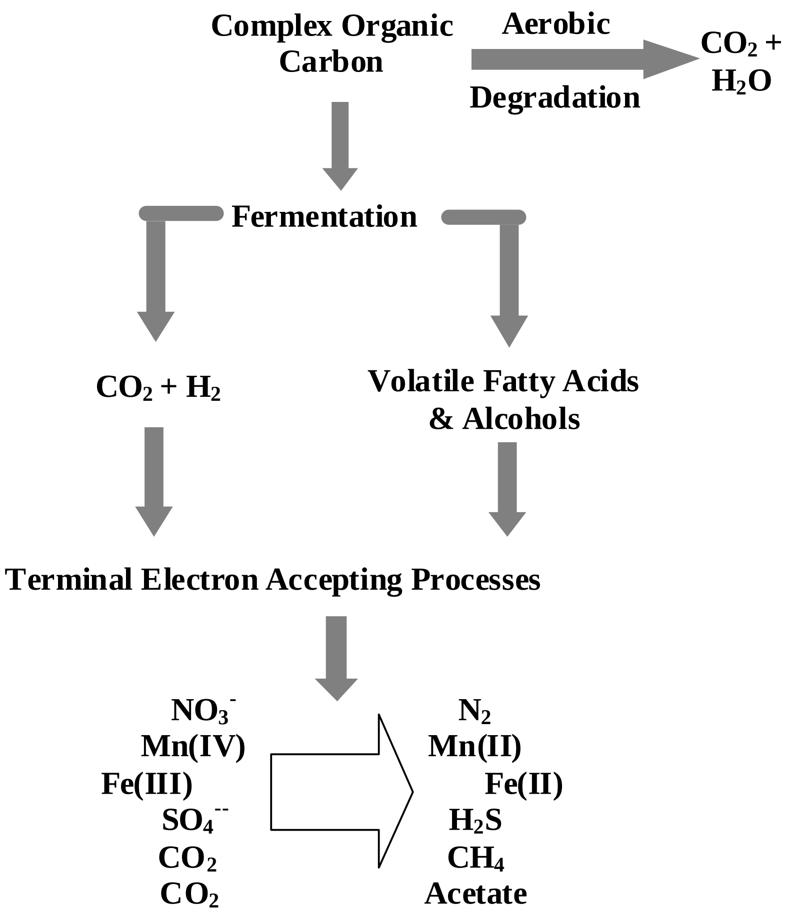

Figure A-2: Microbial degradation of organic materials.

Organic breakdown using oxygen as a terminal electron acceptor or fermentation may be mediated by prokaryotes and the fungi and protozoa. The employment of alternative terminal electron acceptors e.g. nitrate etc, is confined to the prokaryotes. In radioactive waste disposal sites fungi may take the same role as bacteria in organic degradation and particularly the fermentative breakdown of organic materials. Yeast-like microorganisms are a significant component of the microbial population present in groundwater samples from the Äspö HRL (Pedersen, 2000). Anaerobic cellulose-degrading fungi were also detected in the UK LLWR (Lockhart et al, 2006).

Microorganisms may also be characterised by the source of carbon they use to build cellular components. Organisms which obtain cellular carbon from carbon dioxide are known as autotrophs, whereas those which require more complex sources of carbon are known as heterotrophs. Autotrophy is commonly but no exclusively associated with lithotrophic and phototrophic energy strategies.