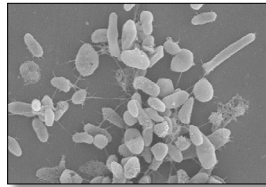


# **M**icrobial Influence on the Performance of Subsurface, Salt-Based Radioactive Waste Repositories

An Evaluation Based on Microbial  
Ecology, Bioenergetics and Projected  
Repository Conditions





Radioactive Waste Management and Decommissioning

**Microbial Influence on the Performance  
of Subsurface, Salt-Based  
Radioactive Waste Repositories**

An Evaluation Based on Microbial Ecology, Bioenergetics  
and Projected Repository Conditions

© OECD 2018  
NEA No. 7387

NUCLEAR ENERGY AGENCY  
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

## ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

The OECD is a unique forum where the governments of 35 democracies work together to address the economic, social and environmental challenges of globalisation. The OECD is also at the forefront of efforts to understand and to help governments respond to new developments and concerns, such as corporate governance, the information economy and the challenges of an ageing population. The Organisation provides a setting where governments can compare policy experiences, seek answers to common problems, identify good practice and work to co-ordinate domestic and international policies.

The OECD member countries are: Australia, Austria, Belgium, Canada, Chile, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Korea, Latvia, Luxembourg, Mexico, Netherlands, New Zealand, Norway, Poland, Portugal, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom and the United States. The European Commission takes part in the work of the OECD.

OECD Publishing disseminates widely the results of the Organisation's statistics gathering and research on economic, social and environmental issues, as well as the conventions, guidelines and standards agreed by its members.

*This work is published on the responsibility of the OECD Secretary-General.*

### NUCLEAR ENERGY AGENCY

The OECD Nuclear Energy Agency (NEA) was established on 1 February 1958. Current NEA membership consists of 33 countries: Argentina, Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Japan, Korea, Luxembourg, Mexico, the Netherlands, Norway, Poland, Portugal, Romania, Russia, the Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom and the United States. The European Commission also takes part in the work of the Agency.

The mission of the NEA is:

- to assist its member countries in maintaining and further developing, through international co-operation, the scientific, technological and legal bases required for a safe, environmentally sound and economical use of nuclear energy for peaceful purposes;
- to provide authoritative assessments and to forge common understandings on key issues as input to government decisions on nuclear energy policy and to broader OECD analyses in areas such as energy and the sustainable development of low-carbon economies.

Specific areas of competence of the NEA include the safety and regulation of nuclear activities, radioactive waste management and decommissioning, radiological protection, nuclear science, economic and technical analyses of the nuclear fuel cycle, nuclear law and liability, and public information. The NEA Data Bank provides nuclear data and computer program services for participating countries.

This document, as well as any data and map included herein, are without prejudice to the status of or sovereignty over any territory, to the delimitation of international frontiers and boundaries and to the name of any territory, city or area.

Corrigenda to OECD publications may be found online at: [www.oecd.org/publishing/corrigenda](http://www.oecd.org/publishing/corrigenda).

#### © OECD 2018

You can copy, download or print OECD content for your own use, and you can include excerpts from OECD publications, databases and multimedia products in your own documents, presentations, blogs, websites and teaching materials, provided that suitable acknowledgement of the OECD as source and copyright owner is given. All requests for public or commercial use and translation rights should be submitted to [neapub@oecd-nea.org](mailto:neapub@oecd-nea.org). Requests for permission to photocopy portions of this material for public or commercial use shall be addressed directly to the Copyright Clearance Center (CCC) at [info@copyright.com](mailto:info@copyright.com) or the Centre français d'exploitation du droit de copie (CFC) [contact@cfcopies.com](mailto:contact@cfcopies.com).

Cover photos: Geological salt beds deep underground in New Mexico (Courtesy of Carlsbad Department of Development); *Halobacterium sp. (noricense)* isolated from Salado halite in the Waste Isolation Pilot Plant (United States).

## Foreword

Because of their ability to effectively isolate radioactive waste from the environment and the public, deep geological formations are considered the optimal choice for the disposal of hazardous waste. Granitic rock, basalt, clay, tuff and evaporite salt beds are among the different types of sites that are being considered for radioactive waste repositories. Since it was determined that organisms are active even in deep geological settings, a great deal of research has been undertaken to determine the influence of microorganisms on repository performance (i.e. safety cases). Nonetheless, few data are available on the microbiology of subterranean salt formations in proposed and active locations for radioactive waste repositories. Because the biogeochemistry of other deep geological settings differs significantly from that of subterranean salt, it is not always possible to extrapolate microbial activity from one type of site to another. In lieu of directly applicable data, repository scientists rely on “indirect” information – such as microbial ecology, genomics and the thermodynamic feasibility of certain types of metabolism in hypersaline environments – to predict microbial activity and the potential impact on the performance of the repository.

The lack of data and the resulting uncertainty surrounding microbial processes in high ionic strength repository settings has meant that performance assessments and safety cases must be conservative in their predictions of potential microbial impact. While such a conservative approach can be defended in a regulatory process, a better understanding of the system would nonetheless alleviate the need to spend engineering resources on what may only be perceived problems.

Microorganisms are predicted to have diverse effects on radioactive waste repository performance. These effects are linked to activities that may affect radionuclide speciation and solubility, or that may enhance mobility and thus the source term used in evaluating repository performance.

## Acknowledgements

The NEA would like to express its appreciation to the principal authors of this report: J.S. Swanson,<sup>1</sup> A. Cherkouk,<sup>2</sup> T. Arnold,<sup>2</sup> A. Meleshyn<sup>3</sup> and D.T. Reed.<sup>1</sup> The Los Alamos National Laboratory (LANL) – Carlsbad Operations Actinide Chemistry and Repository Science Program in Carlsbad, New Mexico – the Helmholtz-Zentrum Dresden-Rossendorf (HZDR) Centre for Resource Ecology in Rossendorf, Germany; and the Gesellschaft für Anlagen und Reaktorsicherheit in Braunschweig, Germany helped in compiling this report.

The NEA also wishes to acknowledge the support of Russ Patterson, the US Department of Energy (DOE) programme manager. LANL research described herein was funded by the Department of Energy-Carlsbad Field Office (DOE-CBFO) as part of the ongoing recertification of the Waste Isolation Pilot Plant (WIPP) transuranic repository. HZDR research described herein was funded by the Young Investigators Grant, MicroSALT, and by the German Federal Ministry for Economic Affairs and Energy (BMWi).

- 
1. Los Alamos National Laboratory – Carlsbad Operations; Carlsbad, New Mexico, United States.
  2. Helmholtz-Zentrum Dresden-Rossendorf; Dresden, Germany.
  3. Gesellschaft für Anlagen und Reaktorsicherheit; Braunschweig, Germany.

## Table of contents

<b>List of abbreviations and acronyms</b> .....	7
<b>Executive summary</b> .....	9
Chapter 1. <b>Repository performance and the possible effects of microbial activity</b> .....	11
Chapter 2. <b>Background</b> .....	13
The subterranean salt environment and the potential for microbial life .....	13
The microbiology of hypersaline systems and the subterranean salt biosphere .....	14
Chapter 3. <b>The potential for microbial activity under projected repository conditions</b> .....	19
Expected conditions .....	19
Expected repository contents .....	24
Chapter 4. <b>Predicted modes of metabolism in the repository setting</b> .....	29
Aerobic respiration .....	29
Nitrate reduction/denitrification .....	30
Fermentation .....	30
Sulphate reduction with incomplete organic oxidation .....	30
Methanogenesis from methylated amines .....	31
Homoacetogenesis .....	31
Other sulphidogenic reactions .....	32
Reduction of other oxyanions .....	32
Metabolism variation in space .....	32
Metabolism variation in time and the ability to adapt and evolve .....	33
Chapter 5. <b>Effects of microbial activity on salt-based repository performance</b> .....	35
Oxidation of repository organic matter .....	35
Complexation of radionuclides with other microbially generated ligands .....	39
Creation of a reducing environment .....	40
Alteration of pH .....	40
Redox reactions with iron and radionuclides .....	41
Biocolloid vectors .....	42

Chapter 6. <b>Incorporating microbial parameters into a repository performance assessment/safety case: Case study of the Waste Isolation Pilot Plant</b> .....	45
Model assumptions.....	45
Incorporated model/safety case parameters at the Waste Isolation Pilot Plant.....	46
Chapter 7. <b>Microbial issues lacking sufficient data</b> .....	47
Presence of sulphate reducers and other sulphidogens.....	47
Presence of methylated amines and methanogenesis.....	47
Low-level waste/transuranic versus high-level waste.....	48
Radionuclide toxicity.....	48
Microbial interactions with barrier components.....	48
Chapter 8. <b>Conclusions</b> .....	51
Chapter 9. <b>References</b> .....	53

### List of figures

1.1: Overview of key repository phases and the microbial issues associated with each phase.....	12
1.2: Halobacterium sp. (noricense) isolated from Salado halite in the WIPP.....	15
1.3: Approximate upper salt concentration limits for the occurrence of selected microbial processes.....	17
3.1: Water activity in pure solutions of sodium chloride and magnesium chloride.....	21
8.1: Repository phases and microbial issues to be considered for salt-based repositories.....	51

### List of tables

3.1: Measurements of brine taken from the United States and German subterranean salt formations.....	22
--	----



## List of abbreviations and acronyms

$a_w$	Water activity
BTEX	Benzene, toluene, ethylbenzene, xylene
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
GWB	Generic weep brine
HLW	High-level waste
HMW	High-molecular weight
LLW	Low-level waste
L/ILW	Low- to intermediate-level waste
LMW	Low-molecular weight (in reference to organic compounds)
MgO	Magnesium oxide (engineered barrier at the WIPP)
NEA	Nuclear Energy Agency
OECD	Organisation for Economic Co-operation and Development
SRB	Sulphate-reducing bacteria
TRU	Transuranic
WIPP	Waste Isolation Pilot Plant (United States)



## Executive summary

The potential for microbiological effects to impact the long-term performance of a salt-based radioactive waste repository was assessed based on the current understanding of microbial processes and microbial communities in high ionic strength systems. Although some potential impacts have been identified, one key conclusion of this report is that the expected environment in a salt repository is unlikely to be conducive to the level of microbial activity needed to incur a significant impact on repository performance. This view contrasts with what has often been observed in near-surface or low ionic strength environs where higher activity and diversity have been predicted. It also contrasts with current salt repository performance assessment projections where, in the absence of sufficient data, conservative assumptions have led to the prediction of high impacts.

In a deep geological setting, both an indigenous microbial population will exist as well as an introduced population from waste emplacement activities and from the waste itself. The assessment of deep geological settings as potential sites for nuclear waste disposal should evaluate and account for the potential impacts of both populations on performance. For this reason, the microbiology of granite, basalt, tuff and clay formations in Europe and the United States has been under investigation for decades, and much has been learnt about the potential influence of microorganisms on repository performance and about deep subsurface microbiology in general. However, a great deal of this knowledge, as is shown in the present report, cannot be projected to the salt repository case because of the unique microbial constraints and limitations introduced by hypersaline (typically  $I > 5 \text{ M}$ ) conditions.

Microbial communities can be found in hypersaline settings. They are, however, limited in both structural and functional diversity when compared to other environmental matrices, such as soil. The reason for this limitation is that in order to survive at high-salt concentrations, such organisms must osmotically balance their internal and external environments, limiting their ability to perform certain modes of metabolism based on the energy required for survival and the energy derived from a given metabolic reaction. The field of repository microbiology has assumed that diverse organisms capable of many metabolic processes will be present and active in the repository setting and will adhere to the usual progression through terminal electron-accepting processes. However, this may not be the case at extremely high-salt concentrations.

At the highest salt concentrations, extremely halophilic archaea are the dominant members of the microbial population because of their ability to balance osmotic pressure using a low-energy strategy. These organisms are almost all aerobic, with limited anaerobic and fermentative capability, and thus their role in repository microbiology may be confined to early oxic periods. They are nonetheless

able to survive tens of thousands of years encased in salt, such that they will be present throughout repository history. Some extremely halophilic bacteria also exist in hypersaline environments. In general, these organisms will have a much more diverse metabolic repertoire, including aerobic, anaerobic and fermentative capabilities. However, these capabilities narrow as salt concentration increases, resulting from the high-energy cost strategy used by most bacteria to maintain osmotic balance. Bacteria present in repository waste or introduced during mining operations are not likely to be halophilic and may not survive long term. However, the role of microorganisms within drums, prior to emplacement and brine inundation, may be significant and would need to be evaluated.

The likely survival and activity of microorganisms has been evaluated for the range of expected conditions in a salt repository (e.g. parameters such as humidity/brine composition, pH, oxygen availability, pressure and temperature) and modes of metabolism. Humid conditions may permit the growth of less halophilic organisms with broader metabolic capability; however, in this case, access to nutrients and substrates is limited. In brine-inundation scenarios, only the most halophilic of organisms will survive, but the lack of oxygen and suitable organic substrates, as well as the combination of low water activity and high concentrations of chaotropic solutes, will constrain this population significantly. Under the expected repository conditions, aerobic degradation of organics may be confined to the short, post-emplacement period that is likely to be oxic. Once oxygen is depleted and brine inundation occurs, the likely dominant processes will be nitrate reduction and fermentation. No strictly anaerobic halophiles have ever been isolated from subterranean salt deposits, although they exist in surficial hypersaline settings, such as brine lake sediments. Thus sulphate reduction is uncertain, but in other settings, rates decrease with increasing salinity. Methanogenesis can only be sustained if methylated amines are present, which is also uncertain.

The potential impacts on actinide or radionuclide biogeochemistry have also been evaluated. Microbial activity can help establish reducing conditions by removing oxygen or other oxidants, or by generating reductants (e.g. hydrogen, sulphide, Fe<sup>2+</sup>) – all of which will help argue for low radionuclide solubility. Respiration via direct bioreduction of radionuclides in the near field is unlikely given the lack of this metabolism in the most extreme halophiles. Although some carbonate and degradation products with complexing capacity may be produced during the oxic phase, this production should not be significant once brine inundation occurs. Sorption onto active cells, dead cells, and/or other cellular material will be possible; however, the biomass available as sorbent will be limited by the environmental conditions and potential toxicity of soluble radionuclides, keeping in mind that there is no guarantee of cellular migration.

The potential projection of these microbial issues onto the assessment of repository performance was evaluated based on what is known about the microbial ecology of hypersaline settings, the bioenergetics of survival at high ionic strength and related repository microbiology studies. Further work is needed beyond that which appears in this report. Although it can be demonstrated that many potential effects are mitigated by hypersalinity, it remains important to evaluate these effects on a site-specific basis, since repository scenarios, substrate and nutrient availability, as well as brine composition, will likely vary from one repository concept to another.

## Chapter 1. **Repository performance and the possible effects of microbial activity**

Deep geological formations are common choices for the disposal of hazardous waste because of their ability to effectively isolate the waste from the environment and the public. Granitic rock, basalt, clay, tuff and evaporite salt beds have been proposed as potential sites for radioactive waste repositories. Much research has been conducted on the influence of microorganisms on repository performance (i.e. safety cases), since it was determined that organisms are active even in such deep geological settings (Pedersen, 2002; Poulain et al., 2008; Anderson et al., 2011).

Although an abundance of research on halophilic microorganisms exists, few data are available on the microbiology of subterranean salt formations that pertain to proposed and active locations for radioactive waste repositories. Because the biogeochemistry of other deep geological (e.g. low ionic strength groundwaters) settings differs significantly from subterranean salt, it is not always possible to extrapolate microbial activity from one site type to the other. In lieu of directly applicable data, repository scientists may rely on “indirect” information – such as microbial ecology, genomics and the thermodynamic feasibility of certain types of metabolism in hypersaline environments – to predict microbial activity and potential impact on performance. Supportive research is nevertheless needed to directly address specific safety case issues.

Because of the lack of data and resulting uncertainty surrounding microbial processes in high ionic strength repository settings, performance assessments and safety cases must be conservative in their predictions of potential microbial impact. While this conservative approach can be defended in a regulatory process, a better understanding of the system might alleviate the need to spend resources trying to solve what may only be perceived problems.

The success of a radioactive waste repository is measured based on its ability to prevent the release of radionuclides into the surrounding environment or to limit that release to levels deemed acceptable by the appropriate regulatory agencies and the public. These mandates have variable lifetimes, which may depend upon the repository location and whether the waste has low, intermediate or high levels of radioactivity.

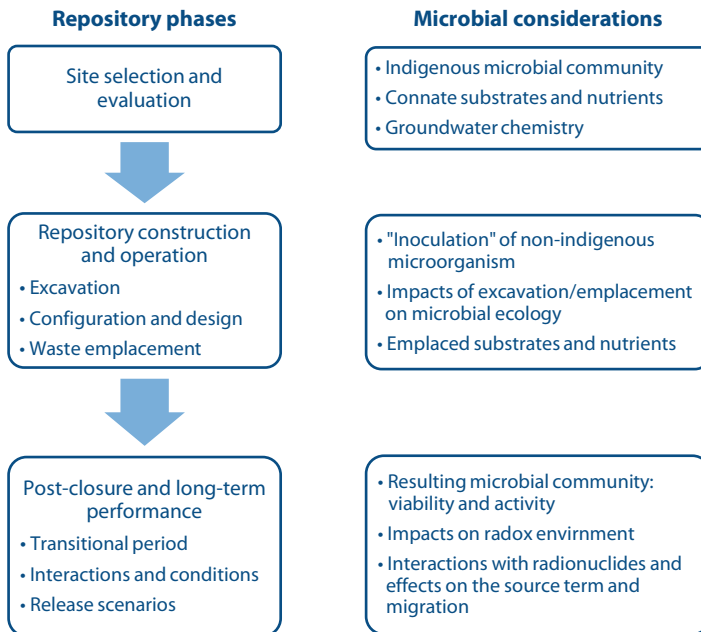
Microorganisms are predicted to have diverse effects on radioactive waste repository performance (McCabe, 1990; Pedersen, 1999; Macaskie and Lloyd, 2002; Pedersen, 2005; Wang and Francis, 2005; Lloyd and Gadd, 2011). These effects are linked to activities that may affect radionuclide speciation and solubility or that may enhance or mitigate mobility, and hence the source term used in evaluating repository performance.

Key microbial processes and effects include:

- complexation with carbon dioxide or organic ligands generated from the breakdown of organic waste;
- complexation with microbially generated ligands;
- creation of a reducing environment through the consumption of oxygen, generation of hydrogen, or formation of reduced metal species;
- alteration of pH;
- redox reactions with metal and/or radionuclide elements;
- any interactions that may indirectly enhance migration, such as surface sorption or internal uptake of radionuclides that may lead to biocolloid transport;
- any activities that may affect the performance of engineered barriers, such as canister corrosion and alteration of backfill or other barrier materials, such as cement or clay.

The relevance of these processes in subterranean salt formations will be discussed in detail in later sections. In general, microbial considerations for radioactive waste repositories can be summarised as shown in Figure 1.1.

Figure 1.1: **Overview of key repository phases and the microbial issues associated with each phase**



Note: This can be applied generically to all permanent geological disposal concepts.

## Chapter 2. Background

### **The subterranean salt environment and the potential for microbial life**

The subterranean salt formations proposed as radioactive waste repository sites exist in different forms – bedded salt and salt domes, or diapirs. Both begin as evaporite beds, of which halite is the dominant component, comprising between 93-97% of the total by weight. Other components include carbonates, anhydrite or gypsum, and chloride or sulphate salts of magnesium and potassium. Impurities, such as sandstones, volcanic materials, and clays may exist as a result of tectonic movement, wind, and run-off, during and after the deposition period (Harvie et al., 1980; Casas and Lowenstein, 1989). An uprise in the salt layer, because of its lower density in relation to the overlying sedimentary rock and subsidence of the sediment, can lead to dome formation.

In contrast to other geological formations that may contain deep aquifers, subterranean salt environments are relatively dry. What fluid there is may be external, from a surrounding water-bearing region via intergranular spaces, or internal, as trapped fluid inclusions or bound water in hydrous mineral impurities, e.g. gypsum, and clays (Roedder, 1984). Because of its low permeability, very little fluid or vapour movement occurs within subterranean salt formations. It has been suggested that salt domes contain less intergranular and inclusional fluid because of textural changes during stress deformation, but they may contain more water in hydrated minerals. This appears to vary with site history and mineralogy, such that site-specific testing is warranted (Knauth and Kumar, 1981; De Las Cuevas and Pueyo, 1995).

Most deep geological environments are dark and oligotrophic. This limits the sources of energy for microbial growth, and although microorganisms may remain viable, they may be inactive or in a state of low activity. Hydrothermal vents in some settings allow a gas-driven biosphere, or deep aquifers may introduce other energy sources. However, like water, this type of external input of potential energy sources for microorganisms is essentially absent in subterranean salt. “Native” substrates and electron donors are limited to syndepositional (e.g. fluid inclusion contents) or potential “migratory” sources (e.g. movement along fractures during past halokinesis, or between salt crystals; Pusch et al., 2014).

Syndepositional substrate sources can include halophilic algae and their associated organic osmolytes trapped within fluid inclusions. These are hypothesised to provide a long-term carbon and energy source to co-located halophilic prokaryotes (Schubert et al., 2010), although others have suggested that those prokaryotes are in a dormant state and are not capable of using these

osmolytes or that the algal release of glycerol alone is insufficient for the entombed halophiles to survive (Fendrihan et al., 2012; Winters et al., 2015). Still, the oldest halite in which entrapped algae were found was 150 000 years old (Sankaranarayanan et al., 2011); this is younger than the Permo-Triassic salt beds currently in use or under consideration for radioactive waste repositories (e.g. Salado Formation in United States; Zechstein Formation in Europe).

Native hydrocarbons have also been found within inclusions (Roedder, 1984; Pironon et al., 1995) but are more common within intergranular spaces and within anhydrite crystals (Hammer et al., 2012; Pusch et al., 2014), and are especially associated with salt domes (Posey and Kyle, 1988). Other organics may be associated with interstitial impurities, such as clays (Pironon et al., 1995), but whether or not these are available or usable substrates for microorganisms remains unknown.

Although there are no hydrothermal vents in subterranean salt to provide gaseous sources of energy, there are trapped gases within fluid inclusions. These may be from the originally trapped atmosphere (e.g. N<sub>2</sub>); may have resulted from transient microbial activity (e.g. CO<sub>2</sub>, H<sub>2</sub>, CH<sub>4</sub>, ethane, CS<sub>2</sub> and H<sub>2</sub>S) suggestive of the degradation of common marine biological compounds or hydrocarbons (Roedder, 1984; Siemann and Ellendorff, 2001); or from the thermal degradation of entrapped hydrocarbons (Kovalevych et al., 2008). Again, it is unknown whether these are either available or significant energy sources.

## **The microbiology of hypersaline systems and the subterranean salt biosphere**

Hypersaline conditions result in a unique microbial ecology, because of as a result of the thermodynamic constraints imposed upon the organisms inhabiting such environments. Survival depends on an organism's ability to maintain osmotic balance with its external environment (Oren, 2006). Two strategies exist by which organisms can do this: i) increasing salt concentrations, usually K<sup>+</sup> or Cl<sup>-</sup>, intracellularly ("salting-in") or ii) generating or taking up small, compatible organic solutes to raise the intracellular ionic strength. The former strategy is limited to archaea (of the order *Halobacteriales*) and a few anaerobic bacteria (orders *Halanaerobiales* and *Bacteroidetes*); while, the latter strategy is used by all other halophilic and halotolerant bacteria and all eukaryotes. As a result, haloarchaea generally tend to be the dominant organisms at the highest salt concentrations, while halophilic bacteria generally tend to dominate low-salt environments.

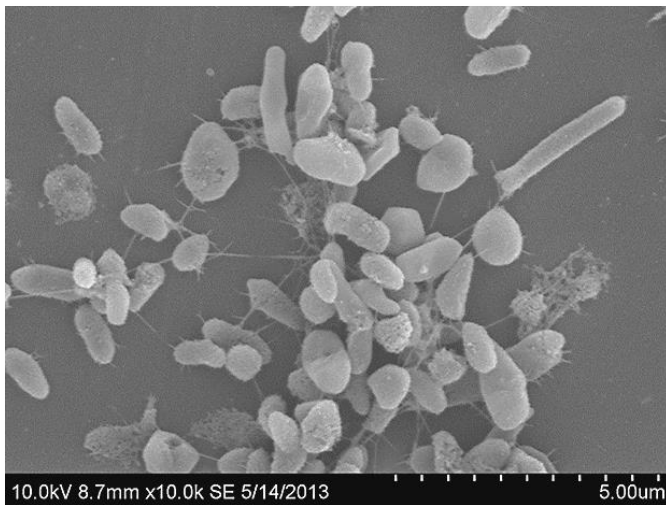
### **The microbial community in subterranean salt**

Although once thought to be sterile environments, it is now accepted that microorganisms inhabit subterranean salt formations. Commonly detected archaeal genera include *Halobacterium*, *Halorubrum*, *Halococcus* and *Natronomonas* spp. (Norton et al., 1993; Stan-Lotter et al., 1999, 2002; Fish et al., 2002; Park et al., 2009; Schubert et al., 2009; Gramain et al., 2011; Swanson et al., 2013a). Less common are organisms belonging to *Haloarcula*, *Halarchaeum*, *Haloterrigena* and



*Halosimplex* spp. (Vreeland et al., 2002; Park et al., 2009; Schubert et al., 2009). Recently described *Halolamina* and *Haloparvum* spp. have also been detected in subterranean salt formations, and many unclassified deoxyribonucleic acid (DNA) sequences may yet result in the descriptions of novel genera (Radax et al., 2001; Park et al., 2009; Gramain et al., 2011; Swanson et al., 2013a; Chen et al., 2016).

Figure 1.2: ***Halobacterium* sp. (noricense)** isolated from Salado halite in the WIPP



Bacteria are less commonly sought from rock salt, and in general, they are the dominant organisms cultivated at lower salt concentrations. Those that have been detected (by DNA signatures) or isolated include members of the phyla *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* (Vreeland et al., 2000; Fish et al., 2002; Roohi et al., 2012). DNA detection does not translate to cell viability, as high-salt concentrations may help preserve nucleic acids (Fish et al., 2002; Borin et al., 2008).

The numbers of organisms that can be cultivated from rock salt vary widely, from 0 to  $10^4$  colony-forming units per gram of salt (Norton et al., 1993; Vreeland et al., 1998; McGenity et al., 2000; Swanson et al., 2013a). This is likely because of the heterogeneous distribution of microorganisms in rock salt, such that often large quantities must be processed before any growth can be seen (Norton et al., 1993; McGenity et al., 2000). There is little documentation of direct microscopic cell counts in halite, possibly because of the low numbers (Swanson et al., 2013a). Microbial numbers in subterranean salt also appear to be lower than those in surficial hypersaline environments, such as brine lakes or solar salterns (e.g.  $10^7$  cells/ml in a crystalliser pond of a solar saltern; Antón et al., 2000), most likely as a result of the input of exogenous carbon and energy sources. Brines found in subterranean halite can also range in cell density (none to  $10^7$  cells/ml)

even within the same formation (Norton et al., 1993; Francis and Gillow, 1993; Vreeland et al., 1998; McGenity et al., 2000; Swanson, unpublished). This may be a function of brine composition, pH, source or length of exposure to mine air and other mine workings.

### ***The microbial community in subterranean salt-based radioactive waste repositories***

The microorganisms present in subterranean salt repositories are either indigenous to the salt formation (i.e. present in fluid inclusions or interstitial brines), indigenous to the surrounding environment (e.g. groundwater infiltrate), introduced in and on the emplaced waste, introduced on mining equipment and personnel, and introduced via air-intake shafts. Their true origin is not as relevant as their ability to survive and be active under projected repository conditions.

### ***Thermodynamic feasibility of microbial metabolism at high-salt concentrations and documented modes of metabolism in subterranean salt environments***

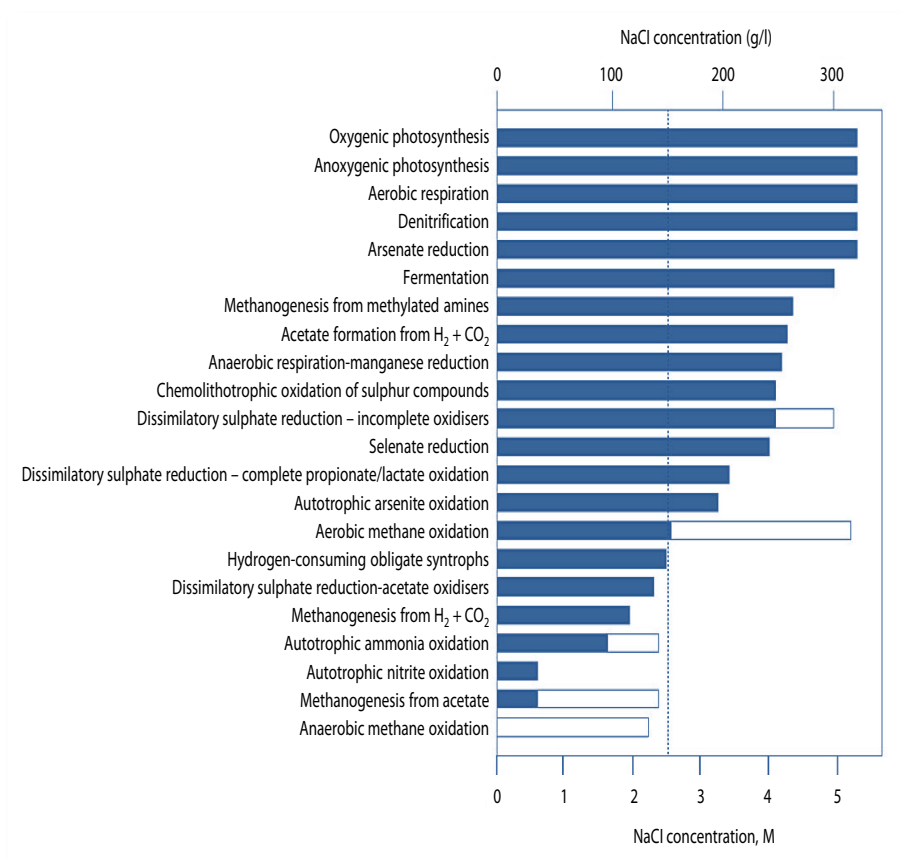
Maintaining osmotic equilibrium with the surrounding high-salt environment is energetically costly to organisms, but the organic osmolyte strategy is significantly more expensive than “salting-in”, especially when the compatible solutes are not available in the surrounding medium and must be synthesised *de novo*. As a result, microbial metabolic processes in hypersaline systems are limited to reaction pathways that result in a high enough energy yield to offset the cost of maintaining this balance.

The following metabolic processes are favourable at salt concentrations greater than 2.5 M NaCl (the cut-off for extremely halophilic microorganisms; see Figure 1.3; for excellent reviews, see Oren 1999, 2011, 2012):

- oxygenic and anoxygenic photosynthesis (energy from light);
- aerobic respiration;
- nitrate reduction/denitrification;
- fermentation;
- manganese, arsenate, and selenate reduction (anaerobic respiration using oxyanions as terminal electron acceptors);
- dissimilatory sulphate reduction with incomplete organic oxidation;
- reduction of elemental sulphur;
- methanogenesis from methylated amines;
- acetogenesis (generation of acetate from H<sub>2</sub> and CO<sub>2</sub>);
- chemolithotrophic oxidation of sulphur compounds (e.g. S<sup>0</sup>, thiosulphate).

All of these processes are either energetically favourable or are performed by organisms that maintain osmotic balance by the less costly strategy of “salting-in”.

Figure 1.3: **Approximate upper salt concentration limits for the occurrence of selected microbial processes**



Source: Oren, 2012.

Note: Solid bars are derived from laboratory experimental data using pure cultures; open bars are taken from in situ measurements of possible microbial activity. Dashed vertical line represents 2.5 M NaCl cut-off for extreme halophiles.

Of these possible modes of metabolism, some can easily be ruled out for subterranean salt settings: e.g. phototrophy. The rest depends upon the presence of electron donors and acceptors and the appropriate carbon substrates and nutrients. There appear to be no studies documenting the successful cultivation of halophilic organisms from subterranean halite under anaerobic conditions and only two documented failed attempts to do so (Michaud and van Demark, 1967; Swanson et al., 2013a). However, haloarchaea isolated under aerobic conditions have been found capable of growing anaerobically with the reduction of nitrate, trimethylamine N-oxide, dimethyl sulfoxide (DMSO) or fumarate; or are able to ferment amino acids, such as arginine (Oren, 2011; Müller and DasSarma, 2005).



## Chapter 3. **The potential for microbial activity under projected repository conditions**

What will happen over time to the resident (i.e. indigenous and introduced) microbial population remains largely uncertain but will depend upon whether or not conditions are favourable for activity or merely for survival. In order for microorganisms to influence repository performance (excluding colloid transport), they must not only be viable, they must be active. Microbial activity is dependent upon the presence and availability of moisture, adequate substrates and nutrients, and sources of energy (electron donors and acceptors), and these requirements may vary according to organism type. While it may be possible for all these basic requirements to be present in a repository environment, it is also possible that they will never come into contact with microorganisms, or that contact will be limited to microenvironments, because of the uncertainty surrounding humid versus inundation scenarios and the integrity of waste canisters.

### **Expected conditions**

Prevailing salt-based repository conditions may vary between sites and/or repository types. For instance, temperatures will differ with waste type (high-level [HLW] heat-generating waste versus intermediate- or low-level waste [LLW]); lithostatic pressures may vary with repository depth; and the presence of introduced constituents may affect the physicochemical environment (e.g. clay, cement, magnesium oxide). The only guaranteed, shared conditions across all salt-based repositories are high-salt content, the presence of radionuclides and an ultimately closed system because of the self-sealing properties of salt.

After excavation and during waste emplacement, the repository site is essentially an “open reactor”. Air-intake shafts introduce an oxygenated atmosphere, moisture and microorganisms. Miners and mining equipment provide another source of non-native microorganisms. The emplaced waste may be a source of organics, inorganics and microorganisms. Additionally, excavation can lead to fluid migration into the disturbed rock zone, and this may introduce brine, hydrocarbons, if present, and microorganisms into the repository horizon. Once sealed, the repository is theoretically limited in “reactants” to connate components and the components that were emplaced.

### **Moisture: Water activity, relative humidity and brine inundation**

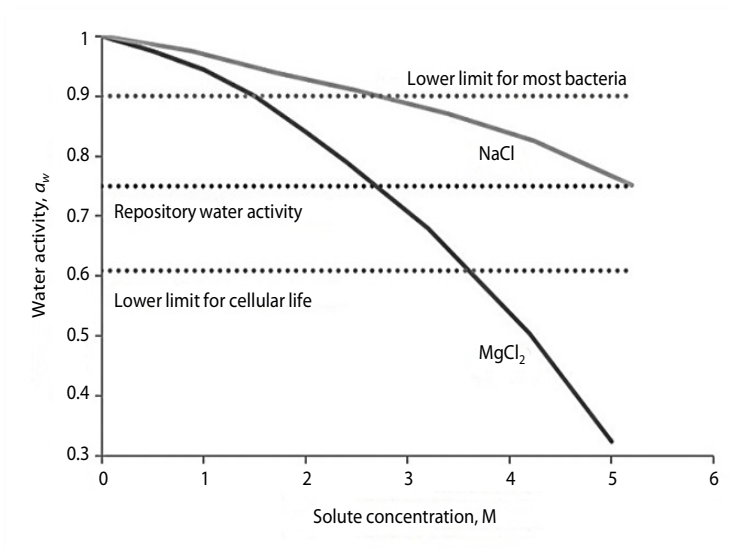
As mentioned earlier, subterranean salt formations are relatively dry – a desirable attribute for a radioactive waste repository. Still, brine can be present in fluid inclusions or as intergranular solutions. The amount of brine in subterranean salt varies with the salt formation and with its impurities, but in general is very low, e.g. 0.3 weight percent in pure halite; 1.5% in argillaceous halite (Hansen and Leigh, 2011). The movement of this fluid may depend upon the nature and extent of the disturbed rock zone, subsequent salt subsidence and creep, and temperature (especially for HLW repositories). Seeps or sumps of significant volume can result from the redistribution of interstitial brine through the ethylenediamine tetraacetic acid (EDTA) during excavation activities (Deal et al., 1995).

The availability of water is a key prerequisite for microbial life. Water activity determines its availability and is defined as the ratio of the vapour pressure of a solution (or solid medium) to the vapour pressure of pure water. In dry environments, water becomes unavailable as it is lost to evaporation. In brines, high concentrations of solutes compete for solvent water, making it effectively less available. The lowest documented water activity ( $a_w$ ) that supports xerophilic life is 0.61 (Grant, 2004). Most bacteria thrive at activities greater than 0.90, but extreme halophiles can thrive under salt (NaCl) saturated conditions,  $a_w = 0.75$ . Saturated  $MgCl_2$  solutions,  $a_w = 0.33$ , have not been shown to support life, but this may be a result of chaotropy as much as low water activity.

Halite deliquescence may occur at a relative humidity (RH) of 75% (25°C), which is equivalent to  $a_w = 0.75$ . Deliquescence can create a moist environment that, although it is halite-saturated, has been shown to stimulate transient microbial activity (Davila et al., 2008). Condensate may also form and persist in halite pore spaces less than 100 nm in diameter, thus providing a sustained higher RH in the immediate environs (Wierzchos et al., 2012). Thin-layer condensate may support the survival of less halophilic microbial species, since organisms at a liquid/air interface are not exposed to high-salt concentrations from all sides. This may explain the isolation of many desiccation-resistant bacteria and fungi from salt that do not otherwise survive at extremely high NaCl concentrations in culture (e.g. *Actinobacteria*, *Firmicutes*; Roohi et al., 2012; Swanson et al., 2013a and unpublished data).

Apart from its availability, “water structure” relationships with solutes and biological macromolecules may also affect microbial survival. For example,  $MgCl_2$  is a chaotropic solute that weakens the electrostatic forces that stabilise biomolecules, while NaCl, a kosmotropic solute, counteracts these effects (Hallsworth et al., 2007; Cray et al., 2013). Thus, microbial survival is unlikely at high Mg concentrations unless a sufficient amount of NaCl is also present. The highest Mg concentration that supports microbial life is thought to be 2.3 M in the presence of NaCl, but this upper limit may be pushed to 3 M based on the detection of possibly live cells in deep-sea, Mg-rich brines with a  $a_w = 0.631$  (van der Wielen et al., 2005; Hallsworth et al., 2011; Yakimov et al., 2015).

Figure 3.1: **Water activity in pure solutions of sodium chloride and magnesium chloride**



Source: Modified from Hallsworth et al., 2007.

The presence of cementitious waste is predicted to significantly influence brine composition in some concepts. Corrosion of cement in Mg-rich brines can lead to high concentrations of calcium chloride. Calcium, like magnesium, is a chaotropic solute that affects protein structures and water activity. *Chromohalobacter salexigens* was found to tolerate up to 1 M CaCl<sub>2</sub> in the presence of NaCl, and *Halomonas* spp. isolated from a CaCl<sub>2</sub>-rich lake were also found to be Ca-tolerant (Tregoning et al., 2015).

The presence of localised moisture – as RH condensate, seeps or sumps – may be conducive to microbial survival only if the water activity is sufficient and the chaotropicity is low. If these conditions are met, activity may also be possible, provided that all other components necessary for growth are also present. Because such heterogeneously distributed areas of activity are unlikely to affect global repository performance and are nearly impossible to model, the use of inundation scenarios becomes necessary. Inundation from uncertain anthropogenic causes, such as oil drilling, may lead to the introduction of groundwater from above or below the repository horizon. Should this happen, the fluid that enters the horizon will become the “solvent” for waste dissolution and the resulting solution will become the medium for microbial growth.

Table 3.1 provides an example of possible brine chemistries that may be encountered by microorganisms in salt-based repository settings. These values are from two brines obtained from the Waste Isolation Pilot Plant (WIPP) environs (generic weep brine [GWB], Energy Research and Development Administration

Well 6 [ERDA-6]), brine from German salt repository investigations (Q), a sample of sump fluid from Asse, and recently sampled brine seepage from the WIPP. These formulations are only examples and, with the exception of GWB and ERDA-6, are not meant to be representative of a given site. Brine composition may vary between and within subterranean salt formations.

The chief determinants for survival and community structure in hypersaline systems are sodium, magnesium and chloride concentrations, but other constituents, such as boron, may also be toxic to some organisms. Thus, the chemistry and water activity of subterranean salt brines alone may be enough to determine which, if any, microorganisms are likely to survive.

Table 3.1: **Measurements of brine taken from the United States and German subterranean salt formations**

Component	Molar Concentrations (except pH)				
	Generic weep brine*	ERDA-6 (underlying brine reservoir)*	Q-brine <sup>^</sup>	Sump fluid** (Asse)	WIPP brine seep <sup>†</sup>
Na <sup>+</sup>	3.530	4.870	3.088	0.174	0.522
Mg <sup>2+</sup>	1.020	0.019	3.781	4.444	3.497
K <sup>+</sup>	0.467	0.097	0.813	0.075	0.499
Ca <sup>2+</sup>	0.014	0.012	---	6E-4	0.001
Cl <sup>-</sup>	5.870	4.800	8.386	7.729	7.339
SO <sub>4</sub> <sup>2-</sup>	0.177	0.170	0.150	0.291	0.394
Br <sup>-</sup>	0.027	0.011	---	0.045	0.074
B(OH) <sub>3</sub> (aq)	0.158	0.063	---	---	0.370
I (M)	7.43	5.34	15.4	13.9	12.0
pH <sub>measured</sub>	7	8	4.5	4.7	4-5
pC <sub>H+</sub>	8.3	8.9	7.9	7.5	6.3-7.3

Notes: ERDA-6 – Energy Research and Development Administration Well 6.

\* Brush and Xiong, 2009.

\*\* Zirnstein et al., unpublished.

† Swanson et al., unpublished. Ionic strength and pC<sub>H+</sub> values were derived in EQ3/6 v. 8.0a (Wolery, 2003); pH to be discussed in more detail at a later stage.

<sup>^</sup> Thies and Schulze, 1996.



## Temperature

Repository temperatures may vary significantly, depending upon waste type (LLW vs. HLW). Low- to intermediate-level waste (L/ILW) repositories will likely maintain ambient temperatures that are conducive to microbial survival. In a HLW repository, temperatures will again depend upon waste type (e.g. defence-related or civilian, spent nuclear fuel) and overall repository configuration. Numerical simulations of HLW waste predict a temperature gradient as the distance increases from a canister, such that organism survival may be possible as little as 2 metres away (Stauffer et al., 2012). However, extreme heat-generating waste may not permit favourable temperatures until a much greater distance (Bracke and Fischer-Appelt, 2015) or time. Also, computer simulations predict fluid movement towards the heat source, which may be detrimental to organisms entrained within the fluid. Lithology and water content may also be influenced by heat generation and, in turn, influence organism viability.

The highest documented temperature for haloarchaeal growth is 61°C (Bowers and Wiegel, 2011), and DNA of non-thermophilic organisms will degrade near 94°C.

## Pressure

Once a repository has been sealed, pressures are expected to reach as high as 10 megapascals (MPa) or greater, depending upon repository depth (e.g. lithostatic pressure in WIPP is 15 MPa; Gorleben, 18 MPa).

The same attributes that make halophilic organisms resistant to osmotic stress also make them resistant to pressure, although the exact mechanisms are not known for all. For example, the low intracellular water activity and high concentrations of K<sup>+</sup> and Cl<sup>-</sup> resulting from the “salting-in” strategy help stabilise proteins in both high-salt and high-pressure environments. For bacteria that use organic compatible solutes instead of ions, these molecules may serve as piezolytes as well, although not always (Martin et al., 2002). These solutes also help maintain membrane fluidity, and hence stability, in bacteria; while the presence of ether lipids and S-layers maintain membrane fluidity in archaea. High pressure has been shown to upregulate responses to oxidative stress, such as redox proteins and reactive oxygen species scavengers. Although long-term survival under high-pressure conditions has not previously been addressed for halophilic organisms, many remain viable and even active after short-term exposures to pressures up to 400 MPa (Kish et al., 2012).

The effect of increased pressure on non-halophilic (e.g. introduced) organisms is less certain. Bacterial spores present in the waste can easily survive expected repository pressures, and their survival under pressure might be enhanced by low water activity (Sale et al., 1970). An increase in medium ionic strength permitted the survival, but not necessarily activity, of a non-spore-forming member of the *Firmicutes* at increased pressures (Molina-Höppner et al., 2004). Thus, although these organisms may survive, they may not necessarily be active. Nonetheless, directed evolution experiments have resulted in adaptation to pressure over time in organisms not known to be either halotolerant or piezophilic (Vanlint et al., 2011).

## pH

The term pH, as used in this document, refers to the pH of a solution as measured with standard pH electrodes but uncorrected for ionic strength. This usage allows a more direct comparison with microbial pH tolerances reported in the microbiology literature. However, corrections for ionic strength are needed to express the pH in terms of the hydrogen ion concentration ( $pC_{H^+}$ ; Rai et al., 1995; Borkowski et al., 2009). The corrected  $pC_{H^+}$  value can be significantly higher at the ionic strengths that are considered during the assessment of salt-based repository performance (see Table 3.1). This distinction has not been incorporated into the microbial literature but must be considered when applying this literature to a salt-based repository concept.

The examples, provided in Table 3.1, of measured and projected pH for repository brines range widely and may be repository-specific. For example, brines high in magnesium chloride tend to be mildly acidic; whereas, an increase in pH is expected in any repository with a significant cement inventory or with added oxides of magnesium. pH optima for extremely halophilic archaea vary with their source environment – thalassohaline (pH 7-8) versus athalassohaline ( $\geq 8.5$ ) – and seem to correlate with the sodium concentrations in those respective environments (Oren, 2006; Bowers and Wiegel, 2011).

Microbial signatures have been detected in acid lakes using cultivation-independent analyses (pH  $\sim 2.3$ ; Mormile et al., 2009), but no organisms have been isolated yet from such settings. Diverse haloalkaliphilic microorganisms can be found in environments where sodium carbonate or bicarbonate contributes to the high ionic strength and where divalent cation concentrations are low (e.g. soda lakes, pH 9.5-11; Sorokin et al., 2014). In general, haloalkaliphilic organisms are not found in thalassohaline settings, such as subterranean salt; however, *Halomonas* spp. have been isolated from soda lakes as well as subterranean halite (Boltyanskaya et al., 2007).

## Atmosphere

During excavation and waste emplacement, the repository atmosphere will contain ambient air that is forced through the air-intake shafts. Once sealed, canister corrosion and microbial activity will consume this residual oxygen to create a reducing atmosphere. The effects of sub- to anoxia on microbial metabolism will be discussed in a later section.

## Expected repository contents

### **Actinides, fission products, activation products and other radionuclides**

Depending upon the waste source, the radionuclide content of parent isotopes (e.g. U, Pu, Am, Np), fission products (e.g. 129-I, 137-Cs, 244-Cm, 90-Sr, 79-Se, 99-Tc), and activated elements (e.g. 36-Cl, 14-C, 3-H, 59-Ni) may differ, as will their relative activities over time (Keesmann et al., 2005; Umeki, 2007; Grambow, 2008; Schwartz,

2012). In considering microbial interactions with these elements, toxicity and biological relevance must also be addressed, and few studies have done this at higher ionic strengths.

Of the numerous elements present as parent radionuclides, fission products and activation products in radioactive waste, the following can be considered biologically relevant in their non-radioactive forms: i) C, H, Cl, S, Ca, Na, K, as major elements in biological systems; ii) Fe, Mn, Co, Ni, Zn, Mo, as major biological transition metals; iii) Se, as an essential trace element; and iv) As, Cd and Ba, as elements with specialised uses. Of these, <sup>14</sup>C, <sup>36</sup>Cl, and <sup>79</sup>Se have been listed as concerns for long-term exposure in nuclear repository concepts (NEA, 2007). In addition, oxides of Mn, As, Tc, Te, Se, U, and I and chelates of Co can serve as electron acceptors for cell respiration for some organisms (Lovley et al., 1991; Ahmann et al., 1994; Gorby et al., 1998; Lloyd et al., 2000; Maltman et al., 2015). These and other radionuclides (e.g. Np, Pu) can also be reduced as a means of resistance or detoxification by microorganisms (Lloyd, 2003). Per technetate, manganese oxides and arsenate respiration have been documented for halophilic organisms, the latter two only under alkaline conditions (Oremland et al., 2000; Khijniak et al., 2003; Sorokin and Muyzer, 2010). The genes for selenite reductase were found in the genome of WIPP isolate, *Halobacterium noricense* and aerobic selenite reduction as a resistance strategy has been shown in a *Halorubrum* sp. (Güven et al., 2013; Swanson, unpublished). *Halomonas* spp. were hypothesised to reduce per technetate at cell surfaces under aerobic conditions (Fujimoto and Morita, 2006).

Additional problems may arise from the ability of some fission products to substitute for biologically essential elements. For example, Cs can replace K, and Sr can substitute for Ca (Bossemeyer et al., 1989). Because extreme halophiles maintain molar concentrations of potassium and chloride intracellularly, these radioisotopes may be taken up into cells. It is unknown what effect such substitutions or other interactions with radioactive, biologically relevant metals may have on active repository organisms. Furthermore, little is known about what effect mere exposure to any radioactive element may have on repository indigenous organisms.

In the early repository phase, these radionuclides and products will not come into physical contact with indigenous microorganisms until the containers are breached. However, in the case of HLW, gamma radiation may exert an adverse effect. Once a container has been breached, exposure may occur, if solvent (brine) is present to enhance bioavailability. Bioavailability is usually a function of the dissolved concentration of a substance and thus will depend upon the radionuclide content of the waste and the nature and extent of intruding brine. (The speciation of radionuclides in repository brine is beyond the scope of this review and will depend upon many factors, such as redox conditions, pH and soluble concentrations of waste ligands.)

Radionuclide toxicity may be either chemically or radiolytically induced. Chemical toxicity requires that radionuclides be soluble in the inundating liquid, such that microbial exposure occurs, at a concentration that causes a given deleterious effect (i.e. effective concentration, or EC). Thus, chemical toxicity is more dependent upon concentration, oxidation state, and the presence or absence

of a complexant, and the effects on microorganisms are presumably similar to heavy-metal induced effects. Radiation effects are a result of oxidative damage to DNA and proteins and are therefore more dependent upon isotope and activity but do not require that a radionuclide be present in solution. Both radiation and chemical effects may vary with the microorganism tested.

Haloarchaea have numerous unique attributes making them more radiation tolerant than other organisms, including protective carotenoid pigmentation, bias against amino acids prone to reactive oxygen species, high intracellular Mn/Fe ratios, redundancy of genes encoding antioxidants, polyploidy, and the ability to differentially regulate genes needed for reactive oxygen species repair functions (Sharma et al., 2012). Moreover, extracellular and intracellular chloride will help confer resistance by scavenging free radicals (Shahmohammadi et al., 1998; Kish et al., 2009). Radiotolerant mutants of *Halobacterium salinarum*, able to survive doses as high as 25 kGy, are thought to be the most radiation-resistant organisms known (DeVeaux et al., 2007). Because the mechanisms involved in desiccation resistance are similar to those involved in radiation resistance, organisms present in LLW/ILW waste drums may also be radiation resistant.

The chemical toxicity of radionuclides to halophiles is not well studied. The growth of *Halobacterium* sp. (*noricense*), isolated from WIPP halite, was significantly inhibited by exposure to  $10^{-6}$  M neptunium(V) and  $10^{-4}$  M plutonium(V/VI), but the organism remained viable (Swanson et al., unpublished). Earlier studies of WIPP-relevant halophiles showed an inhibition of growth at  $10^{-5}$  M Pu,  $10^{-6}$  M Am, and  $10^{-4}$  M of U and Np when all were complexed with EDTA (Francis et al., 1998; Strietelmeier et al., 1999). These effective concentrations approach the solubilities of some actinides in simplified and complex brines (for example,  $\sim 10^{-6}$  M calculated for Am in reacted WIPP brines at  $pC_{H^+}$  8.7-9 in the presence of organics, per Brush and Xiong, 2009;  $\sim 10^{-5}$  M calculated for Am in Gorleben, per Schwartz, 2012;  $\sim 10^{-5}$  M for Nd measured in 5 M NaCl at a molal-based pH of 9, per Kienzler et al., 2012). Thus, a toxic effect may be observed.

The solubilities of fission and activation products in brines tend to be somewhat higher; for example, Ni, Tc and Se were determined to be soluble at 0.1 mM concentrations in brines from the Zechstein formation (Keesmann et al., 2005; Schwartz, 2012). It is possible that an inhibitory effect might be observed for some organisms at these concentrations. However, salinity has been found to enhance metal resistance in some halotolerant bacteria (Amoozegar et al., 2005), and haloarchaea have been shown to resist levels of selenite up to 30 mM (Güven et al., 2013).

### **Carbon substrates and energy sources**

Prior to the loss of container integrity, the sources of carbon within the repository will be limited to connate compounds. As mentioned earlier, organics may be present in the form of native, heterogeneously distributed hydrocarbons. For example in the Gorleben salt dome, these range from light gases to C40 alkanes to aromatics and from as low as 1 parts per million to over 400 parts per million (Hammer et al., 2011; Pusch et al., 2014; Bracke and Fischer-Appelt, 2015). In

general, increasing salinity leads to a decrease in hydrocarbon solubility because of a salting out effect. However, many low-molecular weight (LMW) aromatics (e.g. benzene, toluene) are highly soluble in brine, as are some aliphatics; while, high-molecular weight (HMW) polycyclic aromatics have limited solubility in water, let alone brines.

In many cases, waste drums have been stored for decades, such that within-drum degradation of waste organics may have already occurred and may continue after emplacement, but prior to brine exposure. Moisture and radioactivity would be the chief limiting factors to microbial activity within the canister. The possibility or extent of “pre-degradation” of waste organics has never been measured and is not considered in performance assessments. However, it may yield lower molecular weight compounds, such as organic acids and alcohols, which are more soluble in brine and possibly more readily degradable. “Pre-degradation” may also have generated CO<sub>2</sub> or methane within the drum that could be utilised by some organisms; although not likely once brine inundation has occurred and conditions become anoxic.

Once a container has been breached, repository organisms may enter the compromised container and/or contents may be released. Many low-molecular weight organics (LMW: e.g. EDTA, citrate, oxalate, acetate, tartrate) were used as ligands during weapons clean-up and are included in waste inventories. Other LMW organics may include surfactants or chlorinated solvents; while, HMW organics also include bitumen, graphite-containing materials and sludges. Organic inventories in HLW may contain radiocarbon compounds generated from the spent fuel matrix and cladding (Nübel et al., 2013).

While the organic inventories of L/ILW waste canisters may be very high, their actual solubilities in brine may be much lower, and solubility is a prerequisite for bioavailability. For example, oxalate solubility in high-magnesium WIPP brine was lower than inventory-calculated concentrations, and it influenced the solubility of other LMW ligands (Swanson et al., 2013b). Solubility is a significant issue for the various HMW organics that can be found in LLW repositories (i.e. cellulose, plastic and rubber). These compounds will be discussed in greater detail later.

While many microorganisms have the ability to use a broad range of substrates, not all organic carbon compounds are appropriate substrates or energy sources for all organisms. Thus, the organics present in nuclear waste may not be deemed palatable by resident organisms. Bacteria are better suited for a broader range of organic substrates, but their activity may be limited in high salt. In both cases, there are limits to degradative capability. Many haloarchaea require complex media for growth; that is, they rely on undefined components – such as amino acids, yeast extract or peptone – that cannot be quantified easily. In natural hypersaline environments, these components may derive from the breakdown of other microorganisms or may be synthesised in situ. These supplements will eventually be limited by a closed repository system.

### **Inorganic nutrients and energy sources**

Nitrate and phosphate are generally present in nuclear waste from clean-up operations, and sulphate is a natural component of subterranean salt brines, given the close association of anhydrite. Phosphate will precipitate in high-magnesium brines, thus lowering its effective availability as a nutrient. High levels of certain minerals considered essential for microbial growth (e.g. Mn, Fe, Co, Ca, Zn, Cu, W, Ni) have been measured in vitrified HLW (Meleshyn and Noseck, 2012), but whether or not these will leach into the brine and become bioavailable is uncertain.

Inorganic electron donors may also be present in, or generated from, the repository waste. These include H<sub>2</sub> and Fe(II) from waste canister corrosion or CO<sub>2</sub> from microbial processes. Sulphur exists mostly as sulphate in the surrounding geology and brines; the authors are not aware if other forms of sulphur (e.g. S<sup>0</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup>) have been measured in subterranean salt formations. CO<sub>2</sub> may be utilised by acetogens and sulphur oxidisers, and CO can be utilised aerobically by some haloarchaea.

### **Electron acceptors**

Oxygen will be present immediately after repository closure until levels decrease from the corrosion of iron canisters and microbial activity. Nitrate and organic acids in the waste, and sulphate from the surrounding geology, will be present as potential electron acceptors for any nitrate-reducing or sulphate-reducing bacteria that are capable of surviving in repository brines. If canister iron is oxidised to its +3 valence state, it too may serve as an electron acceptor.

Oxidised radionuclides, such as uranium, pertechnetate or iodate (if present in the near field), may also serve as electron acceptors (Lovley et al., 1991; Lloyd et al., 2000; Amachi et al., 2007). Although manganese oxide is a well-documented electron acceptor, its radioactive form (present as an activation product) has not been tested in this capacity. The use of other radionuclides as terminal electron acceptors for cell respiration has not been definitively proven although it has been postulated. Only manganese and pertechnetate respiration have been shown in halophilic organisms, but both cases were under alkaline conditions (i.e. high carbonate, low sodium chloride; Khijniak et al., 2003; Sorokin and Muyzer, 2010).

## Chapter 4. Predicted modes of metabolism in the repository setting

Apart from the thermodynamic constraints imposed by high salt and the limitations of subsurface existence, the repertoire of potential microbial metabolic pathways within subterranean salt-based repositories may be limited even further by i) physical confinement of the repository without input of exogenous electron acceptors (especially oxygen) and moisture (i.e. brine); ii) high ionic strength; iii) high pH (cement, magnesium oxide [MgO]); and iv) inventory (e.g. nonideal substrates, lack of usable electron acceptors). These factors may restrict or effectively eliminate many capabilities. High ionic strength alone will limit activity to halophiles in an inundation scenario; therefore, only these organisms will be discussed (i.e. processes occurring between 200-300 g/L, or 3.4 to 5 M, of sodium, as depicted in Figure 1.3).

Each type of metabolism is reviewed below for its feasibility in a salt-based repository. This section assumes that all other needs of the organism are being met (e.g. nutrients, carbon/energy source) and only addresses the mode of energy conservation. It must also assume that the waste drums have been breached and have come into contact with either salt or brine. Unlike soil or sediment environments, there may not be a clear-cut order of succession after the depletion of oxygen. The modes of metabolism below are listed in one possible order of importance. Within-drum activity prior to emplacement or breach is not discussed here.

### **Aerobic respiration (dissimilatory metabolism involving oxidation of organic carbon using molecular oxygen as the terminal electron acceptor)**

After repository closure and prior to inundation, aerobic respiration by haloarchaea, halophilic bacteria and fungi may occur and persist until oxygen is depleted. This will likely occur prior to brine inundation. If oxygen is still present after inundation, it will lead to a predominance of aerobic haloarchaea. Although this oxic period is projected to be the shortest in repository lifetime, it is likely to be when the majority of organic degradation takes place. Still, only those organics that are soluble or utilisable by the resident population will be degraded.

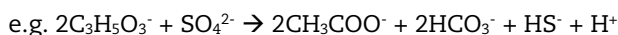
### **Nitrate reduction/denitrification (anaerobic respiration in which nitrate serves as the terminal electron acceptor, often resulting in the formation of nitrogen gas)**

Once oxygen is depleted, nitrate if present in the waste may serve as an electron acceptor. While most haloarchaea are obligate aerobes, two genera are capable of nitrate respiration (*Haloarcula* and *Haloferax*; Mancinelli and Hochstein, 1986). Only the former has been detected in subterranean halite (Norton et al., 1993). Halophilic bacteria that are able to survive in brine may also be capable of this type of metabolism, using either organics or inorganics (e.g. thiosulphate) as electron donors. Examples include *Halomonas* and *Arhodomonas* spp. (Vreeland et al., 1980; Francis and Gillow, 1993; Sorokin et al., 2011), the former having been isolated from numerous subterranean halites.

### **Fermentation**

Fermentation refers to the conservation of energy at the substrate level where the electron donor and acceptor are organics and may even be the same compound. This is the most probable means of energy conservation for extremely halophilic microorganisms within an anoxic, salt-based repository. Low-molecular weight (LMW) organics in the waste (e.g. citrate and tartrate) may serve as both electron donors and/or acceptors for fermenters. Organic waste constituents, such as cellulose, may have already undergone initial hydrolysis reactions in the drums that would yield fermentable by-products, such as glucose. Other indigenous sources of fermentable substrates are the small organics produced by halophilic bacteria for maintaining their osmotic balance in high salt (e.g. glycine betaine, glycerol, trehalose). These can serve as substrates for both haloarchaea and extremely halophilic, anaerobic bacteria (*Halanaerobiales*). This latter group is capable of fermenting a broader range of substrates, including LMW organic acids and sugars, and may be a key player in the repository, if present.

### **Sulphate reduction with incomplete organic oxidation (anaerobic respiration using sulphate as electron acceptor, usually resulting in acetate build-up):**



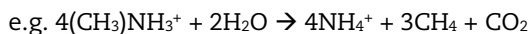
Data on bacterial sulphate reduction at high-salt concentrations are somewhat conflicting. High in situ rates of sulphate reduction have been measured at salinities as high as 25% NaCl (Porter et al., 2007); while, most laboratory studies show a decrease in rates as salinity increases (Sorokin et al., 2011; Brandt et al., 2001; Kulp et al., 2007). Additionally, fewer substrates can be oxidised at the higher salinities, such that incomplete organic oxidation and the generation of acetate as a by-product may occur (Oren, 2011). Finally, the organisms isolated from these environments seem unable to grow when transferred back into media with the same salinity as their source. It is unknown whether this discrepancy is a function of culture bias, the presence of lower-salinity niches within the source environment, or true differences between in situ and in vitro tolerance to salt



(Kjeldsen et al., 2007; Oren, 2011). It is also important to note that the above-mentioned studies investigated sediments (from brine lakes, solar salterns, salt pans). Sediment samples are extremely different from rock salt, in that they are generally richer in organics and other nutrients and are a good source of microbial inocula, especially of anaerobes.

Nevertheless, sulphate-reducing bacteria (SRB) are associated with hydrocarbon pools in salt dome caprocks (Saunders and Thomas, 1996), which emphasises the need for further work in this area. Another matter of interest is that boron has been shown to have an inhibitory effect on SRB, such that subterranean brines (e.g. generic weep brine [GWB] from Waste Isolation Pilot Plant (WIPP), ~158 mM borate) may be detrimental to their growth (Kulp et al., 2007).

### **Methanogenesis from methylated amines:**



Methanogenesis has drawn much attention from deep geological repository scientists and engineers. Methane is produced via three pathways: i) hydrogenotrophy ( $\text{H}_2/\text{CO}_2$ ); ii) acetoclasticism (acetate); and iii) methylotrophy (methylated compounds, such as methanol and methylamines). Only the latter process, using methylamines, has been found to occur in hypersaline systems; while, the first two processes are thermodynamically unfavourable and have not been shown at salt concentrations above 120 g/L, ~2 M (Ollivier et al., 1998; Waldron et al., 2007; Oren, 2011).

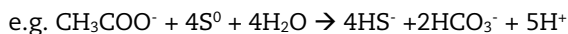
In surficial hypersaline systems, such as salterns and brine lakes, there should be a ready source of methylamines, as they can be formed from the degradation of certain organic osmolytes generated by indigenous microbiota (bacteria, algae, etc.) for their survival in high salt. However, the abundance of methylated compounds may vary in different environments with different salt concentrations (Potter et al., 2009). Studies have shown that substrate limitation is a chief cause of low rates of methanogenesis in hypersaline settings, suggesting that concentrations of methylamines are low (Kelley et al., 2012; Kelley et al., 2015). TMA and other methylated amines are volatile and can be rapidly broken down by microorganisms under both aerobic and anaerobic conditions, which may be a reason for low concentrations in nature.

Methylated amines should provide a non-competitive substrate for methanogens in hypersaline environments, given that they are not utilised by sulphate reducers. To the authors' knowledge, no studies have looked for methylamines in subterranean salt formations.

### **Homoacetogenesis**

Acetate formation from  $\text{H}_2 + \text{CO}_2$  or from lactate in the repository setting would be limited to a small group of anaerobic, halophilic bacteria (*Halanaerobiales*). As noted previously, these organisms are also capable of fermenting betaine, an osmolyte, to methylamines; thereby, generating a possible energy source for methanogens.

## Other sulphidogenic reactions



The generation of sulphides from elemental sulphur can occur at extremely high ionic strengths. Both bacteria (*Halanaerobiales*) and haloarchaea are capable of sulphur reduction, with acetate or pyruvate as the electron donor (Sorokin et al., 2011). A novel genus, and the first and only known obligately anaerobic haloarchaeon, was recently isolated; it is capable of  $\text{S}^0$  reduction as a low-energy form of respiration (Sorokin et al., 2016). Although mineral sulphates may be plentiful in subterranean salt settings, the existence of elemental sulphur or its polysulfide derivatives in these settings is unknown. However, this recent study suggests that there are repository-relevant archaeal metabolic processes yet to be discovered, even though they may be of low-energy yield.

## Reduction of other oxyanions

To the authors' knowledge, the presence of other potential electron-accepting oxyanions (e.g. selenate, arsenate, manganese oxides) in subterranean salt settings has not been investigated. Perchlorate may be a co-contaminant in nuclear wastes because of its use as a solubilising agent. Several halotolerant bacteria and even a haloarchaeon have been shown to reduce perchlorate, but rates of reduction decrease with increasing salt concentration, and nitrate is often required (Okeke et al., 2002; Chung et al., 2009; Ryu et al., 2012).

## Metabolism variation in space

The variation of microbial communities in space concerns the near field versus far field and reflects the possible variation in ionic strength in these spaces. "Far field" is an operational definition; for example, it may describe overlying, water-bearing formations or any area outside of the containment rock zone.

With differing ionic strength comes differing community compositions and, hence, different metabolic potential. Additionally, the far field may contain other, natural, substrates and nutrients that could stimulate microbial activity. Depending upon the ionic strength of the far-field environment and the hypothetical release scenarios for a particular repository concept, these organisms may have a significant impact on released waste and radionuclide migration. At lower ionic strengths, the community predominance will switch from archaea to bacteria, although some haloarchaea may survive in ionic strengths as low as 5% NaCl, because of the ability of some to switch between coping mechanisms, i.e. "salting-in" versus osmolyte synthesis or uptake (Purdy et al., 2004; Youssef et al., 2014).

As outlined earlier, the haloarchaea are restricted metabolically, such that their role in waste transformation will be limited. However, bacteria are more metabolically diverse and their dominance in the far field may lead to significant transformation of waste in that space, should a release occur.

While degradation in the far field will play no role in gas generation scenarios for the repositories, it may generate or further degrade ligands. Metal-reducing organisms may directly reduce radionuclides as well, leading to a less soluble oxidation state. Indirect radionuclide reduction may also occur as a result of reduced iron or sulphide formation or the creation of a reducing environment by fermenters.

## **Metabolism variation in time and the ability to adapt and evolve**

As mentioned earlier, there may be no well-defined succession of modes of metabolism within a salt-based nuclear waste repository. It is presumed that, once oxygen has been depleted, haloarchaeal numbers will decrease and cells will become dormant. If bacteria survive the expected high ionic strength conditions, then anaerobic respiration may occur. Fermentation will likely proceed throughout sub- to anoxic periods. The longevity of haloarchaea entrapped in fluid inclusions or in interstitial brines is well documented; thus, they may be present long into repository history (depending upon mandated lifetime) but are not likely to be active because of unfavourable growth conditions (Norton and Grant, 1988; Mormile et al., 2003; Schubert et al., 2009 and 2010).

Many have questioned whether indigenous or emplaced microorganisms will adapt over the long time frames proposed for nuclear waste repositories ( $10^4$ - $10^6$  years), eventually evolving the ability to survive under projected conditions. Directed evolution experiments (i.e. cell manipulation to mimic natural selection) have shown the ability of some organisms to adapt to specific stressors over time, and this may be the case for some repository organisms (Harris et al., 2009). However, these experiments rarely progress to extreme states of the stressor (e.g. adaptation to increases in salinity  $\neq$  adaptation to hypersaline conditions). Secondly, in order to adapt and evolve, some proportion of the population must initially survive. In many cases, cells only survive because they have become dormant, not because they have mutated; such that if regrown and re-exposed to the stressor, they will exhibit the same response as their parent population. That being said, horizontal gene transfer or genetic mutation may provide the theoretical ability for organisms to evolve specific enzymatic capabilities (e.g. evolution of citrate degradation capability by *E. coli* under aerobic conditions; Bount et al., 2012). However, this is not equivalent to evolving modes of respiration (e.g. aerobic versus anaerobic respiration). Interestingly, haloarchaea are thought to have evolved from primitive methanogens via significant lateral gene transfer events from bacteria throughout geological time (Forterre et al., 2002; Matte-Tailliez et al., 2002; Nelson-Sathi et al., 2015; Groussin et al., 2016). Still, this type of evolution occurred over a time frame much longer than a typical repository's lifetime (hundreds of millions of years), and the anaerobic capability that a haloarchaeal ancestor may have once possessed has long been lost from its genome.



## Chapter 5. Effects of microbial activity on salt-based repository performance

As mentioned in the introduction, microorganisms may influence radionuclide migration via: i) complexation of radionuclides with carbon dioxide generated from the complete oxidation of waste organic matter; ii) complexation of radionuclides with other microbially generated ligands; iii) creation of a reducing environment; iv) alteration of pH; v) redox reactions that affect radionuclide speciation; and vi) serving as colloid vectors after taking up radionuclides. Canister corrosion is also deemed deleterious in some safety cases. Many aspects discussed in this section are specific to a particular repository concept and/or nature of the waste form and may not apply to all cases.

### Oxidation of repository organic matter

A wide range of organic content is possible depending upon the repository concept and nature of the waste. In low-level waste (LLW) and transuranic (TRU) concepts (e.g. Waste Isolation Pilot Plant [WIPP]), very high and diverse organic content is present and is critical in modelling the impact of microorganisms on repository performance. Organic inventories in high-level waste (HLW) may contain radiocarbon compounds generated from the spent fuel matrix and cladding (Nübel et al., 2013). These may include low-molecular weight (LMW) alcohols and organic acids (Kaneko et al., 2003).

When organic waste is present, complete oxidation of these organics will result in the generation of carbon dioxide. Dissolved CO<sub>2</sub> may lower pH, or the resultant carbonate species can act as complexants in the pC<sub>H+</sub> range of 8-10, both of which may enhance radionuclide solubility. Additives, such as MgO, may be used to sequester CO<sub>2</sub>, thereby controlling its fugacity and buffering pH.

In order for carbon dioxide to be problematic, the microbes must be able to metabolise waste organics. As reviewed, there are few substrates present in waste that are palatable to the haloarchaea found in subterranean salt (acetate, oxalate, citrate), and this has been shown only under aerobic conditions. Halophilic bacteria are more competent degraders; however, their survival at the high-salt concentrations and low water activity expected in salt-based repositories is questionable. Of the possible HMW waste forms (cellulose, plastic, rubber and bitumen), cellulose and some LMW constituents of bitumen are the most degradable.

It is probable that waste degradation has already occurred within organic-containing canisters during storage and may continue after emplacement but prior to brine exposure. As mentioned earlier, moisture and radiation may limit the activity of emplaced organisms. The extent of within-drum “pre-degradation”, if any, is not known.

### **Cellulose degradation**

Cellulose degradation is a complex process requiring the concerted efforts of many different groups of organisms, few of which are either found or would survive in hypersaline systems. Additionally, the degradation process differs between aerobic and anaerobic environments, as the organisms within those spaces utilise different mechanisms for hydrolysis (Lynd et al., 2002; Wilson, 2011).

Initial hydrolysis of cellulose is carried out by cellulolytic organisms; following this process, saccharolytic organisms and other opportunists catabolise soluble by-products. Most fungi possess aerobic cellulolytic and lignolytic capability, but their only known anaerobic capability is in animal rumen (Lynd et al., 2002). Both aerobic (e.g. *Actinobacteria*) and anaerobic (*Firmicutes*, *Acidobacteria*) cellulolytic bacteria exist.

In general, laboratory-derived cellulose found in repository waste have been processed (e.g. paper, paper towels, Kimwipes and cardboard), although some natural and pre-treated wood may also be present. In contrast, cellulose sources in nature are derived from plant matter, contain both crystalline and amorphous regions, and are likely to be more amenable to microbial attack than treated products.

Many halophilic microorganisms possessing cellulase activity or capable of growth on cellulosic substrates have been reported. For example, two fungi isolated from subterranean halite, one with documented ligninolytic capability (*Cladosporium*; Gunde-Cimerman et al., 2009), were capable of growth on Kimwipes and carboxymethylcellulose (CMC) as the sole carbon sources (Swanson et al., 2013a).

Still, of the bacteria and fungi capable of cellulose breakdown, most are not halophilic and are unlikely to be active in high ionic strength brines, and all are obligately aerobic. Only one anaerobic, cellulolytic microorganism has been isolated from a hypersaline environment – *Halocella halocellulolytica* (Simankova and Zavarzin, 1992; Simankova et al., 1993). This organism was able to degrade cellulose (filter paper) in concentrations of NaCl up to 20% (3.4 M). It is unknown whether any significant anaerobic cellulose degradation will occur in a salt repository near field; however, lower salinities may permit utilisation in the far field.

In early cellulose degradation experiments carried out for the WIPP, Kimwipes underwent a significant change in appearance, and organic acids were produced during long-term, aerobic (Vreeland et al., 1998) and anaerobic incubations (Gillow and Francis, 2006) at high-salt concentrations. Interestingly, the organism that appeared across all inoculated incubations in the Gillow and Francis study, *Halorhabdus utahense*, is capable of fermenting glucose with the concomitant production of sulphide in the presence of elemental sulphur and also reduces nitrate, although not as a respiratory process (Wainø et al., 2000). Samples incubated with excess nitrate were found to generate more gas. (The presence of

nitrate is often necessary for anaerobic growth of haloarchaea to occur, although it may not be used for respiration.) The only genus identified in those samples that has been found capable of denitrification was *Haloarcula* (Ichiki et al., 2001). It should be noted that these studies used a combined inoculum of salt lake water, salt lake sediment, WIPP brine and WIPP muck pile salt. None of these haloarchaea has been detected thus far in WIPP brine or halite alone, but *Haloarcula* spp. have been found in other subterranean halites (e.g. in the United Kingdom and Spain; Norton et al., 1993; Park et al., 2009).

The presence of exogenous, cellulolytic bacteria introduced in waste drums themselves cannot be ruled out and, in fact, these organisms have been detected in simulated waste pits (Field et al., 2010). If any moisture were present in the drums and if the organisms are radiation resistant, they may have a chance to cause initial cellulose breakdown to products more easily metabolised by cellulase-producing bacteria should they come into contact with these by-products during early oxic periods. Again, these emplaced organisms are unlikely to be active in brine, although some may be halotolerant.

Cellulase-producing haloarchaea, including *Haloarcula*, *Halobacterium* and *Halorubrum* spp., have been isolated previously from hypersaline salt lakes and salterns (Birbir et al., 2007). While these organisms are likely to thrive at high ionic strength, their use of cellulose by-products will be limited, once again, to early oxic periods. Polysaccharide-degrading capability (e.g. xylan) among haloarchaea appears to be associated with those organisms isolated from terrestrial environments, i.e. soil (Anderson et al., 2011), although not all genera have been screened.

The possibility of dramatic increases in pH from cementitious waste will likely have a greater effect on cellulose degradation than indigenous microbiota. Chemical “peeling” reactions of cellulose under alkaline conditions have been found to generate isosaccharinic acid among other organics, only described as “dissolved organic carbon”, or DOC (Pavasars et al., 2003; Glaus and van Loon, 2008). It is possible that such DOC might comprise adequate substrates for microorganisms, but the organisms would still need to be able to survive in haloalkaline conditions.

### **Plastic and rubber degradation**

Plastics and rubber are even less soluble and less degradable than cellulose and are specifically designed to be resistant to degradation. Some organisms (members of the obligately aerobic fungi and bacteria mostly of the order *Actinomycetales* with some few of the phylum *Proteobacteria*) are capable of degrading natural and even vulcanised rubber compounds (Yikmis and Steinbuchel, 2012). It is very possible that these organisms, like cellulose degraders, are present in the emplaced waste and may have already acted on these compounds. Again, this may depend upon moisture (fungi will require less than bacteria), radiation effects, and in the case of fungi, oxygen. Still, it is unlikely that these organisms will be active at high ionic strengths.

### Other carbon sources in waste

Although cellulose, plastic and rubber are the predominant, potentially gas-generating organics listed in most safety cases, it is clear that other organics can be present in much of the LLW/ILW waste. These may include sludges containing chlorinated solvents used as degreasers during weapons clean-up (e.g. CCl<sub>4</sub>) and LMW complexants, such as ethylenediamine tetraacetic acid (EDTA), tartrate or citrate. The degradation of these compounds under hypersaline, anaerobic conditions has not been shown.

### Native hydrocarbons

The degradation of hydrocarbons by haloarchaea has gained recent attention (Al-Mailem et al., 2010; Tapilatu et al., 2010) and should be investigated further for settings in which hydrocarbons are found. Members of the *Halobacteriaceae* (*Haloferax*, *Haloarcula*, *Halococcus*, *Halobacterium* spp.) were found to utilise crude oil vapour, short and medium length *n*-alkanes, and even 2-3-ring aromatics as sole sources of carbon. Additionally, specific genes encoding for enzymes involved in aromatic degradation have been found in some haloarchaea (Erdoğan et al., 2013).

Halotolerant, hydrocarbon-degrading bacteria are well known and are frequently enriched after marine oil spills. A halophilic *Arhodomonas* sp. degraded monoaromatic hydrocarbons in salt concentrations up to 5 M (292 g/L), and other  $\gamma$ -Proteobacteria were reported to degrade LMW aromatics at salt concentrations up to 2.6 M (152 g/L). In both cases, degradation rates and extent were higher at lower salt concentrations (Sei and Fathepure, 2009; Zhao et al., 2009).

It is important to note that some of these studies were set up using hydrocarbon concentrations exceeding their solubility, were incubated in the light, were incubated at elevated temperatures, and almost always required supplementation with a complex nutrient mix. This makes it difficult to rule out precipitation, photo degradation, volatilisation, or even co-oxidation in these cases. Additionally, some haloarchaea were reported to be capable of degrading HMW polycyclic aromatics that even known PAH-degraders isolated from PAH-contaminated soils are incapable of degrading (Bonfa et al., 2011). Clearly, more work is needed to elucidate hydrocarbon degradation processes by haloarchaea, especially as they might relate to expected repository conditions.

The production of surfactants may aid microorganisms in accessing hydrocarbons in brine and overcome the need to maintain a hydrophilic exterior for survival in salt, when a hydrophobic exterior is needed for hydrocarbon uptake. Bio surfactant production by a *Bacillus* sp. was found to dramatically reduce surface tension as salt concentrations increased until it stabilised between 10-15% NaCl, (1.7-2.6 M) (Danilova et al., 2015). Two species of haloarchaea were shown to produce bio surfactants during their lag phase of growth, in response to diesel fuel exposure (Kebbouche-Gana et al., 2009).

To the authors' knowledge, no anaerobic hydrocarbon degradation studies by haloarchaea or halophilic bacteria have been documented. However, other *Arhodomonas* spp. are known to be facultatively anaerobic with nitrate as an electron acceptor, although the BTEX-degrading *Arhodomonas* was not tested for this capability (Saralov et al., 2012; Swanson et al., 2013a).



### **Bitumen degradation**

Some HLW scenarios entail encasement of the waste in a bitumen matrix or using bitumen as a sealant. Additionally, bitumen has been detected in subterranean halite fluid inclusions (Siemann and Ellendorff, 2001; Kovalevych et al., 2008). Constituents of bitumen – saturated hydrocarbons, aromatics, resins, and asphaltenes (Hansen et al., 2013) – will have extremely limited solubility in high ionic strength media. Studies have shown some bitumen fractions to be degradable and to support biofilm formation (Roffey and Norqvist, 1991; Wolf and Bachofen, 1991). In one study, rates of degradation were positively correlated to the surface area of bitumen, suggesting degradation of components that were surface-accessible and/or enhanced degradation by biofilm formation (Wolf and Bachofen, 1991). It is possible that hydrocarbon-degrading, halophilic organisms present in subterranean salt (see above) may be able to degrade certain bitumen fractions. However, it is also clear that once the easily degraded and accessible fraction is oxidised, that rates decrease significantly. The presence of ancient bitumen deposits (and ancient petroleum deposits near salt domes) and bitumen-containing fluid inclusions suggests that, clearly, not all organic carbon is mineralised.

### **Incomplete oxidation of organic carbon**

Organics that are soluble (e.g. LMW ligands) may be consumed and subsequently replaced by other LMW organics going into solution. This suggests that degradation is simply an issue of time. However, assuming that all organics are soluble and degradable, not all carbon is mineralised. Some must go towards the synthesis of biomolecules and subsequent cell growth, and some may be in the form of unusable metabolites. These metabolites may serve as ligands themselves, or their build-up in a closed repository system may be detrimental to further microbial growth.

Most of the organic carbon in waste will be recalcitrant to degradation, and possibly even more so in anaerobic, hypersaline systems. For example, cellulose fibres have been preserved in reportedly ancient fluid inclusions extracted from halite. The authors suggest that this recalcitrance to degradation was due to the lack of ionising radiation, water available for hydrolysis and microbial activity (Griffith et al., 2008).

### **Complexation of radionuclides with other microbially generated ligands**

Apart from the ligands produced as waste metabolites, microorganisms can generate other organic and inorganic ligands. Bacterial mineralisation of uranium via phosphate, as a detoxification mechanism, may result in precipitation and impede migration (Plummer and Macaskie, 1990; Tolley et al., 1992). Phosphate may also serve as a “nucleation focus” on cell surfaces (Lloyd and Macaskie, 2002). A possible association of uranium with phosphate produced by a haloarchaeon has been suggested (Francis et al., 2004).

Sulphides are also potential radionuclide ligands. Sulphidogenesis (from sulphate, thiosulphate or elemental sulphur) decreases with increasing salinity; however, it has been shown in incubations of hypersaline sediments, resulting in enrichment of known sulphate-reducing bacteria (SRB) from the *δ-Proteobacteria*, as well as *Halanaerobiaceae* (Sorokin et al., 2011). Sulphide from elemental sulphur was generated at the highest salinities by a novel haloarchaeon (Sorokin et al., 2015).

Most bacteria can produce siderophores in response to iron-limiting conditions. In contrast, five of seven tested haloarchaeal genera produced only carboxylate siderophores (Davé et al., 2006); while the genus *Halobacterium* was only able to use exogenous siderophores generated by other organisms (Hubmacher et al., 2002). Three haloarchaea isolated from saline soils were found to contain genes for siderophore synthesis, although the ability was not tested (Anderson et al., 2011). Presumably, the waste-emplaced organisms originating from soil can produce siderophores, but again, their activity is uncertain.

### Creation of a reducing environment

Creating a reducing environment may lower the oxidation state of certain multivalent radionuclides, thereby altering their solubility. Microorganisms can do this by consuming oxygen or generating hydrogen or other reductants (e.g.  $\text{Fe}^{2+}$ , sulphides). Canister corrosion is also expected to be a main contributor to hydrogen generation.

Again, in order for microorganisms to change the redox environment, they must be actively respiring or fermenting. Haloarchaea are, for the most part, obligate aerobes; thus, utilisation of oxygen will occur, if all other conditions for growth are met. Once conditions become sub- to anoxic, fermentation reactions will drive the production of hydrogen in this setting. In nature, hydrogen generation is usually coupled with  $\text{H}_2$ -utilising organisms. Of those, only acetogens (producing acetate from  $\text{H}_2 + \text{CO}_2$ ) have been shown to survive at extremely high-salt concentrations. There are no documented hydrogen gas-metabolising or generating haloarchaea (Schwartz et al., 2012). Fermentation is likely to be a dominant form of metabolism occurring in salt-based radioactive waste repositories after oxygen is utilised, and it is also likely that fermentation of waste components will occur within low- to intermediate-level waste (L/ILW) canisters, if all other conditions for survival are met.

### Alteration of pH

Changes in pH due to microbial activity can affect radionuclide solubility. The generation of  $\text{CO}_2$  and organic acids from waste degradation will lower pH and also complex radionuclides. The addition of MgO will buffer the pH of inundating brine by inducing precipitation. In general however, the solubility of  $\text{CO}_2$  in brine decreases with increasing salinity. Changes in pH may also result from hydrogen generation via fermentation, if no concomitant microbial consumption of hydrogen occurs.

## Redox reactions with iron and radionuclides

Microorganisms may influence radionuclide oxidation state (and hence, solubility) indirectly or directly by controlling the redox potential in their immediate environs, generating iron(II), hydrogen, or hydrogen sulphide, or via enzymatic reduction. There are very few data concerning metal reduction in hypersaline environments (Sorokin and Muyzer, 2010; Emmerich et al., 2012). This is likely because of the low solubility of oxidised metal species in these systems; thus, the data are generally limited to lower ionic strength systems, insoluble metal oxides in sediments, or metals associated with particulate organic matter or microbial mats.

An association has been suggested between a haloarchaeon (*Halobaculum gomorrense*) and bacteria (*Virgibacillus* and *Pontibacillus* spp.) in hypersaline sediments showing iron reduction, but this has not been elucidated (Emmerich et al., 2012). Iron reduction by SRB and other fermenters was also shown, and fermentation was presented as a possible mechanism used by the bacterial-archaeal enrichment. In a separate study, incubations of hypersaline groundwater under iron-reducing conditions also yielded cultures of *Virgibacillus* and *Pontibacillus* spp., along with a haloarchaeon, *Haloferax* (Swanson et al., 2013a).

The reduction of metals occurs primarily as a result of bacterial activity; haloarchaea have not been shown to directly reduce metals. However, metal-reducing bacteria may or may not be active in the repository near field because of the high ionic strength. The potential for redox reactions to occur within L/ILW waste drums is unknown but is, as mentioned previously, most likely limited by the absence of water.

If abiotic oxidation of zero-valent canisters occurs, then iron-oxidising microorganisms may contribute further to corrosion by generating reactive ferric oxides. Biotic ferrous iron oxidation occurs under aerobic, microaerophilic and anaerobic conditions, often concomitantly with nitrate, perchlorate, or chlorate reduction (Weber et al., 2006). Nitrate-dependent iron oxidation occurs in a variety of environments, including marine sediments, soils, and sludges, and is thought to lead to transient redox fluctuations that might also affect radionuclide solubility (Benz et al., 1998). Iron oxidisers have been detected in hypersaline lake sediments, but their level of activity could not be established (Emmerich et al., 2012); thus it is unknown whether they will play a role in salt-based repositories.

As mentioned earlier, bio reduction of radionuclides as an anaerobic respiratory process has only been shown for uranium and technetium, but reduction of other radionuclides occurs nonetheless, either advertently as a detoxification mechanism or inadvertently in response to normal cellular processes. Per technetate was reduced by a *Halomonas* sp. isolated from a hypersaline soda lake (Khijniak et al., 2003), but this is the only documented case of direct radionuclide reduction by a halophilic bacterium.

## **Biocolloid vectors**

### **Radionuclide uptake**

Uptake of radionuclides may be extracellular – as in surface sorption or nucleation and precipitation – or intracellular, via active or passive transport. If mobilised, microorganisms may contribute to radionuclide migration; if immobilised, microorganisms may become a radionuclide “sink”.

Although few surface sorption studies have been conducted at high ionic strength, significant progress has been made in this area (Ams et al., 2013; Reed et al., 2013; Bader et al., submitted). Surface sorption of metals occurs through their interaction with anionic functional groups in cell walls. This phenomenon has been shown to be dependent upon many factors, including pH; radionuclide speciation; organism type (bacterium versus archaeon) and biomass concentration; ionic strength of the test matrix, especially magnesium or calcium concentrations; and the presence or absence of strong complexants, such as EDTA. In general, increases in pH initially result in increased metal sorption, when controlled for precipitation and carbonate complexation, presumably from the increased availability of negatively charged sites on the surface of the cell coupled with a decrease in possible competition by cations, such as magnesium. However, once radionuclide speciation becomes predominantly anionic (e.g. as a result of complexation or hydrolysis), a decrease in sorption may be observed. Thus, cell surface-radionuclide interactions are not always straightforward.

Bacteria have been shown, in some studies, to adsorb more than archaea (Reitz et al., 2011), presumably because of differences in cell wall characteristics, especially S-layer structures. However, this may not hold true for all archaea. Neptunium sorption onto a halophilic bacterium in sodium perchlorate media increased with increasing ionic strength, likely as a result of increased Np(V) ion activity at higher ionic strength (Ams et al., 2013). This ionic strength dependence did not hold for complex, magnesium-containing brines, as a result of competition with Mg for cell surface sorption sites and potential changes in Np speciation due to inorganic complexation (e.g. with sulphate, carbonate or borate).

By convention, sorption experiments are generally conducted with resting cells rather than actively growing cells. In hypersaline settings, movement of ions across cell membranes occurs regardless of cell growth (especially for organisms that “salt in”), in order for the organisms to maintain osmotic balance. It is unknown whether this phenomenon has any effect on “uptake”.

The presence of strong complexants, such as EDTA, or other organic ligands may significantly decrease the extent of adsorption across a broad pH range. EDTA also has a “rounding up” effect on microorganisms that can lead to “shedding” of external layers (e.g. extracellular polymeric substance) and alter surface area (and surface area/volume ratios), all of which can affect the extent of uptake.

### **Biomass dependence**

Once again, biomass concentrations will be constrained by repository conditions and thermodynamics. Additionally, biomass concentrations do not increase in perpetuity in closed systems. They will reach a maximum, based on substrate and nutrient supply, in addition to the formation of inhibitory by-products. Haloarchaeal cultures growing under optimum aerobic conditions can reach  $\sim 10^9$  cells/mL. The reality in a salt-based repository setting is likely to be significantly fewer numbers, given a poor starting inoculum, less than ideal conditions, and an anaerobic atmosphere.

Adsorption of metals onto bacterial surfaces does not necessarily depend upon cell viability. Studies using dead archaeal biomass have not been conducted. The constant flow of osmotic-stabilising ions across the membranes of live haloarchaeal cells may lead to a difference between uptake into or onto dead cells.

### **Biomass mobility**

Motility can be used by organisms to move towards an attractive substance or away from a noxious one. Many haloarchaea possess flagella for motility and are capable of swarming. In addition, gas vesicle formation is used appreciably by these organisms in order to move vertically through the water column, presumably to areas of higher oxygen concentration or nutrient load. Still, a sessile existence as a biofilm is of more benefit to microorganisms in that it allows them to retain resources while offering protection from the external environment.

Although cells are predominantly found attached to surfaces in nature, very little research has been conducted on biofilm formation by halophilic microorganisms, especially haloarchaea (Fröls et al., 2012). That being said, over half of the strains tested by this group were able to adhere to surfaces using pili and to form biofilms. A tested *Halobacterium* sp. formed biofilms up to 80  $\mu\text{m}$  thick but was outcompeted for surface area by an introduced *Halorubrum* sp. (DiMeglio et al., 2014).

Given the self-sealing nature of salt and the assumption that organisms remain near a substrate source (i.e. waste), significant movement away from a salt-based repository can theoretically only take place during a pressurised release of brine and repository contents, such as during an intrusion scenario. In this case, inherent motility is not important, as the organisms will be entrained in a pressurised brine flow. However, lysis becomes a realistic possibility, as many haloarchaea will lyse with decreasing ionic strength or as pH's reach extremes. For instance, the Austrian *Halobacterium noricense* isolate cannot survive below 2.1 M NaCl (Gruber et al., 2004); while, the lower limit for the United States (WIPP) isolate is lower ( $\sim 1.4$  M). However, there was a significant lag period (eight weeks) before lysis occurred, suggesting that this organism has potential as a vector for radionuclide transport. These values should be determined for salt-indigenous isolates, in relation to the ionic strength of the surrounding groundwaters. If lysis occurs, the sorbed actinide may or may not precipitate with cell walls; this needs to be verified experimentally. Moreover, this lysis phenomenon may not occur with halophilic bacteria if the change in salt concentration is gradual, although it may occur at pH extremes.

### **Emplaced organisms**

It is probable that radionuclides have already been taken up (either internally or externally) by emplaced organisms within waste drums. The fate of these organisms is unknown during brine inundation. However, preliminary studies on a WIPP TRU waste isolate, *Arthrobacter* sp., have shown that it can survive intact in both generic weep brine (GWB) and ERDA brines for at least one month and can recover when subcultured back into lower ionic strength media (Swanson, unpublished). This suggests that any radionuclides attached to, or taken up by, this organism may remain so and migrate during a pressurised brine release. Furthermore, it is highly likely that there are spores within waste drums; these may also remain intact during inundation. It is unknown how many such cells are present in TRU waste.

### **Microbially induced canister corrosion**

The relative importance of canister corrosion in repository performance may be viewed differently by different regulatory agencies and safety cases. In some concepts (e.g. WIPP), the corrosion process is expected to be self-limiting as available water is completely consumed, and the generation of hydrogen, as well as reduced iron phases or species, is viewed as a benefit, since it establishes a reducing environment in which radionuclide solubility is generally not favoured (DOE/WIPP, 2014). Additionally, canisters are expected to be crushed by salt over time, but the self-sealing salt will prevent radionuclide release. In other regulatory concepts, canister integrity is a significant aspect of performance assessments. For example, corrosion may lead to the release of gaseous (e.g.  $^{14}\text{C}$  as  $^{14}\text{CO}_2$ ) and long-lived radioactive activation products, especially from high-level irradiated fuel assemblies and vitrified waste (Meleshyn and Noseck, 2012).

In either case, the chemical contribution to canister corrosion is predicted to greatly outweigh the microbial contribution, and there is still uncertainty surrounding the latter in high ionic strength systems. In abiotic, humid experiments with  $\text{CO}_2$  added to simulate microbial activity in TRU waste, the corrosion rates of iron and steel coupons were negligible (Roselle, 2013). Hydrogen generation by fermenters and hydrogen sulphide generation by SRB and other sulphidogens may play a role in canister corrosion, but the presence and activity of these organisms, as discussed previously, is uncertain (Telander, 1993). For HLW scenarios, the cycling of heated brine/water vapour may accelerate corrosion (Stauffer et al., 2012), but microbial activity may be inhibited by temperature until later in repository history. It is unknown whether the radiation field at the canister surface will be inhibitory; microbial biofilms have been observed on irradiated spent nuclear fuel cladding (Bruhn et al., 2009), although not in brine.

## Chapter 6. Incorporating microbial parameters into a repository performance assessment/safety case: Case study of the Waste Isolation Pilot Plant

### Model assumptions

Along with geochemical and geophysical parameters, the potential for microorganisms to influence repository performance must be incorporated into a safety case model (e.g. gas generation, biocolloids). Generally, several assumptions must be made about repository conditions that allow for model incorporation. These assumptions can generate simplified, yet “worst-case”, scenarios, thereby building significant conservatism into the model. They may also vary depending upon the intended purpose of the repository (e.g. low- versus high-level waste, mandated lifetime).

In the Waste Isolation Pilot Plant (WIPP) case, a first assumption is that the repository horizon will eventually become a homogeneous “soup”, where all waste components are equally accessible to all organisms. This assumption must be made for purposes of modelling solution chemistry. In reality, unless a repository becomes inundated with brine, there will more likely be localised areas of brine seepage and most waste components will be inaccessible to organisms. Microbial activity, if any in these pockets, presents a large uncertainty and is likely to be much lower than conservatively predicted.

A second assumption is that all basic requirements for microbial survival are optimally met, such that all organisms are viable and active, all types of metabolism will occur, all waste organic matter, and all indigenous carbon sources will be mineralised to carbon dioxide with no apparent contribution to biomass. This assumption has the greatest impact of all on the projected influence of microorganisms in salt-based radioactive waste repositories. Repository conditions, microbial ecology, and energy conservation in high salt have been reviewed here and suggest, again, that model assumptions of activity and capability are extremely optimistic.

A third assumption is that all organisms will take up actinides (either internally or externally; whether alive or dead), and all organisms are mobile. Again, this assumption lends conservatism to a safety case/performance model. Unlike gas generation, however, more data can easily be generated to address this supposition.

## **Incorporated model/safety case parameters at the Waste Isolation Pilot Plant**

### **Gas generation**

It is unknown whether microbial gas generation under realistic, near-field repository conditions can ever be shown. Numerous attempts to do so have failed, but input for performance assessments is necessary. In order to generate input, experiments must be manipulated beyond realistic repository conditions, thus resulting in optimistic and conservative estimates of gas generation. WIPP currently uses gas generation data obtained from experiments using a rich inoculum containing brine lake sediments (Gillow and Francis, 2006).

### **Biocolloid contribution**

Biocolloid formation is a highly variable process and, therefore, difficult to model. If models are based on toxicity data, then uptake by dead organisms must be ruled out. If models are based on actual uptake, then biomass concentration becomes the chief influencing factor. Biomass concentrations measured under optimum growth conditions will, once again, provide a conservative value for the model. The values used for biocolloid transport in the WIPP model are based on the concentration of actinide at which no cell growth was observed during toxicity testing of a *Halomonas* sp. exposed to various actinide-ligand complexes. Such values should be acceptable if the test organisms are the most relevant to the tested space (e.g. near versus far field), and the testing is conducted under expected repository conditions.

### **Near- versus far-field modelling**

Hydrology testing in the overlying Culebra has been ongoing since the inception of the WIPP, with the justification that those water-bearing formations “could potentially transport wastes to the biosphere if the proposed facility were breached” (Mercer, 1983).

Given the certainty of microbial activity in the far-field environment, safety cases should consider the far-field space as a unique “transformation zone”. This requires knowledge of the microbial communities in surrounding groundwaters or water-bearing formations. While gas generation will be difficult and probably unnecessary to model under these conditions, organic transformation, radionuclide reduction and other redox changes, and biocolloid transport can be tested.



## Chapter 7. Microbial issues lacking sufficient data

### Presence of sulphate reducers and other sulphidogens

The generation of sulphide and the precipitation of dolomite are properties of sulphate-reducing bacteria (SRB) that could be beneficial to salt-based nuclear waste repositories, in that they can lead to carbon dioxide or radionuclide sequestration. While sulphate reduction has been deemed thermodynamically feasible at high-salt concentrations, SRB have yet to be isolated or detected in subterranean halites, despite their frequent association with anhydrite and gypsum. However, the presence of oil deposits and high concentrations of hydrocarbons in domal salt formations (e.g. Gorleben) suggests that the growth of SRB may be supported. DNA sequences belonging to SRB were detected in groundwater overlying the Waste Isolation Pilot Plant (WIPP), but none have been isolated. Sulphate reduction has also been reported for the aquifers overlying Gorleben, with up to 200 g/L of total dissolved solids (Klinge et al., 2007).

Sulphidogenesis, unrelated to SRB, may still occur. Fermentation of organics by anaerobic, halophilic bacteria (*Halanaerobiales*) has been shown in the WIPP setting to cause enough change in redox potential to yield sulphide formation via iron reduction (Swanson et al., 2013a). Precipitation of dolomite has been shown at high-salt concentrations by both SRB and a haloarchaeon (Deng et al., 2010; Kenward et al., 2013). Whether or not radionuclides can be sequestered in these minerals remains to be tested, but this type of biomineralisation has been shown for calcite (Lauchner et al., 2013).

### Presence of methylated amines and methanogenesis

Methanogenesis from methylated amines has been measured at concentrations of 250 g NaCl/L, 4.3 M (Zhilina, 1986). These compounds are omnipresent in marine settings and are often derived from the solutes utilised by marine organisms, such as cyanobacteria and algae, to maintain their osmotic balance. Methylated amines provide a non-competitive substrate for methanogens, i.e. one that is not routinely utilised by SRB, allowing for the coexistence of both groups. Methylated amines tend to be volatile and may have disappeared during the formation of evaporite salt beds. Still, it would be prudent to test for the presence of these compounds in subterranean brines and in overlying groundwaters.

## Low-level waste/transuranic versus high-level waste

The majority of US research to date has focused on transuranic waste. The European safety case approaches salt-based repository design from the perspective of both high and low-level waste, and the United States is now considering salt for defence HLW and commercial spent fuel waste disposal. The major factors leading to the inhibition of microbial activity in HLW-salt repositories (apart from those of LLW) are higher temperatures and radioactivity. Both of these areas require further research to determine whether microbial effects on HLW will occur. Repository concepts in which both waste types are present but not segregated should also be addressed for microbial impact.

## Radionuclide toxicity

Since most microbial impacts are dependent upon cell viability, further investigation is warranted into the toxic or radiative effects of radionuclides on salt-indigenous microorganisms. Additionally, studies are warranted on available waste organisms and far-field organisms, as they may play a role in waste transformation prior to repository emplacement and in the far field, respectively.

## Microbial interactions with barrier components

Many repositories use clay and cement as backfill, and salt-based repositories may also use crushed salt or salt/clay mixes. To our knowledge, no work has been done to investigate the impact of salt-indigenous microorganisms on such barrier materials or the impact of the materials on the microorganisms, or the influence of clay-associated microorganisms under high ionic strength conditions.

### Clay

Clay mixed with crushed salt has been investigated as a potential backfill material for waste repositories (Popp et al., 2013). Additionally, clay is found naturally in seams in subterranean salt formations. The heterogeneity, lack of pore space and interconnectivity, moisture, and aeration within clays constrain possible microbial activity and survival. Still, microorganisms have been isolated from and biosignatures have been detected in several subsurface clay formations (Mauclair et al., 2006; Stroes-Gascoyne et al., 2007; Poulain et al., 2008; Lopez-Fernandez et al., 2015). These include clays being considered for, or intended for use in, deep geological repositories (e.g. Opalinus and Boom clays). The organisms isolated and detected are dominated by *Firmicutes* and *Actinobacteria*, but also include *Proteobacteria*. It is hypothesised that these organisms are mostly in a dormant state but that perturbations, such as excavation and transfer to a repository, could revive them (Stroes-Gascoyne et al., 2007). Most of the cultivated organisms were derived from areas exposed to moisture (walls and faults; Boivin-Jahns et al., 1996; Urios et al., 2012).

Sulphate-reducing organisms (*Desulfovibrio* sp.) have been isolated from incubations of MX-80 bentonite, a proposed backfill material (Masurat et al., 2010). The survival of clay-derived organisms at high-salt concentrations remains to be investigated, but the *Desulfovibrio* in this study were only capable of survival at 4% NaCl (0.7 M). Aerobic incubations of argillaceous halite samples from the WIPP resulted in less diverse cultures than those of near-pure halite, possibly because of the effects of clay-associated impurities. However, whether or not this will be the case when large amounts of exogenous clay are introduced is unknown.

Investigations of porosity and compactibility of wetted salt/clay (85%/15%) mixtures showed enhanced compactibility with a dramatic decrease in permeability (Popp et al., 2013). This may serve to confine microbial activity to any remaining pore space, provided all conditions are optimal.

Clays have the potential to adsorb radionuclides, even in hypersaline conditions, (Schnurr et al., 2015). It is unknown whether this leads to an enhanced toxic effect on any surviving microorganisms in the vicinity or whether the sorbed radionuclides become biologically unavailable. Studies at low ionic strength have found that Pu and U sorb preferentially to *Bacillus subtilis* cells rather than clay particles (Ohnuki et al., 2005, 2007).

## **Cement**

The presence of cement may raise the pH of any inundating brine to extremely alkaline levels that may not support microbial life. This will alter the brine chemistry as well as waste organics, specifically cellulose. Cement inventories vary throughout repository concepts.

As discussed earlier, at high enough pH (~12-13), cellulose can undergo abiotic degradation to utilisable glucose monomers and subsequently to lower molecular weight ligands, such as isosaccharinic acid (Pavasars et al., 2003). The presence of glucose at neutral pH could encourage the growth of fermenters (e.g. Halanaerobiaceae), but it is unknown whether these organisms will be viable under alkaline conditions this extreme. Halanaerobium hydrogeniformans, isolated from a soda lake, is capable of cellobiose degradation at pH 11, but other Halanaerobium spp. have circumneutral pH optima (Begemann et al., 2012). Whether or not haloalkaliphilic isosaccharinic acid-degrading organisms exist is unknown, but alkaliphilic degraders have been isolated from contaminated alkaline sediments (Bassil et al., 2014).

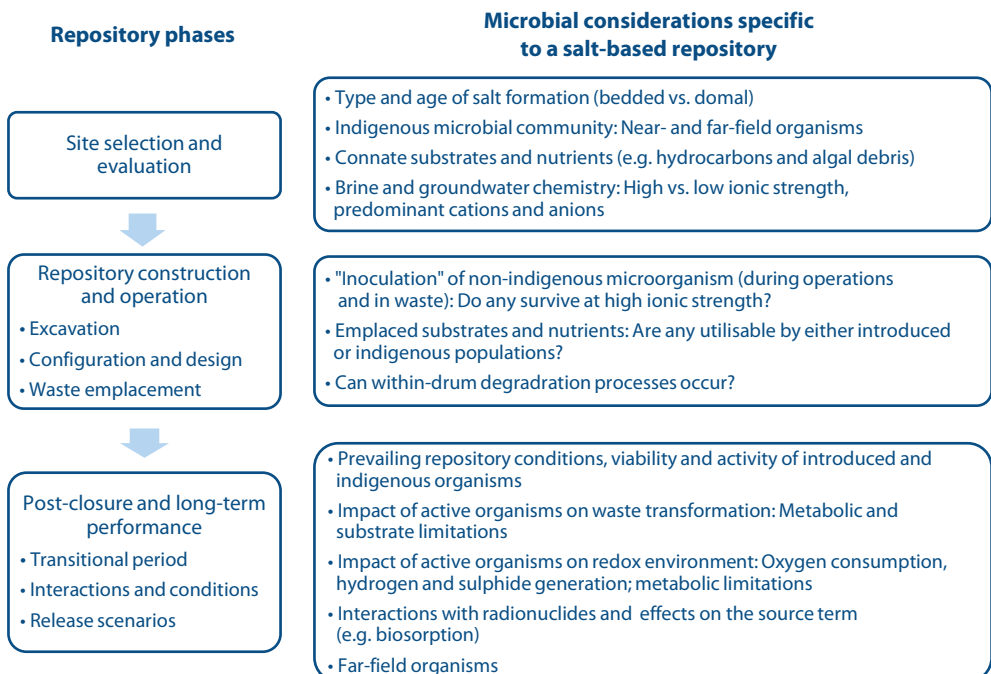


## Chapter 8. Conclusions

In order to have an effect on repository performance, microorganisms must be present and, in most cases, active. Subterranean salt settings contain a unique community of microorganisms with limited metabolic capacity. The additional constraints imposed by repository conditions, waste and barrier constituents suggest that the overall effects of such microorganisms may be severely limited in the near field.

In contrast to other deep geological settings, the usual assumptions about microbial processes do not always apply to salt-based geological settings. Salt-based repositories may have additional microbial considerations, such as those shown in Figure 8.1. The main questions for a salt-based repository concept are: will anything survive and, if it does, will it do anything?

Figure 8.1: **Repository phases and microbial issues to be considered for salt-based repositories**



Although they cannot be incorporated into a performance model, the negative results obtained when trying to grow salt-indigenous organisms under repository conditions should be viewed as meaningful. These negative findings can be supported by community characterisation studies and genome sequencing so as to determine the feasibility of microbial activity under given conditions. Areas in which data can be generated include any microbe-radionuclide interaction studies. Due diligence in all these areas can help mitigate the remaining uncertainty surrounding the effects of microorganisms on salt-based radioactive waste repositories.

## Chapter 9. References

- Ahmann, D. et al. (1994), "Microbe grows by reducing arsenic", *Nature*, Vol. 371, p. 750.
- Al-Mailem, D.M. et al. (2010), "Biodegradation of crude oil and pure hydrocarbons by extreme halophilic archaea from hypersaline coasts of the Arabian Gulf", *Extremophiles*, Vol. 14, pp. 321-328.
- Amachi, S. et al. (2007), "Dissimilatory iodate reduction by marine *pseudomonas* sp. strain SCT", *Applied and Environmental Microbiology*, Vol. 73, pp. 5725-5730.
- Amoozegar, M.A. et al. (2005), "Effect of salinity on the tolerance to toxic metals and oxyanions in native moderately halophilic spore-forming bacilli", *World Journal of Microbiology and Biotechnology*, Vol. 21, pp. 1237-1243.
- Ams, D.A. et al. (2013), "The effect of high ionic strength on neptunium (V) adsorption to a halophilic bacterium", *Geochimica et Cosmochimica Acta*, Vol. 110, pp. 45-57.
- Anderson, C. et al. (2011), "Radionuclide geomicrobiology of the deep biosphere", *Geomicrobiology Journal*, Vol. 28, pp. 540-561.
- Antón, J. et al. (2000), "Extremely halophilic bacteria in crystallizer ponds from solar salterns", *Applied and Environmental Microbiology*, Vol. 66, pp. 3052-3057.
- Bader, M. et al. (2017), "Multistage bioassociation of uranium onto an extremely halophilic archaeon revealed by a unique combination of spectroscopic and microscopic techniques", *Journal of Hazardous Materials*, Vol. 327, pp. 225-232.
- Bassil, N.M., N. Bryan and J.R. Lloyd (2014), "Microbial degradation of isosaccharinic acid at high pH", *The ISME Journal*, pp. 1-11.
- Begemann, M.B. et al. (2012), "A streamlined strategy for biohydrogen production with *halanaerobium hydrogeniformans*, an alkaliphilic bacterium", *Frontiers in Microbiology* 3, online, article 93.
- Benz, M., A. Brune and B. Schink (1998), "Anaerobic and Aerobic oxidation of ferrous iron at neutral pH by chemoheterotrophic nitrate-reducing bacteria", *Archives of Microbiology*, Vol. 169, pp. 159-165.
- Birbir, M. et al. (2007), "Extremely halophilic archaea from Tuz Lake, Turkey, and the adjacent Kaldirim and Kayacik salterns", *World Journal of Microbiology and Biotechnology*, Vol. 23, pp. 309-316.
- Blount, Z.D. et al. (2012), "Genomic analysis of a key innovation in an experimental *Escherichia coli* population", *Nature*, Vol. 489, pp. 513-520.

- Boivin-Jahns, V. et al. (1996), "Bacterial diversity in a deep-subsurface clay environment", *Applied and Environmental Microbiology*, Vol. 62, pp. 3405-3412.
- Boltyanskaya, Y.V. et al. (2007), "*Halomonas mongoliensis* sp. nov. and *Halomonas kenyensis* sp. nov., new haloalkaliphilic denitrifiers capable of N<sub>2</sub>O reduction, isolated from soda lakes", *Microbiology*, Vol. 76, pp. 739-747.
- Bonfa, M.R.L. et al. (2011), "Biodegradation of aromatic hydrocarbons by haloarchaea and their use for the reduction of the chemical oxygen demand of hypersaline petroleum produced water", *Chemosphere*, Vol. 84, pp. 1671-1676.
- Borin, S. et al. (2008), "DNA is preserved and maintains transforming potential after contact with brines of the deep anoxic hypersaline lakes of the Eastern Mediterranean Sea", *Saline Systems*, Vol. 4, pp. 10.
- Borkowski, M. et al. (2009), "Actinide (III) Solubility in WIPP Brine: Data Summary and Recommendations", LA Report LCO-ACP-08, Los Alamos National Laboratory, Carlsbad.
- Borkowski, M., M.K. Richmann and J.F. Lucchini (2012), "Solubility of An(IV) in WIPP Brine: Thorium Analog Studies in WIPP Simulated Brine", LA Report LCO-ACP-17, Los Alamos National Laboratory, Carlsbad.
- Bossemeyer, D., A. Schlosser and E.P. Bakker (1989), "Specific cesium transport via the *Escherichia coli* kup (TrkD) K<sup>+</sup> uptake system", *Journal of Bacteriology*, Vol. 171, pp. 2219-2221.
- Bowers, K.J. and J. Wiegel (2011), "Temperature and pH optima of extremely halophilic archaea: A mini-review", *Extremophiles*, Vol. 15, pp. 119-128.
- Bracke, G. (2013), "Preliminary safety analysis of the Gorleben site (VSG)", presentation given for the Actinide Brine Chemistry-SALT III Workshop, Santa Fe, New Mexico.
- Bracke, G. and K. Fischer-Appelt (2015), "Methodological Approach to a Safety Analysis of Radioactive Waste Disposal in Rock Salt: An Example", in *Progress in Nuclear Energy*, pp. 79-88.
- Brandt, K.K. et al. (2001), "Sulfate reduction dynamics and enumeration of sulfate-reducing bacteria in hypersaline sediments of the Great Salt Lake, Utah, United States", *Microbial Ecology*, Vol. 41, pp. 1-11.
- Bruhn, D.F. et al. (2009), "Microbial biofilm growth on irradiated, spent nuclear fuel cladding", *Journal of Nuclear Materials*, Vol. 384, pp. 140-145.
- Brush, L.H. (2005), "Results of Calculations of Actinide Solubilities for the WIPP Performance Assessment Baseline Calculations", Report ERMS539800, Sandia National Laboratories, Albuquerque.
- Brush, L.H. and Y.L. Xiong (2009), "Results of the Calculations of Actinide Solubilities for the WIPP", CRA 2009 PABC, Report ERMS552201, Sandia National Laboratories; Albuquerque.



- Casas, E. and T.K. Lowenstein (1989), "Diagenesis of saline pan halite: Comparison of petrographic features of modern, quaternary and permian halites", *Journal of Sedimentary Petrology*, Vol. 59, pp. 724-739.
- Chen, S. et al. (2016), "*Haloparvum sedimenti* gen. nov., sp. nov., a member of the family *Haloferaceaceae*", *International Journal of Systematic and Evolutionary Microbiology*, Vol. 66, pp. 2327-2334.
- Chung, J., S. Shin and J. Oh (2009), "Characterization of a microbial community capable of reducing perchlorate and nitrate in high salinity", *Biotechnology Letters*, Vol. 31, pp. 959-966.
- Cray, J.A. et al. (2013), "A universal measure of chaotropicity and kosmotropicity", *Environmental Microbiology*, Vol. 15, pp. 287-296.
- Davé, B.P., K. Anshuman and P. Hajela (2006), "Siderophores of Halophilic archaea and their chemical characterization", *Indian Journal of Experimental Biology*, Vol. 44, pp 340-344.
- Davila, A.F. et al. (2008), "Facilitation of endolithic microbial survival in the hyperarid core of the Atacama Desert by mineral deliquescence", *Journal of Geophysical Research*, Vol. 113, doi:10.1029/2007JG000561.
- Da Silva, F.S.P. et al. (2015), "Unexplored brazilian oceanic island host high salt tolerant biosurfactant-producing bacterial strains", *Extremophiles*, Vol. 19, pp. 561-572.
- De Las Cuevas, C. and J.J. Pueyo (1995), "The influence of mineralogy and texture in the water content of rock salt formations: Its implication in radioactive waste disposal", *Applied Geochemistry*, Vol. 10, pp. 317-327.
- Deal, D.E. et al. (1995), "Brine Sampling and Evaluation Program 1992-1993 Report and Summary of BSEP Data Since 1982", Report DOE-WIPP 94-011, Carlsbad.
- Deng, S. et al. (2010), "Microbial dolomite precipitation using sulfate reducing and halophilic bacteria: Results from Qinghai Lake, Tibetan Plateau, NW China", *Chemical Geology*, Vol. 278, pp. 151-159.
- DeVeaux, L.C. et al. (2007), "Extremely radiation-resistant mutants of a halophilic archaeon with increased single-stranded DNA-binding protein (RPA) gene expression", *Radiation Research*, Vol. 168, pp. 507-514.
- Di Meglio, L. et al. (2014), "Hyperhalophilic archaeal biofilms: Growth kinetics, structure, and antagonistic interaction in continuous culture", *Biofouling: The Journal of Bioadhesion and Biofilm Research*, Vol. 30, pp. 237-245.
- Emmerich, M. et al. (2012), "Abundance, distribution, and activity of Fe(II)-oxidizing and Fe(III)-reducing microorganisms in hypersaline sediments of Lake Kasin, Southern Russia", *Applied and Environmental Microbiology*, Vol. 78, pp. 4386-4399.
- Erdoğan, S.F. et al. (2013), "Aromatic hydrocarbon degradation by halophilic archaea isolate from Camalti Saltern, Turkey", *Water Air & Soil Pollution*, Vol. 224, p. 1449.

- Fendrihan, S. et al. (2012), "Spherical particles of halophilic archaea correlate with exposure to low-water activity – implications for microbial survival in fluid inclusions of ancient halite", *Geobiology*, Vol. 10, pp. 424-433.
- Field, E.K. et al. (2010), "Application of molecular techniques to elucidate the influence of cellulosic waste on the bacterial community structure at a simulated low-level-radioactive-waste site", *Applied and Environmental Microbiology*, Vol. 76, pp. 3106-3115.
- Fish, S.A. et al. (2002), "Recovery of 16S ribosomal RNA gene fragments from ancient halite", *Nature*, Vol. 417, pp. 432-436.
- Forterre, P. (2002), "Evolution of the archaea", *Theoretical Population Biology*, Vol. 61, pp. 409-422.
- Francis, A.J. and J.B. Gillow (1993), "Effects of Microbial Processes on Gas Generation under Expected Waste Isolation Pilot Plant Repository Conditions", Report SAND93-7036, Brookhaven National Laboratory, Upton, New York, Sandia National Laboratories, Albuquerque.
- Francis, A.J. et al. (1998), "Role of bacteria as biocolloids in the transport of actinides from a deep underground radioactive waste repository", *Radiochimica Acta*, Vol. 82, pp. 347-354.
- Francis, A.J. et al. (2004), "Uranium association with halophilic and non-halophilic bacteria and archaea", *Radiochimica Acta*, Vol. 92, pp. 481-488.
- Fredrickson, J.K. et al. (2004), "Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the Hanford site, Washington State", *Applied and Environmental Microbiology*, Vol. 70, pp. 4230-4241.
- Fröls, S., M. Dyall-Smith and F. Pfeifer (2012) "Biofilm formation by haloarchaea", *Environmental Microbiology*, Vol. 14, pp. 3159-3174.
- Fujimoto, K. and T. Morita (2006), "Aerobic removal of technetium by a marine halomonas strain", *Applied and Environmental Microbiology*, Vol. 72, pp. 7922-7924.
- Gillow, J.B. and A.J. Francis (2006), "Microbial Gas Generation Under Expected Waste Isolation Pilot Plant Repository Conditions: Final Report", Report BNL-96148-2011-I.R, Brookhaven National Laboratory, Brookhaven, New York.
- Glaus, M.A. and L.R. van Loon (2008) "Degradation of Cellulose under alkaline conditions: New insights from a 12 years degradation study", *Environmental Science and Technology*, Vol. 42, pp. 2906-2911.
- Gorby, Y.A., F. Caccavo Jr and H. Bolton Jr (1998), "Microbial reduction of cobalt<sup>III</sup>EDTA<sup>-</sup> in the presence and absence of manganese(IV) oxide", *Environmental Science and Technology*, Vol. 32, pp. 244-250.
- Gramain, A. et al. (2011), "Archaeal diversity along a subterranean salt core from the Salar Grande (Chile)", *Environmental Microbiology*, Vol. 13, pp. 2105-2121.

- Grambow, B. (2008), "Mobile fission and activation products in nuclear waste disposal", *Journal of Contaminant Hydrology*, Vol. 102, pp. 180-186.
- Grant, W.D. (2004), "Life at low-water activity", *Philosophical Transactions of the Royal Society of London*, Vol. 359, pp. 1249-1267.
- Griffith, J.D. et al. (2008), "Discovery of abundant cellulose microfibers encased in 250 Ma permian halite: A macromolecular target in the search for life on other planets", *Astrobiology*, Vol. 8, pp. 215-228.
- Groussin, M. et al. (2016), "Gene acquisitions from bacteria at the origins of major archaeal clades are vastly overestimated", *Molecular Biology and Evolution*, Vol. 33, pp. 305-310.
- Gruber, C. et al. (2004), "*Halobacterium noricense* sp. nov., an archaeal isolate from a bore core of an alpine permian salt deposit, classification of *Halobacterium* sp. NRC-1 as a strain of *H. salinarum* and emended description of *H. salinarum*", *Extremophiles*, Vol. 8, pp. 431-439.
- Gunde-Cimerman, N., J. Ramos and A. Plemenitas (2009), "Halotolerant and halophilic fungi", *Mycological Research*, Vol. 113, pp. 1231-1241.
- Güven, K. et al. (2013), "Isolation and identification of selenite reducing archaea from Tuz (Salt) Lake in Turkey", *Journal of Basic Microbiology*, Vol. 53, pp. 397-401.
- Hallsworth, J.E. et al. (2007), "Limits of life in MgCl<sub>2</sub>-containing environments: Chaotropicity defines the window", *Environmental Microbiology*, Vol. 9, pp. 801-813.
- Hammer, J. et al. (2012), *Untersuchungen von Kohlenwasserstoffen im Erkundungsbergwerk Gorleben – Interim Report 2011*, Bundesanstalt für Geowissenschaften und Rohstoffe, Hannover.
- Hansen, F.D. and C.D. Leigh (2011), "Salt Disposal of Heat-Generating Nuclear Waste", Report SAND2011-0161, Sandia National Laboratories, Albuquerque.
- Hansen, J.S. et al. (2013), "Four-component united-atom model of bitumen", *The Journal of Chemical Physics*, Vol. 138, doi: 10.1063/1.4792045.
- Harris, D.R. et al. (2009), "Directed evolution of ionizing radiation resistance in *Escherichia coli*", *Journal of Bacteriology*, Vol. 191, pp. 5240-5252.
- Harvie, C.E. et al. (1980), "Evaporation of seawater: Calculated mineral sequences", *Science*, Vol. 208, pp. 498-500.
- Hubmacher, D., B.J. Matzanke and S. Anemüller (2002), "Investigations of iron uptake in *Halobacterium salinarum*", *Biochemical Society Transactions*, Vol. 30, pp. 710-712.
- Ichiki, H. et al. (2001), "Purification, Characterization, and genetic analysis of Cu-containing dissimilatory nitrite reductase from a denitrifying, halophilic archaeon, *Haloarcula marismortui*", *Journal of Bacteriology*, Vol. 183, pp. 4149-4156.

- Icopini, G.A. et al. (2009), "Plutonium(V/VI) Reduction by the metal-reducing bacteria *Geobacter metallireducens* GS-15 and *Shewanella oneidensis* MR-1", *Applied and Environmental Microbiology*, Vol. 75, pp. 3641-3647.
- Kaneko, S. et al. (2003), "A Study on the Chemical Forms and Migration Behavior of Carbon-14 Leached from the Simulate Hull Waste in the Underground Condition", *Proceedings of the Materials Research Society Symposium 757: II3.8.1-II3.8.7*.
- Kebbouche-Gana, S. et al. (2009), "Isolation and characterization of halophilic archaea able to produce biosurfactants", *Journal of Industrial Microbiology and Biotechnology*, Vol. 36, pp. 727-738.
- Keessmann, S. et al. (2005), *Modellrechnungen zur Langzeitsicherheit von Endlagern I Salz- und Granitformationen*, Report GRS-206, Gesellschaft für Anlagen und Reaktorsicherheit, Germany.
- Kelley, C.A. et al. (2014), "Trimethylamine and organic matter additions reverse substrate limitation effects on the  $\delta^{13}\text{C}$  values of methane produced in hypersaline microbial mats", *Applied and Environmental Microbiology*, Vol. 80, pp. 7613-7323.
- Kelley, C.A., J.P. Chanton and B.M. Bebout (2015), "Rates and pathways of methanogenesis in hypersaline environments as determined by  $^{13}\text{C}$ -labeling", *Biogeochemistry*, Vol. 126, pp. 329-341.
- Kenward, P.A. et al. (2013), "Ordered low-temperature dolomite mediated by carboxyl-group density of microbial cell walls", *American Association of Petroleum Geologists Bulletin*, Vol. 97, pp. 2113-2125.
- Khijniak, T.V., N.N. Medvedeva-Lyalikova and M. Simonoff (2003), "Reduction of pertechnetate by haloalkaliphilic strains of *Halomonas*", *FEMS Microbiology Ecology*, Vol. 44, pp. 109-115.
- Kienzler, B. et al. (2012), "Radionuclide Source Term for HLW Glass, Spent Nuclear Fuel, and Compacted Hulls and End Pieces (CSD-C Waste)", *KIT Scientific Reports 7624*, KIT Scientific Publishing, Karlsruhe.
- Kish, A. et al. (2009), "Salt shield: Intracellular salts provide cellular protection against ionizing radiation in the halophilic archaeon, *Halobacterium salinarum* NRC-1", *Environmental Microbiology*, Vol. 11, pp. 1066-1078.
- Kish, A. et al. (2012), "High-pressure tolerance in *Halobacterium salinarum* NRC-1 and other non-piezophilic prokaryotes", *Extremophiles*, Vol. 16, pp. 355-361.
- Kjeldsen, K.U. et al. (2007), "Diversity of sulfate-reducing bacteria from an extreme hypersaline sediment, Great Salt Lake (Utah)", *FEMS Microbiology Ecology*, Vol. 60, pp. 287-298.
- Klinge, H. et al. (2007), *Description of the Gorleben Site Part 1: Hydrogeology of the Overburden of the Gorleben Salt Dome*, Bundesanstalt für Geowissenschaften und Rohstoffe; Hanover.

- Knauth, L.P. and M.B. Kumar (1981), "Trace water content of salt in Louisiana salt domes", *Science*, Vol. 213, pp. 1005-1007.
- Kovalevych, V.M. et al. (2008), "Geochemical aureoles around oil and gas accumulations in the Zechstein (Upper Permian) of Poland: Analysis of fluid inclusions in halite and bitumens in rock salt", *Journal of Petroleum Geology*, Vol. 31, pp. 245-262.
- Kulp, T.R. et al. (2007), "Effects of imposed salinity gradients on dissimilatory arsenate reduction, sulfate reduction, and other microbial processes in sediments from two California soda lakes", *Applied and Environmental Microbiology*, Vol. 73, pp. 5130-5137.
- Lauchner, E.G. et al. (2013), "Bacterially induced calcium carbonate precipitation and strontium coprecipitation in a porous media flow system", *Environmental Science and Technology*, Vol. 47, pp. 1557-1564.
- Lloyd, J.R. et al. (2000), "Direct and Fe(II)-mediated reduction of technetium by Fe(III)-reducing bacteria", *Applied and Environmental Microbiology*, Vol. 66, pp. 3743-3749.
- Lloyd, J.R. and L.E. Macaskie (2002), "Biochemical basis of microbe-radionuclide interactions", *Interactions of Microorganisms with Radionuclides*, Keith-Roach, M.J. and Livens F.R. (eds), Elsevier Science Ltd, London.
- Lloyd, J.R. and G.M. Gadd (2011), "The Geomicrobiology of radionuclides", *Geomicrobiology Journal*, Vol. 28, pp. 383-386.
- Lopez-Fernandez, M. et al. (2015), "Bacterial diversity in bentonites, engineered barrier for deep geological disposal of radioactive wastes", *Microbial Ecology*, Vol. 70, pp. 922-935.
- Lovley, D.R. et al. (1991), "Microbial reduction of uranium", *Nature*, Vol. 350, pp. 413-416.
- Lynd, L.R. et al. (2002), "Microbial cellulose utilization: Fundamentals and biotechnology", *Microbiology and Molecular Biology Reviews*, Vol. 66, pp. 506-577.
- McCabe, A. (1990), "The potential significance of microbial activity in radioactive waste disposal", *Experientia*, Vol. 46, pp. 779-787.
- Maltman, C., M.D. Piercey-Normore and V. Yurkov (2015), "Tellurite-, tellurate-, and selenite-based anaerobic respiration by strain CM-3 Isolated from gold mine tailings", *Extremophiles*, Vol. 19, pp. 1013-1019.
- Matte-Tailliez, O. et al. (2002), "Archaeal phylogeny based on ribosomal proteins", *Molecular Biology and Evolution*, Vol. 19, pp. 631-639.
- McGenity, T.J. et al. (2000), "Origins of halophilic microorganisms in ancient salt deposits", *Environmental Microbiology*, Vol. 2, pp. 243-250.
- Macaskie, L.E. et al. (2000), "Enzymically mediated bioprecipitation of uranium by a *Citrobacter* sp.: A concerted role for exocellular lipopolysaccharide and associated phosphatase in biomineral formation", *Microbiology*, Vol. 146, pp. 1855-1867.

- Macaskie, L.E. and J.R. Lloyd (2002), "Microbial interactions with radioactive wastes and potential applications", *Interactions of Microorganisms with Radionuclides*, Keith-Roach M.J. and Livens F.R. (eds), Elsevier Science Ltd, London.
- Mancinelli, R.L. and L.I. Hochstein (1986), "The occurrence of denitrification in extremely halophilic bacteria", *FEMS Microbiology Letters*, Vol. 35, pp. 55-58.
- Marsh, K.B. (2010), "Reflections on the solubility of cellulose", *Industrial Engineering and Chemical Research*, Vol. 49, pp. 11121-11130.
- Martin, D.D., D.H. Bartlett and M.F. Roberts (2002), "Solute accumulation in the deep-sea bacterium *Photobacterium profundum*", *Extremophiles*, Vol. 6, pp. 507-514.
- Masurat, P., S. Eriksson and K. Pedersen (2010), "Evidence of indigenous sulphate-reducing bacteria in commercial Wyoming bentonite MX-80", *Applied Clay Science*, Vol. 47, pp. 51-57.
- Mauclaire, L. et al. (2007), "Detection and cultivation of indigenous microorganisms in mesozoic claystone core samples from the opalinus clay formation (Mont Terri Rock Laboratory)", *Physics and Chemistry of the Earth*, Vol. 32, pp. 232-240.
- Meleshyn A. and U. Noseck (2012), *Radionuclide Inventory of Vitrified Waste After Spent Nuclear Fuel Reprocessing at La Hague*, Report GRS-294, Gesellschaft für Anlagen und Reaktorsicherheit, Germany.
- Mercer, J.W. (1983), "Geohydrology of the Proposed Waste Isolation Pilot Plant Site, Los Medanos Area, Southeastern New Mexico", Title 40 CFR Part 191, Compliance Certification Application for the Waste Isolation Pilot Plant, Appendix HYDRO, US DOE-Carlsbad Area Office, Carlsbad, New Mexico.
- Michaud, R.N. and P.J. VanDemark (1967), "The bacterial content of mined, evaporated granulated and solar salt", *Food Technology*, Vol. 21, pp. 89-90.
- Molina-Höppner, A. et al. (2004), "Protective effect of sucrose and sodium chloride for *Lactococcus lactis* during sublethal and lethal high-pressure treatments", *Applied and Environmental Microbiology*, Vol. 70, pp. 2013-2020.
- Mormile, M.R. et al. (2003), "Isolation of *Halobacterium salinarum* retrieved directly from halite brine inclusions", *Environmental Microbiology*, Vol. 5, pp. 1094-1102.
- Mormile, M.R., B.Y. Hong and K.C. Benison (2009), "Molecular analysis of the microbial communities of mars analog lakes in Western Australia", *Astrobiology*, Vol. 9, pp. 919-930.
- Müller, J.A. and S. DasSarma (2005), "Genomic analysis of anaerobic respiration in the *Halobacterium* sp. strain NRC-1: Dimethyl sulfoxide and trimethylamine N-oxide as terminal electron acceptors", *Journal of Bacteriology*, Vol. 187, pp. 1659-1657.
- Nelson-Sathi, S. et al. (2015), "Origins of major archaeal clades correspond to gene acquisitions from bacteria", *Nature Letters*, Vol. 517, pp. 77-80.

- Nicholson, W.L. et al. (2000), "Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments", *Microbiology and Molecular Biology Reviews*, Vol. 64, pp. 548-572.
- Norton, C.F. and W.D. Grant, (1988), "Survival of halobacteria within fluid inclusions in salt crystals", *Journal of General Microbiology*, Vol. 134, pp. 1365-1373.
- Norton, C.F., T.J. McGenity and W.D. Grant (1993), "Archaeal halophiles (halobacteria) from two british salt mines", *Journal of General Microbiology*, Vol. 139, pp. 1077-2081.
- Nübel, A. et al. (2013), *Aspects on the Gas Generation and Migration in Repositories for High Level Waste in Salt Formations*, Gesellschaft für Anlagen und Reaktorsicherheit-303, Germany.
- NEA (2007), *Mobile Fission and Activation Products in Nuclear Waste Disposal – Workshop Proceedings, La Baule, France, 16-19 January 2007*, NEA No. 6310, OECD, Paris.
- Ohnuki, T. et al. (2005), "Interactions of uranium with bacteria and kaolinite clay", *Chemical Geology*, Vol. 220, pp. 237-243.
- Ohnuki, T. et al. (2007), "Chemical speciation and association of plutonium with bacteria, kaolinite clay, and their mixture", *Environmental Science and Technology*, Vol. 41, pp. 3134-3139.
- Okeke, B.C., T. Giblin and W.T. Frankenberger Jr (2002), "Reduction of perchlorate and nitrate by salt tolerant bacteria", *Environmental Pollution*, Vol. 118, pp. 357-363.
- Ollivier, B.J. et al. (1998), "*Methanocalculus halotolerans* gen. nov., sp. nov. isolated from an oil-producing well", *International Journal of Systematic Bacteriology*, Vol. 48, pp. 821-828.
- Oremland, R.S. et al. (2000), "Bacterial dissimilatory reduction of arsenate and sulfate in meromictic mono lake, California", *Geochimica et Cosmochimica Acta*, Vol. 64, pp. 3073-3084.
- Oren, A. (1999), "Bioenergetic aspects of halophilism", *Microbiology and Molecular Biology Reviews*, Vol. 63, pp. 334-348.
- Oren, A. (2006), "Life at high salt concentrations", *The Prokaryotes 2006*, S. Falkow, E. Rosenberg, K.-H. Schleifer, E. Stackebrandt, M. Dworkineds, Springer, pp. 263-282, New York.
- Oren, A. (2011), "Thermodynamic limits to microbial life at high salt concentrations", *Environmental Microbiology*, Vol. 13, pp. 1908-1923.
- Oren, A. (2012), "Life at high salt concentrations", *The Prokaryotes*, E. Rosenberg, E.F. De Long, S. Lory, E. Stackebrandt, F. Thompson (eds), Springer-Verlag, Berlin, pp. 421-440.
- Park J.S. et al. (2009), "Haloarchaeal diversity in 23, 121, and 419 MYA salts", *Geobiology*, Vol. 7, pp. 515-523.

- Pavasars, I. et al. (2003), "Alkaline degradation of cellulose: Mechanisms and kinetics", *Journal of Polymers and the Environment*, Vol. 11, pp. 39-47.
- Pedersen, K. (1999), "Subterranean microorganisms and radioactive waste disposal in Sweden", *Engineering Geology*, Vol. 52, pp. 163-176.
- Pedersen, K. (2002), "Microbial processes in the disposal of high level radioactive waste 500 m underground in Fennoscandian shield rocks", *Interactions of Microorganisms with Radionuclides*, Keith-Roach M.J. and Livens F.R. (eds), Elsevier Science Ltd, London.
- Pedersen, K. (2005), "Microorganisms and their influence on radionuclide migration in igneous rock environments", *Journal of Nuclear and Radiochemical Sciences*, Vol. 6, pp. 11-15.
- Pironon, J. et al. (1995), "Organic inclusions in salt. Part I: Solid and liquid organic matter, carbon dioxide and nitrogen species in fluid inclusions from the Bresse basin (France)", *Organic Geochemistry*, Vol. 23, pp. 391-402.
- Plummer, E.J. and L.E. Macaskie (1990), "Actinide and lanthanum toxicity towards a *Citrobacter* sp.: Uptake of lanthanum and a strategy for the biological treatment of liquid wastes containing plutonium", *Bulletin of Environmental Contamination and Toxicology*, Vol. 44, pp. 173-180.
- Popp, T. et al. (2013), "Improved crushed salt/clay backfill", *Proceedings of the 4<sup>th</sup> US-German Workshop on Salt Repository Research, Design, and Operation*, September, Berlin.
- Porter, D., A.N. Roychoudhury and D. Cowan (2007), "Dissimilatory sulfate reduction in hypersaline coastal pans: activity across a salinity gradient", *Geochimica et Cosmochimica Acta*, Vol. 71, pp. 5102-5116.
- Posey, H.H. and J.R. Kyle (1988), "Fluid-rock interactions in the salt dome environment: An introduction and review", *Chemical Geology*, Vol. 74, pp. 1-24.
- Potter, E.G., B.M. Bebout and C.A. Kelley (2009), "Isotopic composition of methane and inferred methanogenic substrates along a salinity gradient in a hypersaline microbial mat system", *Astrobiology*, Vol. 9, pp. 383-390.
- Poulain, S. et al. (2008), "Microbial investigations in opalinus clay, an argillaceous formation under evaluation as a potential host rock for a radioactive waste repository", *Geomicrobiology Journal*, Vol. 25, pp. 240-249.
- Purdy, K.J. et al. (2004), "Isolation of haloarchaea that grow at low salinities", *Environmental Microbiology*, Vol. 6, pp. 591-595.
- Pusch, M. et al. (2014), "Macro- and microscale distribution of hydrocarbons in the Staßfurt Hauptsalz of the Gorleben salt dome", *German Journal of Geosciences*, Vol. 165, pp. 3-14.
- Radax C., C. Gruber and H. Stan-Lotter (2001), Novel haloarchaeal 16S rRNA "gene sequences from alpine Permo-Triassic rock salt", *Extremophiles*, Vol. 5, pp. 221-228.



- Rai, D. et al. (1995), "Solubilities of Actinide Solids under Oxic Conditions", Report SAND 94-1949, Sandia National Laboratories, Albuquerque.
- Reed, D.T. et al. (2013), "Intrinsic, Mineral, and Microbial Colloid Enhancement Parameters for the Actinide Source Term", LA Report LCO-ACP-18, Los Alamos National Laboratory, Carlsbad.
- Reitz, T. et al. (2011), "Bioaccumulation of U(VI) by *Sulfolobus acidocaldarius* under moderate acidic conditions", *Radiochimica Acta*, Vol. 99, pp. 543-553.
- Roedder, E. (1984), "The fluids in salt", *American Mineralogist*, Vol. 69, pp. 413-439.
- Roffey, R. and A. Norqvist (1991), "Biodegradation of bitumen for nuclear waste disposal", *Experientia*, Vol. 47, pp. 539-542.
- Roohi, A. et al. (2012), "Preliminary isolation and characterization of halotolerant and halophilic bacteria from salt mines of Karak, Pakistan", *Pakistan Journal of Botany*, Vol. 44, pp. 365-370.
- Roselle, G.T. (2013), "Determination of Corrosion Rates from Iron/Lead Corrosion Experiments to be used for Gas Generation Calculations", Report ERMS 559077, Sandia National Laboratories; Albuquerque.
- Ryu, H.W. et al. (2012), "Reduction of perchlorate by salt tolerant bacterial consortia", *Bioresource Technology*, Vol. 103, pp. 279-285.
- Sale, A.J.H., G.W. Gould and W.A. Hamilton (1970), "Inactivation of Bacterial spores by hydrostatic pressure", *Journal of General Microbiology*, Vol. 60, pp. 323-334.
- Sankaranarayanan, K. et al. (2011), "Ancient microbes from halite fluid inclusions: Optimized surface sterilization and DNA extraction", *PLoS ONE*, Vol. 6 (6), doi:10.1371/journal.pone.0020683.
- Saralov, A.I. et al. (2012), "*Arhodomonas recens* sp. nov., a halophilic alkane-utilizing hydrogen-oxidizing bacterium from the brines of flotation enrichment of potassium minerals", *Microbiology*, Vol. 81, pp. 582-588.
- Saunders, J.A. and R.C. Thomas (1996), "Origin of 'exotic' minerals in Mississippi salt dome cap rocks: Results of reaction-path modeling", *Applied Geochemistry*, Vol. 11, pp. 667-676.
- Schubert, B.A., T.K. Lowenstein and M.N. Timofeeff (2009), "Microscopic identification of prokaryotes in modern and ancient halite, Saline Valley and Death Valley, California", *Astrobiology*, Vol. 9, pp. 467-482.
- Schubert, B.A. et al. (2010), "Halophilic archaea cultured from ancient halite, Death Valley, California", *Environmental Microbiology*, Vol. 12, pp. 440-454.
- Schnurr, A. et al. (2015), "Sorption of Cm(III) and Eu(III) onto clay minerals under saline conditions: Batch Adsorption, laser-fluorescence spectroscopy and modeling", *Geochimica et Cosmochimica Acta*, Vol. 151, pp. 192-202.

- Schwartz, E., J. Fritsch and B. Friedrich (2012), "H<sub>2</sub>-metabolizing prokaryotes", *The Prokaryotes*, Rosenberg E., M. Dworkin, S. Falkow, K.-H. Schleifer, E. Stackebrandt, (eds), Springer-Verlag, Berlin, doi 10.1007/978-3-642-30141-4\_65.
- Schwartz, M.O. (2012), "Modelling groundwater contamination above high-level nuclear waste repositories in salt, granitoid and clay", *Radioactive Waste* (R Abdel Rahman ed.)
- Sei, A and B.Z. Fathepure (2009), "Biodegradation of BTEX at high salinity by an enrichment culture from hypersaline sediments of Rozel Point at Great Salt Lake", *Journal of Applied Microbiology*, Vol. 107, pp. 2001-2008.
- Shahmohammadi, H.R. et al. (1998), "Protective roles of bacterioruberin and intracellular KCl in the resistance of *Halobacterium salinarum* against DNA-damaging agents", *Journal of Radiation Research*, Vol. 39, pp. 251-262.
- Sharma, K. et al. (2012), "The *ros R* Transcription factor is required for gene expression dynamics in response to extreme oxidative stress in a hypersaline-adapted archaeon", *BMC Genomics*, Vol. 13, pp. 351.
- Siemann, M.G. and B. Ellendorff (2001), "The composition of gases in fluid inclusions of late permian (zechstein) marine evaporites in northern Germany", *Chemical Geology*, Vol. 173, pp. 31-44.
- Simankova, M.V. and G.A. Zavarzin (1992), "Anaerobic degradation of cellulose from Lake Sivash and hypersaline lagoons of the Arabat Spit", *Microbiologiya*, Vol. 61, pp. 288-292.
- Simankova, M.V. et al. (1993), "*Halocella cellulolytica* gen. nov., sp. nov. a new obligately anaerobic halophilic, cellulolytic bacterium", *Systematic and Applied Microbiology*, Vol. 16, pp. 385-389.
- Sorokin, D.Y. and G. Muyzer (2010), "Bacterial dissimilatory MnO<sub>2</sub> reduction at extremely haloalkaline conditions", *Extremophiles*, Vol. 14, pp. 41-46.
- Sorokin, D.Y. et al. (2011), "Sulfidogenesis in hypersaline chloride-sulfate lakes of kulunda steppe (Altai, Russia)", *FEMS Microbiology Ecology*, Vol. 79, pp. 445-453.
- Sorokin, D.Y. et al. (2014), "Microbial diversity and biogeochemical cycling in soda lakes", *Extremophiles*, Vol. 18, pp. 791-809.
- Sorokin, D.Y. et al. (2016), "Elemental sulfur and acetate can support life of a novel strictly anaerobic haloarchaeon", *The ISME Journal*, Vol. 10, pp. 240-252.
- Stan-Lotter, H. et al. (1999), "Very similar strains of *Halococcus salifodinae* are found in geographically separated Permo-Triassic salt deposits", *Microbiology*, Vol. 145, pp. 3565-3574.
- Stan-Lotter, H. et al. (2002), "*Halococcus dombrowskii* sp. nov., an archaeal isolate from a permian alpine salt deposit", *International Journal of Systematic and Evolutionary Microbiology*, Vol. 52, pp. 1807-1814.

- Stauffer, P., D. Harp and B.A. Robinson (2012), "Model Development and Analysis of the Fate and Transport of Water in a Salt-Based Repository", LANL Report LA-UR-12-25050, Los Alamos National Laboratory, Los Alamos.
- Strietelmeier, B.A. et al. (1999), "Toxicity of actinides to bacterial strains isolated from the Waste Isolation Pilot Plant (WIPP) environment", in *Actinide Speciation in High Ionic Strength Media*, D.T. Reed, S.B. Clark, L. Rao, (eds), Kluwer Academic/Plenum Publishers, New York.
- Stroes-Gascoyne, S. et al. (2007), "Microbial community analysis of opalinus clay drill core samples from the Mont Terri underground research laboratory, Switzerland", *Geomicrobiology Journal*, Vol. 24, pp. 1-17.
- Swanson, J.S. et al. (2013a), "Microbial Characterization of Halite and Groundwater Samples from the WIPP", Report LA-UR-13-26280, Los Alamos National Laboratory, Carlsbad.
- Swanson, J.S. et al. (2013b), "Degradation of organic complexing agents by halophilic microorganisms in brines", *Geomicrobiology Journal*, Vol. 30, pp. 189-198.
- Tapilatu, Y.H. et al. (2010), "Isolation of hydrocarbon-degrading extremely halophilic archaea from and uncontaminated hypersaline pond", *Extremophiles*, Vol. 14, pp. 225-231.
- Telander, M.R. and R.E. Westerman (1993), "Hydrogen Generation by Metal Corrosion in Simulated Waste Isolation Pilot Plant Environments", Report SAND92-7347, Sandia National Laboratories, Albuquerque.
- Thies, A. and J.W. Schultze (1996), "Corrosion and passivity of hastelloy C4 in a high salinity brine", *Materials and Corrosion*, Vol. 47, pp. 146-153.
- Tolley, M.R., P. Smyth and L.E. Macaskie (1992), "Metal toxicity effects the biological treatment of aqueous metal wastes: is a biocatalytic system feasible for the treatment of wastes containing actinides?", *Journal of Environmental Science and Health*, A27, pp. 515-532.
- Tregoning, G.S. et al. (2015), "A halophilic bacterium inhabiting the warm, CaCl<sub>2</sub>-rich brine of the perennially ice-covered Lake Vanda, McMurdo Dry Valleys, Antarctica", *Applied and Environmental Microbiology*, Vol. 81, pp. 1988-1995.
- Umeki, H. (2009), "Holistic assessment to put mobile radionuclides in perspective", *Mobile Fission and Activation Products in Nuclear Waste Disposal – Workshop Proceedings, La Baule, France, 16-19 January 2007*, NEA No. 6310, OECD, Paris.
- DOE/WIPP (2014), "Title 40 CFR Part 191 Subparts B and C Compliance Recertification Application 2014 for the Waste Isolation Pilot Plant", DOE/WIPP-14-3503, Carlsbad.
- Urios, L. et al. (2012), "Microbial diversity of the 180 million-year-old toarcian argillite from Tournemire, France", *Applied Geochemistry*, Vol. 27, pp. 1442-1450.
- Van der Wielen, P.W.J.J. et al. (2005), "The enigma of prokaryotic life in deep hypersaline anoxic basins", *Science*, Vol. 307, pp. 121-123.

- Vanlint, D. et al. (2011), "Rapid acquisition of gigapascal-high-pressure resistance by *Escherichia coli*", *mBio*, Vol. 2(1), online journal.
- Van Soest G.D. (2012), "Performance Assessment Inventory Report (PAIR-2012)", LA-UR-12-26643, Report INV-PA-12, Rev 0, Los Alamos National Laboratory; Carlsbad.
- Vreeland, R.H. et al. (1998), "Distribution and diversity of halophilic bacteria in a subsurface salt formation", *Extremophiles*, Vol. 2, pp. 321-331.
- Vreeland, R.H., W.D. Rosenzweig and D.W. Powers (2000), "Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal", *Nature*, Vol. 407, pp. 897-900.
- Vreeland, R.H. et al. (2002), "Halosimplex carlsbadense gen. nov., sp. nov., a unique halophilic archaeon with three 16S rRNA genes, that grows only in defined medium with glycerol and acetate or pyruvate", *Extremophiles*, Vol. 6, pp. 445-452.
- Wainø, M., B.J. Tindal and K. Ingvorsen (2000), "*Halorhabdus utahensis* gen. nov., sp. nov., an aerobic, extremely halophilic member of the archaea from Great Salt Lake, Utah", *International Journal of Systematic and Evolutionary Microbiology*, Vol. 50, pp. 183-190.
- Waldron, P.J. et al. (2007), "Salinity constraints on subsurface archaeal diversity and methanogenesis in sedimentary rock rich in organic matter", *Applied and Environmental Microbiology*, Vol. 73, pp. 4171-4179.
- Wang, Y. and A.J. Francis (2005), "Evaluation of microbial activity for long-term performance assessments of deep geologic nuclear waste repositories", *Journal of Nuclear and Radiochemical Sciences*, Vol. 6, pp. 43-50.
- Weber, K.A., L.A. Achenbach and J.D. Coates (2006), "Microorganisms pumping iron: Anaerobic microbial iron oxidation and reduction", *Nature Reviews*, Vol. 4, pp. 752-764.
- Wierzchos, J. et al. (2012), "Novel water source for endolithic life in the hyperarid core of the Atacama Desert", *Biogeosciences*, Vol. 9, pp. 2275-2286.
- Wilson, D.B. (2011), "Microbial diversity of cellulose hydrolysis", *Current Opinion in Microbiology*, Vol. 14, pp. 259-263.
- Winters, Y.D., T.K. Lowenstein and M.N. Timofeeff (2015), "Starvation-survival in haloarchaea", *Life*, Vol. 5, pp. 1587-1609.
- Wolery, T.W. and R.L. Jarek (2003), "Software User's Manual: EQ3/6", Version 8.0. Software Document Number 10813-UM-8.0-00, Sandia National Laboratories, Albuquerque.
- Wolf, M, R. Bachofen (1991), "Microbial degradation of bitumen", *Experientia*, Vol. 47, pp. 542-548.

- Yakimov, M.M. et al. (2015), “Microbial community of the deep-sea brine lake kryos seawater-brine interface is active below the chaotropy limit of life as revealed by recovery of mRNA”, *Environmental Microbiology*, Vol. 17, pp. 364-382.
- Yikmis, M, A. Steinbuchel (2012), “Historical and recent achievements in the field of microbial degradation of natural and synthetic rubber”, *Applied and Environmental Microbiology*, Vol. 78, pp. 4543-4551.
- Youssef, N.H. et al. (2014), “Trehalose/2-sulfotrehalose biosynthesis and glycine-betaine uptake are widely spread mechanisms for osmoadaptation in the *Halobacteriales*”, *The ISME Journal*, Vol. 8, pp. 636-649.
- Zhao, B., et al. (2009), “Biodegradation of phenanthrene by a halophilic bacterial consortium under aerobic conditions”, *Current Microbiology*, Vol. 58, pp. 205-210.
- Zhilina, T.N. (1986), “Methanogenic bacteria from hypersaline environments”, *Systematic and Applied Microbiology*, Vol. 7, pp. 216-222.
- Zirnstein, I, T. Arnold and K. Roeske (2016), “Identification of the Microbial Community in Salt Sumps of the Asse II Pit, a Former Salt Mine Used as a Deep Geological Repository for Intermediate- and Low-Level Radioactive Waste” (submitted).

## NEA PUBLICATIONS AND INFORMATION

The full **catalogue of publications** is available online at [www.oecd-nea.org/pub](http://www.oecd-nea.org/pub).

In addition to basic information on the Agency and its work programme, the **NEA website** offers free downloads of hundreds of technical and policy-oriented reports.

An **NEA monthly electronic** bulletin is distributed free of charge to subscribers, providing updates of new results, events and publications. Sign up at [www.oecd-nea.org/bulletin](http://www.oecd-nea.org/bulletin).

Visit us on Facebook at [www.facebook.com/OECDNuclearEnergyAgency](http://www.facebook.com/OECDNuclearEnergyAgency) or follow us on **Twitter** @OECD\_NEA.





# Microbial Influence on the Performance of Subsurface, Salt-Based Radioactive Waste Repositories

For the past several decades, the Nuclear Energy Agency Salt Club has been supporting and overseeing the characterisation of rock salt as a potential host rock for deep geological repositories. This extensive evaluation of deep geological settings is aimed at determining – through a multidisciplinary approach – whether specific sites are suitable for radioactive waste disposal. Studying the microbiology of granite, basalt, tuff, and clay formations in both Europe and the United States has been an important part of this investigation, and much has been learnt about the potential influence of microorganisms on repository performance, as well as about deep subsurface microbiology in general. Some uncertainty remains, however, around the effects of microorganisms on salt-based repository performance. Using available information on the microbial ecology of hypersaline environments, the bioenergetics of survival under high ionic strength conditions and studies related to repository microbiology, this report summarises the potential role of microorganisms in salt-based radioactive waste repositories.

## Nuclear Energy Agency (NEA)

46, quai Alphonse Le Gallo  
92100 Boulogne-Billancourt, France  
Tel.: +33 (0)1 45 24 10 15  
nea@oecd-nea.org [www.oecd-nea.org](http://www.oecd-nea.org)

NEA No. 7387