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Review Article

Radioactive waste microbiology: predicting microbial survival and activity in changing extreme environments

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Abstract

The potential for microbial activity to occur within the engineered barrier system (EBS) of a geological disposal facility (GDF) for radioactive waste is acknowledged by waste management organizations as it could affect many aspects of the safety functions of a GDF. Microorganisms within an EBS will be exposed to changing temperature, pH, radiation, salinity, saturation, and availability of nutrient and energy sources, which can limit microbial survival and activity. Some of the limiting conditions are incorporated into GDF designs for safety reasons, including the high pH of cementitious repositories, the limited pore space of bentonite-based repositories, or the high salinity of GDFs in evaporitic geologies. Other environmental conditions such as elevated radiation, temperature, and desiccation, arise as a result of the presence of high heat generating waste (HHGW). Here, we present a comprehensive review of how environmental conditions in the EBS may limit microbial activity, covering HHGW and lower heat generating waste (LHGW) in a range of geological environments. We present data from the literature on the currently recognized limits to life for each of the environmental conditions described above, and nutrient availability to establish the potential for life in these environments. Using examples where each variable has been modelled for a particular GDF, we outline the times and locations when that variable can be expected to limit microbial activity. Finally, we show how this information for multiple variables can be used to improve our understanding of the potential for microbial activity to occur within the EBS of a GDF and, more broadly, to understand microbial life in changing environments exposed to multiple extreme conditions.

Keywords: extremophiles; microbial survival; radioactive waste; geomicrobiology

Introduction

The use of underground geological disposal facilities (GDFs) is the preferred long-term solution for disposing of radioactive waste. Internationally, a range of disposal concepts have been developed based on the geology of the site and waste type, although all concepts use a combination of natural barriers (the host rock), engineered barriers, and waste packaging to contain the waste. Generally, waste is prepared and then packaged into containers within which it will be transported to the GDF and emplaced. For high heat generating waste (HHGW), a variety of container designs have been considered. Typically, copper or carbon steel have been proposed as container materials, and the waste stored in these containers may well be vitrified. These containers are designed to maintain their integrity and contain the waste for long time periods (e.g. tens of thousands of years) after the closure of the GDF (RWM 2016a). In evaporitic environments, the container is designed to hold the waste until salt creep is complete (RWM 2016b). For lower heat generating waste (LHGW), the purpose of the container is to contain the waste for long periods of interim storage and during the operational phase of the GDF. Container materials include stainless steel, cast iron, and concrete. Depending upon design and environmental conditions, containers for LHGW are expected to maintain integrity for a few years up to thousands of years. This means that waste (including nutrients and energy sources for microbes) is likely to escape from the container into the surrounding barriers much earlier than in the case of HHGW. These wastes contain a variety of materials, including graphite, metals, organics (particularly plastics, cellulosic material, and rubber), concrete, cement, rubble, and sludges and flocs (RWM 2016a). Beyond the lifetime of the containers, other components of the engineered barrier system (EBS) together with the natural barrier of the host rock act to ensure long-term containment of emplaced radioactive waste (RWM 2016b). The EBS, which includes the containers described above, will be designed for a particular geological setting and the quantity and nature of the waste to be disposed (RWM 2016a). A detailed description of the diversity of EBS design is beyond the scope of this review. A brief overview of the key features pertinent to the microbiology is provided, and the reader is directed to other works (Sellin and Leupin 2013, RWM 2016a,b) for further details. Waste type and geology play a key role in the selection of materials in the EBS, and so the diversity of designs can be simplified by considering six combinations of geology and waste type, as outlined in the illustrative disposal concepts described in the UK Radioactive Waste Management (RWM, predecessor organization to Nuclear Waste Services) Generic Disposal Facility Design Report (RWM 2016b). This covers HHGW and LHGW in higher strength rocks (HSR), lower strength sedimentary rocks (LSSR), and evaporites.

(i) Compacted bentonites have been proposed as EBS materials for HHGW and some LHGW waste disposal, typically in HSR and LSSR. The swelling properties of this clay have

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useful hydraulic, chemical, and mechanical properties that prevent the damage to the waste containers and reduce radionuclide transport (due to its low permeability and ability to sorb radionuclides). These properties also minimize the activity of microorganisms due to low pore space and water availability (Sellin and Leupin 2013, RWM 2016a).

(ii) Cementitious materials have been proposed for LHGW and for parts of the Belgian HHGW supercontainer concept and are relevant to HSR and LSSR. Cementitious materials generate a high-pH environment, which reduces the solubility (and therefore mobility) of radionuclides. It maintains a high surface area for sorption of radionuclides and has a relatively high permeability and porosity, which ensures homogeneity of chemical conditions and allows the escape of gas (e.g. generated by metal corrosion or microbial degradation of organic matter within the waste) (RWM 2016a). From a microbial perspective, the high-pH environment presents a challenging environment for microbial activity.

(iii) Magnesium oxide and/or crushed rock (the same as or similar to the host rock) are proposed as the main components of the EBS in evaporitic geologies for HHGW and LHGW. GDFs in evaporites will have an extremely dry environment, and the lack of fluid flow prevents the transport of radionuclides. Magnesium oxide serves to absorb any water that may be present, maintaining the dry environment. Crushed rock isolates the waste from porewaters of the host rock (RWM 2016b). In terms of microbiology, the environment within an evaporitic EBS presents salinity and desiccation stresses.

More detailed information about the design and function of the EBS can be found elsewhere (e.g. RWM 2016b, Marsh et al. 2021), and a summary of disposal concepts considered, planned, and in use around the world can be found on the Nuclear Waste Association webpage (World Nuclear Association 2023).

Potential issues could arise from interactions between microorganisms present within a GDF and the host rock, groundwater, waste, and/or engineered barriers. The implications of these interactions for the safety of the GDF have received considerable attention from waste management organizations. This is because it is recognized that microorganisms may have an impact on processes such as the corrosion of metal canisters and other metallic components of the GDF, the behaviour of bentonite, the production and consumption of gases, and the mobility of radionuclides (Humphreys et al. 2010, Lloyd and Cherkouk 2020). The environmental conditions within the GDF will undergo a complex evolution during operation and following closure, and microbial communities present will be exposed to changing temperature, pH, radiation, salinity, saturation, and availability of nutrients and energy sources (electron donors and acceptors). The manner in which these environmental factors change following closure of a GDF has been examined experimentally and modelled, either individually or in coupled systems, for both the physical changes themselves and, in many cases, the response of microorganisms. Examples of these studies are discussed in the sections that deal with changes of each variable within the EBS. However, there has not been a comprehensive consideration of the effects of the temporo-spatial evolution of these conditions as they relate to the limits of microbial life. The approach developed here for radioactive waste disposal can be applied to understand how changing conditions could affect microbial communities in any other engineered or natural environment that is subject to multiple extreme conditions.

The following sections discuss the currently known limits to life, obtained from published literature for each of the variables considered, and apply these to the environment within an EBS. Although each variable is considered individually, microorganisms will be affected by multiple stresses at the same time, and the effect may be cumulative. Examples of this are highlighted in the relevant sections.

Terminology is important, especially when working on interdisciplinary fields such as the disposal of radioactive waste. Therefore, we would like to draw attention to the following terms used throughout this review:

(i) The term ‘stress’ has different connotations for biologists and those working in the physical or earth sciences. Throughout this review, the term is used to refer to any physical or environmental pressure on a microorganism that elicits a response, e.g. a reduction in growth, rather than the physical sense of a force applied to a rock or other material.

(ii) Throughout this review, ‘survival’ refers to the ability of cells to be active after exposure to a stress, usually measured by the ability to culture cells once the stress has been removed. ‘Activity’ is used in its broadest sense to include growth, particular behaviours such as the production of sulphide by sulphate reducers, or, in some cases, the detection of particular enzymatic activities. Our rationale for using these definitions is that only active cells have the potential to affect the safety functions of the EBS under a particular set of circumstances, but knowledge of whether cells can survive, even if in a dormant state, is valuable as these cells have the potential to become active if the conditions within the EBS become more favourable.

(iii) The EBS environment extends from the waste containers to the host rock wall. Microbial processes occurring within the waste and within the host rock are out of scope for this review. The high temperatures and radiation are generally considered to sterilize the waste in HHGW, and the containers are designed to isolate the waste for long periods of time. Microbial activity is expected to occur within LHGW, as described elsewhere (e.g. Small et al. 2017, Small and Vikman 2021). Although the microbial processes occurring within the waste are not considered here, the LLGW and its breakdown products may be used to sustain microbial activity in the EBS once the container is breached. This is discussed in the ‘Nutrients and energy sources as a limiting factor within the EBS’ section. Similarly, microbiology within relevant host rocks (beyond the EBS) is also out of scope and has been described elsewhere (e.g. Swanson et al. 2012, 2021, Zirnstein and Arnold 2015, Bagnoud et al. 2016, Blomberg et al. 2017, Leupin et al. 2017).

Identifying key microbial groups and defining the limits to life

The UK RWM Status report on the EBS (RWM 2016a) was used as a starting point to identify a number of key factors that can affect microbial survival or activity relevant to EBS environments: temperature, pH, bentonite density, radiation, salinity, saturation/desiccation, and nutrient and energy source (electron donor and acceptor) availability. This RWM report (RWM 2016a) was chosen as it considers a range of options covering the key geological settings. Our aim was to identify and apply currently accepted environmental limits to microbial life in the EBS to un-

Table 1. Functional groups considered in this review and their potential role within the EBS.

Microbial group	Potential effects within the EBS	Reference
Sulphate reducers	Microbiologically influenced corrosion. Interactions with bentonite that could affect swelling capacity.	Rajala (2017), Xu et al. (2023) Liu et al. (2012)
Iron reducers	Interactions with bentonite that could affect swelling capacity. Microbiologically influenced corrosion.	Perdrial et al. (2009), Liu et al. (2012), Vikman et al. (2018), Schütz et al. (2015)
Nitrate reducers/denitrifiers	Nitrate reduction can be coupled to degradation of organic components of clays and wastes (e.g. PVC, superplasticizers). Denitrification produces gases that could increase pressures within the EBS.	Masuda et al. (2013), Nixon et al. (2018), Mijnendonckx et al. (2022), Shrestha et al. (2021)
Methanogens	Consumption of repository gases (hydrogen and carbon dioxide) could reduce pressure. Conversion of radioactive carbon isotopes to gas. Microbiologically influenced corrosion.	Rajala et al. (2019), Taborowski and Pedersen (2019)
Acetogens	Consumption of repository gases (hydrogen and carbon dioxide) could reduce pressure. Production of acetate from volatile fatty acids arising from cellulose degradation could support acetoclastic methanogenesis and heterotrophic metabolisms.	Bengtsson et al. (2017b), Beaton et al. (2019), Abrahamsen-Mills and Small (2021)
Cellulose degraders	Cellulose degradation products such as isosaccharinic acid act as complexants that may enhance the transport of radionuclides.	Bassil et al. (2015), Bassil et al. (2015)

derstand microbial survival and activity in that environment and identify times and locations where microbial life will be more or less likely. For each of the key factors identified, we have reviewed the general microbiology literature to compile currently accepted limits on activity and survival for several microbial groups. The microbial groups selected were based on those of interest to radioactive waste management organizations. The upper limits for the kingdom Fungi and domains Bacteria and Archaea were identified where available. These limits were intended to give a limit for these broad taxonomic groupings without consideration of the specific way in which they might affect the safety function of the EBS. In addition, the literature was searched for limits for methanogens, acetogens, sulphate reducers, and iron reducers. Some sections also include the data on denitrifiers and cellulose degraders, if data are available. Certain functional groups are associated with particular impacts within the EBS (Table 1). In particular, there has been considerable investigation into the survival and activity of sulphate reducers, as they are considered to present a corrosion risk. However, it is recognized that a wide range of organisms are capable of contributing to corrosion via several microbial mechanisms (Rajala 2017, Xu et al. 2023). In addition to these specific functional groups, microorganisms play a wider role in degradation of organic matter, consumption and production of gases, and radionuclide mobility (Lloyd and Cherkouk 2020, Ruiz-Fresneda et al. 2023).

We have focussed primarily on anaerobic microbial processes in this review, as conditions will become anoxic after closure of the GDF. Aerobic microbiology is relevant during the immediate post-closure oxic phase within the EBS; however, this has not been well studied and is not the focus of this review. The oxic period is anticipated to be a relatively short, as microbial activity, geochemical reactions, and corrosion processes are expected to result in reducing conditions establishing over hundreds to thousands of years (McMurry et al. 2004, RWM 2016a). Activity of aerobes could also be possible at specific times and places, if, for example, oxygenated, glacially derived groundwater is driven to repository depths or locally where radiolysis of water is active (Haynes et al. 2021).

Viruses are known to exist at depths relevant to radioactive waste disposal, for example in groundwater around SKB's (the Swedish Radioactive Waste Disposal Company's) Äspö underground research laboratory, where viral predation is thought to play a key role in controlling microbial numbers through the recycling of nutrients as a result of cell lysis (Kyle et al. 2008, Holmfeldt et al. 2021). However, information on viruses is not included in this review for two reasons. Firstly, we were unable to find any studies relating to viruses within the EBS. Secondly, the survival limits of phages are likely to be closely linked to those of their hosts.

For each environmental condition, we collected data on known limits to microbial survival and activity. We did not restrict ourselves to studies where the focus was radioactive waste but gathered information from a variety of sources, including laboratory experiments and natural or perturbed environmental settings, including those that can act as analogue sites for aspects of the environment within the EBS. Such sites can be particularly useful in microbiological studies of radioactive waste disposal because they provide the opportunity to study processes occurring too slowly to measure in the laboratory. Even very slow rates of microbial activity may still have an impact on GDF performance over timescales of thousands to a million years (Butterworth et al. 2021). Next, we reviewed the literature where these limits had been explored in the context of the EBS environment. Finally, studies modelling the evolution of conditions within the EBS of a GDF were identified to determine times and locations within the EBS where these limits to life were likely to be exceeded.

Temperature as a limiting factor in the EBS

After closure of a GDF, the temperature is expected to rise as a result of radioactive decay and/or cement hydration (depending on the waste type or disposal concept). The temperatures reached will depend on the waste inventory, the repository layout, thermal conductivities of the host rock and engineered barriers, saturation of the near field, groundwater flow, and heat transfer by convection and radiation (RWM 2016c). Heat generated from spent fuel (HHGW) is expected to be significant for a few thousand years with temperatures on waste containers potentially in excess of 100°C for hundreds of years and maximum temperatures in some scenarios in excess of 200°C (Fig. 1). Heat generation due to exother-

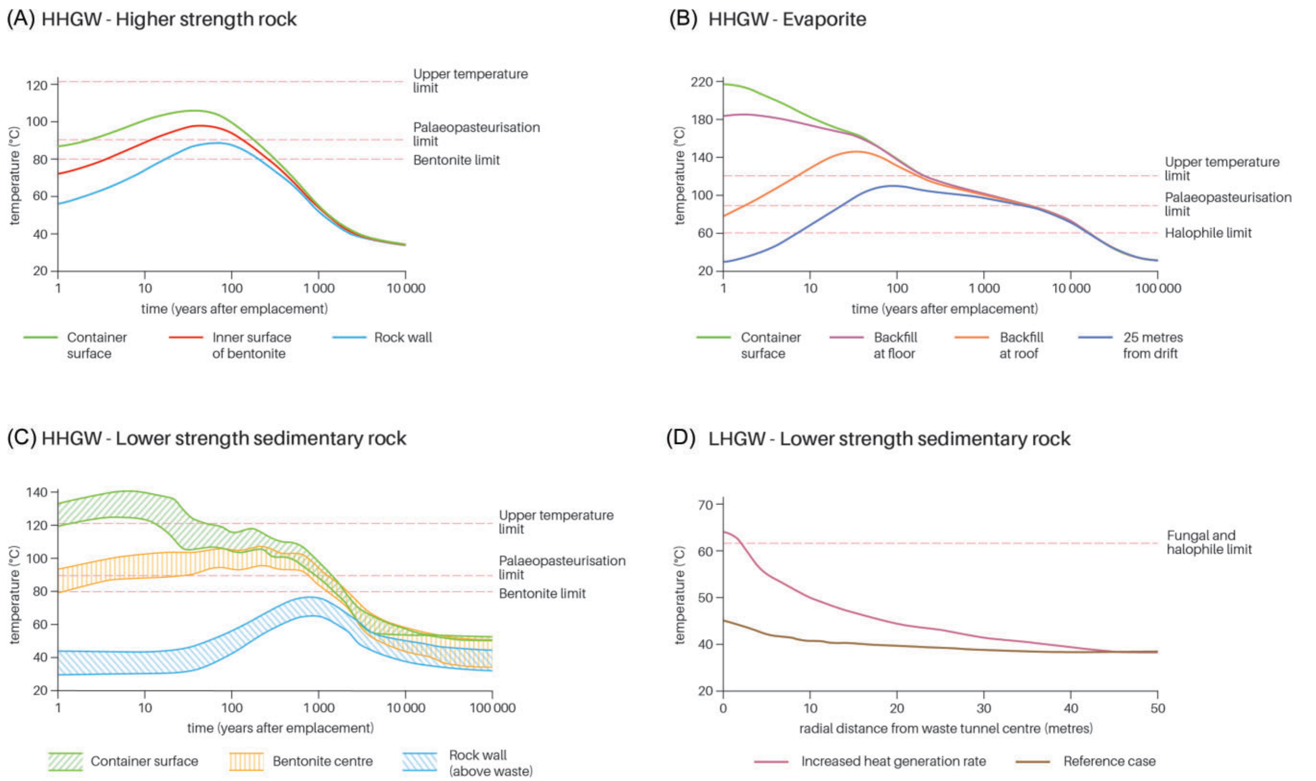

Figure 1. (A) HHGW HSR example: Evolution of temperature over time in the reference case of four pressurized water reactor spent fuel elements in a KBS-3 V type disposal concept, modified from RWM (2016a) © Nuclear Decommissioning Authority 2016. All rights reserved; reproduced with permission from the Nuclear Decommissioning Authority (NDA). (B) HHGW Evaporitic Rock example: Generic Salt Repository for HHGW. Modified from Blanco-Martin et al. (2018), reproduced with permission from Springer Nature. (C) HHGW LSSR example: Evolution of temperature in Nagra's HHGW disposal scenario sited within Opalinus clay (OPA), modified from Nagra (2021) © Nagra 2021. All rights reserved; reproduced with permission from Nagra. (D) LHGW LSSR rock example: Maximum temperatures reached in the increased heat generation case at different distances from the centre of the waste tunnel in intermediate level waste (LHGW) emplacement tunnel within the Opalinus Clay [modified from Johnson et al. (2002) © Nagra 2002]. All rights reserved; reproduced with permission from Nagra). Note that the addition of limits to life on these figures is not meant to imply that they are endorsed by any of the waste management organizations that produced the original figures.

mic cement curing plus any heat generated by LHGW will result in smaller temperature increases, with maxima expected to be in the range of 50–80°C, subsiding within a few years (RWM 2016b). In all cases, heat generation from microbial activity and corrosion are considered insignificant (RWM 2016d).

Temperature limits in natural environments and experimental studies relevant to the EBS

The current known maximum temperature limits for activity or survival of different types of microorganisms and microbial activities are shown in Table 2. The currently recognized upper limit for prokaryotic activity is 122°C for an archaeal methanogen (Takai et al. 2008). The highest confirmed temperature for growth of an isolated bacterial strain is 100°C for a *Geothermobacterium* species (Kashefi et al. 2002). The limit of fungal growth is between 60 and 62°C (Tansey and Brock 1972). The archaeal iron reducer, *Geogemma barossi* strain 121, can grow up to 121°C (Kashefi and Lovley 2003, Lovley et al. 2004), slightly lower than the limit for methanogenesis reported above. Microbial sulphate reduction has been observed in hydrothermal environments up to 110°C (Jørgensen et al. 1992), but the most thermotolerant sulphate-reducing isolate is another archaeon, *Archeoglobus profundus*, which grows at temperatures up to 90°C (Burggraf et al. 1990).

Cells can survive higher temperatures as dormant forms such as spores, and survival rates decrease as the temperature increases. Spores of sulphate reducers have been reported to survive temperatures of up to 180°C for 16 h (Miettinen et al. 2022); 120°C for 48 h (Bengtsson and Pedersen 2016); 110°C for a week (Bengtsson et al. 2017a); and at least 463 days (the longest tested period) at 80°C (Bell et al. 2020). One study reported long-term survival in bentonite at unusually high temperatures (microorganisms could be cultured after heating to 150°C for one year) (Kaspar et al. 2021), but observed that extended periods of incubation were required before cells emerged from dormancy. However, that study was carried out on powdered bentonite rather than compacted bentonite and so is less relevant to GDF environments (see section on Bentonite dry density as a limiting factor in the EBS for details). Of considerable significance to microbial activity in the EBS around heat generating waste is the observation that cooling after prolonged periods at 80°C can trigger spore germination of sulphate reducers. The temperature to which they are cooled has an impact on the species that grow and whether sulphate reduction occurs (Bell et al. 2020). This behaviour might allow any mesophilic sulphate reducers that survive elevated temperatures as spores to become active as the repository cools.

Fungal spores have been reported to survive for short periods (2 h) at up to 115°C (Suryanarayanan et al. 2011), a few days at 100°C, and over 21 days at 80°C, with survival rates higher at lower relative humidity (Palmer et al. 1987). The lower limit for fungal versus bacterial spore survival may simply reflect the fact that less work has been carried out on fungi.

It should be remembered that data of the upper limits of life typically come from environments where these organisms have evolved to live in hydrothermal environments, and it is not clear whether organisms likely to be found in an EBS environment could

Table 2. Maximum temperature limit for activity/survival of different microbial groups.

Microbial group	Upper temperature limit (°C)	Details	Reference
Bacteria (activity)	100	*Geothermobacterium ferrireducens* gen. nov., sp. nov., isolated from a geothermal pool in Yellowstone National Park, USA	Kashefi et al. (2002)
Archaea and methanogen (activity)	122	*Methanopyrus kandleri* isolated from a hydrothermal vent, Gulf of California, USA	Takai et al. (2008)
Fungi (activity)	60–62	species not identified	Tansey and Brock (1972)
Sulphate reducers (activity)	90	*Archaeoglobus profundus* isolated from hydrothermal systems in Guaymas, Mexico can use sulphate, thiosulphate, and sulphite as electron donors.	Burggraf et al. (1990)
Sulphate reducers (activity)	110	Observation of microbial sulphate reduction in sediments	Jørgensen et al. (1992)
Iron reducers (activity)	121	*Geogemma barossii* isolated from a hydrothermal vent in Puget Sound, USA	Kashefi and Lovley (2003)
Bacterial spore (survival)	180	This is a short-term survival limit for some spores within a mixed bentonite community. See notes in text for discussion about longer term survival	Miettinen et al. (2022)

adapt to and colonize the EBS over the timescale of a GDF at such temperatures. Although 122°C is currently understood to represent the temperature limit to life, in subsurface environments, a temperature of 80–90°C is often considered to be the maximum limit of long-term microbial activity in subsurface environments. This is the temperature at which hydrocarbon reservoirs are considered to go through palaeopasteurization (Wilhelms et al. 2001, Head et al. 2003). Above this temperature, microbial activity in the reservoir does not normally affect the recovery of hydrocarbons. This might be a more realistic upper limit of survival in many subsurface environments.

The maximum temperatures that microorganisms can survive for long periods within the EBS in compacted bentonites may be even lower than those quoted above. Based on studies using different organisms over several time frames, the long-term temperature limit is predicted to be somewhere above 55°C but below 80°C (Table 3). Studies on uncompacted bentonites found that sulphate reducers, thiosulphate reducers, nitrate reducers, iron oxidizers, acid producers, and aerobic heterotrophs could be cultured after incubation at 50°C but not at 80°C after 47 days. Anaerobic heterotrophs and iron reducers were not culturable from either temperature (Diler et al. 2021). Fewer studies have been conducted on cementitious EBS materials but one study investigating three grout formulations (pH 10–11) found that out of the above-mentioned microbial groups, only nitrate reducers were above culturable detection limits after a six-month incubation at 80°C (Diler et al. 2023). It should be noted that the microorganisms in these experiments were not adapted to high-temperature environments, and survival of indigenous microorganisms might be different where communities are able to adapt to a gradually (on microbial timescales) increasing temperature regime. Most studies of adaptation to increasing temperatures have concentrated on the relatively small temperature increases associated with global warming but there is evidence from experiments designed to recreate the conditions of subsurface heat storage that indicates microbial communities have the ability to adapt to relatively large temperature changes (at least 60°C) within weeks to months (Bonte et al. 2013, Lienen et al. 2017).

The volume of research carried out on fungi within the EBS is considerably less than for prokaryotes. Interestingly, microscopic evidence of microbial, potentially fungal, activity was observed in non-microbiological bentonite experiments run at 60°C for 5 months (Kaufhold et al. 2015).

Halophillic organisms may be particularly sensitive to high temperatures and will be the only organisms that can survive within brines in evaporitic repositories or other host rocks with saline groundwaters; although the majority of characterized halophilic strains will tolerate temperatures above 50°C, the maximum reported temperature for growth for an aerobic halophile is 61°C (for the archaeal species *Haloterrigena limicola*) and 60°C for an anaerobic halophile (the methanogen *Methanohalobium evestigatum*) (Bowers and Wiegel 2011).

Overall, these data (Table 2 and 3) show that there is no single temperature that can be used as a limit of microbial life, rather several temperature limits could be considered:

The first two limits are general:

(i) 122°C is the currently accepted upper temperature limit for microbial activity. Although some archaea, including methanogens have been shown to survive at this temperature, the upper limit for other groups may be lower. Using this value would give a high degree of confidence that microbial activity will not occur.

(ii) 90°C is the palaeopasteurization temperature limit, and is a more generally accepted limit, for microbial activity in subsurface environments (particularly in the context of hydrocarbon reservoirs). This is broadly consistent with the observations that there is a rapid decrease in microbial activity above 80°C (RWM 2016d). Above this limit, significant microbial activity becomes less likely but cannot be ruled out. Using this value would provide a less conservative estimate of microbial activity in subsurface environments relevant to the EBS.

Additionally, lower limits could be applied that are specific to the particular environment/materials within the repository.

Table 3. Maximum temperature limits for bacterial survival in compacted Wyoming bentonite in experiments of different durations.

Temperature limit	Duration	Details	Reference
80–130°C	2–3 weeks	Survival of bacteria dramatically reduced after incubation at 80°C at a dry density of 1600 kg m^{-3} but not 1800 kg m^{-3}. At the higher dry density, some anaerobes could be cultured after two weeks at 121°C and a further week at 130°C. This was suggested to be due to survival as spores.	Stroes-Gascoyne and Hamon (2010)
80°C	Between 2 and 28 weeks	A selection of aerobic and anaerobic bacteria was tested. *Bacillus* spp. survived up to two weeks but no species survived 28 weeks (including spore-forming sulphate reducers).	(Pedersen et al. 2000a)
Between 50 and 70°C	15 months	A selection of aerobic and anaerobic bacteria was tested. Only spore-forming *Desulfotomaculum nigrificans* and *B. subtilis* survived at 70°C.	(Pedersen et al. 2000b)
Between 55 and 67°C	5 years	No aerobic bacteria were culturable from bentonite incubated at or above 67°C.	Fru and Athar (2008)

(i) 80°C is the approximate, currently accepted upper limit of activity observed in bentonites in long-term laboratory experiments. Individual experiments suggest that the limit could be anywhere between 50 and 80°C. One study found that survival in high-pH grouts may have similar limits (Diler et al. 2023), though more research is required to fully explore how this affects microbiology in cementitious repositories beyond the particular scenario in this study.

(ii) 61°C is currently the upper recognized limit of life for halophilic microorganisms, which could be applied to evaporitic repositories or other environments with hypersaline groundwater.

Changes in temperature within the EBS

Figure 1 provides examples of potential temperature profiles for HHGW in HSR, LSSR, and evaporites and for LHGW in LSSR. In the examples, the maximum temperature will be achieved relatively quickly (on the order of 10 years post-closure). The example of HHGW in HSR (Fig. 1A) shows the temperature at three points: the interface between the bentonite and the rock wall, the inside surface of the bentonite nearest the waste canisters, and the surface of the canister. Between these last two points, there is an air gap with low thermal conductivity when the bentonite is not fully saturated. In this scenario, the upper temperature limit for life is not expected to be exceeded at any point. In some cases, it is a design requirement that the buffer will transfer the heat from the canister efficiently enough to keep the buffer temperature <100°C, which will maintain the thermal and mineralogical stability of the bentonite; the peak bentonite temperature in this case is predicted to be 98°C (RWM 2016e). The palaeopasteurization and bentonite limits could be exceeded during the period of 10–100 years after closure. In the HHGW in LSSR example in Fig. 1C, zones near to the canisters will be above the upper limit for life for 10 years. The temperatures in the outer bentonite will be more suitable for life as temperatures at the edge of the host rock are expected to increase more slowly, peaking at 60–80°C after around 1000 years. If the actual temperatures reached are at the lower end of this range, it might allow a wide range of microorganisms to persist (including halophiles and fungi). In the HHGW example for evaporitic rock (Fig. 1B), the whole of the EBS will exceed the palaeopasteurization limit after 1 year and exceed the upper limit to life after 10 years. Although temperatures of all parts of the repository will drop below the palaeopasteurization limit in <10 000 years, temperatures will not drop below the halophile limit until around 30 000 years. Whether microbial activity can be re-established in any of these cases, assuming it has been extinguished by high temperatures, will depend on two factors: firstly, whether the spores that are present are able to survive and then germinate on cooling, or the contribution of groundwater in introducing new cells into the EBS.

In a cementitious repository for LHGW, the maximum temperature does not exceed the palaeopasteurization limit, but in some scenarios may exceed some of the limits set for fungi and halophiles (Fig. 1D). UK LHGW designs have a guidance temperature maximum of 50°C during the operational phase but allow for the waste package temperatures of up to 80°C for 5 years post-closure in repositories with a cementitious backfill (RWM 2016f). This initial elevated temperature, related to curing of cement, is broadly consistent with the predicted temperatures in GDFs in other countries. For example, maximum temperatures of 72–76°C are predicted in the reference case for Nagra (the Swiss radioactive waste management organization, Nationale Genossenschaft für die Lagerung radioaktiver Abfälle) (Leupin et al. 2016). When a possible scenario incorporating the maximal release rate of hydration heat was modelled, the temperature peak rises to 88°C. Such temperatures could prevent fungal and halophile activity close to the waste, and in the more extreme cases would be close to the palaeopasteurization limit (Fig. 1D). However, after a period of months to years, the temperature of the whole EBS will return to ambient temperatures, and the palaeopasteurization limit is normally applied to longer periods of time. It may be that the most significant effect of temperature in LHGW could be to modify the microbial community rather than limit microbial activity. Work carried out around geothermal energy plants has shown that even moderate temperature changes can alter the dominant metabolisms present, meaning the biogeochemical reactions taking place within the EBS could be affected (Jesußek et al. 2012, Bonte et al. 2013, Lienen et al. 2017).

pH as a limiting factor in the EBS

Extremes of pH are particularly relevant in cementitious GDF designs, where the pH may increase to pH 13.5 as a result of geochemical reactions of the cement with groundwater (Atkinson et al 1988, Berner 1992). In certain cases, low-alkali cements (LAC) may be used, which could limit this maximum to pH ~11 (Nakayama et al. 2006, Vehmas et al. 2020). Cementitious barriers will typically be used for LHGW in LSSR and HSR. A high pH zone is expected to gradually spread away from the GDF along the direction of groundwater flow. It is expected that within the cementitious EBS, the pH will remain above pH 10 for at

Table 4. Maximum pH limit for microbial activity/survival.

Microbial group	Upper pH limit	Details	Reference
Mixed microbial communities (activity)	13.2	Growth of motile rods in controlled pH microcosms containing samples from slag heaps.	Roadcap et al. (2006)
Bacteria (activity)	13.0	Enzymatic activity in *Serratia marcescens* reported at pH 13.	Kaira et al. (2015)
Archaea (activity)	11.5	Growth of *Natronobacterium magadii* at pH 11.5.	Mwatha and Grant (1993)
Fungi (activity)	11.4	Maximum growth rate of *Sodiomyces* spp. was recorded at pH 8.7–10.5, and only a slight decrease in growth at the highest pH tested (pH 11.4).	Grum-Grzhimaylo et al. (2013)
Fungi (survival)	13.5	*Trichoderma gamsii* survived exposure to pH 13.5 for 2 days.	Rinu et al. (2014)
Hydrogenotrophic methanogens (activity)	11.0	Limit of hydrogenotrophic methanogenesis is pH 11. Hydrogenotrophic methanogenesis dominates over acetoclastic at > pH 9 in EBS-relevant experiments.	Wormald et al. (2020)
Acetoclastic methanogens (activity)	10.2	Acetoclastic *Methanocrinis natronophilus* isolated from a soda lake grows up to pH 10.2	Khomyakova et al. (2023)
Single sulphate reducer strain (activity)	11.5	Slow growth *Desulfonatronum buryatense* sp. nov. strain Su2 shows at pH 11.5.	Ryzhmanova et al. (2013)
Mixed sulphate reducer community (activity)	12.3	Sulphate-reducing activity observed up to pH 12.3 but activity decreases above pH 10.5.	Glombitza et al. (2021)
Iron reducers (activity)	11.0–11.7	Iron reduction observed in mixed microbial communities from hyperalkaline sediments. An upper limit of pH 11.7 was found when citrate was used as electron donor.	Rizoulis et al. (2012)
Nitrate reducers (activity)	11.0–12.0	Nitrate reduction observed in mixed microbial communities from hyperalkaline sediments between pH 11 and 12.	Rizoulis et al. (2012), Byrd et al. (2021)
Cellulose degrading microbes (activity)	>12.0	Mixed microbial communities from hyperalkaline site degraded cellulose under various conditions with end point pH 12.	Bassil et al. (2015), Bassil et al. (2020)

least 1 000 000 years (RWM 2016b). Bentonite-based EBS and the crushed salt/backfill used in evaporites are expected to have more moderate pH conditions, and pH effects within these will be discussed briefly.

pH limits in natural environments and experimental studies relevant to the EBS

The upper limits for bacterial growth are reported to be around pH 13; pH 11.5 for archaeal growth; and pH 11.4 fungal growth (Mwatha and Grant 1993, Grum-Grzhimaylo et al. 2013, Kaira et al. 2015) (Table 4). These limits have been determined for aerobic growth, which will be relevant to early post-closure phase when the environment is still oxic. There is some evidence that active bacteria can be found in samples where the bulk porewater pH is above 13 under both oxic and anoxic conditions (Roadcap et al. 2006, Charles et al. 2019). However, measurements of bulk water chemistry do not necessarily reflect the local pH within the vicinity of a microbial cell and the formation of flocs or biofilm. The associated production of extracellular polymeric substances can also reduce local pH and protect cells from the effects of extreme pH. Even when protected by biofilms, microbes may only be able to survive briefly at pH 13 (i.e. days), and pH 12 may still be a more realistic upper limit for longer survival (Charles et al. 2017). The limits for nitrate reduction and iron reduction are also reported to be between pH 11 and pH 12 in experiments relevant to anoxic cementitious repositories. The limit for sulphate reduction in radioactive waste disposal relevant experiments has been reported to be lower than for iron- and nitrate-reduction and may be limited at pH 10 and above (Rizoulis et al. 2012, Bassil et al. 2015, Byrd et al. 2021). However, in serpentinizing environments (those where alteration of ultramafic rocks into serpentine minerals occurs), sulphate-reducing activity occurred up to pH 12.3, although it decreased above pH 10.5 (Glombitza et al. 2021). Hydrogenotrophic methanogenesis has been observed at up to pH 11 but the upper limit of acetoclastic methanogenesis is between pH 9 and pH 10 (Wormald et al. 2020). Understanding this switch is relevant to understanding gas production and consumption in GDF environments. Spore formation is thought to increase resistance to stresses such as high pH conditions; however, there does not appear to be any good data on pH survival limits in the available literature.

Overall, these data suggest three critical thresholds could be considered:

(i) pH 13.2 is upper experimental limit reported for bulk pH in for microbial activity in controlled experimental conditions. This value should be used for an estimate of the absolute limit of microbial activity.
(ii) pH ∼11–∼12 is currently considered to be the pH range that contains the upper limit for many types of microorganisms, including fungi, archaea, hydrogenotrophic methanogens, iron reducers, and sulphate reducers. Microbial activity above this limit is unlikely but cannot be ruled out.
(iii) pH ∼10 represents the limit of acetoclastic methanogenesis.

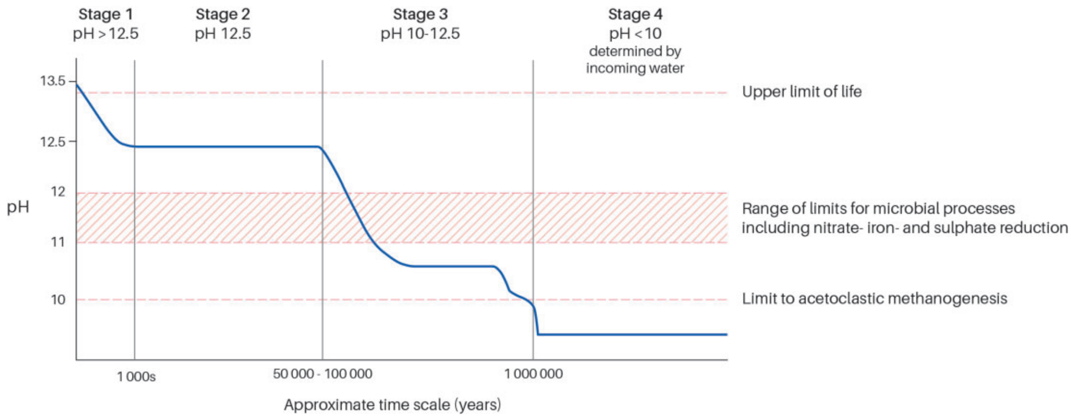

Figure 2. Evolution of pH in a generic cementitious geological disposal facility. Timescales are indicative only as pH is strongly influenced by rate of porewater exchanges, which will be site dependent. Image is based on versions of this schematic in Glasser and Atkins (1994), Kursten and Druyts (2015), RWM (2016a).

Changes in pH within the EBS

In a cementitious repository, the evolution of the pH conditions will be controlled by geochemical interactions of the cement with groundwater and follows three main stages (Fig. 2). The time taken for each stage depends upon the number of porewater exchanges and the times given here are indicative only. Over a period of 1000s years (stage 1), the pH will fall from around 13.5 to around pH 12.4–12.5 (stage 2) which will persist until 50 000 to over 100 000 years after closure (Heath and Hunter 2012, Hoch et al. 2012, Kursten and Druyts 2015). In these pH conditions, microbial activity cannot be completely ruled out (as it is below the current known pH limit of pH 13.2) but is unlikely and limited (as it exceeds the more widely reported limits of pH 11–12). During stage 3, the pH will drop to around pH 10.5, which brings the conditions into those where a range of alkaliphilic microorganisms would be expected to become more active, including methanogens, iron reducers, and sulphate reducers. Stage 3 is expected to persist until at least 1 000 000 years after closure (Kursten and Druyts 2015, RWM 2016b), after which pH will be controlled by inflowing groundwater pH and a wider range of microorganisms will be active.

The outline given above reflects the general evolution of pH for the EBS as a whole. However, there will be heterogeneity within the EBS and localized effects such as the dissolution of calcium silica hydrate (C-S-H) phases at the upstream edge of the backfill or the development of cracks in EBS materials will result in a lower local pH (Swift et al. 2010, Hoch et al. 2012). This could potentially allow microbial activity to become established earlier than in the bulk of the backfill.

In bentonite, elevated pH conditions will be present where the bentonite comes into contact with cement plugs, seals, and concrete in the tunnel lining. Typically, in HHGW bentonite-based repositories, a concrete will be chosen to limit the pH of porewater to < pH 11 (to maintain the swelling capacity of the bentonite). Any elevation of pH is expected to extend millimetres to centimetres into bentonite but persist for <100 000 years (Sellin and Leupin 2013, Savage and Cloet 2018, Jenni et al. 2019). One example of where this contact occurs is in the French design for LSSR, where steel casing will be placed around microtunnels housing the waste, to allow for retrieval if required. In this concept, an alkaline grout and bentonite mixture will be used between the host rock and the steel casing, and a pH of up to 11 is expected. This has been highlighted as an area where microbiologically influenced corrosion could affect retrievability (Diler et al. 2021). Such a pH would exclude acetoclastic methanogens and be close to the limits of activity for several other microbial groups.

In designs for evaporitic geologies, elevated pH is expected in any repository with a significant cementitious inventory or where magnesium oxides are used as buffer materials (Swanson et al. 2018). Detailed data on the anticipated evolution of pH in a GDF in evaporitic geology is not available, but magnesium oxide acts to buffer the pH at alkaline conditions (pH 9–10) (RWM 2016f). This pH would not exclude microbial activity of any of the groups considered in Table 4.

Bentonite dry density as a limiting factor in the EBS

Many disposal concepts for HHGW in HSR and LSSR include bentonite within the EBS because of its low hydraulic permeability, self-sealing capacity, and the durability of properties over long timescales (Sellin and Leupin 2013). Bentonite dry density is the main factor considered by waste management organizations when assessing microbial survival (since it can be controlled during the manufacture of the bentonite blocks). When bentonites hydrate, their swelling behaviour reduces the pore space available for microorganisms to inhabit. Studies express the bentonite densities as either wet- or dry- densities. Here, unless stated otherwise, dry densities are used.

Dry density limits in compacted bentonites from experimental studies relevant to the EBS

The dry density limits for microbial activity or survival are reported to differ, depending on bentonite type. Table 5 reports the currently understood limits for microbial survival and activity at tested dry densities in several bentonites. Typically, for each bentonite type, there is a threshold density above which there is a sharp decline in the survival and activity of microorganisms. This threshold is usually reported as the upper limit but almost all studies find that survival and activity continue above this limit but at a much lower level (Table 5). Only on a few occasions does activity or survival drop below the limits of detection. Most experiments in Table 5 investigated dry density limits for sulphate reducers, and only a few experiments have explored the survival and activity of other key microbial groups (Jalique 2016, Bengtsson et al. 2017b).

Wyoming bentonite (In the literature, Wyoming bentonite is sometimes described as MX-80 bentonite. The former term is used

Table 5. Upper limits of dry density for microbial activity/survival in different bentonites.

Microbial group	Bentonite type	Microbial inoculum (+/-)	Dry density* limit(kg m^{-3})	Details	Reference
Sulphate reducers (survival)	Wyoming	(-)	1171–1406	Survival below detection limit at 1406 and 1562 kg m^{-3}	Bengtsson et al. (2017a,b)
	Wyoming	(+)	>1562	Low levels of survival observed at highest tested dry density.	
	Asha	(-/+)	>1529	Survival in uninoculated tests lower than inoculated tests.	
	Calcigel	(-)	1333–1490	Survival low at 1333 kg m^{-3} but below detection limit at 1490 kg m^{-3}.	
	Calcigel	(+)	>1490	Low survival rates observed.	
	Gaomiaozi	(-/+)	>1469	Survival was 100 times lower at 1469 kg m^{-3} than at 1315 kg m^{-3}.	
	Rockle	(-/+)	>1408	No clear decrease in survival with increasing dry density.	
Sulphate reducers (activity)	Wyoming	(-)	1406–1562	Activity was very low at 1406 kg m^{-3} and below detection limit at 1562 kg m^{-3}.	Bengtsson et al. (2017a,b)
	Wyoming	(+)	>1562	Low levels of activity observed at highest tested dry density.	
	Asha	(-)	1300–1529	Activity was very low at 1300 kg m^{-3} and below detection limit at 1529 kg m^{-3}.	
	Asha	(+)	>1529	One of two tests at 1453 kg m^{-3} was below the detection limit, however, very low activity was detected in both highest dry density tests.	
	Calcigel	(-/+)	>1490	Activity was detected but 4000 times lower than the next lowest tested dry density (1333 kg m^{-3}) for uninoculated samples.	
	Gaomiaozi	(-/+)	>1469	Activity remained high up to the highest tested dry density.	
	Rockle	(-/+)	>1408	Low activity detected at highest tested dry density.	
Denitrifying bacteria (survival)	Wyoming	(-)	1850	Microorganisms capable of reducing nitrate to di-nitrogen did not survive while those caple of reduction to nitrite survived at the single tested density.	Jalique et al. (2016)
Heterotrophic bacteria (survival)	Wyoming	(-)	1850	Aerobes and anaerobes survived at the single tested density.	Jalique et al. (2016)
Acetogens (activity)	Gaomiaozi	(-/+)	>1469	High activity (acetate production) observed at all tested densities.	Bengtsson et al. (2017b)
	Rockle	(-/+)	>1408		

*Dry densities provided were the planned dry densities tested. Actual dry densities may have been calculated after test end, differing from planned dry densities in some tests. For the microbial inoculum column, (+) indicates the experiments were inoculated, and (-) indicates they were uninoculated (-). Limiting dry density is reported as a range; the lower value represents the dry density where microbial survival/activity was not impacted, and the upper value represents the dry density at which impacts could be detected. If information on survival or activity above this range is available, this is noted in the 'details' column. In some cases, conversions from wet to dry densities have been carried out using information within the original papers.

throughout this review for consistency.) bentonite has been the subject of most studies on dry density limits for microbial survival (Table 5). Microbial survival has been shown to decrease above 1600 kg m^{-3} with a few cells surviving up to a maximum tested dry density of 1800 kg m^{-3} (Stroes-Gascoyne et al. 2010a). In a long-term study (8 years), cultivable sulphate reducers and nitrate reducers capable of producing dinitrogen were below detection at 1850 kg m^{-3} but some nitrate reducers capable of producing nitrite and other heterotrophic aerobes and anaerobes (particularly Gram-positive bacteria, including spore formers) could be cultured at the end of the experiment (Jalique et al. 2016). A limit for survival (1406 kg m^{-3}) and activity (1562 kg m^{-3}) of sulphate reducers in Wyoming bentonite has been established for uninoculated tested bentonites (Bengtsson et al. 2017a).

Microbial limits in other bentonites are similarly difficult to pinpoint, but the highest reported limit appears to be between 1333 and 1490 kg m^{-3} for sulphate reducer survival in Calcigel bentonite (Bengtsson et al. 2017a, Haynes et al. 2019). Studies have produced a limit of >1490 kg m^{-3} for inoculated and uninoculated Calcigel (Bengtsson et al. 2017a). For sulphate reducer activity, one study found that the limit in Calcigel was >1490 kg m^{-3} for inoculated and uninoculated bentonite, but a more recent study found the limit to be between 1153 and 1383 kg m^{-3} in inoculated Calcigel (Bengtsson et al. 2017a, Haynes et al. 2019). Threshold densities for the activity of sulphate reducers in many other bentonites have not been determined (Haynes et al. 2021), and the properties of bentonites that affect the limits are not fully understood.

Absolute limits of survival or activity are difficult to establish as they are dependent upon bentonite type and the fact that some microbial survival and activity are often detected at the highest tested dry density. However, some generalizations can be made from studies to date:

(i) 1170–1850 kg m^{-3} is the range at which bentonite dry density limits microbial survival
(ii) 1150–1562 kg m^{-3} is the range at which bentonite dry density limits microbial activity
(iii) Specific dry density limits have been established for indigenous sulphate reducer survival in Wyoming (1406 kg m^{-3}) and Calcigel (1490 kg m^{-3}) bentonite

Changes in bentonite dry density within EBS

Variations of dry density have not been modelled temporally or spatially in the same way that temperature and pH have. *In situ* experiments such as the Alternative Buffer Materials experiment) and the full-scale engineered barriers experiment (FEBEX) have studied the impact of heat generated by waste in a GDF and have identified that the dry density of bentonite decreases (and water content increases) with distance from a heat source. Heat drives out water from the bentonite closest to the heat source (representing the waste container). Consequently, bentonite near to the heat source will swell less and will be compacted by the pressure exerted by greater swelling of bentonite (with higher water content) further from the heater (Villar et al. 2008). In the Alternative Buffer Materials experiment, after one year of exposure to 130°C, dry densities of all tested bentonites were highest (and water content lower) next to the heater [starting dry densities of the bentonites ranged from 1700 to 2100 kg m^{-3}, samples close the heater decreased by ∼180–210 kg m^{-3}, compared to a decrease of 200–480 kg m^{-3} for samples further away from the heater (76.5 mm)] (Svensson et al. 2011). Similar decreases in the outer bentonite dry density, from 1610 to 1450 kg m^{-3}, were reported in the FEBEX experiment (18 years, heated to 100°C) (Samper et al. 2018, Carbonell et al. 2019, Villar et al. 2020). Other factors such as gravity, proximity to the hydration surface, or repository geometry could result in locally reduced dry density (Wieczorek et al. 2017, Villar et al. 2020, 2021). Processes including piping erosion or chemical erosion could also open up space in the bentonite (RWM 2016a) and allow microbial activity. The general trend of reduced dry density with increasing distance from HHGW means that the likelihood of microbial activity increases away from the canister, but the effects of the other factors just mentioned are harder to predict.

Salinity as a limiting factor in the EBS

In evaporitic host rocks, microbial activity will be severely restricted by the high salinity, though it is possible that some microbial activity could occur within some parts of the GDF (Swanson et al. 2018). In other disposal scenarios, increased salinity may arise as a result of interaction with saline groundwater. In this section, salinities are given in either g L^{-1} or as molar concentrations, depending on the units in the original data. Where this relates to a single salt (e.g. NaCl), the units are converted, and both units shown. When it refers to mixed brines, an accurate conversion is not possible, and an approximate conversion that assumes that the mixed brine is comprised solely of NaCl is given so that values can be more easily compared.

Salinity limits in natural environments and experimental studies relevant to the EBS

Many bacteria, archaea, and fungi survive in saturated or near-saturated salt solutions (Table 6). Natural brines contain a variety of salts, and the balance of chaotropic (destabilizing) and kosmotropic (stabilizing) ions in any particular brine will affect the limits of microbial activity (Hallsworth et al. 2007, Oren 2011, Swanson et al. 2021). For example, all but one brine from an evaporite sequence (Boulby Mine, UK) was able to support microbial life. It was suggested that the ionic composition of that single brine (particularly the higher concentration of chaotropic magnesium ions) made it inhospitable rather than ionic strength alone (Payler et al. 2019). Furthermore, the effect of ionic strength is also highly specific to the organisms under investigation, which makes predicting the habitability of saline environments extremely challenging (Stevens and Cockell 2020). Therefore, caution needs to be taken in extending limits determined in experiments that use only NaCl to mixed brines.

Halarsenatibacter silvermanii strain SLAS-1T has been demonstrated to survive up to salt concentrations of at least 5.99 mol L^{-1} (NaCl equivalent) (Blum et al. 2009). Limits of activity vary considerably depending on which metabolic processes are being considered (Table 6). Sulphate-reducing activity in natural environments containing *Desulfonatronovibrio* has been reported at salinities equivalent to NaCl concentrations of 8.13–8.90 mol L^{-1} and pH 10.65 (Oren 1999, 2011, Foti et al. 2007). The limits for acetogens and methylotrophic methanogens are around 4.28 mol L^{-1} (Lai and Gunsalus 1992, Oren 1999, 2011), whereas the upper limits of acetoclastic and hydrogenotrophic methanogenesis are 2.41 and 2.05 mol L^{-1} NaCl, respectively (Oren 1999, 2011).

Fungal tolerance to salinity is less well studied but one species, *Wallemia ichthyophaga*, requires at least 1.5 mol L^{-1} NaCl and grows up to 5.5 mol L^{-1} NaCl (Ma et al. 2010). Studies on *Wallemia hederae* indicate that the limits to growth in $MgCl_2$ are at least 2.0 mol L^{-1} (Jancic et al. 2016). Halophilic archaea are able to survive inside halite crystals in saturated fluids with >5.5 mol L^{-1} NaCl for long periods of time, and viral salinity tolerance shows some pH-dependence (Grant 2004, Demina et al. 2016). Spores are adapted for survival in extreme conditions, including high salinity but as was noted for temperature, survival decreases with exposure time (e.g. *Bacillus subtilis* spore survival decreased by as much as ∼50% after 1 year at 3.6 mol L^{-1} NaCl (Ulrich et al. 2018).

In Wyoming bentonite, salinities of 0.86–1.71 mol L^{-1} NaCl (and $CaCl_2$) (50–100 g L^{-1}) have been shown to suppress the microbial activity of isolates from bentonite and selected laboratory strains (Stroes-Gascoyne and Hamon 2008, 2010, Stroes-Gascoyne et al. 2010b, Stone et al. 2016a,b). These concentrations are considerably lower than the maximum values for any of the microbial groups listed in Table 6, so relatively moderate salinities could contribute to the suppressive effects of high bentonite densities afforded by the high dry density. More research would be required to establish whether the salt tolerance of microorganisms varies depending on bentonite type.

Defining limits to survival requires knowledge of the brine compositions but with that caveat, it is clear that some bacteria, archaea, and fungi are all able to grow in saturated salt solutions (Grant 2004, Blum et al. 2009, Ma et al. 2010). Based on the above literature review, the following limits can be suggested:

Table 6. Maximum salinity limit for microbial activity/survival.

Microbial group	Max salinity (mol L^{-1})	Details	Reference
Bacteria (activity)	6.0 (mixed salt)	*Halarsenatibacter silvermanii* strain SLAS-1T grows at pH 8.7–9.8, 350 g L^{-1} mixed salt, and 28–55°C	Blum et al. (2009)
Archaea (survival)	> 5.5 (NaCl)	Haloarchaea survive long periods inside halite crystals	Grant (2004)
Fungi (activity)	> 5.5 (NaCl)	Some *Wallemia* spp. can grow in saturated NaCl and up to 2 mol L^{-1}MgCl	Ma et al. (2010), Jancic et al. (2016)
Acetogens (activity)	4.4 (NaCl)	Alkaliphilic *Natroniella acetigena* grows up to 260 g L^{-1} NaCl, and between pH 8.1–10.7	Zhilina et al. (1996)
Methylotrophic methanogens (activity)	4.4 (NaCl)	Methylotrophic *Methanohalophilus* strain Z7302 grows at 257 g L^{-1} NaCl	Lai and Gunsalus (1992)
Acetoclastic methanogens (activity)	2.4 (NaCl)	Limit of acetoclastic methanogenesis is 141 g L^{-1}	Oren (1999), Oren (2011)
Hydrogenotrophic methanogens (activity)	2.0 (NaCl)	Limit of hydrogenotrophic methanogenesis (*Methanocalculus halotolerans*) is 120 g L^{-1}	Oren (1999), Oren (2011)
Sulphate reducers (activity)	8.9 (mixed salt)	Sulphate-reducing activity in lake salts at 520 g L^{-1} Individual strains of sulphate reducers have been reported to be active up to 240 g L^{-1} NaCl (*Desulfohalobium utahense*, *D. retbaense*)	Foti et al. (2007), Ollivier et al. (1991), Jakobsen et al. (2006)
Iron reducers (activity)	4.2 (NaCl)	*Fuchsiella ferrireducens* grows between pH 8.5–10.2, 25–45°C	Zhilina et al. (2015)
Spores (survival)	3.6 (NaCl)	*Bacillus subtilis* spore survival decreased ~50% after 1 year	Ulrich et al. (2018)

Unless NaCl is specified all salts should be assumed to be of mixed composition. > indicates that microbial survival or activity was detected at the highest tested salinity, expressed as NaCl molar equivalents if the brine or salt used was not NaCl. Fully saturated NaCl at standard temperature and pressure is equivalent to 5.5 mol L^{-1}; therefore, this value is used where papers (Grant 2004, Ma et al. 2010) describe using saturated NaCl without specifying the molarity.

(i) 8.90 mol L^{-1} NaCl equivalent (520 g L^{-1}) is currently the highest concentration at which sulphate reduction has been observed in the environment, though the sulphate reducing limit for individual strains is lower.
(ii) 5.99 mol L^{-1} is the highest reported salinity for activity of any bacterial strain. Various bacteria, archaea, and fungi show activity in saturated (5.5 mol L^{-1}) and supersaturated conditions.
(iii) 4.28 mol L^{-1} NaCl equivalent is the currently recognized limit for acetogens.
(iv) 4.40, 2.41, and 2.05 mol L^{-1} NaCl equivalents are the currently recognized limits for methylotrophic, acetoclastic, and hydrogenotrophic methanogens, respectively.
(v) 0.86–1.71 mol L^{-1} NaCl or $CaCl_2$ has been suggested as the limit for survival of microorganisms in Wyoming bentonite.

Changes in salinity within the EBS

It is generally considered that the salinity in an EBS within evaporitic geologies will prevent microbial activity occurring at a level that could have a significant impact on repository performance (Swanson et al. 2018, 2021). Where there are no brines (no liquid water), microbial activity will not be possible. In terms of spatial and temporal patterns, the host rock and buffers or backfill (crushed rock and/or magnesium oxide) within evaporitic repositories will make the salinity high throughout and from the outset. These conditions offer little opportunity for microbial activity, though it cannot be completely ruled out (Swanson et al. 2018, 2021).

In the UK, at repository depths, salinity could be in excess of 1.7 mol L^{-1} (100 g L^{-1}) in non-evaporitic rocks, for example, the porewater within the Mercia Mudstone Group (a potential LSSR host rock) can be up to 189 g L^{-1} (3.2 mol L^{-1}) (Bloomfield et al. 2020, Smedley et al. 2023). Other processes, such as permafrost events could increase groundwater salinity through salt exclusion in freezing (Busby et al. 2015, Kilpatrick 2017). Over time, the salinity in the EBS is likely to adjust to the local groundwater salinity as resaturation occurs. Timescales for resaturation are highly dependent upon geology. For example, in the Callovo-Oxfordian Clay in France, timescales of tens to hundred of thousand of years are expected (Andra 2005) but in the Boom Clay in Belgium, complete saturation of the GDF is expected within 50 years (Weetjens et al. 2006). In saturated HSR, saturation of the EBS could be complete within a few years (RWM 2010).

Desiccation as a limiting factor in the EBS

Salinity and desiccation tolerance are linked, as the former reduces water activity and induces similar osmotic stresses on the cells as other forms of desiccation. This section, including the entries in Table 7, focusses on the desiccation that occurs when heat from HHGW drives away groundwater and dries out material close to the waste, as this is most relevant to the EBS environment (see Potts (1994) for a more detailed discussion of the different microbial effects and responses of these two types of desiccation). The EBS within evaporites will also be highly desiccated due to the lack of water within these GDFs.

Water availability is either reported as relative humidity, water activity (a_w), or percentage water content. A water activity of 1 is equal to a relative humidity of 100% but conversion of these units to water content is material-specific. As a rough guide, Wyoming bentonite with a 25% water content equates to approximately $a_w = 0.96$; 20% to $a_w = 0.92$; and 15% = a_w 0.78 (Pedersen et al. 1995). For ease of comparison, units are given as original units and approximate conversions to percentage water content, where possible.

Table 7. Desiccation limits to microbial activity and survival from studies relevant to EBS.

Microbial group	Material tested	Desiccation limit	Details	Reference
Aerobic microorganisms (activity) Aerobic microorganisms (survival)	Wyoming bentonite	Between (a_w) 0.3–0.75	Microbial activity was observed at 75% but not at 30% relative humidity. Survival was 2.8 times higher at the lower relative humidity.	Stone et al. (2016c)
Bacteria (survival)	Granulated OT-9607 bentonite	Water content 12%	Survival of heterotrophic aerobic bacteria ceased. Temperatures up to 100°C likely to contribute to this limit.	Aoki et al. (2010)
Methanogens	50% sand, 50% bentonite	Water content 15%	Up to 10^2 cells g^{-1} dry weight bentonite for methanogens and sulphate reducers survived after 2.5 years at 85°C and 15% moisture content.	Stroes-Gascoyne et al. (1997)
Sulphate reducers	Wyoming bentonite	a_w 0.96	Sulphate reducers did not survive 1 day at 30°C at this a_w.	Motamedi et al. (1996)

Desiccation limits in natural environments and experimental studies relevant to the EBS environments

In studies where strong salt or sugar solutions produce the water stress, water activities of 0.748 (*Halanaerobium lacusrosei*), 0.635 (haloarchaeal strains GN-2 and GN-5) and 0.585 (*Aspergillus penicillioides*) have been reported as minima for bacterial, archaeal, and fungal activity, respectively (Stevenson et al. 2015, 2017). However, data on survival under dried conditions (rather than osmotic stress-induced desiccation), which is more relevant to the desiccation experienced when heat from radioactive waste drives water out of the EBS, is difficult to find. A variety of anaerobic microorganisms, including methanogens, acetogens, and sulphate reducers can survive prolonged periods of desiccation in oxic desert soils (Peters and Conrad 1995, Angel et al. 2011), and many microorganisms, including sulphate reducers, from volcanic tuff can survive for at least eight months of desiccation (a_w 0.36) (Pitonzo 1996). Methanogens can be revived after exposure to both oxic and anoxic desiccation (Liu et al. 2008). Under ideal conditions, which may include low temperatures, desiccation may promote survival of some prokaryotes with survival times being thousands if not millions of years (Potts 1994). In experiments specific to radioactive waste disposal (Table 7), water contents below 12% in granulated OT-9607 bentonite (Aoki et al. 2010) and 15% in a 2.5-year underground research laboratory experiment containing a sand and bentonite mix prevented survival of microorganisms (Stroes-Gascoyne et al. 1997). In both cases, the temperatures enter the range previously identified as detrimental to survival in Wyoming bentonites (Table 3). It is therefore possible that temperature rather than desiccation alone had a role in determining the limits of survival. In another experiment, a reduction from 75% relative humidity (∼15% water content) to 30% (unknown water content) was sufficient to cause microbial activity to cease, but survival rates were higher at the lower water content (based on numbers bacteria and fungi that could be cultured from samples at the end of the test) (Stone et al. 2016c). The authors suggested that the lower relative humidity may have prompted rapid entry to a dormant state and improved survival rates. Higher survival rates at lower relative humidity have also been reported for fungal spores (Palmer et al. 1987). The value of a_w 0.96 [25% water content] in compacted Wyoming bentonite (Pedersen et al. 1995, Motamedi et al. 1996, Stroes-Gascoyne et al. 2010a) given in Table 7 should be treated with some caution as there is little data available on limits for particular microbial groups and it is not clear whether water activity or the compaction of the bentonite was the key limiting factor in those studies. Although desiccation limits microbial activity, it may promote microbial survival, and further work is required to establish the implications of this for the EBS environment. Based on the reports of sulphate reducer and methanogen survival in arid conditions in other environments (Peters and Conrad 1995, Pitonzo 1996, Angel et al. 2011), it is possible that such organisms could survive the desiccated conditions within an EBS.

Although data on defining limits is scarce, the following limits are suggested:

(i) Water activities of 0.748 (bacteria), 0.635 (archaea), and 0.585 (fungi) are the minimum reported for survival under osmotic stress desiccation.
(ii) Within compacted bentonites, <12%–15% water content (approx. a_w < 0.75) can be considered the limit to microbial activity (when bentonite is dried by heating).
(iii) It should also be noted that desiccation may promote the long-term survival dormancy, including as spores.

Changes in desiccation within the EBS

The desiccation of bentonite in the FEBEX experiment was previously discussed in the context of changes to dry density. The relative humidity of bentonite closest to the heater and dropped from 40% to <10% after 1 year. It had not returned to initial conditions after five years but had risen to 60% at 10 cm from the heater after 18 years (Villar et al. 2005, 2020). Another heater experiment (the Alternative Buffer Materials experiment) found that although the water content of bentonites nearest to the heater was 21.0%–39.7% (Svensson et al. 2011), two non-bentonite buffer materials tested did have lower water contents (Friedland Clay down to 14.8% and Callovo-Oxfordian Clay down to 11.9%). In this case, all tested bentonites remained above the 12%–15% dry density limit suggested for microbial survival. Together, these studies indicate that desiccation will be greatest nearest to the waste. Desiccation is unlikely to extend throughout the whole of the bentonite buffer, and the time period over which is a limiting factor may be relatively short. While this has the potential to reduce microbial activity close to the waste, it could promote survival of cells, with the potential for them to be revived if the bentonite becomes resaturated. However, the risk of this may be mitigated if elevated temperatures during the desiccated period reduce survival rates.

Radiation as a limiting factor in the EBS

While waste containers remain intact, the radiation dose in the EBS will be highest adjacent to the waste containers and will drop off rapidly with distance. At the container surface, maximum dose rates are expected to be <0.05 kGy h^{-1} for HHGW (Stroes-Gascoyne et al. 1994a, Pitonzo et al. 1999, SKB 2010a, RWM 2016a, Morco et al. 2017) and several orders of magnitude lower for LHGW. If a canister is breached, radionuclides may be transported to the surrounding environment, releasing radiation that could locally affect microorganisms. The amount and the extent of radiation released will depend upon the type and concentration of radionuclides released as well as the hydrogeological properties of the EBS and surrounding rock (SKB 2006). Due to the complexity of these processes, we focus only on the radiation emitted from intact waste canisters. The majority of experimental studies use γ-sources, so all references discussed here refer to γ radiation, unless otherwise specified.

Radiation limits in natural environmental and experimental studies relevant to the EBS

Defining the radiation limits that microorganisms can withstand is complex because of the different ways that radiation resistance is tested and reported, and the paucity of experimental data from relevant environmental settings or long-term studies. Short exposure to a high dose has a different effect on microorganisms than exposure to lower doses for long periods of time. Therefore, the total (accumulated) dose and the rate at which that dose is applied are important in understanding microbial survival. Most research investigates acute radiation exposure (typically in the context of sterilizing food or medical equipment) and the derived radiation limit expressed in terms of accumulated dose only. The challenge for understanding microbiology in repository environments is that little information exists about survival and activity under long-term radiation with lower dose rates. Several studies (e.g. Janning and Tveld 1994, Stroes-Gascoyne et al. 1994b, Babich et al. 2021, Jroundi et al. 2023) have described microbial communities exposed to long-term radiation in uranium deposits, but dose rates at sampling locations were not reported.

When microorganisms are exposed to radiation, two phases of response can be identified. Up to a certain dose, there is no loss of viable cells or observable DNA damage (DNA damage can be repaired as fast as it occurs). Above that dose, the number of surviving cells decreases to extinction as DNA damage leads to loss of protein function and cell death (Daly 2012). Taking the model radiotolerant bacterium, *Deinoccoccus radiodurans*, as an example, 5 kGy is often quoted at the maximum dose at which no DNA damage accumulates (Battista et al. 1999), while the maximum total dose that can be tolerated is >20 kGy with D_{10} value of up to 12.6 kGy. (The D_{10} value is the radiation dose needed to reduce microbial numbers to 10% of their original). Factors that are likely to be encountered within an EBS such as anoxia and desiccation are known to make cells more resistant to radiation (Mingarro et al. 2005, Bauermeister et al. 2011). Data on the upper limits for different microbial groups is included in Table 8, which reveals a broad range of values reflecting that resistance depends on environmental conditions and a combination of dose rate and accumulated dose.

Exposure to 100 kGy (36 s at 10 000 kGy h^{-1}) decreases the number of culturable bacteria in permafrost soils by two orders of magnitude but some soil bacteria can be cultured after 148 kGy (when applied at 43 h at 3.44 kGy h^{-1}) but not 320 kGy (93 h at 3.44 kGy h^{-1}) (Cheptsov et al. 2018, 2019). It has been proposed that radiation exposure can cause cells to enter a viable but non-culturable (VBNC) state, where they cannot grow under standard culture conditions but show other evidence of life, e.g. respiration (Pitonzo et al. 1999, Cheptsov et al. 2019). This is significant as relying on cultivation-based tests could lead to an underestimation of the number of cells able to persist around a GDF if cells are released from their VBNC state when the conditions become suitable (Pitonzo et al. 1999).

Surprisingly, there is little research on radiation tolerance from a radioactive waste perspective. *Kineococcus radiotolerans* (isolated from a high-level radioactive waste facility at Savannah River Site, USA) with background radiation doses of between 0.00018 kGy h^{-1} and 0.0035 kGy h^{-1}) and an isolate, closely related to *D. radiodurans*, have been shown to survive an accumulated dose of 20 kGy in the laboratory (Phillips et al. 2002, Fredrickson et al. 2004, Bagwell et al. 2008). This site had been receiving radioactive waste for decades at the time of sampling, but it is not clear how long the specific sampling sites had been receiving the stated radiation doses. A variety of microorganisms isolated from the Lanyu low-level waste (LHGW) repository in Taiwan had D_{10} values of up to 2.05 kGy (at 2 kGy h^{-1}). Most showed active growth when exposed to 0.0014 kGy h^{-1}, with the most resistant isolates (*Micrococcus* sp. and *Candida guilliermondii*) only slightly inhibited at the highest tested dose rate (0.0068 kGy h^{-1}). This dose rate is equivalent to 100 times the expected dose at container surfaces in that repository (Chou et al. 2011, Li et al. 2015). In compacted bentonite, viable iron reducers and sulphate reducers survived radiation exposure of 1 kGy (1.45 kGy h^{-1}) (Haynes et al. 2018). There was a large reduction in the diversity of communities in subsequent iron reducer enrichment cultures, but little difference on the diversity in sulphate reducer enrichment cultures. Irradiation of FEBEX bentonite at 0.0258 kGy h^{-1} produced a D_{10} value of 0.5–2.5 kGy (Mingarro et al. 2005). Similar D_{10} values (0.34 to 1.68 kGy) were obtained from Wyoming bentonite irradiated at ~6 kGy h^{-1}, and it was predicted that extinction would be achieved within 9 to 33 days (Stroes-Gascoyne et al. 1994a). To investigate reports of microbial growth in radioactive waste settings (e.g. surface storage pools), Bruhn et al. (2009) exposed metal cladding hulls to 0.0021 kGy h^{1} in experiments up to 99 days. Multispecies biofilms formed and survived for up to 64 days (total absorbed dose of 3.2 kGy). In the longest experiment, no biofilm was detected (49 kGy applied at 1.7–2.1 Gy h^{-1}) but microorganisms could still be isolated from the experiments. While these timescales are still short in the context of radioactive waste disposal, they do point to the potential for longer term survival when radiation doses are comparable to those emitted by waste.

Finally, it is worth noting that, at low dose rates, radiation may actually have a positive effect on microbial activity. For example,

Table 8. Maximum radiation limit for microbial activity/survival.

Microbial group	D_{10} -kGy (dose rate)	Max total radiation—kGy	Details	Reference
Bacteria (survival) (activity)	12.6–dried cells 5.6–cells in phosphate buffered saline	>20	*Deinococcus radiodurans* or closely related isolates. Growth with no loss of viability has been reported at total dose of 5 kGy.	Fredrickson et al. (2004), Cox and Battista (2005), Bauermeister et al. (2011)
Bacteria in soil (survival)	148 (3.44 kGy h^{-1})	Above 148 but below 320	Bacteria from mixed soil communities. 148 kGy reduced the viable cells by an order of magnitude suggesting that this value is close to the D_{10} value.	Cheptsov et al. (2019)
Archaea (survival)	8 (3.6 kGy h^{-1})	30	*Thermococcus gammatolerans* survives the highest total dose for any archaea (N.B. measured when cells were on ice).	Jolivet et al. (2003) Pavlopoulou et al. (2016)
Fungi (survival)	4 (1.8 kGy h^{-1})	>10	*Histoplasma capsulatum* and *Cryptococcus neoformans*.	Nurtjahja et al. (2017), Dadachova and Casadevall (2008), Dadachova et al. (2004)
Methanogen (survival)	12.6 (at 0.9 kGy h^{-1} (X-ray source)	10	*Methanosarcina soligelidi* shows the highest D_{10} but not total exposure limit for any archaea. The physiological D_{10} (based on methane production rates) was considerably higher, 25.3 kGy.	Morozova et al. (2015)

Note that the maximum accumulated dose was often determined by the maximum that could be applied in any particular experiment. Note also that these experiments have been conducted at a range environmental conditions, not all of which are directly applicable to the EBS environment.

microbial iron reduction activity was reduced after exposure to 0.03 kGy h^{-1} for 56 days (accumulated dose 38.6 Gy), but increased after exposure to a lower dose of 0.0005 kGy h^{-1} (accumulated dose of 0.6 Gy). Another study found that that exposure of cellulose and PVC to radiation increased their degradation rates (Nixon et al. 2018, Bassil et al. 2020). Additionally, hydrogen produced by the radiolytic breakdown of water is believed to sustain microbial life in deep marine sediments and basalts (D'Hondt et al. 2009, Dzaugis et al. 2016, Sauvage et al. 2021) and could potentially have the same effect around a GDF.

The limits of microbial survival in response to radiation are difficult to specify as the combination of total accumulated dose and dose rate. Although figures can be obtained for the maximum accumulated dose that microorganisms can survive (Table 1), it will be clear from the discussion presented here that these values are strongly influenced by the dose rate. As a result of this complexity we do not find that it useful to describe specific limits for radiation dose, instead we have interpreted the data in the context of the EBS environment at the end of the next section.

Changes in radiation dose within the EBS

The radiation dose rate at canister surfaces will be variable and may not be known until the point of deposition (Andersson et al. 2017). Much of the experimental work carried out used dose rates considerably higher than the expected maximum dose rate at the surface of HHGW canisters, which are likely to be in the range of 0.0005–0.05 kGy h^{-1} (Stroes-Gascoyne et al. 1994a, Pitonzo et al. 1999, SKB 2010a,b, RWM 2016a, Morco et al. 2017). In the example of the Canadian deep geological repository concept, the cumulative dose on the canister surface will increase rapidly, reaching ~10 000 kGy in a few hundred years with dose rates dropping from 0.001 kGy h^{-1} to 1×10^{-6} kGy h^{-1} over this period (Morco et al. 2017). The waste in the LHGW repository in Lanyu, Taiwan has a surface dose rate of between 1×10^{-7} and 2×10^{-6} kGy h^{-1} (Li et al. 2015). In the absence of other data on expected dose rates on the external surface of LHGW containers, the maximum expected dose rate can be bounded by using the internal maximum dose rate, which is predicted to be 0.00001–0.001 kGy h^{-1} (RWM 2016a).

Calculations of the radiation penetration of bentonite indicate that 6 kGy h^{-1} would sterilize a relatively narrow region (<25 cm) next to the waste source. The thickness of clay required to reduce the intensity of γ-radiation by half has been measured as 4.2 to 7.2 cm, increasing as energy increases (Olukotun et al. 2018). In Boom clay, it is estimated that a dose of 0.4 kGy h^{-1} at the clay-canister interface would reduce to practically zero at 50 cm from the canister (Noynaert et al. 1998).

Extensive data on expected radiation doses in LHGW is not readily available but, in the case of SKB's Final Repository for low- and intermediate-level waste, radiation is not expected to be high enough to influence microbial processes, and therefore the effects of radiation can be disregarded (SKB 2010a, 2014), a principle that can likely be applied to other LHGW.

Combining the available data on likely doses of radiation emitted from waste and the findings of research into microbial responses to radiation, focussing on the longer term lower dose rates, we find that:

(i) Microorganisms isolated from around the Lanyu repository, Taiwan (LHGW), grew best at 0.0014 kGy h^{-1} and were only slightly inhibited at 0.0068 kGy h^{-1}.
(ii) There are several examples of microorganisms surviving long periods of time around natural radiation sources. While many of these reports do not include data on dose rates, bacteria isolated from from the vicinity of radioactive waste storage sites have survived long periods with a dose rate of up to 0.0035 kGy h^{-1}.
(iii) Surface dose rates for HHGW are predicted to be between 0.0005 and 0.05 kGy h^{-1} and even if the external dose rates for LHGW were the same as the expected maximum dose rate inside LHGW (0.001 kGy h^{-1}) (RWM 2016a), then in

Table 9. Examples of low electron donor and acceptor concentrations for important microbial processes in a repository environment.

Microbial group	Electron donor threshold (μmol L^{-1})	Electron acceptor threshold (μmol L^{-1})	Reference
Methanogens	4.0×10^{-4} hydrogen, 2.0×10^{2} acetate	4.44×10^{1} dissolved inorganic carbon	Chen et al. (2019), Jetten et al. (1992), Karadagli and Rittmann (2007), Chen et al. (2019)
Sulphate reducers	7×10^{-4} hydrogen, 4–6 for formate and acetate, and 5×10^{-1} for propionate	1×10^{1} in seafloor sediments, $<1.04 \times 10^{1}$ in petroleum-contaminated aquifer	Karadagli et al. (2023), Frank et al. (2015), Vroblesky et al. (1996), Glombitza et al. (2015)
Iron reducers	3×10^{-5} hydrogen	Iron reducers can use Fe^{3+} in solid material, e.g. ferrihydrite	Karadagli et al. (2023)

most cases, the radiation emitted from radioactive waste should have minimal impacts on microbial survival and activity. Even if dose rates are as high as 0.05 kGy h^{-1}, the rapid reduction of radiation as it travels through bentonite (combined with radioactive decay rates) is likely to mean that any effect will be spatially and temporally limited.

Nutrients and energy sources as a limiting factor in the EBS

Even if none of the limits described so far are exceeded, all microbial life requires access to a number of chemical elements and compounds for growth and metabolism. Within an EBS, these could come from the groundwater (influenced by the dissolution of surrounding host rock and engineered barrier materials), materials and contaminants entering the GDF during the operational phase, the waste itself, and potentially directly from mineral surfaces on engineered barrier materials (Humphreys et al. 2010). Concentrations and compositions of these nutrients will vary depending on site and concept.

Nutrient and energy limits in natural environments and studies relevant to the EBS

A complete review of nutrient and energy requirements is beyond the scope of this paper, but some examples of minimum energy source (electron donors and acceptors) requirements for microbial growth are given in Table 9. Very low concentrations of hydrogen can sustain microbial activity (3×10^{-5} to 7×10^{-4} μmol L^{-1}) with iron reduction being about an order of magnitude lower than sulphate reduction and methanogenesis (Karadagli and Rittmann 2007, Karadagli et al. 2023). Higher concentrations of organic compounds are needed if they are being used as electron donors. Known electron acceptor thresholds are in μmol L^{-1} concentrations (see Table 9). Iron reduction often occurs when microorganisms are in direct contact with minerals, there is also evidence that sulphate reducers can be supported by dissolution of mineral sulphates (Maanoja et al. 2020), and methanogens can use mineral carbonates (at high pH) (Wormald et al. 2022).

In general, fungi have the highest nutrient requirements and nutrient thresholds, and archaea typically have lower thresholds than bacteria. Less is known on minimal nutrient requirements in similar natural environments. However, the approach of comparing the elemental composition of a type of cell to known groundwater composition has been used to predict a theoretical maximum microbial biomass in the context of subsurface hydrogen storage (Thaysen et al. 2021). This approach assumes that cell growth can occur until the first essential nutrient reaches zero, and can be used to predict limiting nutrients. This may be an approach that could be used in the future to estimate the amount of biomass that could be supported within the EBS if an assessment of the environmental limits indicates that microbial life can be supported.

Although not necessarily nutrients or energy sources, an excess of certain elements can limit microbial activity. For example, anaerobic citrate degradation in hyperalkaline repository conditions has been shown to be inhibited by elevated nickel and uranium (Byrd et al. 2023). Due to the number of potentially inhibitory substances that could be found in radioactive waste inventories, and the paucity of data available that is specific to EBS conditions, this is beyond the scope of this review.

Changes in nutrient and energy sources within the EBS

The distribution of nutrients in time and space within a the EBS is the result of multiple factors, e.g. material introduced in the construction phase, the waste components, EBS material, and groundwater from the host rock.

The EBS is likely to be more nutrient depleted in HHGW disposal (compared to LHGW), as the waste in the latter typically contains a considerable organic component, summarized in Abrahamsen-Mills and Small (2021), which can support microbial activity and growth. For example, bitumen (a significant component of the Belgian waste inventory) could be a source of nitrates supporting microbial denitrification. It has been shown that nitrate is consumed quickly in the presence of elevated electron donors in the Boom and Opalinus Clays, but complete denitrification does not occur without the addition of electron donors (Bleyen et al. 2016). In most LHGW concepts, the breakdown of cellulose at high pH produces isosaccharinic acid, which can serve as sole carbon and energy sources for microorganisms (Bassil et al. 2015). Other less common components such as plasticizers may be included in encapsulated waste and contain organic polymers (RWM 2019) that could support microbial activity (Nixon et al. 2018). As HHGW does not contain the same organics rich waste as LHGW it is not likely to support microbial life in the same way. However, hydrogen produced by various mechanisms, including as a result of metal corrosion, may be an important electron donor source in the EBS and host rock around a GDF (Libert et al. 2011, Bagnoud et al. 2016, Boylan et al. 2019).

Leaching of any waste components is unlikely to occur until the surrounding groundwater reaches the container itself, at this point, release of certain components will be more or less instantaneously (Marsh et al. 2021). Leaching behaviour depends on the specifics of the waste inventory, EBS, and GDF design. It is not possible to give general descriptions of when or where additions from the waste will make a previously nutrient- or energy-limiting environment habitable. The same is true of minimal amounts of hydrogen released by corrosion.

Considering the EBS materials as sources of nutrients and energy, the concentrations of sulphur and organic carbon in Wyoming-type bentonites may be sufficient to support some microbial activity, including sulphate reduction (Maanoja et al. 2020, 2021, Miettinen et al. 2022). Information is not available for other types of bentonites. Total organic carbon and sulphur concentrations may be limited in bentonites used in the EBS; e.g SKB has set limits for both to <1% dry mass at their SR site (Karnland 2010). The evolution of cementitious materials as they react with groundwater, especially in terms of effects on pH, is well understood, but rigorous quantitative assessments of the nutrients and energy sources that could be available to microorganisms have not been undertaken.

Any biomass that accumulates as a result of the utilization of any available nutrient and energy sources will eventually die, and necromass could support further microbial activity in and around the GDF (Bagnoud et al. 2016, Leupin et al. 2017).

Microbial populations have been found in all three geological settings considered as host rocks for a GDF (Humphreys et al. 2010), indicating presence of some nutrients and electron donors in groundwater. In some cases, for example, the Swiss Opalinus Clay (an LSSR host rock), the organic matter appears to be recalcitrant to microbial oxidation (Leupin et al. 2017). In other cases microbial activity can be stimulated by the presence of organic acids and hydrogen by coupling to iron minerals found in Posiva's ONKALO facility in Olkiluoto, Finland (HSR) (Johansson et al. 2019). The issues surrounding nutrient and energy sources around a GDF that may stimulate microbial activity have been addressed in specific disposal concepts but tend to be qualitative rather than quantitative. This means a view on when and where minimum requirements have been met has not been explored. However, the following general statements provide an overview:

(i) HHGW containers are designed to contain the waste for long periods, but as they corrode they will release hydrogen into the EBS, which can act as an electron donor for microbial processes such as sulphate reduction or methanogenesis. It is not expected that large amount of other energy sources or nutrients will be released from HHGW.

(ii) As LHGW is biologically and chemically degraded, a range of nutrients and energy sources are likely to be released into the EBS once containers are breached. Although these processes are broadly understood, the quantities and types will vary depending upon the waste inventory and disposal concept and GDF design. Work has focussed on the more abundant compounds in the waste inventory (e.g. cellulose breakdown products) rather than focussing on components of the inventory nearer the minimum limits for life.

(iii) EBS materials, such as cements and bentonite, may provide nutrients and energy sources, and there is evidence that microbes can use organics and sulphate from bentonites. The geochemical evolution of cements is relatively well understood, but not in terms of how this affects their ability to support microbial life.

Discussion

Although the stresses have been considered in turn above, microbial communities within the EBS will be subject to multiple, simultaneous (and often coupled) stresses that could act synergistically to limit life in EBS environments (Capece et al. 2013). For example, radiation from HHGW also generates heat that will drive water in the bentonite away from the waste. This has the effect of increasing the desiccation and dry density due to the greater swelling pressure exerted in more distal clay with a higher water content, compressing the drier bentonite. In this scenario, the microorganisms closest to the waste container will be exposed to greater heat, desiccation, elevated dry density, and radiation than those further away. While the desiccation can reduce microbial activity, it may also increase the survival rates of dormant microorganisms, which could potentially become reactivated if conditions become suitable again.

The co-occurrence of certain conditions in natural environments (e.g. high salinity and hyperalkalinity in soda lakes) has led to some organisms developing co-tolerance (Capece et al. 2013). In other cases, the cause and even existence of co-tolerance are less clear. There is some evidence that radioresitance has developed as a coincidental result of desiccation resistance (Pavlopoulou et al. 2016), but other studies have found no clear evidence of co-tolerance developing (Beblo-Vranesevic et al. 2018). Although this means that in specific cases, microorganisms may be able to tolerate the co-occurrence of certain stresses, it is also true that multiple stresses may act together and reduce the possibility of microbial activity (Beblo-Vranesevic et al. 2018). For example, few microbial species have been identified that can survive both high pH (>pH 10–11) and high temperature (>50–60°C), despite neither of these individually being at the upper limits of survival (Bowers and Wiegel 2011, Harrison et al. 2013, Nixon et al. 2022). A similar observation has been made for the lack of cultivated strains that can grow at temperatures above 55°C and salinities >1.7 M (Thaysen et al. 2021). The nature of laboratory experimentation means that stresses are usually considered individually, even though it is recognized that in an EBS environment, multiple stresses may be operating as part of complex coupled processes. Consequently, to increase the confidence in the data presented here, additional research is required to investigate the survival and activity of microorganisms under combinations of stresses. Particular attention should be given to ensuring that the combination of environmental stresses is relevant to a specific disposal concept so that the data generated can form part of a safety assessment.

An understanding of the limits of microbial life, combined with an understanding of the evolution of the conditions in the EBS, can be used to predict times and locations where microbial activity and survival are more or less likely. Although the specific conditions will vary according to geology, waste type, and repository design, some common features can be outlined.

For HHGW within a bentonite-based EBS (in HSR or LSSR), temperatures and radiation levels will be highest in the early stages of GDF, potentially coinciding with the period when oxygen has not yet been completely consumed. The heat will limit, if not exclude, microbial activity in a zone extending from the waste canister surface to some distance into the bentonite buffer. This may result in some or all of the EBS being above palaeopasteurization temperatures in the early stages. Conditions are likely to drop below the predicted limit for survival in bentonite on the order of hundreds of years. It is not certain whether temperatures could return to levels supporting life before all oxygen is consumed and conditions become reducing. If this is the case, then understanding of the potential impacts of processes such as aerobic microbiologically influenced corrosion in these environments would need to be improved. In these early stages, post-closure radiation on the canister surface may allow microbial survival. This is another area where data is sparse and, generally, radiation is not considered to

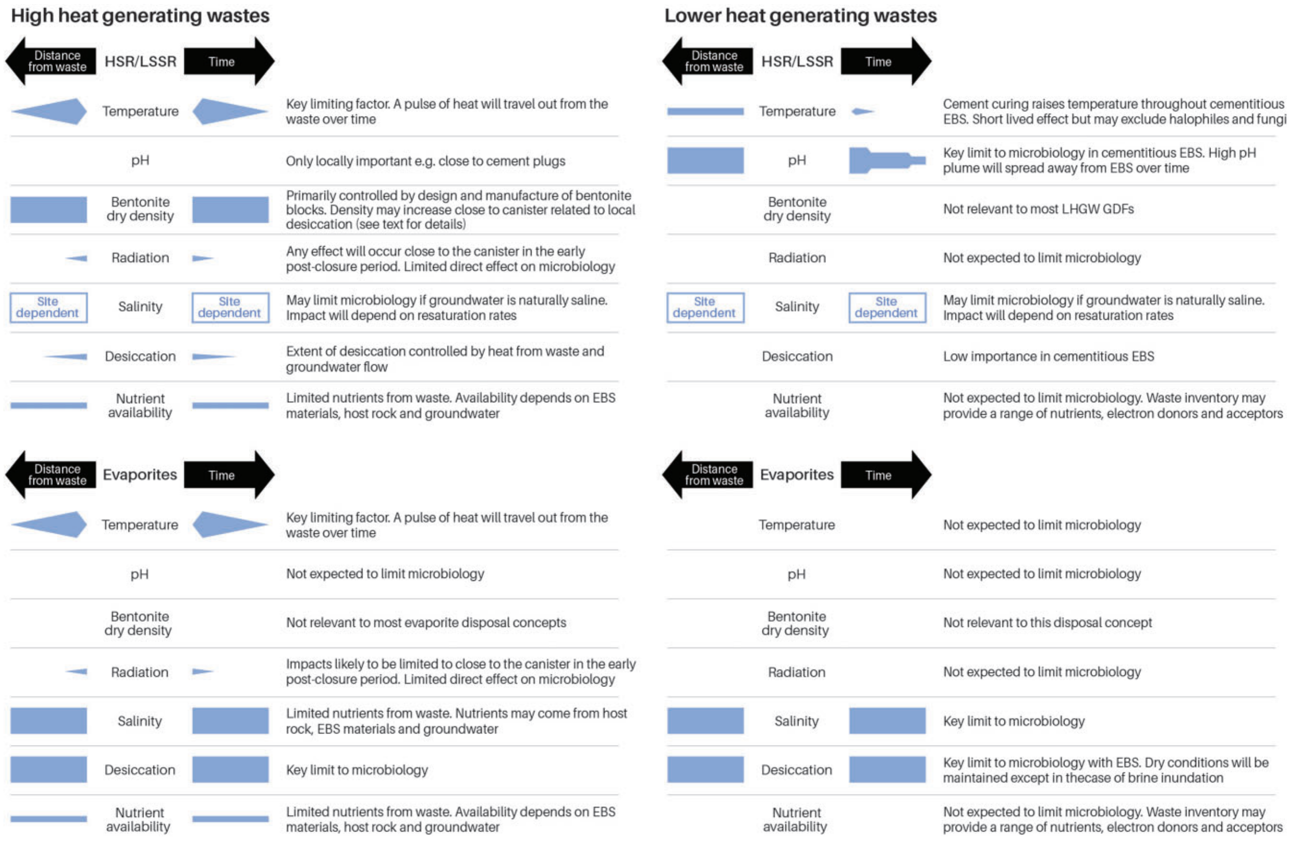

Figure 3. Summary of the extent to which the variables considered in this review may limit microbial survival and activity for GDFs containing HHGW (upper) and LHGW (lower). HSR and LSSR are grouped together at the top of each section and evaporites are considered separately below. The blue bars give a qualitative indication of impact (thicker bars indicate greater potential to limit microbial activity and survival). Length of bar gives an indication of duration/distance of effect. Tapering indicates a gradual decrease or increase of effect.

impact microbial processes in the EBS. Any effects that do occur will be limited to the near-canister environment, as the radiation dose decreases rapidly as it penetrates the bentonite. Heating of the bentonite will result in desiccation and further limit microbial activity in the zone nearest to the waste. As the heat dissipates and the groundwater hydrates the bentonite, the swelling of the clay, and resultant decrease in pore space will further reduce microbial activity. The precise point of exclusion will depend on bentonite type and be affected by heterogeneity of hydration, particularly at interfaces such as at the edges of the bentonite buffer. Extremes of pH are not expected to impact microbial activity in bentonite EBS (with the exception of specific examples given within this review). Salinity may reduce microbial activity where the GDF is situated in geology with saline groundwater, and its influence will be dependent upon hydrogeological factors.

In cementitious repositories, typically intended for LHGW (in HSR or LSSR), the key stress is likely to be high pH. Bulk pH is expected to remain above pH 12.5 for on the order of 50 000–100 000 years, which is anticipated to exclude most microbial activity throughout the cementitious material except where local pH conditions are lower (e.g. within cracks on the outer edge of the EBS). Temperature increases resulting from the curing of cement are expected to alter the prokaryotic community but not exclude them completely. These temperatures could potentially exclude fungi or halophiles for a period of tens to hundreds of years. Microbial activity may also be affected if the groundwater is saline. As pH and salinity resistance may have co-evolved, the hyperalkaliphilic community that will develop may also be resistant to any increases in salinity. After around 50 000 to 100 000 years, pH will drop to around pH 10.5 and an increasing variety of alkaliphiles can become active. Eventually, pH will return to that of the surrounding groundwater, and increasing diversity of microorganisms will become active. As this will occur on the scale of hundreds of thousands up to a million years, this may have less of an effect on the safe operation of the GDF due to the significant radioactive decay that will have occurred over this timescale. Radiation from LHGW is not expected to significantly impact microbial activity in the EBS.

In evaporitic geologies, the salinity will be the primary limiting factor in the EBS (for both HHGW and LHGW). Although microbial activity cannot be ruled out in even the most saline environments, activities are expected to be low and not be detrimental to the performance of the GDF (Swanson et al. 2018). In addition to this stress, very high temperatures could be generated by HHGW. Combined with the relatively low temperature limits for characterized strains of halophilic anaerobes, this means that a zone of considerable distance around the waste could remain too hot for microbial activity for thousands of years.

If none of the environmental limits are exceeded, microbial activity may still not occur if the environment is limited in specific elements, nutrients, or sources of energy required for building and maintaining microbial cells. Assessments of this are likely to remain a challenge due to the complexities of the possible contributions from groundwater, host rock, EBS materials, and waste and how these vary as the environmental conditions evolve. If the exercise described here can narrow down the times and

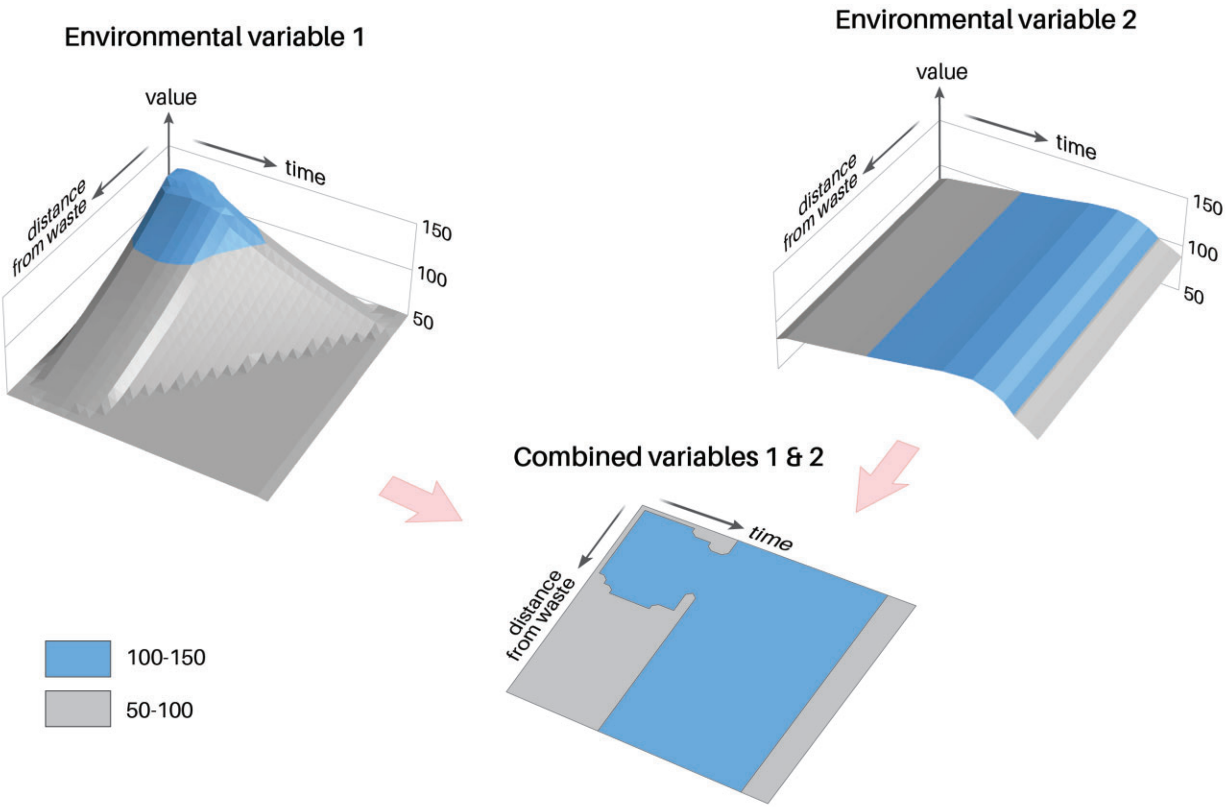

Figure 4. Illustration of how microbial limits approach could be incorporated into modelling of the evolution of conditions within an EBS. The top two illustrations represent how two hypothetical variables change with distance from the waste source and time since the closure of GDF. In each case, a limit for life has been set at 100 arbitrary units and is indicated by the change from grey (below the limit to life) to blue (above limit to life). Data from multiple variables can be superimposed (here only two are shown) to show where, in space and time, the conditions might allow microbial activity (grey zone). In this example, variable 1 alone indicates that the environment becomes habitable beyond a certain distance from the waste and after a certain period. However, the second variable limits life throughout the EBS for a period of time. When the two are combined (in the lower image), a better impression of the habitable space/time can be gained. This information can be used alongside other data to determine whether microbial activity at those times/places poses a potential risk (or benefit) to the safety case, and decisions can be made about whether additional research needs to be conducted to confirm the type and magnitude of microbial activity, e.g. through laboratory experimentation or modelling.

places where microbial activity could occur, the task of predicting whether there will be sufficient nutrients and energy sources will become less challenging than predicting their levels throughout the entire of the post-closure period.

In this work, we have intentionally taken a broad approach to demonstrate, in principle, how a knowledge of the environmental limits to microbial life could be used to predict where, when, and what type of microbial activity could occur within the environment of an EBS for a GDF. As such, it has not been conducted with any specific geology, waste type, or disposal concept in mind. Some general principles of the periods and locations where different environmental factors might exert their influence are shown in Fig. 3. This figure is meant to be illustrative and there may be factors specific to the site or EBS design that mean the situation differs for a particular GDF.

To apply this to any proposed GDF, the limits we have outlined should be used as input parameters for system models of expected changes to temperature, pH, resaturation, etc. Incorporating microbial limits into such models will identify the times and places when microbial activity could occur. An illustration of how this might be done for two variables is shown in Fig. 4. This approach could be expanded to include the ranges of multiple variables to identify a habitability space around a GDF as conditions over time. This habitability space can then be cross-referenced against safety documents to assess whether activity at those times and places has the potential to affect the safe containment of waste. If it could, then a decision can be made about whether further experimental work is required to confirm or exclude the possibility of relevant microbial activity under those conditions.

Conclusions

We have outlined an approach to determining the likelihood for microbial activity in relation to the disposal of radioactive waste. This approach is based upon the environmental limits of microorganisms for stresses likely to be encountered during the lifetime of a GDF and which is broadly applicable to predicting microbial activity in other environments over long periods under variable environmental conditions. Where data is available, different types of microbial activity can be incorporated so that the limits of particular microbial groups can be refined by taxonomic group (e.g. bacteria or fungi) or microbial process (e.g. sulphate reducers or methanogens). This approach could inform safety considerations by providing a series of limits to life that could be incorporated into existing or new models of the environmental conditions. In this way, a more targeted approach to understanding microbial impacts can be integrated into safety planning to identify whether there is potential for negative or positive impacts on the safe containment of waste, and the outputs of this exercise could be used to identify any gaps in understanding microbiology within the EBS and inform the design of future research that will improve our understanding of the microbiological processes at critical times and locations within the EBS that have the potential for positive or negative impacts on the safe containment of waste.

Author contributions

Simon P. Gregory (Conceptualization, Project Administration, Investigation, Writing – original draft, Writing – review & editing), Megan J. Barnett (Investigation, Writing – original draft, Writing – review & editing), and Jessica R.M. Mackie (Investigation, Writing – original draft, Writing – review & editing)

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References

Abrahamsen-Mills L, Small JS. Organic-containing nuclear wastes and national inventories across Europe. In: *The Microbiology of Nuclear Waste Disposal*. Amsterdam, Netherlands: Elsevier, 2021, 1–20.

Andersson JV, Salo J-P, Morén L *et al.* Safety functions, performance targets and technical design requirements for a KBS-3 V sepository; Svensk Kärnbränslehantering AB: Forsmark, Sweden; Posiva Oy: Eurajoki, Finland, Posiva SKB Report 01. 2017.

Andra. Evaluation of the feasibility of a geological repository in an argillaceous formation. Andra Report INIS-FR-19-1357. 2005. pp.237. https://inis.iaea.org/collection/NCLCollectionStore/_Public/50/056/50056449.pdf?r=1 (17 January 2024, date last accessed).

Angel R, Matthies D, Conrad R. Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen. *PLoS One* 2011;**6**:e20453.

Aoki K, Sugita Y, Chijimatsu M *et al.* Impacts of thermo-hydro-mechanical experiments on the microbial activity in compacted bentonite at the Kamaishi Mine, Northeast Japan. *Appl Clay Sci* 2010;**47**:147–54.

Atkinson A, Everitt NM, Guppy RM. Time dependence of pH in a cementitious repository. In: Ludz W, Ewing RC (eds.), *Scientific basis for nuclear waste management XII. Materials Research Society Symposium proceedings. Pittsburgh, PA (USA); 12th international symposium on the scientific basis for nuclear waste management; 10-13 Oct 1988*. **Vol. 127**, Berlin, Germany: Materials Research Society, 1988, 439–46. https://inis.iaea.org/search/searchsinglerecord.aspx?recordsFor=SingleRecord&RN=21037560. ISBN 0-931837-97-9.

Babich TL, Semenova EM, Sokolova DS *et al.* Phylogenetic diversity and potential activity of bacteria and fungi in the deep subsurface horizons of an uranium deposit. *Microbiology* 2021;**90**:607–20.

Bagnoud A, Chourey K, Hettich RL *et al.* Reconstructing a hydrogen-driven microbial metabolic network in Opalinus Clay rock. *Nat Commun* 2016;**7**:1–10.

Bagwell CE, Bhat S, Hawkins GM *et al.* Survival in nuclear waste, extreme resistance, and potential applications gleaned from the genome sequence of *Kineococcus radiotolerans* SRS30216. *PLoS One* 2008;**3**:e3878.

Bassil NM, Bryan N, Lloyd JR. Microbial degradation of isosaccharinic acid at high pH. *ISME J* 2015;**9**:310–20.

Bassil NM, Small JS, Lloyd JR. Enhanced microbial degradation of irradiated cellulose under hyperalkaline conditions. *FEMS Microbiol Ecol* 2020,**96**:fiaa102.

Battista JR, Earl AM, Park MJ. Why is *Deinococcus radiodurans* so resistant to ionizing radiation? *Trends Microbiol* 1999;**7**:362–5.

Bauermeister A, Moeller R, Reitz G *et al.* Effect of relative humidity on *Deinococcus radiodurans*' resistance to prolonged desiccation, heat, ionizing, germicidal, and environmentally relevant UV radiation. *Microb Ecol* 2011;**61**:715–22.

Beaton D, Pelletier P, Goulet RR. Microbial degradation of cellulosic material and gas generation: implications for the management of low-and intermediate-level radioactive waste. *Front Microbiol* 2019;**10**:204.

Beblo-Vranesevic K, Bohmeier M, Perras AK *et al.* Lack of correlation of desiccation and radiation tolerance in microorganisms from diverse extreme environments tested under anoxic conditions. *FEMS Microbiol Lett* 2018;**365**:fny044.

Bell E, Sherry A, Pilloni G *et al.* Sediment cooling triggers germination and sulfate reduction by heat-resistant thermophilic spore-forming bacteria. *Environ Microbiol* 2020;**22**:456–65.

Bengtsson A, Blom A, Hallbeck B *et al.* Microbial sulphide-producing activity in water saturated MX-80, Asha and Calcigel bentonite at wet densities from 1500 to 2000 kg m^{-3}. SKB TR-16-09. 2017a. https://www.skb.com/publication/2487851/TR-16-09.pdf

Bengtsson A, Blom A, Johansson L *et al.* Bacterial sulphide-producing activity in water saturated iron-rich Rokle and iron-poor Gaomiaozi bentonite at wet densities from 1750 to 1950 kg m^{-3}. SKB TR-17-05. 2017b. https://www.skb.se/publikation/2488913/TR-17-05.pdf (17 January 2024, date last accessed).

Bengtsson A, Pedersen K. Microbial sulphate-reducing activity over load pressure and density in water saturated Boom Clay. *Appl Clay Sci* 2016;**132**:542–51.

Berner UR. Evolution of pore water chemistry during degradation of cement in a radioactive waste repository environment. *Waste Manag* 1992;**12**:201–19.

Blanco-Martín L, Rutqvist J, Battistelli A *et al.* Coupled processes modeling in rock salt and crushed salt including halite solubility constraints: Application to disposal of heat-generating nuclear waste. *Transp Porous Media* 2018;**124**:159–82.

Bleyen N, Vasile M, Marien A *et al.* Assessing the oxidising effect of $NaNO_3$ and $NaNO_2$ from disposed eurobitum bituminised radioactive waste on the dissolved organic matter in Boom Clay. *Appl Geochem* 2016;**68**:29–38.

Blomberg P, Itävaara M, Marjamaa K *et al.* Metabolic pathways of deep groundwater microbiomes and sulphide formation at Olkiluoto. Posiva Oy, Working Report 2017-11, 2017. https://www.posiva.fi/en/index/media/reports.html (17 January 2024, date last accessed).

Bloomfield JP, Lewis MA, Newell AJ *et al.* Characterising variations in the salinity of deep groundwater systems: a case study from Great Britain (GB). *J Hydrol Reg Stud* 2020;**28**:100684.

Blum JS, Han S, Lanoil B *et al.* Ecophysiology of *Halarsenatibacter silvermanii* strain SLAS-1T, gen. nov., sp. nov., a facultative chemoautotrophic arsenate respirer from salt-saturated Searles Lake, California. *Appl Environ Microb* 2009;**75**:1950–60.

Bonte M, Roling WF, Zaura E *et al.* Impacts of shallow geothermal energy production on redox processes and microbial communities. *Environ Sci Technol* 2013;**47**:14476–84.

Bowers KJ, Wiegel J. Temperature and pH optima of extremely halophilic archaea: a mini-review. *Extremophiles* 2011;**15**:119–28.

Boylan AA, Perez-Mon C, Guillard L *et al.* H2-fuelled microbial metabolism in Opalinus Clay. *Appl Clay Sci* 2019;**174**:69–76.

Bruhn DF, Frank SM, Roberto FF *et al.* Microbial biofilm growth on irradiated, spent nuclear fuel cladding. *J Nucl Mater* 2009;**384**:140–5.

Burggraf S, Jannasch HW, Nicolaus B *et al.* *Archaeoglobus Profundus* Sp.Nov., represents a new species within the sulfate-reducing archaebacteria. *Syst Appl Microbiol* 1990;**13**:24–28.

Busby JP, Lee JR, Kender S *et al.* Modelling the potential for permafrost development on a radioactive waste geological disposal facility in Great Britain. *P Geologist Assoc* 2015;**126**:664–74.

Butterworth SJ, Stroes-Gascoyne S, Lloyd JR. The microbiology of natural analogue sites. In: Lloyd JR, Cherkouk A (eds.), *The Microbiology of Nuclear Waste Disposal*. Amsterdam, Netherlands: Elsevier, 2021, 21–39.

Byrd N, Lloyd JR, Small JS *et al.* Microbial degradation of citric acid in low level radioactive waste disposal: impact on biomineralization reactions. *Front Microbiol* 2021;**12**:565855.

Byrd N, Lloyd JR, Townsend LT *et al.* Anaerobic biodegradation of citric acid in the presence of Ni and U at alkaline pH; impact on metal fate and speciation. *Env Sci Adv* 2023;**2**:1196–209.

Capece MC, Clark E, Saleh JK *et al.* Polyextremophiles and the constraints for terrestrial habitability. In: Seckbach J, Oren A, Stan-Lotter H (eds.), *Polyextremophiles*. Dordrecht: Springer, 2013, 3–59.

Carbonell B, Villar MV, Martin PL *et al.* Gas transport in compacted bentonite after 18 years under barrier conditions. *Geomech Energy Envir* 2019;**17**:66–74.

Charles CJ, Rout SP, Patel KA *et al.* Floc formation reduces the pH stress experienced by microorganisms living in alkaline environments. *Appl Environ Microbiol* 2017;**83**:e02985–16.

Charles CJ, Rout SP, Wormald R *et al.* *In-situ* biofilm formation in hyper alkaline environments. *Geomicrobiol J* 2019;**36**:405–11.

Cheptsov V, Belov A, Vorobyova E *et al.* Viability of the soddy–podzolic soil microbial community after 148-1250 kGy gamma irradiation. *Planet Space Sci* 2019;**172**:8–13.

Cheptsov VS, Vorobyova EA, Osipov GA *et al.* Microbial activity in Martian analog soils after ionizing radiation: implications for the preservation of subsurface life on Mars. *Aims Microbiol* 2018;**4**:541–62.

Chou FI, Li CC, Chen TY *et al.* Microbial occurrence in bentonite-based buffer materials of a final disposal site for low level radioactive waste in Taiwan. *Proceedings of the 13th International Conference on Environmental Remediation and Radioactive Waste Management, 2010*. 2011, 229–36. https://asmedigitalcollection.asme.org/ICEM/proceedings-abstract/ICEM2010/54525/229/350279 (17 January 2024, date last accessed).

Cox MM, Battista JR. Deinococcus radiodurans - the consummate survivor. *Nat Rev Microbiol* 2005;**3**:882–92.

D'Hondt S, Spivack AJ, Pockalny R *et al.* Subseafloor sedimentary life in the South Pacific Gyre. *Proc Natl Acad Sci USA* 2009;**106**:11651–6.

Dadachova E, Casadevall A. Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. *Curr Opin Microbiol* 2008;**11**:525–31.

Dadachova E, Howell RW, Bryan RA *et al.* Susceptibility of the human pathogenic fungi *Cryptococcus neoformans* and *Histoplasma capsulatum* to γ-radiation versus radioimmunotherapy with α- and ß-emitting radioisotopes. *J Nucl Med* 2004;**45**:313–20.

Daly MJ. Death by protein damage in irradiated cells. *DNA Repair (Amst)* 2012;**11**:12–21.

Demina TA, Atanasova NS, Pietila MK *et al.* Vesicle-like virion of *Haloarcula hispanica* pleomorphic virus 3 preserves high infectivity in saturated salt. *Virology* 2016;**499**:40–51.

Diler E, Leblanc V, Gueune H *et al.* Potential influence of microorganisms on the corrosion of carbon steel in the French high- and intermediate-level long-lived radioactive waste disposal context. *Mater Corros* 2021;**72**:218–34.

Diler E, Leblanc V, Gueune H *et al.* Potential influence of microorganisms on the corrosion of carbon steel in the French high-level long-lived radioactive waste disposal context at 80°C. *Mater Corros* 2023;**74**:1795–1810.

Dzaugis ME, Spivack AJ, Dunlea AG *et al.* Radiolytic hydrogen production in the subseafloor basaltic aquifer. *Front Microbiol* 2016;**7**:76.

Foti M, Sorokin DY, Lomans B *et al.* Diversity, activity, and abundance of sulfate-reducing bacteria in saline and hypersaline soda lakes. *Appl Environ Microbiol* 2007;**73**:2093–100.

Fredrickson JK, Zachara JM, Balkwill DL *et al.* Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the Hanford Site, Washington State. *Appl Environ Microbiol* 2004;**70**:4230–41.

Fru EC, Athar R. In situ bacterial colonization of compacted bentonite under deep geological high-level radioactive waste repository conditions. *Appl Microbiol Biotechnol* 2008;**79**:499–510.

Glasser FP, Atkins M. Cements in Radioactive Waste Disposal. *MRS Bulletin* 1994;**19**:33–8.

Glombitza C, Putman LI, Rempfert KR *et al.* Active microbial sulfate reduction in fluids of serpentinizing peridotites of the continental subsurface. *Commun Earth Environ* 2021;**2**:81.

Grant WD. Life at low water activity. *Philos Trans R Soc Lond B Biol Sci* 2004;**359**:1249–66.

Grum-Grzhimaylo AA, Debets AJM, van Diepeningen AD *et al.* *Sodiomyces alkalinus*, a new holomorphic alkaliphilic ascomycete within the Plectosphaerellaceae. *Persoonia* 2013;**31**:147–58.

Hallsworth JE, Yakimov MM, Golyshin PN *et al.* Limits of life in $MgCl_2$-containing environments: chaotropicity defines the window. *Environ Microbiol* 2007;**9**:801–13.

Harrison JP, Gheeraert N, Tsigelnitskiy D *et al.* The limits for life under multiple extremes. *Trends Microbiol* 2013;**21**:204–12.

Haynes HM, Bailey MT, Lloyd JR. Bentonite barrier materials and the control of microbial processes: Safety case implications for the geological disposal of radioactive waste. *Chem Geol* 2021;**581**:120353. https://doi.org/10.1016/j.chemgeo.2021.120353.

Haynes HM, Nixon S, Birgersson M *et al.* Verification of microbial sulfide-producing activity in calcigel bentonite at wet densities of 1750 and 1900 kg m^{-3}. SKB Report P-10-07, 2019. https://skb.se/publikation/2493577/P-19-07.pdf.

Haynes HM, Pearce CI, Boothman CL *et al.* Response of bentonite microbial communities to stresses relevant to geodisposal of radioactive waste. *Chem Geol* 2018;**501**:58–67.

Head IM, Jones DM, Larter SR Biological activity in the deep subsurface and the origin of heavy oil. *Nature* 2003;**426**:344–52.

Heath TG, Hunter FMI. Calculation of near-field pH buffering: effect of polymer encapsulant. Serco Report SA/ENV-0909 Issue 2, 2012. https://webarchive.nationalarchives.gov.uk/ukgwa/20211004151355/https://rwm.nda.gov.uk/publication/calculation-of-near-field-ph-buffering-effect-of-polymer-encapsulant/?download.

Hoch AR, Baston GMN, Glasser FP *et al.* Modelling evolution in the near field of a cementitious repository. *Mineral Mag* 2012;**76**:3055–69.
Holmfeldt K, Nilsson E, Simone D *et al.* The Fennoscandian Shield deep terrestrial virosphere suggests slow motion 'boom and burst' cycles. *Commun Biol* 2021;**4**:307.
Humphreys PN, West JM, Metcalfe R. Microbial effects on repository performance. *Quintessa Report to the Nuclear Decommissioning Authority, Radioactive Waste Management Directorate (RWMD), QRS-1378Q-1, Version 2.0*. 2010. https://eprints.hud.ac.uk/id/eprint/7613/1/Microbial_Effects_on_Repository_Performance.pdf.
Jakobsen TF, Kjeldsen KU, Ingvorsen K. *Desulfohalobium utahense* sp nov., a moderately halophilic, sulfate-reducing bacterium isolated from Great Salt Lake. *ISME J* 2006;**56**:2063–9.
Jalique DR, Stroes-Gascoyne S, Hamon CJ *et al.* Culturability and diversity of microorganisms recovered from an eight-year old highly-compacted, saturated MX-80 wyoming bentonite plug. *Appl Clay Sci* 2016;**126**:245–50.
Jalique DR. Microbial abundance, diversity, and potential activity in bentonite clay. Thesis, Department of food and bioproduct Sciences, University of Saskatchewan Saskatoon, 2016. https://harvest.usask.ca/items/45b2bdbc-bc28-4a3e-8ed1-7ef6368179e8/full.
Jancic S, Zalar P, Kocev D *et al.* Halophily reloaded: new insights into the extremophilic life-style of *Wallemia* with the description of *Wallemia hederae* sp nov. *Fungal Divers* 2016;**76**:97–118.
Janning B, Tveld PHI. Susceptibility of bacterial strains to desiccation—a simple method to test their stability in microbiological reference materials. *Anal Chim Acta* 1994;**286**:469–76.
Jenni A, Wersin P, Mäder UK *et al.* Bentonite backfill performance in a high-level waste repository: a geochemical perspective NTB-19-03. 2019. https://nagra.ch/wp-content/uploads/2022/08/e_ntb19-003.pdf.
Jesußek A, Grandel S, Dahmke A. Impacts of subsurface heat storage on aquifer hydrogeochemistry. *Environ Earth Sci* 2012;**69**:1999–2012.
Johansson L, Stahlén J, Taborowski T *et al.* Microbial release of iron from Olkiluoto rock minerals. *Posita Oy*, Working Report 2018-30, 2019;110. https://www.posiva.fi/en/index/media/reports.html (17 January 2024, date last accessed).
Jørgensen BB, Isaksen MF, Jannasch HW. Bacterial sulfate reduction above 100°C in deep-sea hydrothermal vent sediments. *Science* 1992;**258**:1756–7.
Jroundi F, Povedano-Priego C, Pinel-Cabello M *et al.* Evidence of microbial activity in a uranium roll-front deposit: unlocking their potential role as bioenhancers of the ore genesis. *Sci Total Environ* 2023;**861**:160636.
Kaira GS, Dhakar K, Pandey A. A psychrotolerant strain of *Serratia marcescens* (MTCC 4822) produces laccase at wide temperature and pH range. *AMB Express* 2015;**5**:1–8.
Karadagli F, Marcus A, Rittmann BE. Microbiological hydrogen (H_2) thresholds in anaerobic continuous-flow systems: effects of system characteristics. *Biotechnol Bioeng* 2023;**120**:1844–56.
Karadagli F, Rittmann BE. Thermodynamic and kinetic analysis of the H_2 threshold for *Methanobacterium bryantii*. *Biodegradation* 2007;**18**:439–52.
Karnland O. Chemical and mineralogical characterization of the bentonite buffer for the acceptance control procedure in a KBS-3 repository. SKB TR-10-60. 2010. https://www.skb.com/publication/2137257.
Kashefi K, Holmes DE, Reysenbach AL *et al.* Use of Fe(III) as an electron acceptor to recover previously uncultured hyperthermophiles: isolation and characterization of *Geothermobacterium ferrireducens* gen. nov., sp nov. *Appl Environ Microbiol* 2002;**68**:1735–42.
Kashefi K, Lovley DR. Extending the upper temperature limit for life. *Science* 2003;**301**:934.
Kaspar V, Sachlova S, Hofmanova E *et al.* Geochemical, geotechnical, and microbiological changes in Mg/Ca bentonite after thermal loading at 150°C. *Minerals* 2021;**11**:965.
Kaufhold S, Baille W, Schanz T *et al.* About differences of swelling pressure—dry density relations of compacted bentonites. *Appl Clay Sci* 2015;**107**:52–61.
Kilpatrick AD. Geochemical modelling of permafrost processes. Contractor Report BGS/CR/16/144 v6. 2017.
Kursten B, Druyts F. Assessment of the uniform corrosion behaviour of carbon steel radioactive waste packages with respect to the disposal concept in the geological Dutch Boom Clay formation. OPERA-PU-SCK513. 2015. https://www.covra.nl/app/uploads/2019/08/OPERA-PU-SCK513.pdf.
Kyle JE, Eydal HSC, Ferris FG *et al.* Viruses in granitic groundwater from 69 to 450 m depth of the Aspo hard rock laboratory, Sweden. *ISME J* 2008;**2**:571–4.
Lai MC, Gunsalus RP. Glycine betaine and potassium ion are the major compatible solutes in the extremely halophilic methanogen *Methanohalophilus* strain Z7302. *J Bacteriol* 1992;**174**:7474–7.
Leupin OX, Bernier-Latmani R, Bagnoud A *et al.* Fifteen years of microbiological investigation in Opalinus Clay at the Mont Terri rock laboratory (Switzerland). *Swiss J Geosci* 2017;**110**:343–54.
Leupin OX, Smith P, Marschall P *et al.* Low- and intermediate-level waste repository-induced effects. *NTB 17-14*,2016;89. https://nagra.ch/wp-content/uploads/2022/08/e_ntb14-014.pdf.
Li CC, Chung HP, Wen HW *et al.* The radiation resistance and cobalt biosorption activity of yeast strains isolated from the Lanyu low-level radioactive waste repository in Taiwan. *J Environ Radioactiv* 2015;**146**:80–87.
Libert M, Bildstein O, Esnault L *et al.* Molecular hydrogen: an abundant energy source for bacterial activity in nuclear waste repositories. *Phys Chem Earth* 2011;**36**:1616–23.
Lienen T, Lüders K, Halm H *et al.* Effects of thermal energy storage on shallow aerobic aquifer systems: temporary increase in abundance and activity of sulfate-reducing and sulfur-oxidizing bacteria. *Environ Earth Sci* 2017;**76**:261.
Liu CT, Miyaki T, Aono T *et al.* Evaluation of methanogenic strains and their ability to endure aeration and water stress. *Curr Microbiol* 2008;**56**:214–8.
Liu D, Bishop H, Dong ME *et al.* Microbial reduction of structural iron in interstratified illite-smectite minerals by a sulfate-reducing bacterium. *Geobiology* 2012;**150**:1472–4677.
Lloyd JR, Cherkouk A. *The Microbiology of Nuclear Waste Disposal*. 1st edn. Amsterdam: Elsevier BV, 2020.
Lovley DR, Holmes DE, Nevin KP. Dissimilatory Fe(III) and Mn(IV) reduction. *Adv Microb Physiol* 2004;**49**:219–86.
Ma Y, Galinski EA, Grant WD *et al.* Halophiles 2010: life in saline environments. *Appl Environ Microb* 2010;**76**:6971–81.
Maanoja S, Lakaniemi AM, Lehtinen L *et al.* Compacted bentonite as a source of substrates for sulfate-reducing microorganisms in a simulated excavation-damaged zone of a spent nuclear fuel repository. *Appl Clay Sci* 2020;**196**:105746.
Maanoja S, Palmroth M, Salminen L *et al.* The effect of compaction and microbial activity on the quantity and release rate of water-soluble organic matter from bentonites. *Appl Clay Sci* 2021;**211**:106192.
Marsh AI, Williams LG, Lawrence JA. The important role and performance of engineered barriers in a UK geological disposal

facility for higher activity radioactive waste. *Prog Nucl Energy* 2021;**137**:103736.

McMurry J, Dixon DA, Garroni JD *et al. Evolution of a Canadian deep geologic repository*. No. 06819-REP. Ontario Power Generation Report, 2004. https://unene.ca/wp-content/uploads/file/courses/un602/McMurry%20Evoln%20of%20a%20waste%20vault.pdf.

Miettinen H, Bomberg M, Bes R *et al.* Transformation of inherent microorganisms in Wyoming-type bentonite and their effects on structural iron. *Appl Clay Sci* 2022;**221**:106465.

Mijnendonckx K, Bleyen N, Van Gompel A *et al.* pH and microbial community determine the denitrifying activity in the presence of nitrate-containing radioactive waste. *Front Microbiol* 2022;**13**:150–62.

Mingarro M, Garcia-Gutierrez M, Quinones J. Radiation resistance of the natural bacteria population in Febex bentonite. In *Clays in Natural & Engineered Barriers for Radioactive Waste Confinement International Meeting, March 14-18, 2005,* Tours, France: 2005. pp. 723, 405-406. https://www.osti.gov/etdeweb/biblio/20703125.

Morco RP, Joseph JM, Hall DS *et al.* Modelling of radiolytic production of HNO_3 relevant to corrosion of a used fuel container in deep geologic repository environments. *Corros Eng Sci Techn* 2017;**52**:141–7.

Motamedi M, Karland O, Pedersen K. Survival of sulfate reducing bacteria at different water activities in compacted bentonite. *FEMS Microbiol Lett* 1996;**141**:83–87.

Mwatha WE, Grant WD. *Natronobacterium vacuolata* sp-nov, a haloalkaliphilic archaeon isolated from Lake Magadi, Kenya. *Int J Syst Bacteriol* 1993;**43**:401–4.

Nakayama M, Iriya K, Fujishima A *et al.* Development of low alkaline cement considering pozzolanic reaction for support system in HLW repository construction. *MRS Proceedings* 2006;**932**:159.

Nixon SL, Bonsall E, Cockell CS. Limitations of microbial iron reduction under extreme conditions. *FEMS Microbiol Rev* 2022;**46**:fuac033.

Nixon SL, van Dongen BE, Boothman C *et al.* Additives in plasticised polyvinyl chloride fuel microbial nitrate reduction at high pH: implications for nuclear waste disposal. *Front Environ Sci* 2018;**6**:97.

Noynaert L, De Carrière P, Volckaert G *et al.* The CERBERUS project: study of the effect of heat and radiation on the near field of a HLW or spent fuel repository. In: *Waste Management Symposium*, 1998, pp. 1–7. https://archivedproceedings.econference.io/wmsym/1998/html/sess27/27-17/27-17.htm.

Olukotun SF, Gbenu ST, Ibitoye FI *et al.* Investigation of gamma radiation shielding capability of two clay materials. *Nucl Eng Technol* 2018;**50**:957–62.

Oren A. Bioenergetic aspects of halophilism. *Microbiol Mol Biol R* 1999;**63**:334–48.

Oren A. Thermodynamic limits to microbial life at high salt concentrations. *Environ Microbiol* 2011;**13**:1908–23.

Palmer FE, Emery DR, Stemmler J *et al.* Survival and growth of microcolonial rock fungi as affected by temperature and humidity. *New Phytol* 1987;**107**:155–62.

Pavlopoulou A, Savva GD, Louka M *et al.* Unraveling the mechanisms of extreme radioresistance in prokaryotes: lessons from nature. *Mutat Res Rev Mutat Res* 2016;**767**:92–107.

Payler SJ, Biddle JF, Lollar BS *et al.* An ionic limit to life in the deep subsurface. *Front Microbiol* 2019;**10**:426.

Pedersen K, Motamedi M, Karnland O *et al.* Mixing and sulphate-reducing activity of bacteria in swelling, compacted bentonite clay under high-level radioactive waste repository conditions. *J Appl Microbiol* 2000a;**1038**:1364–5072.

Pedersen K, Motamedi M, Karnland O *et al.* Cultivability of microorganisms introduced into a compacted bentonite clay buffer under high-level radioactive waste repository conditions.*Eng Geol* 2000b;**58**:149–61.

Pedersen K, Motamedi M, Karnland O. Survival of bacteria in nuclear waste buffer materials: the influence of nutrients, temperature and water activity. SKB-TR-95-27. 1995;1–22. https://www.skb.com/publication/12036/SKB95-27_OCR.pdf.

Peters V, Conrad R. Methanogenic and other strictly anaerobic-bacteria in desert soil and other oxic soils. *Appl Environ Microbiol* 1995;**61**:1673–6.

Phillips RW, Wiegel J, Berry CJ *et al. Kineococcus radiotolerans* sp nov., a radiation-resistant, gram-positive bacterium. *Int J Syst Evol Micr* 2002;**52**:933–8.

Pitonzo BJ, Amy PS, Rudin M. Effect of gamma radiation on native endolithic microorganisms from a radioactive waste deposit site. *Radiat Res* 1999;**152**:64–70.

Pitonzo BJ. Characterization of Microbes Implicated in Microbially-influenced Corrosion from the Proposed Yucca Mountain Repository. PhD Thesis, University of Nevada, Las Vegas, 1996.

Potts M. Desiccation tolerance of prokaryotes. *Microbiol Rev* 1994;**58**:755–805.

Rajala P. Microbially-induced corrosion of carbon steel in a geologic repository environment. PhD Thesis, University of Helsinki, Department of Food and Environmental Sciences. 2017. https://publications.vtt.fi/pdf/science/2017/S155.pdf.

Rizoulis A, Steele HM, Morris K *et al.* The potential impact of anaerobic microbial metabolism during the geological disposal of intermediate-level waste. *Mineral Mag* 2012;**76**:3261–70.

Roadcap GS, Sanford RA, Jin QS *et al.* Extremely alkaline (pH >12) ground water hosts diverse microbial community. *Ground Water* 2006;**44**:511–7.

RWM. Geological Disposal: waste package evolution status report. NDA Report no. DSSC/451/01. 2016a. https://webarchive.nationalarchives.gov.uk/ukgwa/20211004151355/https://rwm.nda.gov.uk/publication/geological-disposal-near-field-evolution-status-report-december-2010/?download.

RWM. Geological Disposal: engineered barrier system status report. NDA Report no. DSSC/452/01. 2016b. pp. 146. https://assets.publishing.service.gov.uk/media/5a822846ed915d74e34020a5/NDA_Report_no_DSSC-452-01_-_Geological_Disposal_-_Engineered_Barrier_System_Status_Report.pdf.

RWM. Geological Disposal Generic Disposal Facility Design. NDA Report no. DSSC/412/01. 2016c. https://assets.publishing.service.gov.uk/media/5a81e6c7e5274a2e8ab56709/NDA_Report_no_DSSC-412-01_-_Geological_Disposal_-_Generic_Disposal_Facility_Designs.pdf.

RWM. Geological Disposal: gas status report. NDA Report no. DSSC/455/01. 2016d. https://assets.publishing.service.gov.uk/media/5a81d90740f0b623026997bd/NDA_Report_no_DSSC-455-01_-_Geological_Disposal_-_Gas_Status_Report.pdf (18 January 2024, date last accessed).

RWM. Geological Disposal High Heat Generating Wastes Project: Final Report NDA/RWM/13.. 2016e. pp. 150. https://webarchive.nationalarchives.gov.uk/ukgwa/20211004152408/https:/rwm.nda.gov.uk/publication/geological-disposal-high-heat-generating-wastes-project-final-report/.

RWM. Geological Disposal part B: technical specification generic disposal system specification. NDA Report no. DSSC/402/01. 2016f. https://assets.publishing.service.gov.uk/media/5f0f16f13a6f40038437ba42/DSSC_-_402_-_01_-_Geological_Disposal_-_Disposal_System_Specification_-_Part_B_-_Technical_Specification.pdf.

Ruiz-Fresneda MA, Martinez-Moreno MF, Povedano-Priego C *et al.* Impact of microbial processes on the safety of deep geological repositories for radioactive waste. *Front Microbiol* 2023;**14**:1134078.

RWM. Geological disposal: guidance on the use of polycarboxylate ether superplasticisers for the packaging of low heat generating wastes. Nuclear Decomissioning Agency Report WPS/926/02. 2019. https://assets.publishing.service.gov.uk/media/5e85f33186650c744620780f/Guidance_on_the_use_of_SP_for_packaging_LHGW_Issue_2.pdf.

RWM. Geological Disposal: near-field evolution status report. Nuclear Decommissioning Authority Report NDA/RWMD/033. 2010. https://webarchive.nationalarchives.gov.uk/ukgwa/20211004151355/https://rwm.nda.gov.uk/publication/geological-disposal-near-field-evolution-status-report-december-2010/?download.

Samper J, Mon A, Montenegro L. A revisited thermal, hydrodynamic, chemical and mechanical model of compacted bentonite for the entire duration of the FEBEX in situ test. *Appl Clay Sci* 2018;**160**:58–70.

Sauvage JF, Flinders A, Spivack AJ *et al.* The contribution of water radiolysis to marine sedimentary life. *Nat Commun* 2021;**12**:1297.

Savage D, Cloet V. A review of cement-clay modelling. Nagra Report NAB-18-24. 2018, 1–56. https://www.researchgate.net/publication/328233095_A_review_of_cement-clay_modelling?channel=doi&linkId=5c934744299bf111693b9711&showFulltext=true.

Schütz MK, Schlegel ML, Libert M. Impact of iron-reducing bacteria on the corrosion rate of carbon steel under simulated geological disposal conditions. *Environ Sci Technol* 2015;**49**;7483–90.

Sellin P, Leupin OX. The use of clay as an engineered barrier in radioactive-waste management—a review. *Clays Clay Miner* 2013.;**61**:477–98.

Shrestha R, Cernousek T, Stoulil J *et al.* Anaerobic microbial corrosion of carbon steel under conditions relevant for deep geological repository of nuclear waste. *Sci Total Env* 2021;**800**:149539.

SKB. Buffer, backfill and closure process report for the safety assessment SR-site. SKB TR-10-47. 2010a. https://www.researchgate.net/publication/328233095_A_review_of_cement-clay_modelling?channel=doi&linkId=5c934744299bf111693b9711&showFulltext=true.

SKB. Geosphere process report for the safety assessment SR-Site. SKB TR-10-48. 2010b. https://skb.se/publikation/2151483/TR-10-48.pdf.

SKB. FEP report for the safety assessment SR-PSU. Techincal Report TR-14-07. 2014. https://skb.se/upload/publications/pdf/TR-14-07.pdf (18 January 2024, date last accessed).

SKB. Long-term safety for KBS-3 repositories at Forsmark and Laxemar-a first evaluation. Main Report of the SR-Can project. SKB TR-06-09. 2006. https://www.skb.com/publication/1192585/TR-06-09.pdf.

Small JS, Nykyri M, Vikman M *et al.* The biogeochemistry of gas generation from low-level nuclear waste: modelling after 18 years study under in situ conditions. *Appl Geochem* 2017;**84**:360–72.

Small JS, Vikman M. Microbial impacts on gas production in LLW/ILW. In: LLoyd JR, Cherkouk A (eds.), *The Microbiology of Nuclear Waste Disposal*. Amsterdam, Netherlands: Elsevier, 2021, 193–211.

Smedley PL, Bearcock JM, Newell AJ *et al.* Guide to reference groundwater and porewater compositions in support of the UK GDF Programme NWS-CR-23-007. 2023;155. https://assets.publishing.service.gov.uk/media/6569f2879462260013c5684a/NWS-CR-23-007_-_Guide_to_Reference_Groundwater_and_Porewater_Compositions_in_Support_of_the_UK_GDF_Programme.pdf.

Stevens AH, Cockell CS. A Systematic study of the limits of life in mixed ion solutions: physicochemical parameters do not predict habitability. *Front Microbiol* 2020;**11**:1478.

Stevenson A, Cray JA, Williams JP *et al.* Is there a common water-activity limit for the three domains of life? *ISME J* 2015;**9**:1333–51.

Stevenson A, Hamill PG, O'Kane CJ *et al. Aspergillus penicillioides* differentiation and cell division at 0.585 water activity. *Environ Microbiol* 2017;**19**:687–97.

Stone W, Kroukamp O, Korber DR *et al.* Microbes at surface-air interfaces: the metabolic harnessing of relative humidity, surface hygroscopicity, and oligotrophy for resilience. *Front Microbiol* 2016a;**7**:1563.

Stone W, Kroukamp O, Moes A *et al.* Measuring microbial metabolism in atypical environments: bentonite in used nuclear fuel storage. *J Microbiol Meth* 2016b;**120**:79–90.

Stone W, Kroukamp O, McKelvie J *et al.* Microbial metabolism in bentonite clay: saturation, desiccation and relative humidity. *Appl Clay Sci* 2016c;**129**:54–64.

Stroes-Gascoyne S, Lucht LM, Borsa J *et al.* Radiation resistance of the natural microbial population in buffer materials. *MRS Online Proceedings Library* 1994a;**353**:345–52.

Stroes-Gascoyne S, Francis AJ, Vilks P. Microbial research. In: Cramer J, Smelle JAT (eds.), *Final Report of the AECL/SKB Cigar Lake Analog Study*. Atomic Energy of Canada Limited Report AECL-10851, also COG-93-147 and SKB TR-94-04, Stockholm: Swedish Nuclear Fuel and Waste Management Co, 1994b, pp. 261–65. https://inis.iaea.org/collection/NCLCollectionStore/_Public/27/057/27057231.pdf?r=1.

Stroes-Gascoyne S, Hamon CJ, Maak P *et al.* The effects of the physical properties of highly compacted smectitic clay (bentonite) on the culturability of indigenous microorganisms. *Appl Clay Sci* 2010a;**47**:155–62.

Stroes-Gascoyne S, Hamon CJ, Dixon D *et al.* The effect of $CaCl_2$ porewater salinity (50-100 g/L) on the culturability of heterotrophic aerobic bacteria in compacted 100% bentonite with dry densities of 0.8 and 1.3 g/cm^3. NWMO TR-2010-06. 2010b. https://www.nwmo.ca/-/media/Reports---Reports/1547_nwmotr-2010-06_cacl2_r0b.ashx?sc_lang=en.

Stroes-Gascoyne S, Hamon CJ. The effect of intermediate dry densities (1.1-1.5 g/cm^3) and intermediate porewater salinities (60-90 g NaCl/L) on the culturability of heterotrophic aerobic bacteria in compacted 100% bentonite. NWMO TR-2008-11. 2008. https://www.nwmo.ca/-/media/Reports---Reports/1410_nwmotr-2008-11_effectofinterme.ashx?sc_lang=en.

Stroes-Gascoyne S, Hamon CJ. The effects of elevated temperatures on the viability and culturability of bacteria indigenous to Wyoming MX-80 bentonite. NWMO TR-2010-08. 2010.

Stroes-Gascoyne S, Haveman SA, Hamon CJ *et al.* Occurrence and identification of microorganisms in compacted clay-based buffer material designed for use in a nuclear fuel waste disposal vault. *Can J Microbiol* 1997;**43**:1133–46.

Suryanarayanan TS, Govindarajulu MB, Thirumalai E *et al.* Agni's fungi: heat-resistant spores from the Western Ghats, southern India. *Fungal Biol* 2011;**115**:833–8.

Svensson D, Dueck A, Nilsson U *et al.* Alternative buffer material: status of the ongoing laboratory investigation of reference materials and test package 1. SKB TR-11-06. 2011. https://www.skb.com/publication/2442994/TR-11-06.pdf.

Swanson J, Reed D, Ams D *et al. Status Report on the Microbial Characterization of Halite and Groundwater Samples from the WIPP. LA-UR-12-22824*. Los Alamos National Laboratory. 2012. https://permalink.lanl.gov/object/tr?what=info:lanl-repo/lareport/LA-UR-12-22824.

Swanson JS, Bader M, Cherkouk A. Potential microbial influence on the performance of subsurface, salt-based nuclear waste repositories. In: Lloyd JR, Cherkouk A (eds.), *The Microbiology of Nuclear Waste Disposal*. Amsterdam, Netherlands: Elsevier, 2021, 97–117.

Swanson JS, Reed DT, Cherkouk A *et al.* Microbial influence on the performance of subsurface, salt-based radioactive waste repositories. *An Evaluation Based on Microbial Ecology, Bioenergetics and Projected Repository Conditions. NEA–7387*. Organisation for Economic Co-Operation and Development. 2018. https://read.oecd-ilibrary.org/nuclear-energy/microbial-influence-on-the-performance-of-subsurface-salt-based-radioactive-waste-repositories_9789264303133-en#page1.

Swift BT, Bamforth PB, Hoch AR *et al. Cracking, Flow and Chemistry in NRVB*. SERCO Report SERCO /TAS/000505/001, 2010, pp. 155. https://webarchive.nationalarchives.gov.uk/ukgwa/20210401103154/https://rwm.nda.gov.uk/publication/tn-17701-serco_tas_000505_001_cracking_flow_and_chemistry_in_nrvb_issue_3/.

Taborowski T, Pedersen K. Methanogens in SFR. SKB Report R-18-08, 2019. https://www.skb.com/publication/2492456/R-18-08.pdf.

Takai K, Nakamura K, Toki T *et al.* Cell proliferation at 122°C and isotopically heavy CH_4 production by a hyperthermophilic methanogen under high-pressure cultivation. *Proc Natl Acad Sci USA* 2008;**105**:10949–54.

Tansey MR, Brock TD. The upper temperature limit for eukaryotic organisms. *Proc Natl Acad Sci USA* 1972;**69**:2426–8.

Thaysen EK, McMahon SA, Strobel O *et al.* Estimating microbial growth and hydrogen consumption in hydrogen storage in porous media. *Renew Sust Energ Rev* 2021;**151**:111481.

Ulrich N, Nagler K, Laue M *et al.* Experimental studies addressing the longevity of *Bacillus subtilis* spores—the first data from a 500-year experiment. *PLoS One* 2018;**13**:e0208425.

Vehmas T, Montoya V, Alonso MC *et al.* Characterization of Cebama low-pH reference concrete and assessment of its alteration with representative waters in radioactive waste repositories. *Appl Geochem* 2020;**121**:104703.

Vikman M, Matusewicz M, Sohlberg E *et al. Long-term experiment with compacted bentonite*. VTT Technical Research Centre of Finland, VTT Technology No. 332, 2018. https://cris.vtt.fi/ws/portalfiles/portal/19390120/T332.pdf.

Villar MV, Fernández AM, Martín PL *et al.* Effect of heating/hydration on compacted bentonite: tests in 60-cm long cells. Ciemat Technical Report 1146. 2008. https://www.osti.gov/etdeweb/servlets/purl/21135062

Villar MV, Garcia-Sineriz JL, Barcena I *et al.* State of the bentonite barrier after five years operation of an in situ test simulating a high level radioactive waste repository. *Eng Geol* 2005;**80**:175–98.

Villar MV, Iglesias RJ, Garcia-Sineriz JL *et al.* Physical evolution of a bentonite buffer during 18 years of heating and hydration. *Eng Geol* 2020.;**264**:105408.

Villar MV, Iglesias RJ, Gutierrez-Alvarez C *et al.* Pellets/block bentonite barriers: laboratory study of their evolution upon hydration. *Eng Geol* 2021.;**292**:106272.

Weetjens E, Sillen X, Valcke E. Deep disposal of eurobitum bituminised waste: scoping calculations of nitrate release. *MRS Proceedings* 2006;**932**:735.

Wieczorek K, Gaus I, Mayor JC *et al.* In-situ experiments on bentonite-based buffer and sealing materials at the Mont Terri rock laboratory (Switzerland). *Swiss J Geosci* 2017;**110**:253–68.

Wilhelms A, Larter SR, Head I *et al.* Biodegradation of oil in uplifted basins prevented by deep-burial sterilization. *Nature* 2001;**414**:1034–7.

World Nuclear Association. Storage and disposal of radioactive waste. 2023. https://world-nuclear.org/information-library/nuclear-fuel-cycle/nuclear-waste/storage-and-disposal-of-radioactive-waste.aspx#:~:text=Radioactive%20wastes%20are%20stored%20so,about%2050%20years%20before%20disposal (30 October 2023, date last accessed).

Wormald RM, Hopwood J, Humphreys PN *et al.* Methanogenesis from mineral carbonates, a potential indicator for life on Mars. *Geosciences* 2022;**12**:138.

Wormald RM, Rout SP, Mayes W *et al.* Hydrogenotrophic methanogenesis under alkaline conditions. *Front Microbiol* 2020;**11**:614227.

Xu DK, Gu TY, Lovley DR. Microbially mediated metal corrosion. *Nat Rev Micro* 2023;**21**:701–8.

Zhilina TL, Tatjana N, Zavarin GA *et al. Natroniella acetigena* gen. nov. sp. nov., an extremely haloalkaliphilic, homoacetic bacterium: a new member of Haloanaerobiales. *Curr. Microbiol* 1996;**32**:320–6.

Zirnstein I, Arnold T. Insights into the biodiversity of salt sumps in the Asse II salt mine, Germany. *Proceedings of the Third International Workshop on Actinide and Brine Chemistry in a Salt-Based Repository (ABC-Salt III)*. In: Reed D, Altmaier M (eds.), 2015. https://www.ptka.kit.edu/ptka-alt/downloads/ptka-wte-e/6%20Proceedings%20of%20the%206th%20USGerman%20Workshop_SAND2016-0194R-reduziert.pdf.

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