Chapter 7
Microbiology of the Deep Subsurface Geosphere and Its Implications for Used Nuclear Fuel Repositories

J.R. McKelvie, D.R. Korber, and G.M. Wolfaardt

Abstract A number of countries are actively working toward the siting and development of deep geological repositories (DGR) for used nuclear fuel. Given their ubiquity and metabolic capabilities, it is assumed that with sufficient time and appropriate conditions, microorganisms could alter the geochemistry of the repository. As such, the DGR concept provides an invaluable opportunity to evaluate the evolution of subsurface conditions from "disturbance" back to original state. The design concept involves the use of steel or copper/steel used fuel containers, surrounded by a low-permeability, swelling clay buffer material within a low-permeability, stable host rock environment. Within a newly constructed DGR, conditions would be warm, oxidizing, and dry. With sufficient time, these conditions would gradually revert to the original state of the surrounding geology. This chapter discusses how microbes and their metabolic activity may change over time and discusses the potential effects they may have on the engineered barrier system (EBS) that serves to isolate the used fuel containers and on the used fuel itself. The widespread support for the development of underground facilities as a means to ensure safe, long-term storage of increasing inventory of nuclear waste underscores the pressing need to learn more about the impacts of microbial activity on the performance of such facilities over the long term.

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7.1 Introduction

An active and diverse community of microorganisms is commonly found in deep subsurface crystalline and sedimentary rock environments (Ghiorse and Wilson 1988; Balkwill 1989; Pedersen and Ekendahl 1992a, b; Baker et al. 2003; Newby et al. 2004). In fact, the abundance of organisms (~6.1×10^{30} cells) in the subsurface, or geosphere, has been estimated to be approximately the same as the photosynthetically derived green plant biomass present on the earth's surface (Whitman et al. 1998; Amend and Teske 2005; Harvey et al. 2007). The diversity of microorganisms is now known to be considerably greater than previously thought, providing a possible explanation for the range of biological reactions observed within the geosphere.

Factors influencing numbers, diversity, and activity of subsurface microbes, such as pore size, water activity, temperature, pH, available carbon sources, hydrostatic pressures, and electron acceptors often differ notably from those in the biosphere surface. The prevalent geochemistry largely dictates the physiological potential of the resident microbes, as well as the biogeochemical reactions that they catalyze (Fredrickson and Balkwill 2006). In turn, the response of microbes to their physical-chemical environment will determine how they affect their surrounding macro- and micro-environments. When subsurface environments are disturbed through anthropogenic activities, conditions are changed from the original state: the atmosphere becomes more oxidizing, pressures are reduced, and pore space becomes less restrictive. Overall, these generally trend toward becoming more permissive for microbial growth. Any anthropogenic disturbances would also introduce microorganisms previously considered foreign. Even if the host rock originally contained a relatively inactive and sparse indigenous microbial population, a variety of allochthonous microorganisms with a range of metabolic capabilities could come to inhabit a disturbed underground system.

Internationally, many countries are actively working toward the siting and construction of deep geological repositories (DGR) for used nuclear fuel. The DGR concept provides an invaluable opportunity to evaluate the evolution of subsurface conditions from "disturbance" back to original state. Within a newly constructed DGR, located at a depth of 500 m, conditions would be warm, oxidizing, and dry. With sufficient time, these conditions would gradually revert to the original state of the surrounding geology. Data capturing these changes can provide insight into how microbes and their metabolic activity may change over time with potential effects on the engineered barrier system (EBS) that serves to isolate the used fuel container and the used fuel itself. The widespread support for the development of underground facilities as a means to ensure safe, long-term storage of increasing stockpiles of nuclear waste underscores the pressing need to learn more about the impacts of microbial activity on the performance of such facilities over the long term.

7.2 Microbial Diversity in the Deep Subsurface

Interest in subsurface microbiology has increased over the last few decades, originally through culture-dependent approaches, and more recently through culture-independent analyses that have revealed new information about the diversity and range of metabolic activities in this poorly explored environment (Ghiorse and Wilson 1988; Balkwill 1989; Fredrickson and Balkwill 2006; Biddle et al. 2008).

Microorganisms living in geological formations near the surface are influenced by the activity of higher organisms and their products, as well as by above ground climatic conditions that select for generalist microbes capable of responding to environmental change. Under conditions of increasing isolation, the physical and chemical properties of the geologic matrix tend to be more stable, such that microorganisms found deeper in the subsurface tend to be more selective in terms of their metabolism. In particular, the supply of carbon and energy sources has the potential to be growth limiting due to the absence of primary photosynthetic production. Similarly, oxygen becomes increasingly unavailable with depth, giving rise to a corresponding increasing dependence on alternative (oxygen-independent) metabolic strategies (i.e., anaerobic respiration, fermentation, and chemoautotrophy).

Concentrations of microorganisms found in different deep subsurface environments appear to vary with location, ranging from none detected in high salt, low-permeability rock formations (e.g., 750 m salt deposits at Asse, Germany) to diverse and abundant microbial communities living in fractures (West and McKinley 2002). In the deep subsurface, microorganisms may exist as free-living individuals, as cell aggregates in liquid suspension, or as surface-attached colonies called biofilms (Costerton et al. 1978, 1995). Due to their small size and simple, often specialized, metabolism, many microbes have the potential to survive as free-living individuals within the small matrices of subsurface environments, as evidenced by numerous reports of various members of Bacteria (Gram-positive and Gram-negative bacteria, DeFlaun et al. 2007; Rastogi et al. 2009) and Archaea recovered from subsurface samples.

Yeast and fungi have also been detected in subsurface environments and the existence of a food chain that includes higher level bacterivorous predators is not beyond the realm of possibility, as gene sequences from flagellated and ciliated protozoa have been recovered from the deep subsurface (Pedersen 1999a, b). More recently, Borgonie et al. (2011) reported the discovery of a species of the phylum Nematoda from fracture water 0.9–3.6 km deep in South African mines. Interestingly, the nematodes were not detected in the mines' service waters, but in palaeometeoric fracture water with an age of 3000–12,000 years based on carbon-14 data. The authors suggested that these subsurface nematodes might control microbial population density by grazing on biofilms on fracture surfaces. They also noted that the nematodes were able to enter anabiosis (a state of suspended animation) for extended periods and metabolize aerobically when oxygen partial pressures were as low as 0.4 kPa. Even though these larger organisms are confined to fractures and fissures, their discovery demonstrates that the metazoan biosphere reaches much deeper than previously recognized and, more generally, that deep ecosystems are more complex than formerly supposed. Although many reports suggest that the complexity of subsurface communities rivals that of surface environments, this clearly is not the case for deep subsurface consolidated habitats, especially those where oxygen is not readily available. Under anoxic conditions, the diversity of organisms is much lower and their mode of survival more specialized: i.e., involving (chemolitho-) autotrophy and anaerobic respiration, which tends to revolve around hydrogen, bicarbonate, methane, and acetate. Further, bacteriophages, or bacterial viruses, have been found in deep granitic groundwater in Äspö, Sweden (Kyle et al. 2008). Up to 10^7 virus-like particles were detected per milliliter and the authors suggested that these agents could control microbial populations in the deep subsurface.

As discussed further in Sect. 7.4, the excavation of a repository would perturb the natural system given the likely introduction of microorganisms associated with various construction materials and with worker activities (e.g., species from workers, surface dust and airborne/aerosolized microorganisms, machinery), as well as physical and chemical effects. There would also most certainly be long-term equilibration of the installation with its surrounding environment: biologically, chemically, and physically. It is expected that with the passing of sufficient time after emplacement of used nuclear fuel, the repository would eventually return to the natural conditions where low potentials for microbial growth and activity dominate. As such, characterization of the existing microbial community and its relationship to the hydrogeochemistry of the system is a component of DGR site investigations.

7.2.1 Microbial Growth, Activity, and Survival

Microbial cells are capable of exponential growth. A typical first-order exponential growth equation (Eq. 7.1) can accordingly predict the change in the number of microorganisms over a given period of time (t), providing that the initial number (N_o) of bacteria is known as well as the organism's specific growth rate (μ).

$$N = N_o e^{\mu t} \tag{7.1}$$

Microbial growth in any habitat is dependent on nutrient availability and is usually controlled by the concentration of the most limiting factor (Kieft and Phelps 1997). Limiting factors could be major macronutrients (e.g., carbon, nitrogen, or phosphorus), but could also be physical and chemical conditions such as osmotic pressure, temperature, or pH. In general, microbial growth rates in most deep subsurface environments have been estimated as quite low, more than 1000s of times lower than rates observed for surface environments (Thorn and Ventullo

1988), with doubling times on the order of centuries or longer (Chapelle and Lovley 1990; Phelps et al. 1994; Fredrickson and Onstott 1996).

It has been speculated that in some deep subsurface environments, microbial metabolism would primarily be directed toward cell maintenance rather than growth (Stevens 1997). Considering the in situ growth rate potential for microbes in low-nutrient, subsurface habitats, the activity and reproductive potential for microbes is expected to be low in the host rocks being considered for DGRs. Within the context of the subsurface, the growth, activity, and survival of microorganisms therefore take on the following specific and concise meanings:

- Growth—indicated by the increase in the number of microbial cells.
- Activity—reflected by the outcome of metabolic processes of living cells.
- Survival—the prolonged persistence of cells in a non-growing state, where with the return of appropriate conditions, cells could return to a growing state.

Figure 7.1 is a generalized representation of the continuum between cells that are alive and dead, as described by Davey (2011), where the exact "point of no return" between alive and dead is difficult to determine. In most laboratory studies, or in clinical or industrial settings where microbial presence is determined, the emphasis is on obtaining data in a relatively short time, seldom longer than a few weeks. Because the microorganisms evaluated in these instances typically would be those which have short generation times, such timescales are realistic; in fact, there are sustained efforts to develop more rapid analyses. There has been relatively little discussion, however, about the effectiveness of commonly used microbiological techniques to account for the remarkably slow rates of microbial growth in the subsurface environment due to factors such as low concentrations of nutrients.

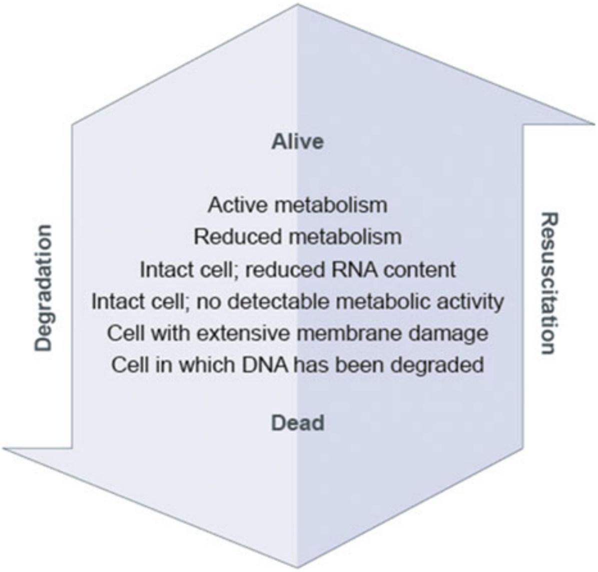
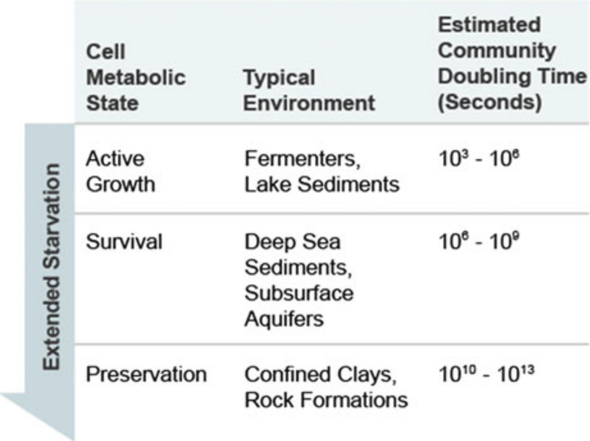


Fig. 7.1 The route from live to dead for a microbial cell, adapted from Davey (2011). Light end of the *arrow* is where the process is less likely to occur



Cell Metabolic State	Typical Environment	Estimated Community Doubling Time (Seconds)
Active Growth	Fermenters, Lake Sediments	10^3 - 10^6
Survival	Deep Sea Sediments, Subsurface Aquifers	10^6 - 10^9
Preservation	Confined Clays, Rock Formations	10^{10} - 10^{13}

Fig. 7.2 Metabolic state and estimated community doubling times for cells in various environments

Under these circumstances, the relative position of the perceived point of no return is likely different from that generally applied in everyday (high nutrient, diagnostic) microbiology. For example, cells in consolidated subsurface environments enter a state of preservation in which the calculated doubling time, based on predicted rates of flux of growth requirements, can be on the order of centuries, as summarized in Fig. 7.2.

The increasing number of successful resuscitations of ancient microbial cells sampled from the deep subsurface is a clear indication that a time frame for the point of no return from alive to dead cannot be definitively assigned for microbial cells; rather, environmental conditions have a strong influence on microbial persistence. There is a wide range of conditions under which microorganisms are uncultivable, and there is, thus, a need to expand on the potential offered by genomic and proteomic techniques to delineate the viability of cells from these extreme environments. As with efforts aimed at deriving actual in situ metabolic rates in laboratory columns, there is a justifiable concern that these methods may overestimate rates. This further emphasizes the importance of applying appropriate methods when performing subsurface microbiology and of interacting with specialists from other disciplines.

7.2.1.1 Microbial Energetics

A recurring theme in subsurface microbiology is the source of energy available for sustained microbial growth. A link between the characteristics of a subsurface environment and the presence of specific microorganisms has already been demonstrated (Fredrickson and Balkwill 2006; Lovley 2006). Where subsurface microbial activity is high, an active or recent linkage of the subsurface environment with surface terrestrial processes can usually be shown. For example, subsurface microbial communities may be linked with the aerobic or anaerobic breakdown of dissolved or particulate photosynthetically derived organic matter that either

(1) percolates via surface water into subsurface habitats or (2) was deposited via sedimentary processes (e.g., kerogen, petroliferous deposits, lignin, etc.).

Deeper in the geosphere, chemoautotrophic systems may also become established independent of photosynthesis (Stevens 1997). For such a chemoautotrophic microbial community to develop and be sustained, a sufficient source of energy must be available. Relatively new evidence points to the existence of subsurface ecosystems based on the geochemical production of hydrogen via radiolysis. Primary production supporting this system would be provided by chemoautotrophic homoacetogens, as well as acetoclastic and hydrogenotrophic methanogens, and subsequent heterotrophic organisms based on the autotrophic production of organic carbon (Pedersen 1997, 1999a, b; Stevens and McKinley 1995; Fredrickson and Balkwill 2006). This suggestion is not improbable because conditions in the deep subsurface (high temperatures, reducing gases, etc.) resemble conditions that persisted when life first developed on earth. Still, the energy requirements of microbial cells may be considerable in comparison to the small energy yields and fluxes that dominate in much of the deep subsurface. These differences in energy requirements, along with the maximum potential rates of energy supply, impose a significant constraint on the habitability of consolidated materials, and, as indicated by Hoehler (2004), this important determinant of the presence, distribution, and productivity of life in photosynthesis-independent subsurface environments has seldom been tested. Thus, in the absence of the comparatively high-energy light- and oxygen-based metabolic processes that are the primary drivers of the earth's surface biosphere, life in the deep subsurface is relegated to low-energy anaerobic processes dependent primarily on the mineral geochemistry of the host environment. Jones and Lineweaver (2010) described terrestrial waters that are uninhabited due to limitations in nutrients and energy, and, among others, restrictions on pore space. Whether certain habitats are indeed uninhabited remains a topic for discussion, given our inability to detect very low numbers of organisms with stringent growth requirements.

Hoehler (2004) described the magnitude of the energy required to sustain basic biochemical integrity and function in terms of two concepts: biological energy quantum (BEQ) and maintenance energy (ME). With respect to BEQ, for free energy to be usefully harnessed it must be available at levels equal to or larger than the specific required finite minimum energy needed to drive the synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP): i.e., the minimum free energy that must be available in a given environment to sustain life. Importantly, this free energy change $\left(\Delta G_{ADP \rightarrow ATP}\right)$ is sensitive to prevailing physical and chemical conditions, and the parameters with specific relevance to the environment of interest in the deep subsurface are (1) magnesium concentration, (2) ionic strength, and (3) pH. Many subsurface environments have high magnesium content, which has a strong affinity for ATP, ADP, and phosphate, and the $\Delta G_{ADP \rightarrow ATP}$ increases with ionic strength. Similarly, $\Delta G_{ADP \rightarrow ATP}$ becomes more positive with increases in pH. It should be noted that in contrast to BEQ requirements described above, Jackson and McInerney (2002) indicated that some fermentative bacteria do have the ability to couple substrate metabolism directly to

ATP synthesis to obtain energy for growth via reactions in which the change in free energy is less than what is needed for ATP synthesis. However, with reference to earlier observations, and based on a combination of theoretical and experimental observations, Hoehler (2004) suggested that the BEQ required for actively growing populations would be twice as much as that required for static populations in a maintenance mode. Further, the emergence and proliferation of a population are prerequisites to its survival in maintenance mode. Given the stability of the deep subsurface geosphere, however, it is not clear how such proliferation could occur in stages other than deposition and early consolidation or some major geologic disturbance.

To preserve life, organisms require a minimum rate of energy intake to maintain molecular and cellular function in addition to integrity. As discussed above, energy is used by microorganisms in one of three broad categories: growth, maintenance of basic metabolism without growth (i.e., metabolic activity), or survival that involves little or no metabolic activity and where energy is primarily used to preserve the integrity of amino acids and nucleic acids (that may be operative in consolidated materials). Ignoring the important constraints on energy flux in the deep subsurface geosphere and failing to carefully consider maintenance requirements of microbial cells in the context of this important control of energy availability could result in a significant overestimation of related microbial activity. Indeed, Morita (1999) suggested that when active bacteria are reported in ancient materials, it is possible that the suspended animation state has been broken either by giving the cells a substrate or electron acceptor or by creating different conditions from the in situ environment or from some other perturbation of the sample.

This concern has been discussed in a number of seminal publications (e.g., Chapelle and Lovley 1990; Phelps et al. 1994; Kieft and Phelps 1997; Krumholz 1998). In brief, the observations are as follows: (1) that the use of inappropriate methodology can lead to a significant difference between potential activity and actual in situ activity of microbes; (2) that laboratory estimates of microbial activity are often orders of magnitude higher than actual in situ rates, potentially by factors up to 10^6 over what geochemical models and knowledge of groundwater flows would substantiate; and (3) that calculated rates of microbial activity are typically averaged over time and distance, while the actual rates of activity are temporally and spatially heterogeneous. Considering the extremely slow rates of energy flux and the levels required for growth, it is probable that the slow rates of metabolism in the deep subsurface will be directed primarily toward *survival* rather than *growth*. The adaptive ability of microorganisms, when stressed by one set of conditions (e.g., starvation) to initiate cross-protection against other stressors, such as osmotic stress, heat stress, or temperature extremes, generally contributes to survival success to the degree that microorganisms in lithotrophic ecosystems have apparently developed mechanisms for survival and extended periods of anabiosis (Krumholz 1998). Another theory now emerging is that individual microorganisms can survive in subsurface environments for millennia while carrying out cellular metabolism at an extremely slow rate (Morita 1999).

The selective pressures on organisms or groups of organisms, as well as how they will survive and potentially proliferate, should also be taken into account (Davey 2011). Baas-Becking's (1934) eloquently stated "everything is everywhere, but the environment selects." For example, organisms that require complex organic materials for their carbon and energy requirements (heterotrophic organisms) cannot grow in the absence of such compounds. However, heterotrophy may occur aerobically (using oxygen as terminal electron acceptor) or anaerobically (by facultative anaerobes using a range of alternate electron acceptors of varying oxidative states). Under saturated conditions, the deep subsurface environment is typically reducing, thus anaerobic processes predominate in these zones (Lovley 2006). Anaerobic chemotrophs can use various chemicals as sources of energy (generally, redox reactions where electrons are shuttled between donor molecules to microbial electron acceptors, with liberation of potential energy) and therefore have the potential for being highly active, depending on the chemistry of the system. The type of chemotrophy that occurs is dependent on the microbes, with some organisms requiring pre-formed organic materials as a source of energy (chemoorganotrophs or chemoheterotrophs), and others requiring or being able to use inorganic materials (chemolithotrophs). In the case of chemolithoautotrophic organisms (e.g., *Acidithiobacillus ferrooxidans* previously known as *Thiobacillus ferrooxidans*, and *Nitrosonomas spp.*), the source of reducing energy comes from the geosphere and includes reduced inorganic electron donors such as H_2, NH_3, NO_2^-, Fe^{2+}, S^o, and H_2S and the utilization of relatively oxidized compounds as electron acceptors (e.g., O_2, NO_3^-, SO_4^{2-}).

To sustain a photosynthesis-independent ecosystem, a primary reductant is necessary; in the subsurface, this reductant could be geologically evolved hydrogen. Stevens and McKinley (1995) offered proof for the existence of such a system and presented evidence where autotrophic organisms outnumbered heterotrophs, and where stable carbon isotope analysis indicated that autotrophic methanogenesis was linked to the disappearance of inorganic carbon. They further demonstrated that production of H_2 from the reaction of basaltic rock with anaerobic water supported microbial growth in laboratory experiments. Pedersen (1999a, b) offered support for a hydrogen-driven microbial ecosystem, as hydrogen (termed by Pedersen as "geogas") can be generated from basaltic rocks by hydrolytic reactions of naturally occurring radioisotopes with water. For example, the decay of alpha-emitting particles, such as radon and radium, causes water hydrolysis, yielding hydrogen. Pedersen's model explains how the hydrogen thus produced could support growth of methanogens and acetogens, as described in Eqs. (7.2 and 7.3), respectively:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \tag{7.2}$$

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \tag{7.3}$$

The products of these reactions would then provide the reduced carbon necessary to support other microorganisms in the H_2-driven ecosystem: e.g., production of methane and CO_2 from acetate; production of H_2S, CO_2, and water from acetate and sulfate; and production of water, ferrous iron, and CO_2 from acetate and ferric iron (and acid), as respectively, shown in Eqs. (7.4, 7.5, and 7.6):

$$CH_3COOH \rightarrow CH_4 + CO_2 \tag{7.4}$$

$$CH_3COOH + SO_4{}^{2-} + 2H^+ \rightarrow 2CO_2 + 2H_2S + 2H_2O \tag{7.5}$$

$$CH_3COOH + 8FeOOH + 16H^+ \rightarrow 2CO_2 + 8Fe^{2+} + 14H_2O \tag{7.6}$$

Fermentation reactions are also common under anaerobic conditions, but rely on the presence of organic compounds that function as either electron donors or electron acceptors. Typical fermentative end products include CO_2, H_2, acetate, propionate, butyrate, ethanol, lactate, and formate, with typical substrates including cellulosic and other carbon-rich materials (e.g., carbohydrates). Fermentation often occurs in syntrophic association with organisms that use H_2 and CO_2 for methane generation (in the rumen, for example), as well as those acetoclastic organisms that strictly use acetate for methanogenesis. Thus, where sufficient organic material is found, these organisms will play a functional metabolic role.

In summary, the deep subsurface imposes significant physical (e.g., extremely small void space) and chemical (e.g., low available energy and energy flux) constraints on microorganisms, and as discussed by McCollom and Amend (2005), a rigorous accounting of controls, such as energy flow, is needed to improve our understanding of the potential biological productivity of chemolithoautotrophic communities and to better describe limits to habitability in subsurface environments. Such information is needed to derive more accurate estimates of microbial contribution to geochemical evolution.

7.2.1.2 Spores, Cell Dormancy, and Death

Microorganisms are known to survive under extreme conditions, including cells entering either an inactive state (also known as moribund, latency, dormancy, and cryptostatic) or a resting state (sporulation) involving chemical-morphological adaptations (Davey 2011). If such cells are present in the surrounding matrix of a DGR, the possibility that bacteria may survive must be considered. Repositories positioned in clay deposits create conditions likely conducive for cell entry into dormancy because of accumulation of endogenous waste and low availability of water. The best known “resting-state” mechanism is the formation of an endospore (spore): that is, heat- and desiccation-resistant, non-vegetative structures formed by members of genera such as *Bacillus* and *Clostridia* that have undergone stress or change in their growth conditions. Spores have a complex, layered wall containing high amounts of calcium, dipicolinic acid, and peptidoglycan, as well as a full copy of the cell’s genome, and are much more capable of surviving stress conditions than are their vegetative counterparts. Accordingly, spores undergo extended periods of dormancy until conditions are once again appropriate for vegetative growth.

Reportedly, spores have been recovered from archeological samples as old as 15,000–40,000 years (Grant et al. 2000). Reports of million year-old spores revived from insects entrapped in amber (Cano and Borucki 1995; Greenblatt et al. 1999), though highly controversial, have also been published.

7.2.2 Biofilms

A generic description of a biofilm is the aggregation of microbial cells and their EPS (extracellular polymeric substances) on a surface. Numerous research articles and reviews describe biofilm formation as a sequence of events. Most of these descriptions have the common features described by Busscher and Van der Mei (2006): microbial adhesion to surfaces is the onset of biofilm development, which is typically preceded by formation of a conditioning film of macromolecular components that enables initial microbial adhesion. The initial stages of biofilm formation are typically described as reversible, but formation becomes less reversible once the cells anchor themselves through EPS matrix production. Within this matrix, the cells start to grow and form microcolonies that ultimately develop into a mature biofilm from which viable cells are released back to the environment. Microbial transport to the substratum is typically enabled by microbial motility, as well as by mass transfer processes such as convection, diffusion, or sedimentation.

In the deep subsurface, biofilm research to date has primarily focused on groundwater within fractures of crystalline rock. Anderson et al. (2007) pointed out that in the case of nonporous crystalline rock, an influencing factor is groundwater flow through fractures, which could support biological growth on fracture walls. Earlier studies examining the potential for biofilm formation in granitic systems have employed recirculating flow cells and biofilm samplers connected to boreholes with outlet valves set to maintain pressures close to those found in the boreholes (Vandergraaf et al. 1997; Stroes-Gascoyne et al. 2000; Anderson et al. 2006). In their study, Anderson et al. (2006) described observations on biofilms cultivated in flow cells connected in a closed loop with a borehole that intersected a hydraulically conductive fracture zone. They showed significant biofilm formation (up to 1.86×10^4 cells/mm^2) accompanied with the production of EPS. The EPS had functional groups involved in adsorption, which led the authors to suggest that biosorption was mediated by functional groups on the microbial cell wall and the EPS matrix, without the need for energy from metabolically active cells. A subsequent publication by Jägevall et al. (2011) reported significant numbers of microorganisms on fracture surfaces of a natural hard rock aquifer, as measured by quantitative polymerase chain reaction analyses and clone libraries.

An interesting question is whether the advantages frequently attributed to the biofilm mode of growth will be of relevance in view of the very scarce nutrient supply and limited liquid flow expected in low-permeability host rock environments under consideration for DGRs. For example, increased EPS production by biofilm cells has been described as a mechanism to expand microbial habitat range by trapping nutrients and preventing desiccation; however, the energy requirements for EPS production are poorly described, especially in environments with limited water supply. Among the few articles on energy expenditure related to EPS production, Harder and Dijkhuizen (1983) calculated that in aqueous environments bacteria may invest more than 70 % of their carbon and energy for production of EPS. Delineating the survival mechanisms of the surface-associated cells in oligotrophic, dense matrices could indeed reveal information relevant to biofilms as a survival mechanism, which may provide more credible information to our understanding of survival in the deep subsurface environment.

7.3 Deep Geological Repositories

When solid used nuclear fuel bundles are removed from reactors, they are highly radioactive. Although the radioactivity of used nuclear fuel will decrease with time, its chemical toxicity will persist such that it will remain a potential health risk essentially indefinitely. Sources of radiation include reaction products that form upon splitting of the fissionable U^{235} uranium atoms: radioactive isotopes, including iodine, molybdenum, cesium, technitium, palladium, etc., and those elements that are formed when the uranium atoms that absorb bombarded neutrons do not split—elements with atomic numbers of 93 or greater such as neptunium and plutonium—known as transuranic actinides. The radioactive material is unstable and undergoes decay or breakdown over time, during which radiation is emitted. Some of this radiation is gamma radiation, which is highly energetic and penetrating, like X-rays, and thus damaging to biological (living) material due to direct and indirect (formation of damaging radicals) effects. Those fission products with very short half-lives tend to rapidly emit large amounts of radiation energy, and thus are typically very hazardous, but do not require long-term management. However, due to the presence of significant amounts of radionuclides with long half-lives in used nuclear fuel, safety assessments for deep geological repositories consider a time frame on the order of one million years.

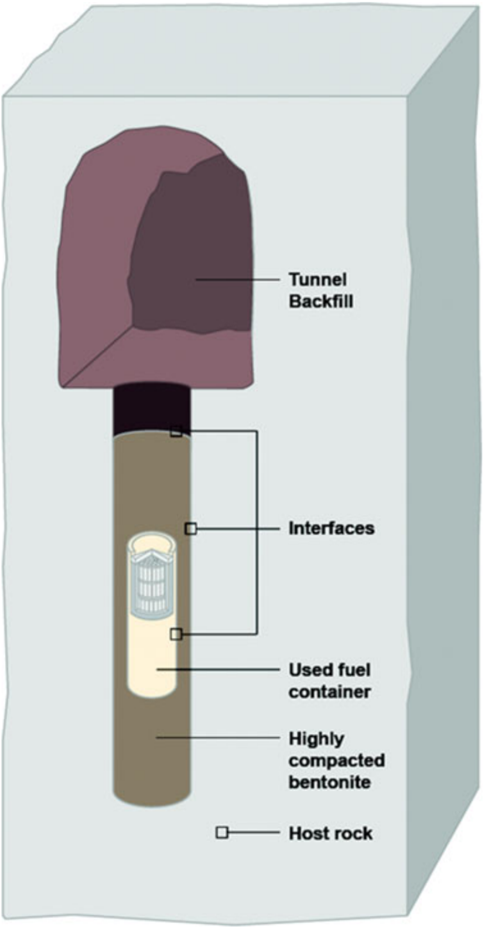
On initial emplacement of used nuclear fuel, the repository environment would experience a sharp increase in temperature because of the release of energy from fission products with short half-lives. Based on known decay rates and proposed density of used nuclear materials, container surface temperatures of 100 °C (Maak et al. 2010), or possibly higher in some designs (120 °C; Jolley et al. 2003), would occur approximately 10 years after placement and then begin to drop. Peak temperatures and time frames will be design and site specific. However, temperatures of the host rock in a DGR would likely range from 55 to 75 °C, and these elevated temperatures would persist for hundreds to a few thousands of years.

Nuclear waste management agencies have considered a number of concepts for the disposal of used nuclear fuel that employ a system of engineered barriers: clay, seals, and metal containers. For example, the design concept of SKB and POSIVA, the Swedish and Finnish nuclear waste management agencies, respectively, uses copper-iron containers, consisting of an outer copper shell and a cast iron insert, for encapsulation of used nuclear fuel prior to emplacement in their deep geological repository. Similarly, the Canadian program design concept employs a steel inner vessel surrounded by copper. In contrast, the engineered barrier system design of NAGRA, the Swiss nuclear waste management agency, presently uses a steel container within a sedimentary host rock design. Corrosion of DGR-relevant materials has undergone considerable study (King 2007) and shows the potential for corrosion will change as repository conditions evolve from relatively warm, dry, and aerobic (in the initial phase) to cooler, wet, and anaerobic (over the longer term). Despite different container designs, both concepts surround the used nuclear fuel containers with bentonite clay to protect the containers and limit groundwater access and potential transport of microbial metabolites, such as sulfide, via groundwater, to the container surfaces (Bennett and Gens 2008; Pedersen 1996, 1999b; Smart et al. 2011). Bennett and Gens (2008) have reviewed repository concepts and EBS designs for high-level waste and used nuclear fuel disposal in European countries.

There are several emplacement options being considered internationally for used fuel containers, including vertical and horizontal boreholes, and horizontal tunnels (Maak et al. 2010). Used nuclear fuel containers would be placed in rooms or tunnels constructed from main access tunnel leads or in boreholes. A clay-based buffer (100 % compacted bentonite rings) would surround containers. The facility would be tailored to the geological matrix in which the DGR is situated, with horizontal and vertical boreholes generally adopted for crystalline rock repositories (as well as hard sedimentary rock), and the horizontal tunnel option preferred for a soft sedimentary rock repository (e.g., analogous to the Opalinus Clay Formation in Switzerland).

The deep geological repository concept for used nuclear fuel involves multiple barriers to ensure long-term safety of humans and the environment. Figure 7.3 shows an example of a multibarrier system conceptual design. Considerable research and development has been undertaken to investigate the potential for microbial activity within each region of the repository. Specifically, work has been undertaken to (1) characterize the microbial communities present in low-permeability, stable geologic formations under consideration as host rocks for a DGR; (2) minimize microbiologically influenced corrosion of the used fuel containers; (3) inhibit microbial growth and activity in the high-density clay barrier that surrounds the used nuclear fuel containers; and (4) create low-permeability seals and fill to backfill the repository. Hence, the multiple protective barriers in the DGR concept include both naturally occurring, low-permeability, stable geologic formations and the engineered barrier system (EBS), which consists of the used nuclear fuel containers and surrounding clay buffer, seal, and backfill materials.

Fig. 7.3 Regions in a repository where microbial activity is evaluated



7.3.1 *The Host Rock Environment*

The host rock is the natural barrier component of the repository. International siting activities are focused on finding low-permeability host rocks that can isolate and contain used nuclear fuel. The type of host rock influences whether in-floor boreholes, horizontal boreholes, or tunnels are most suitable as a waste container emplacement method (Maak et al. 2010). The character of the host rock also determines conditions for microbial growth and represents a possible source of microorganisms. Thus, the potential for microorganisms to affect the performance of the EBS located in either host rock type warrants discussion.

In design concepts where the used nuclear fuel would be deposited within fractured igneous/crystalline rock, more stringent performance criteria of the EBS (particularly with respect to container life expectancy) would be required versus emplacements in intact rock. Groundwater movement through the fractured rock matrix could transport viable microbes and nutrients into and out of the proximity of the EBS where they could exert effects, including (1) affecting waste container integrity (e.g., via microbiologically influenced corrosion), (2) altering radionuclide mobility (in the event of a container failure) by complexation with various microbial components, such as biofilms and their extracellular products (Pedersen and Albinsson 1992), or (3) alteration of the redox state, thereby influencing radionuclide solubility (Pedersen 1996; Anderson and Lovley 2002). These effects could occur in the DGR (e.g., between the buffer material or backfill and the host rock) or within the cracks and fissures in the surrounding geologic matrix.

Repository concepts where the host rock is sedimentary rock or clay typically place greater emphasis on the host rock as a low-permeability barrier against radionuclide movement, and thus the required container lifetimes are relatively shorter. Such sedimentary rock typically does not support extensive microbial growth; evidence to support the existence of bacteria in these formations that would have been emplaced millions of years before the present time is still a matter of conjecture. For example, a preliminary study of microbial communities in potential Canadian sedimentary host rock types with low water activity suggested low to negligible microbial biomass (Stroes-Gascoyne and Hamon 2008a), which in turn suggests that a large contribution of microorganisms from sedimentary host rock types to the EBS is not likely. Others predicted that the small pore space (e.g., <0.02 μm diameter) and low water availability within clay matrices would significantly restrict the number and activity of microorganisms (Stroes-Gascoyne et al. 2007a, 2010b; Poulain et al. 2008). In such an environment, movement of nutrients to, and metabolic wastes from, microbial cells would similarly be hindered, causing nutrient limitation and end-product inhibition, and resulting in a low frequency of cellular division. Small pore throats would further prevent any significant bacterial translocation. Possible mechanisms by which bacteria would survive under the above conditions are as yet unknown. It follows that the potential impact of these scarcely distributed microorganisms within a clay formation would likely be negligible relative to the potential effects of introduced organisms at the time of DGR construction.

The hydrogeochemistry associated with the deep subsurface is site specific; however, there are identifiable trends. Overall, the potential influence of the chemical and physical conditions, and related microbial activity, in the host rock (as discussed by Sherwood Lollar 2011) on the biotic reactions in the repository appears to be minimal, which indeed is the objective of EBS (i.e., to isolate these two zones). Nevertheless, the numbers, distribution, and activities of microorganisms in the host rock provide a realistic scenario of the endpoint toward which these characteristics may evolve in the repository.

Overall, current knowledge of microbial distribution and activity in previously undisturbed deep subsurface environments that were subsequently disturbed, for example, by mining activities, provides indications as to what may happen in a deep geological repository. It is noteworthy that gradients of water activity, pressure, pore space, and temperature will occur locally within the EBS as well as over the larger repository (e.g., it is expected that temperatures in the center of the repository will be higher than those attained at the periphery of the installation). Realistically, we can assume that there will be (1) indigenous microorganisms that may be stimulated by the disturbance and (2) microorganisms introduced during EBS installation. Given this, the impact of microbes on repository performance requires evaluation. Specifically, what are the potential outcomes with regard to microbial behavior and how much of an effect could microorganisms have considering the in situ physical-chemical properties and geochemical evolution? Over what timescale are microorganisms active before transforming from active metabolism—to survival—to preservation, or the reverse scenario? Establishing the boundaries of these behaviors and their effects remains a goal of research on potential microbial outcomes on a DGR.

7.3.2 The Engineered Barrier System

Many countries have adopted clay-based materials as essential components of various EBS designs in both sedimentary and crystalline rock. Bentonite is expected to serve as a buffer between the used nuclear fuel containers and host rock, where it will influence hydraulic, mechanical, thermal, and chemical processes, as well as radionuclide diffusion (Stroes-Gascoyne 2005). Most designs will have used nuclear fuel containers encased in 100 % high-density compacted bentonite when placed within a hole or a tunnel within the host rock. Subsequently, the bentonite clay will swell following saturation by groundwater. Microorganisms indigenous to the water and bentonite will be present in the zones spanning from the container surface to the surrounding host rock. Redox conditions of the infiltrating groundwater may be oxic initially, with oxygen being introduced in the repository atmosphere during installation, but will return to anoxic conditions due to minor container corrosion, mineral oxidation, or microbial respiration. However, once all the oxygen has been consumed, the next most oxidized, relatively abundant alternate electron acceptor (based on the chemistry of the geologic system) would then be used (e.g., NO_3^-). After all the NO_3^- has been consumed, the electron acceptor would switch to NO_2^- followed by species such as manganese (IV), ferric iron, sulfate, and lastly, CO_2. Organisms generally use electron acceptors that yield the greatest amount of energy (Amend and Teske 2005) providing that these acceptors are sufficiently abundant.

7.3.2.1 Metal Containers

It is highly unlikely that biological processes will have an impact on the used nuclear fuel itself, or the inside of the containers holding the used fuel, due to the combination of high temperature, high radiation, absence of water, and lack of nutrients (Meike and Stroes-Gascoyne 2000). Nuclear waste management agencies around the world have considered a variety of container designs and components as part of their engineered barrier systems. For example, Canada is examining two used fuel container designs. One design, which similar to the 4 m long, cylindrical Swedish and Finnish designs, consists of a 10 cm thick carbon-steel, structural inner vessel within an outer copper corrosion-resistant shell approximately 5 cm thick. The other design involves a smaller container, approximately 2 m in length, consisting of a 4 cm thick carbon-steel vessel directly coated with 3 mm of copper. Other international containers, such as the Swiss and French designs, involve carbon-steel only. All of the containers, when located within the anaerobic environment of the deep geological repository, are designed to withstand corrosion from a variety of mechanisms, including microbiologically influenced corrosion (MIC), as discussed further in Sect. 7.4.2.3.

7.3.2.2 Bentonite

Since the activity of microorganisms within the repository has the potential to cause microbiologically influenced corrosion, and may also contribute to the generation of gases, much attention has been focused on the ability of the clay buffer to inhibit microbes and their activity. Microorganisms can grow over a large range of water activities (a_w of 0.75–0.999), but most favor a_w values of 0.98 or above (corresponding to a salinity of water of ~3.6 % or less) (Jay et al. 2005). Studies have shown that pure bentonite that has been compacted to 2 Mg/m^3 (corresponding to a dry density of about 1.6 Mg/m^3) and is water saturated (approximately 26 % v/w) has an a_w of 0.96, which is sufficient to inhibit the activity of the large majority of bacteria likely to be problematic in a repository (Stroes-Gascoyne et al. 2011). Further, water-expanded bentonite clay physically restricts the movement of water and whether emplaced or naturally occurring, hydrated clay matrices are known to result in a low-permeability environment, with hydraulic conductivities in the range of 10^{-12}–10^{-14} m/s (Pusch and Weston 2003). This would serve to directly limit the diffusion of radionuclides, as well as that of nutrients needed by microorganisms for metabolic activity and growth. Given that the average pore size of the clay matrix is on the order of hundreds of times smaller than a bacterial cell, cell growth and movement would also be physically restricted.

In the current Canadian EBS reference design, highly compacted 100 % bentonite buffer material surrounding the waste containers is proposed to prevent or minimize potential negative consequences of microbial activity, such as damage to the container or barrier integrity. Early Canadian EBS design options were based on a blended mix of sand and clay; however, findings reported by Stroes-Gascoyne (2010) related to microbial behavior recommended the use of 100 % bentonite. For the highly compacted 100 % bentonite to inhibit the activity of bacteria and germination of bacterial spores, it has been established that the bentonite would need to meet one or both the following criteria (Stroes-Gascoyne et al. 2006, 2007a, b): (1) have a water activity of less than or equal to 0.96, resulting from either a bentonite dry density of at least 1.6 Mg/m^3 or a porewater salinity of greater than 60 g NaCl/L; or (2) yield a swelling pressure of at least 2 MPa.

7.3.2.3 Seals and Tunnel Backfill

Repository seals isolate the disposal room from the repository access shaft and prevent the backfill mass comprised of either clay alone or clay and rock from expanding into the access tunnels. The repository seal enables the swelling clay surrounding the used fuel containers to meet density and water activity specification targets. Overall, clay-based backfill and sealants play a number of important functional roles (Stroes-Gascoyne 2005), including (1) limiting the rate of liquid movement by diffusion, (2) providing mechanical support to the container, thereby protecting it from movement or shifting of the surrounding host rock, (3) retention of radionuclides in the event of container failure, (4) provision of a thermally conductive medium to transmit heat to the surrounding host rock, and (5) limiting numbers, activities, and transport of microorganisms near the container. Options for seals include bulkheads of cement or compacted clay plugs placed at the entrance to the waste repository rooms.

Clay-based sealants have been the focus of intense research as related to EBS performance, including comprehensive research programs by Canada and Sweden over more than two decades. Clay-based seal and backfill materials have been shown to contain indigenous aerobic and anaerobic microflora, including sulfate reducing bacteria (SRB). For example, *Desulfovibrio africanus* has been found in MX-80 bentonite (Masurat et al. 2010a, b). Many studies have focused on showing how buffer and backfill parameters are likely to affect survival and activity of these and other organisms under relevant EBS conditions (see e.g., Stroes-Gascoyne 2010; Stroes-Gascoyne et al. 1997b, 2007a,b, 2010b; Pedersen 1993a; Pedersen et al. 2000a,b).

Dense backfill that consists of crushed rock, non-swelling clay, and bentonite (dry bulk density of 2.1 $\mathrm{Mg/m^3}$) would be used to fill remaining excavation spaces around the bentonite buffer. Pellets consisting of 50:50 bentonite/crushed rock or 100 % bentonite will be blown into areas where the dense backfill cannot be emplaced. Backfill material serves to (1) support the containers and the 100 % compacted bentonite buffer, (2) aid in creation of anaerobic conditions, and (3) slow saturation of the repository. Upon saturation, the hydraulic conductivities of these buffer and backfill would be low, typically 10^{-12}–10^{-14} m/s for 100 % bentonite buffer and 10^{-10}–10^{-11} m/s for backfill (Pusch and Weston 2003). Seals or bulkheads constructed out of concrete or expandable clays (or both) would be used to seal emplacement rooms and repository openings.

High-performance cement (Portland cement) is an option for seals, grouts, tunnel liners, bulkheads, and floors. Repository applications rely on cement primarily for its mechanical support and sealant properties in the repository, rather than the direct containment of used nuclear fuel containers. Concrete forms the backbone of the building industry and its use goes back for centuries. Thus, there is a relatively large body of data available on the susceptibility of concrete to microbial attack. Pedersen (1999b) has questioned whether "relevant microorganisms survive at pH equivalent to that of repository concrete and can they possibly influence repository performance by concrete degrading activities such as acid production." Evidence from surface (e.g., roads, bridges) and underground cement structures (e.g., sewer pipes) has shown that the integrity of concrete over extended timescales can indeed be influenced by microorganisms, particularly the well-known sulfur-oxidizing bacteria such as *Acidithiobacillus thiooxidans* (previously *Thiobacillus thiooxidans*), which produces sulfuric acid under aerobic conditions through the oxidation of reduced sulfate, sulfide, and thiosulfate compounds. Sulfuric acid contributes to the degradation of concrete by dissolving the calcium silicate hydrate and calcium hydroxide cement matrix constituents. Nitric acid, produced by the combined action of nitrosifying and nitrifying bacteria that use inorganic nitrogen compounds (i.e., ammonium, nitrite), may similarly lead to acid-mediated concrete deterioration. It is noteworthy that within a DGR, oxygen would be in finite supply, in sharp contrast to the situation of roads and bridges; thus, once utilized during corrosion, mineral dissolution, and microbial redox reactions, oxygen would no longer be able to contribute to the formation of sulfuric acid.

The detrimental effects of microbial activity on concrete have been described in a number of reviews. However, the emphasis in most literature on the biodeterioration of concrete and other cement-based products relates to conditions conducive to microbial proliferation. In contrast, the repository environment poses a number of challenges to microbial activity, including the extremely low porosity that will exclude all but those with an ultrasmall cell size. It is recognized though that the study of microbes in the deep subsurface and in highly consolidated materials is at a relatively early stage, and there are likely microbes to be discovered in addition to ultrasmall bacteria adapted for persistence in these extreme environments.

Cement has a high pH (~13) and contains relatively low concentrations of carbon-containing nutrients to support microbial growth. Cement compounds for repository applications typically use 0.5–1 % of a superplasticizer (abbreviated SP and typically made of sulfonated naphthalene condensates) which functions to reduce the amount of water required for cement mixing and to improve the workability and strength of the cement for repository applications. These plasticizers are carbon-containing compounds and thus they may be subject to microbial breakdown as a possible carbon source. The study of plasticizer leaching from concrete still remains incomplete, and despite the small quantities of SP added, there is evidence to suggest that some microorganisms use these compounds as a carbon source (Haveman et al. 1996; Stroes-Gascoyne 1997).

Microbial activity can accelerate other processes in the cement components and potentially affect other repository component requirements (e.g., the ability to maintain swelling pressure of the buffer, and limiting water activity in the clay matrix). It is also known that disturbance of an environment (e.g., introduction of air and nutrients) results in a temporary increase in microbial activity; therefore, the period during emplacement and soon after may be critical to the potential impact of microbial activity and may warrant consideration (Wersin et al. 2011).

7.3.2.4 Repository Interfaces

Interfaces and transition zones between the repository components may support increases in microbial diversity and metabolic activity (Fig. 7.3). Such areas pertain to (1) the area between the used fuel container and the highly compacted bentonite, (2) the area between the highly compacted bentonite and the host rock, and (3) areas where the backfill comes into contact with the bentonite buffer and host rock.

There is a compelling body of research (Stroes-Gascoyne 1997; Stroes-Gascoyne and West 1997; Pedersen et al. 2000a, b), indicating that microbial growth and activity is not expected on the container surface or in the bentonite close to the used fuel container, primarily due to heat and radiation, which will inhibit microbes in this area. Furthermore, the low permeability of the bentonite is expected to impede repopulation of the region near the container as long as the clay buffer remains undamaged.

Of more concern is the area between the bentonite and the host rock, between the backfill and bentonite, or between the backfill and host rock. As discussed above, the low water activity and swelling pressure of sealing materials is integral to impeding microbial growth. However, it is possible in some very low-permeability host rocks that a very long time will be needed to saturate the bentonite materials, offering areas where localized microbial activity may occur. However, the generalized biofilm model, sometimes described as a community of cells, is not likely relevant to this environment due to limitations in water and nutrients. Nonetheless, nuclear waste management agencies are investigating the potential for microbial growth and activity in (1) fractures in the host rock due to excavation damage, (2) areas of incomplete sealing at interfaces between different zones, or (3) fissures that form as a result of gas formation or uneven drying in the bentonite buffer.

If biofilms were to form, they could significantly affect subsurface geochemical interactions. However, it is also possible that biofilms located at interfaces between the host rock and the buffer matrix, or the container and buffer, could function to effectively plug these regions and decrease fluid transport. Because biofilm formation can have a significant impact on the porosity and permeability of fractures and porous media (Coombs et al. 2010 and references therein), it is also important to consider the potential impacts of attachment–detachment dynamics on fluid flow. Biofilms can reduce fluid flow by constricting pore throats and increasing tortuosity of pore flow paths and can also alter pH, redox, as well as groundwater and rock chemistry (Coombs et al. 2010). Porosity and permeability can be reduced by microbiologically mediated precipitation, i.e., biomineralization, resulting in possible plugging or cementation of pore spaces (Coombs et al. 2010). However, biofilm growth that covers mineral surfaces can also potentially block access to sorption sites and decrease sorption (Bass et al. 2002). The relationship between planktonic cells (which could sorb and then transport radionuclides) and attached microbial communities in the subsurface is complex and poorly understood (Onstott et al. 2009). Overall, the assessment for the potential for biofilm formation, and any associated impacts, is design and site specific and will warrant further investigation.

7.3.3 Environmental Factors

Tolerance of microorganisms to nearly every example of extreme conditions is well documented. West and McKinley (2002) summarized the specialized microbes, which have evolved within various extreme habitats of relevance to deep geological repositories for used nuclear fuel (Table 7.1), revealing that there is hardly any foreseeable condition under which microorganisms might not be expected to survive. Thus, the potential for resistant microbes to become established within certain regions of a DGR is not only possible but likely. However, the potential for microorganisms to develop a poly-extremophilic phenotype (e.g., to become multiply resistant to heat, pressure, low water activity, and radiation) so as to survive and proliferate in the immediate proximity of the used nuclear fuel containers is considered most improbable. The extreme conditions would occur almost instantaneously on placement of the used nuclear fuel, thus not allowing microorganisms the time necessary for evolutionary or adaptive change.

A growing body of research (Stroes-Gascoyne et al. 2002; Stroes-Gascoyne 2010) suggests that conditions within the EBS will result in a significant reduction in the number of surviving, viable microorganisms or cause cells to enter a dormant, relatively inactive state. The range of conditions expected to exist in the repository environment after emplacement of used nuclear fuel (e.g., after saturation of the excavated zone) would include (1) extreme antimicrobial conditions at or near the surface of used nuclear fuel containers due to the combined effects of radiation (especially with thin-walled containers), heat, and desiccation; (2) low water activity, heat, and restricted pore space within the compacted bentonite clay material; and iii) higher water activity, pore space, and some nutrients (carbon, nitrogen, and oxygen) within the backfill, as well as the EBS-host rock interface regions (Stroes-Gascoyne 2010; Pedersen 2010). For microbes to have any impact on the emplaced barriers or fuel, they will first need to survive long-term exposure to these harsh conditions. The survival and potential activity of bacterial cells will be dependent upon their distance from the waste container and local conditions of

Table 7.1 Range of tolerances of bacteria to a variety of subsurface conditions

Condition	Range of tolerance
Temperature	−20–113 °C
a_w	Minimum a_w of 0.62
Radiation	Dosages of 17–30 kGy
Salinity	Up to 50 % w/w
Pressure	180 MPa
Pore size	0.2 μm

Adapted from West and McKinley (2002)

moisture, temperature, nutrients, pore space, and time. From Stroes-Gascoyne (2010), it can be concluded that heterogeneities existing at boundaries in the EBS will likely be critical in determining microbial survival; results from this in situ emplacement repository study revealed that interfaces between the bentonite-based buffer, backfill, and sealing materials contained higher numbers of culturable heterotrophic aerobic bacteria and anaerobic bacteria—including SRB—than did the bulk materials themselves. Fractures and incomplete seals between adjacent blocks of expanded bentonite, the expanded bentonite and the host rock walls of the repository, and the expanded bentonite and the surface of the waste container could all therefore offer refuges to microbial cells.

7.3.3.1 Temperature and Water Activity

In a large-scale underground experiment, an electric heater was installed in 50 % sand and 50 % bentonite buffer material at 240 m depth for 2.5 years as part of the Buffer Container Experiment (BCE) at the Atomic Energy of Canada Limited's Underground Research Laboratory (AECL URL) to simulate container emplacement (Stroes-Gascoyne et al. 1997b). Heat surrounding the experimental container resulted in creation of a gradient of moisture within the buffer matrix, ranging from 24 % at the host rock wall to 13 % at the container surface, where temperatures of 50–60 °C were maintained. Following decommissioning of the experiment, it was found that viable microbes, including heterotrophs and specialized organisms, could only be recovered from heated buffer matrix materials where the moisture was >15 % (an $a_w \geq 0.96$), suggesting that the buffer and backfill materials could be populated or repopulated when higher moisture levels prevailed.

A limiting water activity value of 0.96 on SRB survival in bentonite was also reported by Motamedi et al. (1996) using *Desulfomicrobium baculatum* and *Desulfovibrio* sp. over a 60-day incubation at 30 °C. However, the presence of an indigenous SRB (e.g., *Desulfovibrio africanus*, a common corrosion-causing microorganism) isolated from dry Wyoming bentonite MX-80 powder suggests that strategies employed by SRB include the formation of a "dormant" state (Masurat et al. 2010a). In this case, organisms became active upon addition of growth medium containing 4 % salt and temperatures of 40 °C. Notably, viable *D. africanus* cells could still be recovered from dry bentonite powder even after heat treatment of 100 °C for 20 h (but not after treatment of 120 °C for 20 h), providing evidence of considerable tolerance to heat, and suggesting that viable SRB are likely to be present in the emplaced buffer material and can be expected to be active if other controls on their activity are not imposed.

Enhanced survival of desiccated cells is a well-known phenomenon and is likely linked, in part, to the increased efficacy and penetration of moist heat compared to dry heat on various cell components (e.g., protein denaturation effects versus oxidation and dehydration) (Fine and Gervais 2005; Jay et al. 2005). The potential for spores to survive under conditions of elevated temperature and low moisture, which may occur close to the used fuel container, must also be considered in light of evidence of organism survival at temperatures of 120 °C in Yucca mountain tuff (Horn et al. 1998). However, given that the surface temperatures of a used nuclear fuel container would not drop to <60 °C for ~10,000 years, in combination with increasing swelling pressure and low water activity within the compacted bentonite, the potential for microbial activity within the zone most affected by temperature is considered extremely remote (King et al. 2010).

7.3.3.2 Radiation

A series of experiments were conducted by AECL from 1991 to 1997 to evaluate the extent to which radiation emitted from used nuclear fuel would impact microorganisms in situ (Stroes-Gascoyne 1997). Several scenarios were explored with respect to microbial survival in the container-buffer zone, including (1) effects of radiation under near-saturated conditions, (2) effects of different radiation dosages, (3) effect of radiation at three temperature regimes (30, 60, and 90 °C), and (4) effect of radiation under various moisture conditions (0, 23, and 47 % saturation). The studies revealed that radiation and desiccation effects within the bentonite buffer used to surround nuclear fuel waste containers would essentially create a sterilized zone extending a few centimeters and a microbe-depleted zone extending tens of centimeters (Stroes Gascoyne and West 1997). After ~40 cm, radiation levels would decrease by several orders of magnitude, no longer inhibiting organisms solely based on radiation effects (Stroes-Gascoyne et al. 1995). However, there is also evidence suggesting that microbes could survive at elevated cumulative dosages of radiation. For example, radiation-tolerant bacteria have been found in the reactor core at Three Mile Island, where 10 Gy/h radiation was received by the organisms which were also regularly exposed to biocides and hydrogen peroxide in the system (Booth 1987).

Other reports have shown that specialized organisms have the capacity to survive normally lethal conditions of radiation exposure. For example, *Deinococcus* species isolated from Sonoran Desert soils were demonstrated to survive up to 30 kGy radiation (Rainey et al. 2005) and survival of chronic IR (irradiation) of up to 60 Gy/h has also been reported (Daly 2000). Work by Fredrickson et al. (2008), and further reviewed by Meike and Stroes-Gascoyne (2000), described a link between protein oxidation (desiccation) resistance and IR resistance, posing interesting questions about the evolution of IR resistance in bacteria beyond that of genome copy number and DNA repair. In a DGR, those regions closest to the used nuclear fuel container will be lethal given the combined effects of temperature and radiation; however, microbes located further from the containers will be subjected to less extreme conditions. Due to shielding provided by the used nuclear fuel container and surrounding buffer material, the effects of radiation will not, in fact, extend very far from the actual container surface. Thus, other controls in the EBS (i.e., clay swelling pressure, water availability, temperature) will primarily be responsible for limiting the activity and potential effects of microorganisms as the DGR evolves.

7.3.3.3 Salinity, Pressure, and Pore Size

Due to the water adsorption capacity of expandable clays, such as the bentonite used as buffer material, there is less available water for microbial growth. There is also a positive relationship between clay swelling pressure and buffer compaction density; the more compacted the clay (i.e., the more expandable clay per unit volume), the greater the resultant swelling pressure (Craig 1987). The swelling pressure exerted by water-saturated (26 % v/w) 100 % bentonite compacted to a density of 2 Mg/m^3 (dry density of Mg/m^3) is approximately 5 MPa (Pedersen et al. 2000a). It has been demonstrated that SRB isolates *Desulfovibrio aespoeensis* and *Desulfomicrobium bacalatum* become non-cultivable at clay densities higher than 1.8 Mg/m^3. Similarly, results from Hedin (2006) indicated that swelling pressures of 2 MPa or greater were sufficient to eliminate microbial activity within compacted bentonite.

Increasing salinity results in a decrease in water availability due to interaction of water molecules with solute ions. While microorganisms have been documented to survive a_w ranges of 0.74–0.99, most bacteria prefer an a_w of 0.98 or higher (Jay et al. 2005). Several studies have assessed the effect of salinity (NaCl and CaCl) on microbial survival in commercial Wyoming MX-80 bentonite clay (75 % montmorillonite) (Stroes-Gascoyne et al. 2010a, b; Stroes-Gascoyne and Hamon 2008b). Stroes-Gascoyne et al. (2010b) examined the effect of salinity (0, 50, 100, 150, or 200 g/L NaCl) on microbial survival over a 40–90-day period in saturated MX-80 bentonite clay over a range of dry densities (0.8, 1.3, 1.6, 1.8, or 2.0 Mg/m^3). When porewater salinity was $\leq$50 g/L NaCl, a relatively high bentonite dry density of 1.6 Mg/m^3 was required to achieve an a_w of 0.96 and swelling pressure of 2 MPa in order to suppress microbial growth (i.e., no increase in culturable cell numbers compared to dry uncompacted bentonite with ~200 CFU/g). Higher porewater salinities were more effective at inhibiting microbial culturability. For example, when $\geq$60 g/L NaCl porewater was used, a water activity of less than 0.96 was achieved regardless of the bentonite dry density used (Stroes-Gascoyne and Hamon 2008b). Studies evaluating $CaCl_2$ salinity on culturability of aerobic bacteria up to 100 g/L yielded results similar to the NaCl studies (Stroes-Gascoyne et al. 2010a).

A similar limiting water activity value was obtained by Motamedi et al. (1996) for some species of SRB in compacted pure bentonite. Pedersen et al. (2000a, b) examined the effect of MX-80 bentonite buffer density on various microorganisms, including SRB, and their ability to produce sulfide over a 28-week incubation period, and determined that the bulk density of buffer started to exert an inhibitory effect on sulfide generation at a bulk density of ~1.5 Mg/m^3. The authors postulated that microbes would be present to within a few centimeters of the container, either due to spores originally in the clay or subsequently transported by groundwater during saturation, but that the conditions within the compacted bentonite clay would be inhibitory—possibly against all microorganisms—after full swelling of the clay had been achieved. Ultimately, the key microbial control parameters of water activity and swelling pressure are determined by the dry density of the compacted bentonite and the porewater salinity (Stroes-Gascoyne et al. 2010b).

From the porewater salinity effects (Stroes-Gascoyne et al. 2010a) described above, it follows that the performance of clay barriers would be influenced by the ambient solution chemistry; for example, as the buffer and backfill zones saturate, there will be an increase in salinity over time (Dixon et al. 2002). The concentration of total dissolved solids (TDS) within the groundwater will vary depending on the surrounding environment, but will affect the swelling ability of the montmorillonite clay in the bentonite, thereby influencing the hydraulic conductivity of the matrix. Using a maximum TDS of 100 g/L salts, the highly compacted 100 % bentonite matrix would be minimally affected, with less than a one-log effect on hydraulic conductivity (Dixon 2000). Within the backfill, however, there is the possibility of microbial movement during saturation of this zone, but the swelling properties in the compacted bentonite would remain, more or less, unchanged.

It is possible that during the saturation of the EBS buffer and backfill with groundwater, microbes could be transported to the fuel container if heat and desiccation cause radial cracking or other physical damage to the buffer integrity. Stroes-Gascoyne and West (1997) considered cell repopulation events in a series of studies involving penetration of viable *Pseudomonas stutzeri* cells into compacted bentonite buffer plugs (50:50 bentonite:sand; dry densities of 1.2–1.8 Mg/m^3) pre-saturated with sterile water. Their results revealed that cell mobility was restricted to less than 5 mm in all cases, the smallest sampling interval used, but that rapid movement did occur along the metallic holder–buffer interface. Their results suggested that any interfaces or zones with reduced density could provide preferential pathways for cell migration.

In a similar study, Fukunaga et al. (2001) determined that *Escherichia coli* suspensions only penetrated <5 mm into compacted 70 % bentonite 30 % sand buffer over a three-week period. Together, these studies provide evidence that as long as the buffer matrix remains intact, microbial regrowth or colonization of the used nuclear fuel container surface and surrounding regions will be very slow or that these areas will remain devoid of active microorganisms.

Given the small clay particle size, the availability of pore space in water-saturated clays is also highly limiting and appears to have a direct effect on microbial activity. For instance, Fredrickson et al. (1997) found no evidence of metabolic activity, as determined by anaerobic mineralization of [^{14}C]-acetate and [^{14}C]-glucose, and $^{35}SO_4^{2-}$ reduction, in intact shale cores with pore throats <0.2 μm in diameter collected in northwestern New Mexico. Subsequent enrichments revealed the presence of SRB and $^{35}SO_4^{2-}$ reduction in the shale materials after 14 days of incubation. Comparatively rapid rates of metabolic activity were found in sandstone core samples with a large percentage of pore throats >0.2 μm in diameter. From these results, the authors concluded that while viable bacteria can be maintained and stimulated in materials such as shales with pore throats smaller than the size of known bacteria, subsurface bacteria require interconnected pore throats greater than 0.2 μm diameter for sustained activity. A further observation was that the detrital organic matter in the small-pore-diameter shales is not subject to direct microbial attack. In contrast, bacteria in adjacent sandstones with a more open pore structure are likely sustained by endogenous nutrients that are slowly released from the shale. Extrapolating these observations to the DGR environment, it may be possible that similar nutrient exchange takes place between dense and more porous materials, emphasizing the need to avoid the inclusion of components that may serve as nutrients, even in dense EBS materials.

In general, there is a growing body of research (e.g., the Boom Clay at Mol, Belgium, and the Opalinus Clay at Mont Terri, Switzerland) that suggests while argillaceous matrices can host microbial populations (Stroes-Gascoyne et al. 2007c; Mauclaire et al. 2007), their activity has been limited over a much longer time frame than the times currently under consideration for a DGR. Thus, the engineered barrier system is anticipated to function in restricting microbial growth and metabolism in this application.

7.3.3.4 Carbon and Energy Sources

Sources of nutrients within a DGR will vary depending on whether the repository is located within a granitic or sedimentary rock formation. For example, Canadian granitic rock has been categorized as "nutrient poor" (Stroes-Gascoyne 1997); deep groundwaters in the Canadian Shield are similarly limited by availability of organic carbon, which is typically <2 mg/L (Loewen and Flett 1984; Stroes-Gascoyne 1989). Inorganic carbon, in the form of carbonates, would range from 0.01 to 0.1 g/L and concentrations of sulfate could be as high as 3 g/L (McMurry et al. 2003) in these groundwaters.

It is inevitable that DGR construction will introduce a variety of materials that could be used by microorganisms for growth, including those associated with intentionally placed repository materials (e.g., the used nuclear fuel and container, buffer, and backfill), as well as those materials which are inadvertently placed during construction activities, including fuels, detergents, lubricants, wastes from human activities, etc. (Hallbeck 2010). Stroes-Gascoyne and West (1996) performed an analysis of the nutrients that would be available to heterotrophic and chemolithotrophic microorganisms in intentionally emplaced EBS materials and determined that the major nutrients, N and P, would be growth limiting, even using the unlikely scenario of all of the carbon being available for growth.

Bentonite clay proposed for use as buffer and backfill does contain small quantities (up to 1.5 %; Sheppard et al. 1997) of carbon-containing compounds (e.g., humic and fulvic acids), which overall would not be readily available to microorganisms but which may be extractable (Lucht et al. 1997). It has been proposed that heat, along with effects of radiation, may cause the degradation of clay-bound organic compounds rendering them more bioavailable (Stroes-Gascoyne et al. 1997a). In an experiment designed to explain this, heating (60 and 90 °C) and irradiation (25 and 50 kGy) of a 50:50 Avonlea bentonite:silica sand buffer preparation, followed by extraction with a 3:1 water:buffer ratio, resulted in an approximate two-log greater enhancement of cell growth (relative to control extracts not exposed to heat and irradiation) when supplemented with granitic groundwater. While this suggests the potential for stimulation of microbes within a repository exists, any microbial utilization of extracted carbon within these low hydraulic conductivity (~10^{-12} to 10^{-14} m/s) environments would occur far more quickly than the substrate could be replenished by diffusion, inhibiting any sustained effect on microbial growth. Subsequent work has examined other possible nutrient additions to the EBS, with particular focus on the blasting components as potential sources of N and C (e.g., ammonia nitrate fuel oil; ANFO) (Stroes-Gascoyne et al. 1996; Stroes-Gascoyne 1997). Within blast rubble, a significant amount of nitrogen and carbon could remain as a result of incomplete combustion or detonation failure of explosive materials, ranging from 4 to 5 % of the total ANFO (Stroes-Gascoyne et al. 1996) to as much as 10–20 % (Forsyth et al. 1995). Excavated rock from repository construction may therefore constitute a significant potential nutrient source for microorganisms if incorporated into EBS backfill (Stroes-Gascoyne et al. 1996; Stroes-Gascoyne and Gascoyne 1998). Factors affecting nutrient concentration in blast-rubble rock include surface area of rock exposed to the blast and the amount of explosives used, as well as the amount of blasting materials and gases entering the rock via fissures. Direct measurements on leachates from freshly broken rock at the AECL URL indicated that the amount of N in freshly excavated rock varied from 6.5 to 47 % of the total N present in the initial blasting materials (Stroes-Gascoyne et al. 1996; Stroes-Gascoyne and Gascoyne 1998) and could potentially contribute to a two-log increase in microbial numbers.

Concentrations of organic carbon, as high as 120 μg/g of rock matrix, in old and fresh broken rocks could originate from a variety of anthropogenic sources, including leaked oil, greases, and paints. Surface waters used during drilling operations similarly present a potential source of carbon (Stroes-Gascoyne and Gascoyne 1998). Because the net effect of stimulation of microbes within the EBS is unknown, it has been suggested that measures (e.g., washing, leaching) should be taken to reduce the potential for nutrient addition via excavated rock or surface-processed waters (Stroes-Gascoyne and Gascoyne 1998).

Lastly, superplasticizer (SP) ingredients in high-strength cement used in bulkheads, or as grouts to seal groundwater leaks within the repository, are not thought to likely play a significant role as a source of carbon due to the low relative abundance compared to the major components of the EBS, along with the low concentrations and leachability (10^{-16} kg/m^3) of the SP (Onofrei et al. 1991).

It is expected that any labile carbon source introduced in the bentonite buffer or backfill material, due to construction activities, or transported into the repository with the groundwater would be consumed during either aerobic or anaerobic respiration (Wang and Francis 2005). The largest contribution of organic matter in the EBS is likely to be from the buffer and backfill. As such, once materials for buffer and backfill have been chosen, their organic matter content and potential bioavailability for microbial processes should be further assessed (OECD 2012).

7.4 Perturbation and Deep Subsurface Successional Change: Deep Geological Repositories for Used Nuclear Fuel as a Perturbation Model

Discussion of potential impacts of microbial activity in the context of the EBS should include two broad questions: (1) can microbial metabolism realistically have an impact on repository function; and (2) if yes, what can be done to mitigate the impact, or control microbial activity? The specific environment associated with each design and placement method, together with the conditions of the site where they will be applied, can potentially present different outcomes in terms of microbial persistence and activity. This information should allow the development of a "microbial potential" index for different materials under conditions typical of the area at and near the used-fuel containers. The DGR concept provides an invaluable opportunity to evaluate the evolution of subsurface conditions from "disturbed" back to original state. This section discusses the expected evolution of the repository that can be expected, as well as the potential impacts that microbial activity could have on a DGR.

7.4.1 Sources of Microbes and Their Activity

A relatively large variety of microbial functional groups that may affect the overall performance of deep geologic storage facilities for used nuclear fuel has been described, including (1) those with the potential for directly damaging storage containers, (2) those organisms with potential for creating corrosion-aggressive environments where the diffusion of metabolic end products to the container could result in indirect container damage, (3) those that produce metabolites that could lead to the deterioration of EBS components such as concrete and seals, and (4) those that may impact the mobility of released radionuclides.

Specific bacterial groups have received much attention in the literature for their known involvement in metal deterioration, such as the SRB and metal reducing bacteria. The potential for microbiologically influenced corrosion of used nuclear fuel containers is a significant consideration in safety assessments for DGR performance. However, to obtain realistic data, it is important that these metabolic reactions be considered in the context of the EBS environment (and also from a microbial ecology perspective). For example, as pointed out by Chen et al. (2011), sulfide and bisulfide (formed by either mineral dissolution or produced by SRB) presumably are the most likely corrosive agents in the groundwater to which containers will be exposed. In their experiments performed in solution, Chen and coauthors (2011) showed that once HS^- became depleted in solution, its diffusion in the bulk solution became rate determining. Considering the extremely slow rate of microbial metabolism that prevails in low-permeability environments, it is highly improbable that the source values for microbiologically produced sulfide would be sufficient to create a notable concentration gradient (Chen et al. 2011). While the SRB may derive some benefit from the presence of other microorganisms (e.g., specifically those that lower the redox potential to levels conducive for SRB growth), they would also compete with these cells for nutrients in this oligotrophic environment, further limiting the source rates.

As well as physical and chemical controls on microbial activity, microbial metabolic activity could also create conditions conducive to growth of other specialized functional microbial groups, or even organisms that might compete with them for scarce resources. Typically, these microorganisms would be adapted for purposes other than just survival in oligotrophic environments and would span a variety of functional groups. One example is halophilic and halotolerant microorganisms. The potential for halophiles to be present within subsurface microbial communities is not surprising, as halophiles have long been isolated from most environments (particularly within soils where salts periodically become concentrated during dry periods) (Stewart 1938). Extremely halophilic organisms have also been detected, albeit in low numbers, in subsurface environments associated with highly saline environments including the Waste Isolation Pilot Plant located 650 m below ground surface in a bedded salt formation located in Calsbad, New Mexico (Vreeland et al. 1998).

In a long-term test experiment of buffer performance, the moderately halophilic bacterium, *Desulfovibrio salexigens,* isolated from the deep groundwater of the Äspö hard rock laboratory (HRL), was used to evaluate bacterial survival under different clay swelling pressures (Pedersen 2000). This organism did not survive as well, or penetrate as deeply into the clay (~6 mm), as did a *Bacillus subtilis* test strain. It should be pointed out, however, that it is almost impossible to realistically simulate in situ conditions, including the role of microbial species interaction, when performing pure culture studies. A guide for future considerations in this respect is the comprehensive study by Stroes-Gascoyne et al. (2007b) that examined the effect of salinity on the fate of microorganisms existing within bentonite buffers of differing dry densities relevant to the EBS environment.

While there is little evidence to suggest a strong presence of eukaryotes in the deep subsurface, there is some indication that eukaryotes are present at depth. For example, investigations of groundwater from the Äspö HRL have suggested that small fungi may inhabit Fennoscandian Shield igneous rock aquifers, although this would not likely have any direct relevance to a DGR (Pedersen 2000). Given the unique metabolic processes in which eukaryotes participate, fungal chelating agents could offer possible mechanisms for radionuclide transport, and more generally, could affect the activity of those functional microbial groups of interest in the EBS environment.

Concentrations of microbes in the deep subsurface vary widely, ranging up to 10^8 CFU per mL of water or gram of sediment (Fredrickson and Onstott 1996; Balkwill 1989; Ghiorse and Wilson 1988) and comprise a diverse variety of predominantly Gram-negative microbes and Archaea, including SRB, methanogens, and acetogens (Kotelnikova and Pedersen 1997). Backfill and buffer materials undergo handling and processing and therefore will not be sterile. These materials have been reported to contain various organisms (up to 10^4 CFU/mL in as-bought bentonite; Haveman et al. 1995), including those with potential to impact the EBS, such as SRB (Masurat et al. 2010a, b). The intrusion and saturation of backfill by ground or service waters could serve to both inoculate and activate organisms present in, or introduced into, the porous matrix. It is not anticipated that these microorganisms will penetrate through the compacted bentonite buffer, as the average pore throat diameter in clays would be too small for bacterial cells to pass through (Chapelle 1993); however, groundwater, and associated microorganisms, will inevitably reach the backfill and the backfill-host rock interface.

Given the presence of viable microorganisms within the EBS, the only questions remaining are how active these organisms will be, and what effects their activity will have on the DGR. Following closure of the DGR, there would likely be a strong chemical gradient between the repository and host rock because they would be oxidizing and relatively reducing (−225–0 mV; McMurry et al. 2003), respectively. Redox fronts will likely play a key role in defining the types of reactions that organisms would mediate (McKinley et al. 1997) and would create suitable conditions for lithoautotrophic (chemoautotrophic) organisms. Chemoautotrophic organisms are predominantly aerobes and thus would benefit by being positioned at the aerobic–anaerobic interface where the conversion of Fe(II) to Fe(III) by *Acidithiobacillus ferrooxidans*, for example, would yield energy for the fixation of CO_2. Alternative oxidizing electron acceptors (e.g., NO_3^-) would similarly function within a repository environment as conditions evolve from the initial oxidizing state.

A variety of microbial end products could have an impact on a DGR, including production of organic acids and other metabolites (e.g., formate, acetate, lactate, butyrate, nitrite, ammonia), as well as gases such as sulfide, CO_2, and methane, and an assemblage of various extracellular products that might influence both the repository or radioelements. Hydrogen may also exist or be produced by microbes, but in any environment where it is produced, its evolution tends to be coupled with consumption by other organisms (i.e., SRB) so that free microbiologically produced H_2 gas is not common.

It has been speculated that hydrogen-driven ecosystems could exist in the deep subsurface (Pedersen 1993b, 1996, 1999a; Stevens and McKinley 1995; Kotelnikova and Pedersen 1997). If this is the case, the presence of high amounts of sulfate would most certainly drive sulfidogenesis rather than methanogenesis, due to the higher substrate affinity of SRB for hydrogen relative to methanogens (Uberoi and Bhattacharya 1995). However, it is not thought likely that the activity of SRB and other microorganisms would cause corrosion to occur within the buffer region near the used fuel container. Accordingly, sulfide gas or other corrosive metabolites would need to diffuse through the EBS buffer and backfill in order to reach the copper container surface, where possible stress corrosion cracking (SCC) could occur. King and Kolář (2006) previously determined through modeling that the likelihood of significant SCC occurring is very low.

In the absence of an abundance of organic materials in the repository, it is not expected that microbial fermentation will be a dominant process. Only minor quantities of fermentation end products (e.g., acetate, formate, propionate, ethanol, butyrate, lactate) are expected in a used nuclear fuel repository. Because the 100 % highly compacted bentonite buffer is expected to be strongly inhibitory to microbial growth and metabolism, reduced end products resulting from any microbial fermentation that did occur would be produced mainly in the backfill regions.

While there will be microorganisms in both the backfill and compacted bentonite, the backfill zone is significantly more likely to undergo microbiologically mediated changes. To date, relatively few studies have specifically targeted backfill, which will contain a mixture of bentonite and crushed rock, but it is evident that the material will contain a diversity of microorganisms which may be active on saturation with water, with possible short-term benefits to the repository redox conditions by using up the O_2 introduced into the repository (Stroes-Gascoyne et al. 1997a; Stroes-Gascoyne and West 1996).

7.4.2 Impact of Microbes on Introduced Materials

Complex carbon sources are likely of limited availability in the subsurface (Loewen and Flett 1984; Stroes-Gascoyne and West 1996). However, it is likely that construction of an engineered barrier system (EBS) would introduce some sources of carbon. Thus, there may be an enhanced capacity for metabolic activity of heterotrophic organisms in the EBS versus that occurring within the unperturbed host rock. The DGR environment is expected to gradually become more reducing as organisms present in this zone consume O_2 as a terminal electron acceptor and produce CO_2. Development of reducing conditions could enable anaerobic organisms like SRB to become active, providing that sufficient sulfate is present, as well as appropriate electron donors such as hydrogen or organic carbon. This may lead to the production of bisulfide/HS^-, which could diffuse through the clay buffer and contribute to corrosion of metal containers.

7.4.2.1 Microbial Metabolites and Extracellular Materials

Gasses could be generated as a consequence of (1) microbial degradation of organic materials present in the backfill and buffer (e.g., yielding CO_2, hydrogen sulfide, or methane), (2) anaerobic corrosion of the metal container (evolving copper sulfides and hydrogen gas), or (3) abiotic radiolysis of water, yielding hydrogen gas. In general, production of significant quantities of microbial gas within the compacted bentonite buffer of an EBS is thought unlikely for two main reasons: (1) minimal organic material and (2) low numbers of microorganisms and the controls on their activity within the buffer region (i.e., bentonite swelling pressure and water activity). The majority of studies on gas generation, as related to nuclear waste repositories, have been centered on disposal of carbon-rich (often cellulose-rich) intermediate- and low-level wastes, as summarized by Humphreys et al. (2010).

The backfill region and associated interfaces have higher hydraulic conductivities than the host rock and can potentially support notable microbial activity. As discussed by Stroes-Gascoyne (1997), the availability of organic materials contained in bentonite clays is expected to be very low, as evidenced by their extreme stability since the time of their deposition (approximately 75–85 Ma for Wyoming and Saskatchewan-Avonlea bentonites). The effects of exposure of clays in backfill to aerobic conditions are not well understood; hence, any contribution to in situ gas production is speculative (Lucht et al. 1997; Stroes-Gascoyne and West 1997). Organic materials arising from EBS construction (e.g., blasting residues, drilling fluids, machine exhaust, etc.) in the repository could also represent sources of organic carbon for supporting microbial growth.

Microbial activity within the backfill zone following closure of the repository will drive the gas phase from oxidizing to reducing at some point post-closure. Data from the long-term tunnel sealing experiment (TSX) at the AECL URL (Stroes-Gascoyne et al. 2007a) offers indirect evidence of this; samples retrieved from the 10 % bentonite/90 % sand backfill region of the tunnel seal contained higher numbers of SRB and a notable decrease in the number of heterotrophic aerobic, nitrate respirers and nitrate reducing bacteria, as compared to the 70 % bentonite/30 % sand highly compacted buffer material. It was proposed that the high moisture, nutrients, and space associated with this region of the backfill could stimulate aerobes, thereby depleting oxygen and creating conditions conducive for the strict anaerobes. An alternate explanation for the increase in anaerobe numbers is that the elevated temperatures obtained during the second phase of the TSX could have inhibited the aerobes and facultative anaerobes more than the strict anaerobes.

Gas analysis of samples from the isothermal test (ITT), part of a long-term (6.5 year) study on the evolution of buffer gas and microbiological parameters (Stroes-Gascoyne et al. 2002), implied that sulfide production may have just been initiated (only about 0.02–0.5 % of total available bulk sulfate was converted to sulfide) and that evolution of reducing conditions was not yet significant. Analysis of gas from the buffer material showed that gas composition remained relatively unchanged from initial conditions (there was some slight evidence of O_2 reduction and small increases in H_2 and CH_4 levels). This may reflect a loss of viability within the resident microorganisms, as a comparison of culture-based and biochemical analysis of the buffer samples indicated that the viable population of cells was approximately two logs higher than what could be cultured. In general, development of a reducing anaerobic backfill zone after repository closure will likely retard oxidative copper corrosion, thereby serving to stabilize oxidative copper corrosion of the fuel containers over the short term (Pedersen 2000).

The most common of the microbiologically derived gasses likely to be found in the EBS would include CO_2, hydrogen, hydrogen sulfide, and methane. While initially most abundant, CO_2 will be reduced in subsequent microbiologically mediated reactions. Hydrogen and methane are thought to have the greatest potential for having impacts on the EBS environment, since these are reduced compounds that may function as microbiological energy sources (and in the case of methane, a carbon source also). It is likely that SRB would effectively outcompete methanogens at higher sulfate concentrations, given their greater affinity for hydrogen (Lovley and Klug 1983; Uberoi and Bhattacharya 1995). Indeed, Sheppard et al. (1997) showed that methanogenesis would not start until sulfate levels had dropped considerably. Other organisms may oxidize H_2 using nitrate, iron, manganese, and CO_2 as electron acceptors in the absence of methanogens and SRB (Schwartz and Friedrich 2006).

The Belgium and Swiss designs may incorporate the use of stainless steel (e.g., AISI 316 L) for high-level waste disposal, and it has been estimated that this would represent the largest gas generation source term within their EBS (Ortiz et al. 2002). The authors proposed that the hydrogen evolved due to anaerobic corrosion of the steel container surfaces would be microbiologically transformed and potentially reduced into either methane or sulfide/thiosulfate. Modeling results suggested that diffusion would not be sufficient to offset gas generation, which could create preferential pathways in the EBS. However, this is not a likely outcome due to the plasticity of clay buffer materials that would likely self-seal. In general, the nature of the gas phase will depend on the kinetics of its production, with rapid gas evolution leading to formation of bubbles or a separate gas phase, whereas slow evolution of gasses may simply diffuse away or be metabolized by other microorganisms at the time of production.

7.4.2.2 Effect of Microbes on Permeability in the EBS

Following repository closure, the presence of oxygen could stimulate aerobic metabolic processes and drive oxidative precipitation of dissolved metals such as Fe and Mn (Tufenkji et al. 2002; Haveman et al. 2005), which could lead to plugging of flow paths and pore spaces (Howsam 1987; Goldschneider et al. 2007). Ultimately, heterotrophic metabolism would deplete the available O_2 requiring use of alternate electron acceptors. Under anaerobic conditions, reductive dissolution of Fe and Mn oxides in the repository environment could then occur (Lovley 1987, 2006). These reactions tend to occur at interfaces between aerobic and anaerobic environments, or during transition from aerobic to anaerobic conditions. The resulting geochemical changes have the potential to affect backfill permeability.

It is well known that biofilms contribute to the plugging or fouling of flow paths in fractures (Wolfaardt et al. 2007; Characklis 1990; Sharp et al. 1999). Accordingly, it has been hypothesized that biofilms could clog or plug larger pore spaces within the EBS backfill. Evidence to support this is lacking, however. A study by Lucht et al. (1997) at the Whiteshell Laboratories (Pinawa, MB) infused low-nutrient groundwater into columns containing different backfill preparations for a 180-day period and found no evidence to support pore clogging, based on permeameter data, even though there were 10^6–10^7 CFU/mL of viable microbes and up to 30 mg/L of DOC present in the column effluent. Studies demonstrating microbiologically mediated (biofilm) plugging of crushed rock and sediment/sand matrices have been conducted, although it must be emphasized that these simulated highly permeable environments involving the continuous flow of nutrient-rich liquid to support sufficient growth to cause plugging (Hama et al. 2001; Brydie et al. 2005; Coombs et al. 2010). Liquid flow, generally considered a stimulatory factor for biofilm formation, along with sufficient nutrient concentrations, would not be present within the backfill and buffer region of a repository. At the host rock–EBS interface, a potential limiting role could be played by biofilms in terms of scavenging nutrients being transported from the host rock into the EBS, as well as through the direct complexation of biofilms with radionuclides that might be migrating from the repository (via adsorption, uptake, or precipitation reactions; see below) (Anderson et al. 2007).

Due to its importance to the EBS, the integrity of the bentonite clay buffer over time is of primary significance; any functional change in the bentonite could potentially affect its ability to undergo swelling upon saturation and subsequently could affect its capacity to control microbial growth and migration of radionuclides. The transformation of montmorillonite to illite (Eq. 7.7) has been studied primarily from an abiotic point of view (Huang et al. 1993; Wersin et al. 2007); high temperatures (~150 °C), elevated pressure (100 MPa), and time are required.

$$\begin{aligned} &\mathrm{Ca^{2+}/Na^{+} - montmorillonite + K^{+} + \left(Al^{3+}\right)} \\ &\quad \rightarrow \mathrm{illite + silica + Ca^{2+}/Na^{+}} \end{aligned} \tag{7.7}$$

In general, the scarcity of illite in naturally occurring bentonite deposits suggests that this conversion is not common (McMurry et al. 2003). Biologically mediated changes (biotransformation) of montmorillonite (smectite) to illite have been shown to occur also in the absence of extremes of temperature and pressure, instead by relying upon microbial reduction of Fe(III). In this case, the authors suggested that microbes dissolved smectite at room temperatures, at 101 kPa, over 14 days (Kim et al. 2004). In a more recent study by Jaisi et al. (2011) using X-ray diffraction and high-resolution transmission electron microscopy, it was demonstrated that formation of illite from nontronite by the mesophile *Shewanella putrefaciens* and the thermophile *Thermus scotoductus* occurred at basic pH (8.4) and high temperature (65 °C). While the conversion of Fe(III) to Fe(II) is known to be mediated by various iron-reducing bacteria, as well as by SRB, it is notable that conditions for microbial growth and activity within the compacted bentonite matrix will be severely inhibitory, as indicated elsewhere in this review. The montmorillonite to illite bioconversion is not thought, therefore, to be of potential significance to the functioning of the clay barrier.

A biodeterioration process of potential significance to repository functioning relates to the integrity of cement-containing materials, particularly use of cement bulkheads for sealing tunnels. Within the repository cement, bulkheads play two key roles. The first is to provide a barrier between the used nuclear fuel vault and the main tunnel system. Secondly, but perhaps more importantly, is a role in which the cement bulkhead provides a brace against which expandable clays present in the buffer and backfill may exert swelling pressure. Evidence from the Tunnel Sealing Experiment (Stroes-Gascoyne et al. 2007a) suggests that the interface between the cement bulkheads and the backfill zone is a likely region where the preferential conditions for microbial growth would exist, even possibly supporting a biofilm community of attached microorganisms. Sources for the growth of the biofilm would comprise microbes and materials contained in the backfill and groundwater, as well as those carbon compounds present in the superplasticizers that leach out of the cement. Metabolites of these biofilms would include organic acids, which could accelerate cement weathering and biodeterioration.

7.4.2.3 Microbiologically Influenced Corrosion of the Used Fuel Container

Conditions under which microbiologically influenced corrosion (MIC) of copper-steel or steel containers may occur are obviously a consideration for emplacement options. MIC of metals can occur through direct interaction of microbes on the surface of the container, or indirectly, where microbes growing in another place produce chemicals (i.e., acetate, ammonia, or sulfide, as above) that diffuse to the container. Given the compelling body of research (Stroes-Gascoyne 1997; Stroes-Gascoyne and West 1997; Pedersen et al. 2000a, b) indicating that the container surface and high-density clay buffer surrounding the container would likely be biologically inactive (due primarily to heat, radiation, and water activity effects) for a period of hundreds to thousands of years or longer, there is little evidence to suggest that a biofilm of SRB could develop on a used fuel container, provided that the EBS remained intact. Further, transport studies, such as that conducted by Stroes-Gascoyne and West (1997) using *Pseudomonas stutzeri*, suggest that repopulation of the region near the container is highly unlikely as long as the clay buffer remains undamaged. The rate of SRB-induced corrosion would therefore be limited by the rate of diffusion of sulfide (HS^-) produced allosterically outside of the container zone to the container surface (Kwong 2011). The kinetics of this diffusion would need to be sufficient for continued MIC to be sustained. Given this, MIC rates expected in a DGR are notably slower than when microbes are directly associated with metal surfaces (Sheng et al. 2007; Xu et al. 1999). In addition, they are dependent on the type of metal being corroded, as described below.

Corrosion of Iron and Steel

MIC processes are facilitated by electrochemical reactions on the metal surface. Electrons produced in the oxidation reaction at the anode are consumed in the reduction reaction at the cathode because the two reactions occur at equal rates. Examples of anodic and cathodic reactions (Eqs. 7.8 and 7.9) on ferrous metals in aerobic environments are as follows:

$$\mathrm{Fe} \rightarrow \mathrm{Fe}^{2+} + 2\mathrm{e}^- \tag{7.8}$$

$$2\mathrm{H_2O} + \mathrm{O_2} + 4\mathrm{e}^- \rightarrow 4\mathrm{OH}^- \tag{7.9}$$

Products of these reactions (i.e., Fe^{2+}, OH^-) will further react to produce, for example, ferrous hydroxides, in the presence of water, and ferric hydroxides in the presence of oxygen and water (Eqs. 7.10 and 7.11, respectively):

$$\mathrm{Fe}^{2+} + 2\left(\mathrm{OH}^-\right) \rightarrow \mathrm{Fe}\left(\mathrm{OH}\right)_2 \tag{7.10}$$

$$\mathrm{Fe}\left(\mathrm{OH}\right)_2 + \tfrac{1}{2}\,\mathrm{H_2O} + \tfrac{1}{4}\,\mathrm{O_2} \rightarrow \mathrm{Fe}\left(\mathrm{OH}\right)_3 \tag{7.11}$$

When dehydration or partial dehydration reactions are considered, many oxides or oxyhydroxides can also be formed, including from Eq. (7.10), FeO, and from Eq. (7.11), Fe_2O_3 and FeOOH, as well as the mixed-species Fe_3O_4 magnetite. Different atomic arrangements of the ferric species (Eq. 7.11) include hematite (α-Fe_2O_3), maghemite (γ-Fe_2O_3), to a lesser extent β- and δ-Fe_2O_3, goethite (α-FeOOH), and lepidocrocite (γ-FeOOH), among others. In addition, when oxygen is present, the solution near the steel surface will contain quantities of Fe^{2+} and Fe^{3+}; the specific solubilities of these components are highly dependent on pH.

Following the consumption of oxygen by corrosion reactions (Eqs. 7.9 and 7.11) and microbial processes, steel corrosion can continue anaerobically in the presence of water, which reduces according to the half reaction shown in Eq. (7.12), to produce hydrogen gas.

$$2\mathrm{H_2O} + 2\mathrm{e}^- \rightarrow 2\mathrm{OH}^- + \mathrm{H_2} \tag{7.12}$$

When coupled with iron oxidation (Eq. 7.8), ferrous hydroxides will be produced (as per Eq. 7.10); however, the lower oxidizing power of water compared to hydrogen mitigates formation of ferric hydroxides or ferric oxides listed above (or solution-based Fe^{3+}). Only the mixed-oxide species magnetite (Fe_3O_4) will be formed that contains Fe^{3+}, owing to the high lattice energy of this oxide as a surface species. Thus, the steel surface will consist of significant quantities of magnetite and ferrous oxides or hydroxides, and an absence of ferric oxides, while the solution near the corroding surface will contain only Fe^{2+}.

While the combination of Eqs. (7.8 and 7.9) does constitute corrosion reactions, the iron oxides and hydroxides possibly affect the actual corrosion rates, depending on how they deposit on the surface.

Corrosion of Copper

During the early phase of the DGR life cycle, when conditions are warm and oxidizing, roughening in the form of under-deposit corrosion and fast uniform corrosion of the copper containers will occur only as long as sufficient oxygen is available (Stroes-Gascoyne and West 1996; Kwong 2011). While corrosion is a chemical process, microbes may enhance the process by altering the local chemical environment (e.g., creation of differential aeration cells), in particular pH and Eh, and by production of corrosive metabolites, such as sulfide that would diffuse from a remote location and may impact the durability of the metal waste containers in a repository. It was suggested by Akid et al. (2008) that up to one-third (~33 %) of material loss that arises from copper corrosion may be attributed to microbial activity. In contrast, Kwong (2011) reported that after 1 million years MIC would conservatively contribute up to 1 mm of total predicted copper container wall loss (1.27 mm; i.e., MIC would contribute ~80 %). This value was determined using an extreme-value calculation, based on a HS^- concentration of 3 ppm continuously being supplied by SRB to the container surface in a crystalline rock repository environment, corresponding to an estimated corrosion rate of 1 nm/year (King and Stroes-Gascoyne 1995; King 1996). In summary, the predicted extents of MIC are both design and site specific and require appropriate study accordingly.

King et al. (2001, 2010) noted that under anaerobic conditions, it is expected that copper corrosion, along with H_2 evolution (Eq. 7.13), would occur in the presence of sulfide in solution.

$$2\text{Cu} + \text{H}^+ + \text{HS}^- \rightarrow \text{Cu}_2\text{S} + \text{H}_2 \tag{7.13}$$

After examining HS^- effects on copper corrosion up to 167 days under anaerobic conditions using dilute 5×10^{-5} mol/L Na_2S in 0.1 mol/L NaCl, Chen et al. (2011) concluded that the concentration of HS^- at the copper–water interface would ultimately limit corrosion, given that the diffusion coefficient in compacted clay buffer would be on the order of 10^{-7} cm^2/s.

Overall, conclusions from laboratory experiments suggest that the main MIC effects on copper corrosion would occur indirectly from the diffusion of sulfides produced in regions of the repository (backfill and interface) where high temperature and other conditions would not result in microbial death or inactivation (e.g., Stroes-Gascoyne and King 2002). Diffusion of the sulfide will be limited through the compacted buffer, however, making this source of corrosion a minor component (Wersin et al. 1994; Masurat et al. 2010a; Pedersen 2010; Kwong 2011).

Corrosion of Copper-Iron

Smart et al. (2011) installed miniature copper–cast iron used nuclear fuel containers with 1 mm diameter "defects" in the outer container shell and exposed these to ambient granitic groundwater in SKB's Äspö Hard Rock Laboratory in Sweden. The main aim of the study was to determine how the container would perform over time should the outer copper barrier fail, as part of a worst-case scenario study on galvanic coupling between steel and copper components. The authors came to three general conclusions: (1) water analysis showed that there were compositional differences between water from inside the support cages compared with the external borehole water, and these differences were attributed to enhanced corrosion of iron components in their experimental system, as well as increased microbial activity inside the cages that surrounded their experimental container assemblies; (2) microbiological analysis showed that SRB were active in the boreholes and the support cages, with the microbial activity higher inside the support cages compared with the boreholes outside the support cages; and (3) over time, electrochemically measured corrosion rates for both iron and copper increased in the experimental systems containing low-density bentonite, while increased rates were observed only for iron in the absence of bentonite. It was suggested that microbiologically produced sulfides were responsible for the increased rates. However, the authors noted that this effect has not yet been confirmed in fully compacted bentonite and that the increased microbial activity by SRB could have been stimulated by the corroding iron. Furthermore, because hydrogen may be generated by the anaerobic corrosion of the used fuel containers, as well as by the radiolysis of water, there would seem to exist the potential for these processes to fuel further SRB growth and sulfide generation, thereby accelerating overall corrosion.

7.4.2.4 Impact of Microbes on Radionuclide Speciation, Fate, and Movement

In the early years following a DGR closure, it is expected that any viable microorganisms within the clay buffer immediately surrounding the used nuclear fuel containers would be almost completely inactivated as a consequence of the combined effects of heat, low water activity, limited pore space, and radiation (Motamedi et al. 1996; Stroes-Gascoyne et al. 2010b). On cooling, the region around the containers would eventually become saturated with groundwater, at which time conditions might enable surviving cells to once again become active. However, the swelling pressure exerted by the bentonite buffer is expected to inhibit bacterial growth.

In the event of container failure, microbes can interact with both toxic and nontoxic radionuclides dissolved from the used nuclear fuel. The reaction of microorganisms with inorganic metals is well understood and includes metal oxidation/reduction (redox) reactions, biosorption of metals to cell surfaces and extracellular components, intracellular accumulation of metals, and extracellular precipitation (Vieira and Volesky 2000; Suzuki and Banfield 2004; Frazier et al. 2005; Merroun and Selenska-Pobell 2008). In addition to generating energy, the microbially mediated reactions and microbial products may detoxify compounds. Consequently, microbe–radionuclide interactions may affect the mobility of the radionuclide by mediating its immobilization either onto or within the cell or by altering the radionuclides solubility.

A variety of microorganisms are known for their abilities to involve metal elements, including redox-sensitive radionuclides (i.e., uranium, plutonium, neptunium, technetium), in a wide range of redox reactions. Much of the available literature centers on application of microbial biotechnology for the remediation of uranium-contaminated sites associated with the shallow subsurface (see review by

Anderson and Lovley 2002). Because toxic metals cannot be degraded, remediation strategies have focused on stabilizing these elements so they become less mobile and thus less biologically available. These include using microorganisms to reduce soluble-phase actinides (e.g., U(VI)) to their insoluble (i.e., U(IV)) state, as proposed by Lovley et al. (1991) as a strategy for preventing movement of soluble U into groundwater systems. The initial phase of this approach depends upon creation of anaerobic environmental conditions through stimulation of microbial metabolism, which itself requires having an adequate supply of either organic carbon or hydrogen to serve as electron donors. A variety of organisms that mediate similar metal reduction reactions includes SRB (*Desulfovibrio* spp.) and Fe-reducing bacteria (*Geobacter and Shewanella* spp.). In typical reactions of uranium, U (VI) behaves as an electron acceptor (dissimilatory U reduction) with the production of low-solubility uranium minerals, thereby decreasing the concentration of soluble U(VI).

It is noteworthy that this reaction may proceed in the opposite direction upon interaction of reduced uranium compounds under oxidizing environments (Merroun and Selenska-Pobell 2008), a microbiologically mediated process that has been exploited to recover uranium via bioleaching from low-grade ore deposits (Brierley 1978). In these reactions, addition of Fe(III), under acidic conditions, functions as a U(IV) oxidant and yields the more soluble U(VI). Re-oxidation of the reduced Fe(II) is performed by *Thiobacillus ferrooxidans* (reclassified as *Acidithiobacillus ferrooxidans* in 2000), which use reduced iron as an electron donor for energy production during CO_2 autotrophy.

More relevant to DGR environments, naturally occurring redox cycling by anaerobic microbes has been demonstrated by Wilkins et al. (2006, 2007) to influence the speciation and mobility of radionuclides associated with low-level radioactive waste at the Drigg low-level storage site in the UK. In that system, the removal of both soluble U(VI) and Tc(VII) from groundwaters [via reduction to their respective insoluble forms, U(IV) and Tc(IV)] was correlated with microbial Fe(III) reduction in microcosms constructed with sediment. Wilkins et al. (2007) further confirmed that the potential for nitrite reduction to re-oxidize and hence re-mobilize these elements was radionuclide specific (i.e., uranium underwent oxidative re-solubilization, whereas technetium did not).

The high sorption capacity of clay-based buffer and backfill materials is key to retardation of radionuclide migration. The conditions in compacted bentonite (radiation, water activity, swelling pressure, and temperature) make it unlikely that microbes would be involved in the migration of radionuclides from the zone nearest the used nuclear fuel container. However, there is an increased possibility that fractures within the outer regions of the backfill zone, or at the interface between the backfill and the host rock, could be colonized by microorganisms, which could then potentially affect actinide mobility. One consideration is that soluble radionuclides or radionuclides sorbed to bentonite colloids could diffuse out of the repository and reach attached biofilm communities (West et al. 2002), which could function to retard colloid-facilitated nuclide diffusion by sorbing either the clay–radionuclide complex or free radionuclides. Subsequently, biofilm cells bearing clay-adsorbed radionuclides could themselves be transported through fissure conduits along the backfill-host rock interface, mobilizing radioactive materials from repository into the host rock (Kurosawa and Ueda 2001).

Ohnuki et al. (2010) examined U(VI) and Pu(VI) with *Bacillus subtilis* in kaolinite clay. The authors determined that both U(VI) and Pu(VI) sorbed to bacterial cells in the presence of kaolinite, but that U(VI) became directly sorbed to the cells, whereas Pu(VI) underwent sequential reduction to Pu(VI), Pu(V), and then Pu(IV) before sorbing to cells. The microbial cells were determined to compete directly with clay colloids for radionuclide binding, and similar mechanisms utilized by attached biofilms on rocks in situ may be expected to retard radionuclide migration.

Such a mechanism could explain the stability of uranium species (i.e., no chemical evidence suggesting a conduit for radionuclide migration) associated with the Cigar Lake (Canada) UO_2 deposit, despite the existence of large fractures spanning the clay dome overlying the UO_2 deposit (Brown and Sherriff 1999; Smellie et al. 1997). While there is abundant evidence that microbes sorb radionuclides along with a variety of other metal elements (Merroun and Selenska-Pobell 2008), there are also reports (Anderson et al. 2007) that biofilms in fractured subsurface (granite surfaces) decrease the capacity for adsorption of mobile radionuclide compounds. Using neutral pH anaerobic groundwater conditions in a microcosm, Anderson et al. (2007) determined that biofilms suppress the capacity for the fluid-rock interfaces to act as barriers (i.e., to sorb) to specific nuclear material migration offsite (e.g., ^{60}Co(II), ^{241}Am(III), ^{234}Th(IV), but not trivalent species such as Pm and Am).

Evidence for the indirect facilitated transport of radionuclides via the metabolic products of microbial cells is also available. Frazier et al. (2005) reported that bacterial siderophores promoted dissolution of UO_2 at fivefold greater net dissolution rates than by simple proton-promoted dissolution. Siderophores normally function to sequester iron under limiting conditions, where low-solubility iron exists as iron oxides (Boukhalfa and Crumbliss 2002). The fact that normally stable tetravalent actinides (e.g., U(IV)) held under anaerobic conditions may in fact be mobilized by soluble microbial organic ligands offers an additional route for radionuclide transport. Siderophore-coupled accumulation of radionuclides (Pu (IV)) was also observed for *Microbacterium flavescens* (JG-9), which produces the siderophore desferrioxamine-B (DFOB) (John et al. 2001). In that study, it was shown that only living and metabolically active *M. flavescens* were observed to take up Fe(III)-DFOB and Pu(IV)-DFOB complexes.

Overall, it is evident that the potential for microbes to influence radionuclide migration is complex and will be site specific since it is dependent on the microorganisms present in the repository and host rock, as well as the geochemical conditions of the system (e.g., pH and Eh).

7.5 Concluding Remarks

International nuclear research programs have provided a considerable body of evidence that a diverse community of microorganisms can be expected in the deep geological repository (DGR) environment. Within the engineered barrier system (EBS), there will be a substantial gradient effect with respect to zones of inhibition, from most inhibitory at or near the container surface, decreasing out toward the bentonite clay into the host rock. The primary controls on microbial growth in the bentonite buffer will be water activity, clay swelling pressure, temperature, and radiation. Available evidence to date suggests that these controls will create a biologically inactive zone extending from the container to tens of centimeters into the compacted clay buffer. Microbes positioned further from the container, particularly in the backfill region of lower density clay, would not be subjected to the same inhibitory factors, and hence, could remain viable. The extent of their activity will be governed, in part, by temperature and availability of nutrients, as well as by mass transport conditions dominant within the backfill and surrounding geologic formations. The activity of viable organisms within the nonlethal regions of the EBS would serve to consume nutrients using oxygen as a terminal electron acceptor and help drive redox conditions from oxidizing to reducing within the repository. Ultimately, a combination of biological and chemical reactions would be expected to create a stable anaerobic environment. These effects would lead to the gradual return of the repository conditions to that of the surrounding host rock. However, the time periods required for this to be achieved remain unclear.

Interfacial regions between the various EBS components, primarily the buffer- and backfill-host rock interfaces, as well as interfaces between cement components and the backfill, may support greater microbial activity and hence larger populations than the highly compacted bentonite buffer. Water movement along these interfaces will be governed by saturation rates imposed by the enclosing low-permeability host rock. Microbes positioned at interfaces would be expected to produce greater amounts of soluble and gaseous end products than elsewhere in the repository. If gases are produced in sufficient quantity, so as to accumulate more rapidly than they dissolve, diffuse, or are consumed, the integrity of the EBS could potentially be affected by creation of preferential pathways for groundwater, and hence microbial transport.

While SRB-mediated MIC will probably not occur in the oxidizing phase of repository evolution, allochthonously produced HS^- could migrate to the container surface once conditions become anoxic, after which indirect corrosion could proceed at a rate limited by sulfide diffusion through, and precipitation (as FeS) in, the buffer. In the event of failure of the used fuel container, other potential microbiologically mediated processes would similarly rely on the integrity of the compacted bentonite surrounding the used fuel containers, including microbial movement to and from the container, as well as transport of microbe–radionuclide complexes or radionuclide–siderophore complexes. Water activity within the compacted bentonite is expected to remain sufficiently limiting so as to prevent significant microbial activity.

The overall potential for microbial activity in low-permeability crystalline and sedimentary rock formations considered for deep geological repositories is low compared with soil, surface, and aquatic environments. In a review, Fredrickson and Onstott (1996) stated that average population doubling times within subsurface environments may be so slow (e.g., 100 years or more; see Fig. 7.2) that, realistically, metabolic activity would scarcely meet the requirements of cell preservation, let alone proliferation. For some subsurface environments, it is not yet clear whether viable cells may even be recoverable, and that perhaps cells present in those environments, while metabolically functional, may have lost the ability to replicate. One would expect that with the passing of sufficient time after nuclear waste emplacement, the repository would eventually return to natural host rock conditions where low potentials for microbial growth and activity dominate. Given this, questions that require consideration are (1) to what extent will biotic reaction rates increase as a consequence of the disturbances that would accompany construction of a DGR and (2) how long will it take for microbial rates to return to original levels? Seeking answers to these important questions provides a unique opportunity to advance environmental microbiology and to expand our understanding of microbial behavior under extremely restrictive conditions.

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