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NWMO BACKGROUND PAPERS
4. SCIENCE AND ENVIRONMENT

4-6 REVIEW OF THE IMPLICATIONS OF MICROBIOLOGICAL FACTORS ON THE LONG-TERM MANAGEMENT OF USED NUCLEAR FUEL

D. Roy Cullimore, Ph.D. R.M., Droycon Bioconcepts Inc.

NWMO Background Papers

NWMO has commissioned a series of background papers which present concepts and contextual information about the state of our knowledge on important topics related to the management of radioactive waste. The intent of these background papers is to provide input to defining possible approaches for the long-term management of used nuclear fuel and to contribute to an informed dialogue with the public and other stakeholders. The papers currently available are posted on NWMO's web site. Additional papers may be commissioned.

The topics of the background papers can be classified under the following broad headings:

1. **Guiding Concepts** – describe key concepts which can help guide an informed dialogue with the public and other stakeholders on the topic of radioactive waste management. They include perspectives on risk, security, the precautionary approach, adaptive management, traditional knowledge and sustainable development.
2. **Social and Ethical Dimensions** - provide perspectives on the social and ethical dimensions of radioactive waste management. They include background papers prepared for roundtable discussions.
3. **Health and Safety** – provide information on the status of relevant research, technologies, standards and procedures to reduce radiation and security risk associated with radioactive waste management.
4. **Science and Environment** – provide information on the current status of relevant research on ecosystem processes and environmental management issues. They include descriptions of the current efforts, as well as the status of research into our understanding of the biosphere and geosphere.
5. **Economic Factors** - provide insight into the economic factors and financial requirements for the long-term management of used nuclear fuel.
6. **Technical Methods** - provide general descriptions of the three methods for the long-term management of used nuclear fuel as defined in the NFWA, as well as other possible methods and related system requirements.
7. **Institutions and Governance** - outline the current relevant legal, administrative and institutional requirements that may be applicable to the long-term management of spent nuclear fuel in Canada, including legislation, regulations, guidelines, protocols, directives, policies and procedures of various jurisdictions.

Review of the Implications of Microbiological Factors on the Long-term Management of Used Nuclear Fuel

"A severe blow will be inflicted upon the coming centuries, if the gulf between philosophy and science widens constantly from both sides; if, upon the one side, confused speculation, and, upon the other, narrow specialization constantly prevail, and prevent a mutual approach toward a beneficent common laboring ground" Max Verworn (1899) quoted by Woodger, J.H.(1929)

D. Roy Cullimore, Ph.D. R.M.
Droycon Bioconcepts Inc.,
315 Dewdney Avenue, Regina, Saskatchewan

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Executive Summary

This review focuses on aspects of used nuclear fuel management that can be impacted by the microorganisms present in the biosphere. While to some this may seem to be an insignificant part of the challenge, there is a growing recognition that single-celled microorganisms do have a major role in the functioning of atmospheric, aquatic and terrestrial systems, including soil and geospheric processes. Traditionally, microorganisms have been viewed by the public as the pathogens causing serious diseases in humans, animals and plants. Less attention has been paid to the other very significant roles that microorganisms play in influencing environmental processes.

In dealing with the long-term management of used nuclear fuel, most studies and proposals have emphasized protecting humans, animals and plants from the potential risks associated with safely storing and disposing of the used nuclear fuel. In the last two decades, there has also been a growing awareness of the need to protect the environment, or more specifically the surface biosphere, from the activities of humans. This perspective has tended to over-simplify and artificially compartmentalize the approaches to used nuclear fuel management. For example, the preoccupation with protecting the surface biosphere from uncontrolled dispersal of radionuclides has led to a definition of used nuclear fuel disposal within the Earth's crust which describes the geosphere as being located below the biosphere. A major flaw in this definition is the lack of recognition that the subsurface biosphere extends into the geosphere. Thus, there is a need to recognize and include factors relating to the subsurface biosphere in the design and establishment of any used nuclear fuel storage or disposal concepts which may be developed.

Clearly, interactions between the geosphere and the subsurface biosphere can be expected to occur. For example, at the disposal site, within the containment envelope, it can be expected that the generation of heat and radiolysis would act as a stimulant to some types of microbial activity, particularly where free liquid water is present, and an inhibitor to other types. For the purposes of this review, the containment envelope is considered to be the used nuclear fuel and all engineered systems for providing protection and support to the fuel within a natural environment. Liquid water for the support of microbial activity within the envelope would occur anywhere from saturation down to 2 percent of the saturation capacity. The form of microbial incursion into the envelope would be dependent not just on available

water, but also available nutrients and the physical conditions relating particularly to pH and the oxidation reduction potential (ORP). Radiolytic effects emanating from the fuel are likely, in the presence of water, to significantly affect the ORP towards oxidative conditions that would be supportive for aerobic (oxygen-utilizing) bacteria. One effect of this would be the focused generation of microbial biomass at the juncture between reductive and oxidative conditions (redox front). Such accumulations would have a multiplicity of implications for the chemical stability, solubility and subsequent mobility of the used nuclear fuel within the containment envelope, including:

- accumulation of metal (commonly with iron dominating) and other chemicals (such as carbonates, oxides and hydroxides) within the biomass that could then cause shielding from the radiolytic affects,
- accumulation in the biomass of escaping radionuclides, increasing corrosion risks to metal and concrete components within the envelope due to products from the biomass (such as hydrogen sulphide and various acidic products of growth),
- changes to the normal hydraulic flows as a result of bio-occlusive processes (plugging),
- gas evolutions from the biomass that could cause fracturing within the porous media components in the envelope and the more rapid movement of water through pneumatic processes, and
- mutagenesis of microbial cells leading to adaptation to growth under more challenging conditions.

Given that the local environment within a containment envelope would likely be too extreme for the activities of plants (i.e., no light source for photosynthesis) and animals (i.e., inadequate oxygen for respiration), the subsurface biosphere is likely to be dominated by microorganisms that have a greater versatility than either plants or animals to function in extreme environments. For plants and animals, the very nature of the complex and sophisticated cellular forms involved render these organisms less adaptable to change and such changes that do occur are intergenerational in their nature. For microorganisms, the simple form of the cell and the nature of cellular organisations, including, in some cases, the coating of the cell with matrices of bound water held in place by a complex network of thread-like molecules (polymers), provide a greater level of adaptability to extreme environments. This adaptability encompasses a variety of different potential challenges, including changes in barometric or hydrostatic pressure, temperature, concentrations of dissolved salts (up to saturation), pH, and ORP. Depending upon the extremity of the parameters applied, microorganisms may either adapt quickly, enter into a transient survival stage that can last for millennia (as can occur with a spore or an ultra-microbacterium), become traumatized and enter a suspended animation state, or be killed if the environment is sufficiently extreme. Given the large number of independent single cells involved (commonly measurable in millions and billions), the impact of any extreme environment has to effectively have killed at least six orders of magnitude of cells before the impact is seen and even then the potential still exists for the survivors to become active. Possibly the simplest validation of the versatility of microorganisms is the fact they dominate the subsurface biosphere and remain active in the geosphere under conditions of increasing pressure, temperature, and salt concentration, and where the ORP falls into highly reductive conditions.

In zoology and botany, it is common to consider each species as occupying a particular range of environmental niches with little ability to adapt beyond those conditions. Microorganisms are distinctly different in two ways: (1) species tend to function in consortia with other species in a mutualistic manner; and (2) component species within the consortium tend to function differently at various places within the consortium. This interdependence means that there is a remarkable ability for these consortia to adapt and locate to functionally significant points (e.g., at the redox front) within the environment where growth can occur. These consortia have the advantage of being able to bioaccumulate chemicals within the bound water around the cells for the purpose of storing future nutrients beyond immediate needs and protecting the cells from possibly harmful chemicals by “locking” them into these extracellular slimes (bound water in a polymer matrix). Should the consortium detach from a surface and move into the flowing liquid water phase, then these slimes (biocolloids) could also carry these excess chemicals, which could include radionuclides moving from the used nuclear fuel due to deterioration in the containment envelope. Once in the biocolloids, then the movement of such entrapped radionuclides would be more a function of the movement of the biocolloid with the ground water systems around the envelope. It could no longer be considered controlled by a first- or second-order reaction, but more by the functional ability of the carrier biocolloid to locate a new attachment point where growth may occur.

A discussion of these various topics is provided in the subsequent chapters of this review. Following an introductory chapter, Chapter 2 gives an overview of the science of microbiology, particularly from the environmental and ecological perspectives. Chapter 3 considers the critical microbiological issues with respect to the storage and disposal of used nuclear fuel. The influences of natural phenomena on microbiological aspects of the containment are addressed in Chapter 4, and Chapter 5 summarizes the potential forms of microbiologically mediated movement of the radionuclides from containment.

The key points arising from this review are that:

- Microbial populations (particularly the Archea) are found throughout the biosphere and several kilometers deep into the geosphere.
- These communities exist anywhere they can exploit an energy gradient (pH gradient, redox gradient,) that may be driven by radiation, temperature, etc.
- The introduction of a used-fuel disposal facility deep into the geosphere will create local gradients that will act as a source of energy for Archea and bacteria capable of exploiting these gradients.
- The consequences of this exploitation could involve increasing the rate at which radionuclides contained in the facility are solubilized, the chemical form of the solubilized radionuclides and as a consequence their potential mobility through the geosphere.
- This in turn may influence the flux of these radionuclides into the receiving surface biosphere where human beings and the ecosystem on which our species depends is adversely affected.
- Any consideration of a concept and associated design for a deep geological repository should include consideration of the effect of microbial processes.

1. **Introduction**

> *"The goal of scientific endeavor is to learn the truth of nature and not to win debates" David Noel, 1988*

This review considers the influence microbial populations may have on the decisions that have to be made concerning the long-term management of used nuclear fuel. It examines the potential impacts that could be generated by microbiological factors upon the long-term management of used nuclear fuel. The review considers the influence of microbes on both extended storage and disposal management issues. For the extended storage option, there would be an ongoing reactive management to minimize challenges to the storage facility that could result in elements of the used nuclear fuel impacting on the environment outside of the containment facility. With the disposal option, the used fuel becomes contained within a structure that has been designed and engineered to prevent significant releases of components of the used nuclear fuel that could impact the environment surrounding the disposal facility. Disposal therefore implies (unlike extended storage) that the various potential challenges to the concept have to be effectively addressed, preferably prior to the completion of the design phase for the selected disposal option, but certainly before the completion and closure of the disposal facility.

Potential challenges in maintaining secure management of the contained used nuclear fuel can be mitigated by giving adequate and appropriate consideration to all of the physical, chemical and biological factors that could affect the engineered containment concept.

The review is limited to the microbiological factors that need to be addressed in the proactive development, design and construction of an effective used nuclear fuel repository. It addresses the following issues in a conceptual manner, indicating where strengths and weaknesses presently exist in the science as it affects our ability to design an effective, robust and durable repository. These issues include:

- the state of understanding of the science of microbiology;
- an evaluation of the key considerations relevant to the design of a repository concept;
- the weighting of microbiological factors in the design and implementation of any preferred repository concept;
- prediction of the impact of natural events such as weather patterns, geological disturbances and glaciation on microbiological events that may affect the operational characteristics of a used nuclear fuel management facility;
- consideration of the microbiological events and processes that would accelerate or retard the movement of elements of the used nuclear fuel from

the repository to the biosphere (note that for the purposes of this review the biosphere is considered as encompassing the geosphere); and

- the state of the art with respect to understanding the various aspects of microbiology that could be significant to the repository.

A discussion of these various topics is provided in the subsequent chapters of this review. Chapter 2 gives an overview of the science of microbiology, particularly from the environmental and ecological perspectives. Chapter 3 considers the critical microbiological issues with respect to the storage and disposal of used nuclear fuel. Chapter 4 addresses the potential implications of significant environmental changes (such as global warming and glaciation) on the microbiological populations and the associated management challenges. In Chapter 5, the potential pathways for contaminant releases from the used nuclear fuel are examined, including how they can be moderated or magnified by microbiological activity.

The key issue to be addressed is how our current understanding of microbial populations and processes influence decisions that need to be made regarding the long-term management of Canada's used nuclear fuel.

2. The Science of Microbiology

Microorganisms are small organisms, which cannot generally be seen with the naked eye. Cell sizes range from 0.5 to 20 microns (10^{-6}m). This is well beyond human vision, limited to objects greater than 100 to 200 microns. This small size means that these organisms only become visible as conglomerates of cells along with associated supporting structures. These growths become visible to the naked eye as slimes (or biofilms), floating colloidal/aggregated structures in water (commonly seen as sea snow in the oceanic environment), encrustations, tubercles, nodules, rusticles and crystalline structures. As a result of their small size, microorganisms were not commonly recognized until the middle of the nineteenth century and many of the structures produced by them were considered to be chemical in origin. It was not until the later part of the nineteenth century that the work of Pasteur, Koch, Winogradski, Molisch and many others developed a broader-based understanding of the diversity and function of microorganisms. Scientific breakthroughs in the identification of many bacterial diseases in animals created a focus for the future development of microbiology in the diagnostic and therapeutic applications in the medical and veterinary fields. From the late 1920s onwards, there was growing interest in the chemical aspects of microorganisms, leading to the growth of immunology (by 1940), microbial generation of antibiotics (by 1960) and molecular and genetic aspects of microbiology (beginning in the 1960s). As a consequence of this bias, microbial ecology that had originally been developing in parallel to medical microbiology became largely ignored. This led to the persistence of chemical explanations for many microbiological phenomena and a lack of understanding of the activities of microorganisms within the biosphere.

In the development of conceptual understandings of the challenges facing the disposal of used nuclear fuel in the latter part of the twentieth century, chemical explanations were still being applied to resolve essentially microbiologically-induced events. Consequently, potential impacts on the environment tended to be projected as chemical and/or physical events occurring within a geosphere that was considered to be free from significant microbiological activities (i.e., functionally sterile and therefore microbial activities were not considered to be significant and largely ignored).

In general, humankind has a natural preoccupation with the surface biosphere associated with the exposed crust of Earth (i.e., the land masses). This habitat is diverse, with many species of plants and animals functioning in a complex and dynamic manner. In reality, this land habitat (by occupancy volume) is very small in depth (from 1 m deep in the soil to a height of approximately 30 m for trees) compared to that of the sea habitat (with an average oceanic depth of 3,802 m). Ground water as a habitat (with depths reaching 16,000 m and pressures reaching greater than 1,300 kPa.) has been largely ignored as a part of the biosphere even though it can contain significant microbiological biomass.

The crustal elements of the planet have been historically viewed as being biologically inert and the "biosphere" viewed as extending down only to the shallow groundwater and soil systems. Current researches in deep sub-surface microbiology now reveal that microorganisms exist in various forms at all depths, while there is still liquid water present regardless of pressures and temperatures. There does appear to be some shift in the make-up of the microbial communities with depth (Fliermanns et al, 1989) and a tendency for strictly anaerobic bacteria and Archea forms to dominate deeper down. The total biomass may be estimated from a theoretical model with depths divided into sections (with intervals of 1, 10, 100, 1,000, 5,000 and 10,000 meters, porosities (%) of 0.35, 0.3, 0.25, 0.15, 0.05 and 0.01 and incumbencies of 500,000, 5,000, 500, 100, 100 and 50 cfu/mL respectively). The tonnage per hectare from 100 mm below the surface to 10,000 meters has been calculated to be 4.72tonnes/hectare. This assumes the mass of the cells present to have a wet cell weight mean of 2 x 10^{-12} g. Based on this, differentiation of the biomass by depth is calculated to be: 74.2% of total sub-soil biomass at 1 meter; 5.7% of total sub-soil biomass at 10 meters; 4.8% of total sub-soil biomass at 100 meters; 5.7% of total sub-soil biomass at 1000 meters; 8.5% of total sub-soil biomass at 5000 meters; 1.1% of total sub-soil biomass at 10000 meters. Given that the mass may be expanded by "bound" water absorbed within extracellular polymeric structures (EPS) excreted around the cell, the total “biomass” including this bound water could rise to as much as 180 tonnes/hectare or more.

The Earth can be viewed as possessing a biosphere that is laterally layered and extending deep into the geosphere. For both the surface oceanic and land environments, the plant kingdom tends to dominate through the governance of photosynthesis from solar radiation as the prime energy driver. In contrast, the animal kingdom is essentially limited to surface terrestrial and aquatic environments (including deep-ocean) and is restricted to those habitats where oxygen is available. Microorganisms have a distinct advantage over plants and animals in that the diversity includes many species that can grow in reductive (anaerobic) environments where there is no oxygen. Thus the lower lateral layers in the biosphere are dominated by various types of anaerobic microorganisms. In the management of used nuclear fuel there is a need to consider potential interactions with the biosphere that would vary with the location of the repository facility. In all circumstances, it is most likely that the early biological interactions would be with microorganisms active within, or close to, the facility.

Over the last two decades, there has been a growing interest in the microbiology of the deep subsurface biosphere. This includes the publications of texts directly relevant to the microbiological aspects (i.e., Amy et al, 1997; and Hurst et al, 2002). From a microbiological perspective, the challenge for the long-term management of used nuclear fuel is the lack of scientific knowledge about the potential interactions that can result from the long-term storage and final disposal options. Consequent evaluation of the risk potentials from these microbiological challenges rely extensively on conceptual hypotheses. Management of these risks can provide advantages through the manipulation of some microbiological events such as occlusion (plugging) and the localized bioaccumulation of elements of concern being released from the used nuclear fuel that would restrict movement of those elements. A further set of challenges is created by the microbiologically accelerated movement of elements of concern from the used nuclear fuel.

In reviewing the "science of microbiology", several key points can be made:

(1) The biological world consists of:
    a. Eukaryotes (you and I and most other things we can see – trees , elephants etc., and everything else that has a nuclear membrane surrounding its genetic material). These organisms tend to be multi-cellular and interact with their environment through specialized cells (skin, bark, etc.), and
    b. Prokaryotes (bacteria, Archaea, viruses, etc. that we cannot see (for the most part) that do not have a nuclear membrane, genetically are extremely adaptable (swapping DNA and RNA easily) and interact with the environment through specialized cell membrane chemistries. Archaea are as distantly related to bacteria as we are. That is, we are more closely related to a corn plant than bacteria are related to Archaea.
(2) It is the Archaea that dominate the deep geosphere and form the majority of the chemolithic community (the microbial community that makes its living from energy gradients deep in the subsurface).

(3) The microbial biomass in the subsurface is approximately equivalent to the biomass of all life at the surface. The average productivity at the surface is higher than in the subsurface, as the energy gradients and availability of essential nutrients are higher. Local zones of high productivity can, however, exist in the subsurface, depending upon the availability of energy and essential nutrients.

(4) Whenever humans disturb the environment so that an energy gradient is created in the presence of essential nutrients, microbial populations will flourish and exploit the energy gradient whether it be in a rock pile (as is the case for acid mine drainage) or a used-fuel repository.

(5) Microbial communities are everywhere and will adapt rapidly to exploit any energy gradient in the presence of essential nutrients (i.e., water, carbon, P, K, etc.). This fact must be taken into account in the design, development and long-term operation of any used fuel management facility.

3. Key Microbiological Considerations Relevant to the Long-Term Management of Used Nuclear Fuel.

> "The intelligence of the universe is social. Accordingly it has made inferior things for the sake of the superior, and it has fitted the superior to one another. Thou seest how it is subordinated, co-ordinated and assigned to everything its proper proportion, and has brought together into concord with one another the things which are the best" Marcus Aurelius, 180CE.

> "Microbial life is widespread at depth in the crust of the earth - the deep subterranean biosphere. The obvious consequence is that microbes may be involved in many subterranean geochemical processes, such as diagenesis, weathering, precipitation, and in all oxidation and reduction reactions of metals, carbon, nitrogen and sulfur - just as they are in surface environments" Pederson, 1993.

Marcus Aurelius observed the interconnectedness of all aspects of any intelligent occurrence having a social impact. He noted the challenge of the “fit” between parts that are perceived to be of superior importance and those that may be considered inferior. To assign everything in a manageable manner, there needs to be both an understanding and a tolerance to achieve an agreement that would allow the tasks to be properly assigned and managed. In the handling of used nuclear fuel, there is a very high probability that the engineering of the handling (as the superior) may tend to ignore the less obvious factors such as the often covert microbiological activities (inferior). Regarding the microorganisms present in the biosphere, there remains a lack of understanding of the many roles they play. Pederson (quoted above) considered the microorganisms in the geosphere playing roles as active as those witnessed in the surface biosphere. Given that it is highly probable that the ongoing storage and final disposal of used nuclear fuel is likely to take place within the realm of the biosphere, an understanding of the microbiological populations inhabiting these areas becomes an essential part of the successful management of the used nuclear fuel.

Most microbiological investigations relate to applied industrial and medical aspects of the discipline, while the deep ocean, deep crust and the atmosphere remain to be explored.

Aspects considered to be potentially significant are addressed in Sections 3.1 to 3.6 below. These include: the significance of gas generation and organic loading (3.1); radionuclide transportation potential (3.2); sorption characteristics of radionuclides (3.3); the potential impacts of nutrients on containment (3.4); radiolytic effects within an impacted biosphere (3.5), and the potential effects of extreme environments on microbial activities (3.6).

The impact of microorganisms and their associated activities and products on a used nuclear fuel management facility could present a significant challenge. This is in part because the true extent of microbiological activities within and surrounding such a facility remains unclear. For animals and plants, the focus tends to be within the surface and the deep ocean parts of the biosphere, but for microorganisms, activity extends deep into the geosphere, into the deep saturated zones. It is generally believed that microorganisms form the dominant component of the subsurface biosphere, to the almost total exclusion of plants and animals. Recent evidence at excavated geologic sites has shown microorganisms at considerable depths utilizing the nutrients associated with the natural environment and the excavation activities. This would support the concept that the biosphere extends well into the geosphere – in all probability to the fringes of where liquid water is found, regardless of the pressures and temperatures. This is in contradiction of previous thinking that placed the bulk of the biosphere over land masses above the water table. While this premise would be applicable to plants and animals, it would not be relevant to the microbial components in the biosphere.

Like other living organisms, microorganisms possess the ability to migrate from old environments that are no longer conducive for the survival and growth of the species (for whatever reason). However, movement from these environments would mean that some microorganisms could arrive at new environments sufficiently attractive enough to be allow for ongoing growth and survival. Such scales of microbial migration would be very different in both time and distance covered compared to those that could occur amongst species of animals or plants. One key factor that affects the migratory ability of microorganisms (as compared to plants and animals) is the ability of the microbial cells to enter into passive survival modes that can effectively extend both the transitory time during passage and the consequent distances that can be covered indefinitely. Survival forms of microorganisms vary ranging from suspended animates (ultramicrobacteria or metabolically passive cells) to resistant specialized cells (endospores, exospores, and cysts). In such forms, microorganisms have the potential to survive millennia in a passive state. Generally, the survival cells are electrically neutral (possessing no charge), which reduces the potential for absorption onto any charged surfaces, which could reduce the rate of out-migration.

Microorganisms may therefore be considered relatively robust and durable compared to plants and animals and also occupy a much larger domain of more diverse environments. This would mean that the size of the biosphere may be dictated much more by the presence of microorganisms than by the more restricted domains occupied by plants and animals. Essentially, the biosphere including microorganisms would extend up into the trophosphere and down into the geosphere. It would be reasonable to consider that any storage and/or disposal of used nuclear fuel is likely to be within the biosphere and subject to intimate interactions with the surrounding microorganisms located within that region of the biosphere.

3.1 The Potential Formation of Gases from Organic Sources

There are biological mechanisms that cause recycling of some primary elements through gaseous phases. Common gaseous molecules associated with the dynamic organic chemical cycles include: carbon dioxide (CO_2, generated under oxidative conditions primarily as a result of respiration), methane (CH_4, generated under reductive conditions primarily through the degradation of fatty acids), hydrogen sulphide (H_2S, generated under reductive conditions from sulphates and sulphur), hydrogen (H_2, generated reductively through fermentative and electrolytic processes), nitrogen (N_2, generated primarily by reduction of nitrates) and volatile organics (VO_x, generated reductively as short-chained hydrocarbons and fatty acids). All of these gases can be generated from various organic materials and the sources are addressed in Section 3.4.

Gas generation in a storage or disposal scenario can have a dramatic effect on the containment of the used nuclear fuel for a number of reasons.

Hydrogen sulphide can be generated from organic sources (in particular the sulphur containing amino acids) and from inorganic sources of sulphur and sulphate. This gas is well recognized as being a prime cause of electrolytic corrosion of metals and also the production of rotten egg odours, as well as blackened slimes and concretions. If this gas was to be released within a containment zone, it would be expected that there would be an increased risk of corrosion of any impacted metals that could include pitting and perforation of the metal.

Carbon dioxide is the most common product of respiratory processes that can, in confined spaces, lead to radical declines in oxygen. Once the solubility of the water is exceeded at the ambient pressures, then bubbles of carbon dioxide can form perched within fracturing zones or collecting in overhanging impermeable structures. These accumulations of gas in porous media can compromise the structural integrity of porous materials such as sands and also change hydraulic flow characteristics. Under alkaline conditions, particularly where calcium is present, forms of calcium carbonate (e.g., calcite) may be formed as crystalline structures that could encrust the used nuclear fuel containers.

Methane, also referred to as “natural gas”, is generated by methane-producing bacteria under very reductive conditions, with fatty acids, carbon dioxide and hydrogen forming the feedstock. This gas remains recalcitrant under reductive conditions and relatively non-reactive. However, when the methane enters the reduction-oxidation gradient at the redox front, there are many bacteria able to degrade methane oxidatively with carbon dioxide as the gaseous end-product. Such activity could trigger significant growth, primarily as biofilms maturing through to slimes and encrustations that could then cause plugging that would affect the movement of methane and water through the impacted site. In the event that the methane is being generated into a limited space, then the risk of explosion becomes a real concern when the methane reacts with oxygen from the air.

Hydrogen gas may be formed as a final gaseous end-product of the fermentation of organic materials that were degraded reductively to fatty acids. It may also be produced bio-electrolytically or geo-chemically in the deeper regions of the crust that are highly reductive. Evidence for the biogenesis of hydrogen under these conditions was first suggested by T.O. Stevens and J.P. McKinley (1993). They reported that there was an influence of microorganisms on the bulk chemistry through enhancing rock dissolution, which led to increases in the rate of hydrogen production. They speculated that microorganisms could stimulate the rate of hydrogen production in geochemical processes. There is now a growing recognition for the biogenic production of hydrogen. When this gas enters an oxidative region (such as in a redox front), this hydrogen may act as an energy driver for microbiological metabolism and growth with the consequent generation of biomass.

Nitrogen is a major part of the atmosphere, but is also subjected to various fixation activities (biological, meteorological and volcanic) with ammonium being a primary end product. Nitrification is a common microbiologically-driven process where, under oxidative conditions, the ammonium is converted to nitrate. Under reductive conditions, this nitrate is converted back to nitrogen gas (or ammonium) through the process of denitrification. These processes are likely to occur within a storage or disposal facility where organic material is present, and both oxidative and reductive conditions exist. Nitrogen is a major nutrient for all microorganisms and its presence is essential if biomass is to be generated.

Volatile organics, while not strictly gaseous, are capable of moving through the atmosphere and can be biologically accumulated within the biomass, particularly, but not exclusively, under oxidative conditions. Where present, these organics are most likely to be taken up by the oxidative biomass which may then compromise the design of the long-term used nuclear fuel storage and/or disposal facilities.

In summary, there is potential for the microbiologically-driven formation of gases within a storage or disposal facility for used nuclear fuel. These gases will differ in types, depending upon the conditions within the facility. For example, managed storage will probably be in a dry oxidative environment, while passive disposal will probably take place in a wet reductive one. If the conditions are oxidative, then the dominant gas is likely to be carbon dioxide, associated primarily with the degradation of any intrinsic or extrinsic organics that enter the facility. There is also likely to be a significant biomass generated. At the redox front (interface between oxidative and reductive conditions), there is commonly a focussing of microbial biomass. Gases produced here are likely to be carbon dioxide (on the oxidative side of the front) and hydrogen sulphide, nitrogen, methane, hydrogen and various volatiles. Some of these gases would enter into the biomass at the front and be utilized, but some may also migrate away from the front into the reductive regions. This migration may result in the generation of corrosive processes and, at lower hydrostatic pressures, regions occluded by pockets of gas in a manner that may interfere with the engineered performance characteristics for the containment facility.

3.2 Radionuclide Sorption onto Biocolloids

Biocolloids are becoming recognized as a potentially significant component in liquid waters. Vinebrooke and Cullimore (1998) reviewed the scientific literature and found that there was potential for bound waters to exist within aquatic ecosystems. These bound waters would be associated with biofilms (sessile and attached to surfaces) and suspended materials that may be in a free colloidal mass or accumulated onto particles. In both cases (attached or suspended), the bound water would be held by charged extracellular polymeric substances (EPS) that had been generated as a part of microbial activity. Suspended colloidal forms have the ability to accumulate cationic metal ions. These accumulates do not necessarily become metabolised quickly, but are retained within the floating colloids. Iron bioaccumulation within yellow colloids and suspended "red dust" was described by Cullimore et al (2001) occurring both at the wreck site of the *RMS Titanic* and in a series of laboratory experiments examining various aspects relating to the growth of rusticles. Here it was commonly observed that yellow colloidal suspensions would commonly be generated with an 8% content of iron (dry weight).

Given the nature of biocolloidal materials it would be likely, where they are present and active, that cationic ions have a significant likelihood of being accumulated within the colloidal matrices associated with the bound water. Radionuclide sorption into these biocolloidal structures is likely to have a significant effect on the movement of these chemicals. Various events may occur if sorption does occur. These are:

(1) the radionuclides continue to concentrate until there are traumatic effects causing collapse of the supporting EPS and release of these chemicals;
(2) bioaccumulation continues to occur until the EPS becomes saturated with radionuclides without impacting on the biocolloid;
(3) the suspended biocolloidal particle carrying the radionuclide moves away by the biocolloidal density shifting or is transported away by hydraulic flows;
(4) as the biocolloidal entity accumulates more materials including radionuclides, the entity develops a greater density causing settling (if not attached) and the formation of an encrusted state in which the mass hardens and loses viability;
(5) the biocolloidal entity undergoes a structural collapse releasing radionuclides back into the environment;

(6) gas is generated within the biocolloidal entity that radically lowers the density of the entity, causing vertical movement of the radionuclide along with the biocolloid; and

(7) when microorganisms within the biocolloid die thus impacting on the EPS structures leading to a fragmentation which dissipates the radionuclide still absorbed onto the shattered EPS.

These various events may occur concurrently within biocolloids that have accumulated radionuclides. It should be remembered that scientific knowledge regarding the relationship between inanimate colloids and animate biocolloidal particles, and sorption of radionuclides remains in its infancy and the concepts described above represent probable sequences of likely events that would occur in an interactive manner. Such radionuclide behaviour patterns would be very difficult to model in a defensible mathematical manner. The challenges to this would arise from the dynamic characteristics of biocolloids as reactive products of complex microbiological interactions being impacted by the local and regional environments as well as the potential effects from the emission of radiation by the bioaccumulated radionuclides.

3.3. Microbiological Influences on Radionuclide Transportation

Microbial effects on radionuclide transportation go beyond the processes described in Section 3.2, which are limited to biocolloidal events only. Transportation of radionuclides from the used nuclear fuel primarily has to involve a passageway for conveyance. In a water-saturated medium, the transportation is likely to involve a hydraulic vehicle such as water flow. In a semi-saturated water condition, hydraulic conveyance may become restricted to localized movement in "perched" water or though gravity-induced primary descent. Under more arid conditions, where water is present in very limited amounts, radionuclide transportation would be the result of biological and physical processes not reliant on water. Of these three water-based transportation mechanisms, it is the saturated state in which water dominates any free void space that creates the highest probability for microbiological influences on radionuclide transportation. For the purpose of this review, these three states are addressed separately as posing different challenges to the integrity of a storage and/or disposal facility for used nuclear fuel.

Used nuclear fuel within a water-saturated medium can be subjected to a range of microbiological influences that can lead to the transport of some of the radionuclides from the fuel. These influences can be grouped into:

(1) corrosive effects leading to the degradation of the structural integrity of the fuel;
(2) occlusive effects in the surrounding medium leading to changes in the local hydraulic characteristics;
(3) gassing effects leading to the potential for transportation along fractures; and
(4) changes in the pH and redox (oxidation – reduction potential, ORP) causing changes in the natural geochemical characteristics of the supporting medium.

Multi-barrier concepts are designed to restrict these microbiological influences through the separation of the used nuclear fuel from direct interaction with water in the saturated environment. The use of a protective envelope incorporating durable containers and restrictive barriers can be engineered to reduce the potential for these microbiological influences, but not necessarily eliminate them over the fullness of time.

Microbially induced corrosion (MIC) is a major recognized factor in affecting the lifespan of many metal structures. Corrosion is mostly reductively driven with two major initiators: sulphur or degradable organic materials. Sulphur in the form of sulphates or elemental sulphur can provide the feedstock for the reductive generation of hydrogen sulphide through the activities of dissimilatory sulphate and sulphur-reducing bacteria (Cullimore, 2000). Corrosion can also be initiated by the acid-producing bacteria. These bacteria function in reductive conditions where degradable organics are present. Fatty acids are major products of these fermentative activities, leading to the pH falling into the acidic range. Acidic conditions can increase the solubility of many metals and compromise metal-based barriers. Such acidic conditions can also compromise concretes that are cured for use under neutral or alkaline conditions. In the industries where acidic conditions become a concern to the integrity and performance of steel-based equipment (such as in oil and gas extraction wells, heat exchangers and pipelines), there are some disparities as to what are the critical pH values below which acid-induced corrosive processes become significant. In the development of the acid-producing bacteria BART tester, it was found there was no consistency on this critical pH value. Consequently, the critical pH set for this tester was to detect acid production over the pH range of 4.2 and 5.4 as being the critical range of concern (technical manual, APB-BART, 2002, Droycon Bioconcepts Inc. Regina). The presence of sulphur, sulphates or degradable organic material in a water-saturated reductive environment is likely to create corrosive challenges to the used nuclear fuel and its containment envelopes, particularly when the ORP values range between 0 and -200millivolts. Under more reductive conditions than this, there is likely to be competition between the SRB and methane-producing bacteria for the product fatty acids (generated by the fermentative microbial activities on any intrinsic degradable organic matter). Under these more reductive conditions, the challenge is likely to shift to one of gas production that has consequences (i.e., slime growth, plugging and explosions) when methane reaches oxidative conditions.

Another challenge that exists in water-saturated conditions is the generation of microbial biomass. In porous media and fractured rock, the generation of biomass can cause sufficient plugging of the pores and/or fractures to restrict and even stop hydraulic flow. Commonly, the bulk of the biomass forms close to, or at, the redox front where the ORP values shift from reductive (negative millivolts) to oxidative (positive millivolts). Cullimore (1999) showed that this biomass actually consisted of lateralised layers of different microbial bacteria consortia with a typical pattern being (reductive to oxidative): methane-producing, sulphate-reducing, heterotrophic, slime-forming, methane-consuming and iron-related bacteria. This growth impacts on hydraulic flows in a step-wise manner that is in harmony with the natural cycle within the growing biomass and involves three functions (Cullimore, 1993): growth (hydraulic flows are reduced), stabilization (hydraulic flows are constant), and compression (hydraulic flows increase). It may therefore be expected that, where there are adequate nutrients, these occlusive biomass will be generated at any redox front. Such fronts are commonly generated around producing water wells, and the generating biomass can plug up the well if it is a producing or monitoring well (Alford et al, 1999). When this form of occlusive biomass is generated, there are secondary impacts relating to the bio-accumulation of chemicals (such as metals and radionuclides). Accumulation will continue until either the biomass becomes saturated or destabilizes. With saturation, there would be a return in the downstream concentrations of the biomass to the concentrations found upstream, since bioaccumulation ceases to occur. With destabilization, there would be a sloughing of biomass, including the accumulated chemicals downstream. This would initially cause spiking of downstream concentrations (coincident with sloughing) followed by a "bleeding" event when the sheering becomes continuous. This would in turn lead to constantly higher downstream concentrations for the chemicals of concern relative to concentrations upstream of the biomass. In the rehabilitation of plugged water wells, particularly where these are associated with hazardous waste sites, the extracted biomass often contains very high levels of accumulated chemicals of concern and has to be treated as hazardous waste (US Army Corp of Engineers, 2004).

Given the nature of the biosphere with oxidative conditions existing above reductive conditions, it can normally be expected that redox fronts will form in saturated waters at positions where reductive conditions interface with oxidative conditions. Where this occurs, it may be expected that biomass will be generated and bioaccumulation will take place. For a compromised containment envelope, this would mean that some of the disrupted used nuclear fuel is likely to become accumulated and held for a period of time within biomass resident in the redox fronts separating reductive from oxidative conditions. Modelling such events is challenging because of the complexity of microbiological interactions within the environment at the redox front. These interactions cannot be simplified to a first- or second-order reaction, some form of event prediction such as "Monte Carlo" modelling (using randomized sequencing of events) or simplified to a Monad formulation. In the transportation of radionuclides, it may be commonly expected that the sequence of events will include:

1. release from the used nuclear fuel;
2. transport through hydraulic flows in a reductive environment;
3. bioaccumulation within occlusive biomass focussed at redox fronts;
4. release from the biomass on the oxidative side of the redox front;

(5) incorporation into biofilms and biocolloids within the oxidative hydraulic flows;
(6) ingestion of biofilms and biocolloids by various animals (including, but not limited to, protozoa, nematodes and insects);
(7) entry into the food chain.

Some of the radionuclides (^{135}Cs, ^{129}I, ^{79}Se, ^{126}Sn, ^{99}Te, ^{14}C and ^{3}H) were considered in the AECL disposal concept documentation as being "instant release factors" for the purposes of modelling potential impact events. Of these, particular attention was addressed concerning both ^{129}I and ^{99}Te, given the potential that these radionuclides would not be accumulated significantly by any occlusive biomass forming along the pathway to entry into the food chain.

Semi-saturated conditions are likely to occur above the water table in porous media such as soils and in the vadose zone above aquifers. These conditions would involve some direct or indirect interfacing with the atmosphere and are likely to be oxidative. Similar semi-saturated conditions were examined by Komlos et al (1998) to determine the persistence of biofilms as barriers under these conditions. Such events are likely to occur within the crust as a result either of water displacement by gases (commonly under reductive conditions), or extraction of water through geothermal or hydrologic events. Where the environment is semi-saturated with water under oxidative conditions, the dominant micro-organisms are likely to be Fungi that are able to produce mats (mycelium) of thread-like growths (hyphae). These organisms can also bioaccumulate recalcitrant chemicals such as radionuclides but to a much lesser degree because the mycelium does not incorporate bound water in EPS outside of the cells (unlike bacteria which can). There is therefore a much lower potential for radionuclide retention within this fungal growth within the semi-saturated zones than for the bacteria dominating the biomass at the redox front. Radionuclide transportation is therefore more likely to be retarded in the redox front biomass than in the oxidative semi-saturated zone above.

In summary, many of the radionuclide transportation processes are affected by the presence of microbes, specifically the bacteria at the redox fronts wherever these occur, and the fungi in the semi-saturated oxidative zones. It should be recognized that microbes are ubiquitous in the biosphere and their activities will influence the behaviour and transport of nuclides by changing their characteristics with respect to diffusion (through water) and retention (through chelation into biocolloids). These changes would secondarily affect the manner and form with which the radionuclides would be taken up into the food chain (beyond bioaccumulation into the EPS) and assimilated in the food chain animal and plant biota.

3.4 Nutrient Sources for Sub-Surface Biosphere Activities

Recognized major nutrients are classically considered in the field of agronomy to be nitrogen (N), phosphorus (P) and potassium (K). However, in the field of microbiology, the dominant major nutrients shift to include carbon (C) and sulphur (S) but exclude potassium (K). This is because the dominant microorganisms of interest are catabolic, obtaining energy from the degradation of organic materials (like all animals) rather than synthesizing organics primarily utilizing sunlight (like all plants).

In the subsurface biosphere, energy can be extracted from the degradation of organics flowing down from the surface biosphere. This flow-down moves from oxidative to increasingly reductive conditions leading to the generation of recalcitrant forms of reduced organics: oils, tars, natural gas and coals. This generation of a range of compounds, dominated by carbon and hydrogen, and essentially stripped of the other nutrients (N, P and S) during that flow-down process (Cullimore, 1999). From a microbiological perspective, to achieve maximum bacterial activity, the three most critical nutrients (C, N and P) can be placed in a ratio ranging from 100 to 500(C): 1(N): 0.25(P) (Cullimore, 1993). This fluctuation in the carbon is caused by the amount of carbon utilized in the EPS varying considerably from consortium to consortium and species to species.

At the same time, there are some microorganisms that can capitalize on exothermic geochemical events to obtain energy. Prescott et al. (1996) summarized the energy yields generated by chemolithotrophs employing the Calvin cycle to fix CO_2. Species of *Alcaligenes, Hydrogenophaga* and *Pseudomonas* were found to be able to oxidize hydrogen to water with the release of 57 kcal/mole; *Nitrobacter* oxidized nitrite to nitrate with a release of 17 kcal/mole; *Nitrosomonas* oxidized ammonium to nitrite with a release of 65 kcal/mole, *Thiobacillus denitrificans* oxidizes sulphide to sulphuric acid with a release of 119 kcal/mole; and *Thiobacillus ferrooxidans* oxidises ferrous to ferric forms of iron with the release of 11 kcal/mole. While these chemolithotrophic bacteria can generate energy by the oxidation of reduced compounds with the fixation of CO_2, the energy yields are lower than could be obtained by a catabolic (organotrophic) degradation of an organic compound to carbon dioxide (686 kcal/mole). The chemolithotrophic bacteria therefore function with energy returns of 8%, 2%, 9%, 17% and 2% respectively for a heterotrophic degradation of organic carbon. Thus, in the subsurface biosphere, it may be expected that where there is organic carbon present, energy generation would be dominated by these organotrophic bacteria when electron acceptors are available for respiration. These electron acceptors are dominated by oxygen, the common “fuel” for respiration. When oxygen becomes depleted, there is a range of alternative electron acceptors, including nitrate, nitrite, sulphate, CO_2, sulphur and the ferrous form of iron.

In the subsurface biosphere, respiratory activities can continue even after the oxygen has become depleted through the utilization of these alternative electron acceptors. Once these are depleted, the degradation of the organic carbon becomes less efficient, with smaller energy outputs and daughter products dominated by fatty acids. Given these conditions, it may therefore be expected that the chemolithotrophic bacteria will be clustered where suitable substrates exist to allow for growth to occur. Essentially, the subsurface biosphere may be as a series of lateral layers descending through oxidative to reductive conditions with organics being depleted to fatty acids before being reduced further.

For carbon, the chemolithotrophic bacteria would utilize primarily CO_2, while the organotrophic bacteria would utilize organic forms of carbon. In the subsurface biosphere, it can be projected that as the organic forms of carbon are respired away in the upper regions of the biosphere, the by-product CO_2 becomes available to the chemolithotrophic bacteria.

Beyond carbon, the other critical nutrient elements that may control the level of bacterial activity within the subsurface biosphere are nitrogen and phosphorus. Nitrogen is an essential part of the constituents making amino acids, RNA and DNA while phosphorus is a major driver of energy storage. These two nutrients are addressed separately below.

Phosphorus is often the critical nutrient controlling the amount of biological activity, particularly in eutrophic aquatic systems in the surface environment. This is in part because of the key role of phosphorus in adenosine triphosphate that has the ability to carry a high energy charge and act as the cellular mechanism for stored energy. Surplus phosphorus is also stored as polyphosphates in significant quantities to ensure future growth for several generations. This means that any phosphate in an aquatic system is likely to be taken up by the microbial cells and held as polyphosphate. If microbiological activity is to be restricted within the used nuclear fuel containment envelope, then control of phosphorus as a potential nutrient becomes critical.

The occurrence of phosphorus in the Earth's crust was first discussed by Washington et al (1951) and found to be widely distributed. Potts et al (1992) looked specifically at phosphorus in granitic rocks and found that the P_2O_5% weight was 0.28%. This would mean that if granitic rock were to be selected for the containment envelope, then phosphorus may not be such a limiting nutrient, depending upon the rate of weathering and phosphorus release. Phosphorus can also be found in various crustal and water phases within fresh water and ocean floor sediments in significant quantities. The movement of phosphorus has been noted by Baturn (1982), suggesting that the control of phosphorus in the containment envelope may be a critical issue, given the amount, its availability, and the intrinsic environmental conditions. Since phosphorus can exist in recalcitrant forms as well as available forms, and there are phospholytic bacteria that are able to "mine" these recalcitrant forms, the projection of potential nutrient impact would be dependent upon the total amount of phosphorus available within the containment envelope and the ability of microorganisms to extract and utilize that phosphorus. Given the prolonged timeframes involved, availability of this nutrient may become a key component in the determination of the rate of biomass generation that may be developed.

Intrinsic levels of carbon and nitrogen (in all forms) can also have a dramatic effect on the amount of biomass that may challenge the integrity and security of the used nuclear fuel containment envelope. With the observance of good housekeeping and mining practices during the construction and maintenance of the containment envelope, some control can be achieved in the management of carbon and nitrogen inputs.

Good housekeeping relates to the careful control of the normal activities associated with the construction of an engineered structure whether it be installed above-, at- or below-grade in an unsaturated, semi-saturated or saturated environment within which people are able to work. Two functional components of any such endeavour is the provision of potable and industrial grades of water (piped, ponded or stored) and sanitary wastes. This latter form of water will contain high levels of carbon, nitrogen and phosphorus that would require effective treatment and removal from the envelope environment if these nutrients are to be excluded as contributors to the containment challenge. Typically, the water demand would require storage of both potable and industrial grade waters with allowances for any storm run-off. If the engineering includes significant water demand, that water may enter into the containment envelope and become an ongoing source of nutrients to enhance biomass generation in a manner that could be predictable.

A second challenge would be the handling of the sanitary wastewaters that would include very significant nutrient levels. Such waters would require effective treatment to ensure no significant nutrients contaminate the containment envelope in a manner that could generate biomass and compromise the designed objectives. For example, in the projection of sanitary wastewater production for the AECL environmental impact statement for the disposal of used nuclear fuel in the Canadian Shield, it was projected that these inputs would be equivalent to 62,179 person-years.

Another major potential source of nutrient inputs into the containment envelope are the various forms of garbage that would be created during construction which would likely be placed and contained in some form of landfill until only recalcitrant materials remained. The siting of such a landfill operation should be away from any direct or indirect hydraulic contact with the construction of the containment envelope. Surface and groundwater movements around and under the landfill would need to be carefully monitored, with emergency planning and zero tolerance for nutrient releases beyond designed specifications.

In the construction stage of the envelope, caution should also be taken to ensure a minimum of contamination of nutrients in confined air spaces. These may originate from permeation of fumes and condensation of the products from activities associated particularly with the operation of internal combustion engines. Nutrients likely to be of significance from the fumes and subsequent condensate include volatile and combusted organics and some inorganic forms of nitrogen.

Another factor that could provide significant nutrients is residual debris from the detonation of explosives during "drill and blast" operations if these become a part of the construction of the containment envelope. Forsyth et al, (1995) reported that in mine operations "the handling of explosive product has the most significant influence on the quantity of nitrogen entering the water system” and, in addition, “in the case of ANFO (ammonium nitrate fuel oil), losses occur as spillage during filling of explosives loading equipment, actual loading of blast holes and disposal of excess product”. In support of this statement, it was found by Wober (1991) that these losses could amount to between 5% and 15% of total ANFO used in a “drill and blast” operation. In further support of this concern, Golder Associates found in 1993 at one particular mine, nearly one tonne of ANFO was entering the water system of the mine per month, amounting to 5.2% of the total use of ANFO at the mine site.

Secondary effects from “drill and blast” can be linked to the impact that the explosive forces have on the surrounding rock. Here, the forces from the explosion are likely to cause micro-fractures in the rock mass, weakening its structural integrity. This is a significant concern, commonly understood in the industry. However, not so evident but equally important from the viewpoint of nutrient generation, is the blast forcing carbonaceous and nitrogenous products and uncombusted ANFO into the fractures. Within these freshly impacted fractures would be smeared products of the explosion (successful or failed) within micro-fissures that may well be oxidative with at least a water film. Under these conditions, microbial activity is likely to commence utilizing the carbon and nitrogen products from the blasting activities and extracting phosphorus from the rocks through the activities of phospholytic bacteria and/or acidic products of bacterial fermentation.

Even if the “drill and blast” operations did not cause any impact on the surrounding rocks, there is still the concern about residual ANFO and blast products in, and on, the piles of rock created by the activity. Regardless of whether these operations result from a surface stripping or some tunnelling activity, these piles of rock have the potential to generate carbonaceous and nitrogenous nutrients. To reduce the risk, these rock piles have to be moved away from the construction site sufficiently far that there can be no movement of these nutrients into the constructed envelope from the relocated rock piles. Rock washing can be used to remove nitrates and fines, but this poses a risk of nitrate releases (if there is a significant burden of ammonium in the rock) and sulphuric acid (if there is a significant burden of sulphides in the rock). Nitrates are highly mobile in oxidative waters but are degraded rapidly to nitrite and nitrogen gas under reductive conditions. For the sulphuric acid, this can cause acidic leaching around the rock piles, and can interact with and weaken concrete structures and grouting. Furthermore, it can provide a source of sulphur downstream once the acidity has become neutralized through dilution and/or liming. Given the scale proposed in the AECL environmental impact statement that called for 1,310 Mg/d of rock to be removed every 20 working days with 3.2 blast rounds per day, there would need to be an appraisal of the impact of drill, blast, rock removal and rock washing procedures on potential nutrient loadings within the containment envelope, given the evidence of only an 85 to 95% efficiency in the application of the ANFO explosive. A mass balance would need to be conducted to determine the true challenge to the final form of the containment envelope for the used nuclear fuel. This mass balance study could then be used to predict the level of microbiological challenges to the envelope containment system.

Good housekeeping practices would be essential to minimize the impact of such products as ammonium, gases and hydrocarbons generated by the detonation of the explosives (e.g., detonated to gases and particulates, failed-to-explode and impacted into the rocks, and misplaced) that may subsequently become nutrient sources for microbial activities. Similarly, the possible impact of using internal combustion engines within the vault for various purposes could also lead to further nutrient sources becoming resident within the containment envelope. These sources could include condensed exhaust gases and spilt oils. Human activities may also add to the nutrient load through unhygienic practices of convenience. Shifts in the hydrologic web around the vault need also to be considered since there may be nutrient inputs from the surrounding ground- or surface waters.

3.5 Potential Radiolytic Effects of Used Nuclear Fuel on the Sub-Surface Biosphere

Silliker et al (1988) critically examined the applications of radiolytic effects in the food industry. At that time, it was believed that there was a very significant future for utilizing radiolysis for the control of microorganisms causing spoilage in various foods and beverages. Consideration centred on gamma radiation since this is a very intense radiation with great penetrating power, produced during the decay of radioactive isotopes. During the third quarter of the twentieth century, the food industry focused upon the use of gamma radiation to control microbial spoilage which led to extensive scientific research on the sensitivity of various pathogenic and nuisance microorganisms. The range of lethal radiation levels were determined to be (dose in krad): animals and humans, 0.5 – 1.0; insects, 1 – 1,000; vegetative microorganisms, 50 – 1,000; bacterial spores, 1,000 – 5,000; and viruses, 1,000 to 20,000.

Caldwell (1995) summarized physiological and biochemical impacts of radiation depending upon energy level and form of the radiation and the extent to which particular microorganisms are able to withstand the radiation. However, an issue that has not been fully addressed is that of extracellular shielding due to properties of chemicals bioaccumulated within the EPS that is able to absorb radiation and so protect the microbial cells within these structures. Caldwell (1995) mainly concentrated on the function of structures within the cell that could absorb the radiation and the mechanisms that could allow radiation damage repair. Another concern was the probability that radiation can also cause damage to the RNA. In addition to this, it was asserted that: "These radiations induce formation of detrimental entities that range from singlet oxygen to free radicals, superoxide and peroxide anions, and other highly reactive molecular and ionic species. Collectively, these elements are highly detrimental to cells via the destruction and alteration of DNA and generalized oxidative damage to essential cell components."

Risks from radiation thus extend to mutagenic effects that could allow strains of microorganisms to develop that have a greater resistance. Of the vegetative bacteria, there is one species, *Deinococcus radiodurans* that has a much greater resistance to radiation than other bacteria. Madigan et al (1997) considered that this greater resistance (into the realm of 3,000 krad) documented for bacterial spores and viruses was because of a number of unique properties possessed by the cells in these species. These properties included cell walls that were several layers thick, incorporating heptoses and lipids not found in other related genera. Additionally, this species shows a remarkable resistance to highly mutagenic chemicals and enhanced ability to repair radiation-damaged DNA. It is not surprising that this species has been isolated from atomic reactors and other potentially lethal radiation sources. As validation of the robustness of this species, it has also been found to be very resistant to desiccation.

There is a considerable body of evidence from within the food industry and in scientific studies that indicate that the radiation released from the used nuclear fuel could impact severely on the natural microflora exposed to these effects. Given that any storage and disposal will need to involve a containment envelope around the used nuclear fuel, the design and engineering of that envelope would need to take into account effects that are not necessarily limited to recognition of the potential direct effects of radiation on microbial cells. Other effects would include the releases of heat and chemical products from the interaction between the generated radiation and the local waters. A series of potential impacts can be postulated through these various interactions that would need to be addressed in any used nuclear fuel management facility. These interactions are listed below and a first-level assessment is included of the potential impact. It is considered that the interaction between radiation and local waters would be dependent upon the location of the envelope in relation to the local waters. Given that liquid water is assumed to be present within the containment envelope of a repository, then the following events are most likely to need to be considered:

(1) the generation of singlet oxygen to free radicals, superoxide and peroxide anions, and other highly reactive molecular and ionic species;
(2) the generation of a thermal gradient; and
(3) the focal formation of redox fronts that would generate biomass at peripheral locations.

Radiation is likely to generate products containing oxygen (singlet oxygen, peroxide and superoxide) and hydrogen from water, leading to the generation of a series of secondary reactions. These reactions generate oxygen and hydroxyl radicals that can shift the impacted environment to a more oxidative condition (Arrage et al, 1993) which can include the generation of redox fronts where biomass can accumulate. In the event that the containment envelope was placed in very reductive conditions, one effect of radiation could be the generation of oxygen-rich redox fronts. For biomass to be able to grow at these fronts, there would need to be some mechanisms to protect the microorganisms growing at these fronts. These mechanisms could include the growth of radiation-resistant microorganisms such as *Deinococcus radiodurans*, mutated strains of other impacted species that have adapted to the conditions, and the growth of radiation-sensitive strains of microorganisms at sites shielded by the bioaccumulates surrounding the growth. Furthermore, radiation-resistant strains may arise through the processes of mutation and environmental adaptation, forming bioaccumulates within the biomass. These would act to shield the radiation being generated by the used nuclear fuel within the containment envelope.

A second likely event of microbiological significance would be the generation of thermal gradients away from the used nuclear fuel that could significantly influence the shorter-term growth of microorganisms. In the longer term, adapted microorganisms will likely develop optimally under the locally-stabilized conditions that may be generated. At this stage, the major factor would be the presence of adequate liquid water, a favourable nutrient environment and time to adapt. Initially, however, there are known optimal temperatures at which microbial activity can occur. Cullimore (2000) described the standard categorization from optimal growth temperatures as peaking at 10-14^{o}C, 28-32^{o}C, 35-45^{o}C, and then incrementally upwards to boiling water. These categories were based primarily on laboratory studies with isolated cultures and conducted over short time periods that did not allow for long-term adaptation that may take decades or centuries to develop. Given the nature of the containment envelope, it is probable that the ORP (and position of any redox front) would have a greater influence on microbial activity than the intrinsic temperature.

Creation of a redox front usually relates to the movement from a reductive to an oxidative condition with the ORP moving from a negative to a positive millivolt value (Cullimore, 1999). This, however common in groundwater around a producing borehole, does not have to be the only condition for this to occur. Rowe et al (1995), working with leachate from Ontario sanitary municipal landfills, found that bacterial consortia tended to occur clustered around different ORP values going from (reductive to oxidative): methane-producing, sulphate-reducing, denitrifying, heterotrophic, slime-forming, to iron-related bacteria on the oxidative side of the front. Similar consortial structures were determined around water wells and led to the use of zones of interrogation projections (ZIP) for the determination of the relationship of these consortia based upon their presence and activity levels in sequentially pumped samples (BART-SOFT™ beta version 4.1, Droycon Bioconcepts Inc., Regina, Canada). Evidence therefore suggests additional redox fronts can be generated in a manner that could be important to the integrity of the containment envelope. For example, it is probable that a shift in ORP from -200 to -50 millivolts would cause a shift from methane-producing to sulphate-reducing bacterial consortia if other environmental factors were favourable, particularly the presence of fatty acid products from fermentation.

The effects of radiolysis on surrounding microorganisms cannot be simplified to a determination of kill-rates. Consideration has to be also given to the manner in which the impacted environment will change to provide more supportive conditions for survivors. These conditions could include the generation of a favourable redox front, protection from the radiolytic effects, optimized temperature regimes, generation of organics through the radiolytic degradation of some of the organics, and above all else, the presence of water.

3.6 Microorganisms in Extreme Environments

> "Everything is everywhere, the environment selects" Beijerinck, M. (Prescott et al., 1990)

> “Microbes are supremely successful survivors and the physiological attributes they possess undoubtedly mean that they can survive without the Earth if liquid water and the right energy supplies come along” Cockell, C.S. 2003

Martinus Beijerinck was a pre-eminent microbiologist at the end of the nineteenth century who is credited with the postulation that viruses could exist (1889) and went on to be credited with the development of enrichment culture media in 1901. Beijerinck’s broad-spectrum microbiological investigations ended with his consideration that life is ubiquitous and that the environment will select the growth forms capable of growing in that environment. It means that if an environment is favourable, then the microorganisms are everywhere and will find it. Cockell in 2003 considered that the durability and adaptability of members of the microbial world is on such a scale that would make extinction virtually impossible. Growth, on the other hand, would be minimally dependent upon the presence of liquid water.

Traditionally, little consideration has been given to developing a scientific understanding of the extreme conditions under which microorganisms can exist in the biosphere on Earth. Preoccupation has not unnaturally concentrated on the pathogenic, useful and nuisance microorganisms occurring within the surface biosphere that had some level of significance to human society. As mining activities extended deeper into the crust, deep ocean research moved into the extreme environments of high salinity and pressures with low and high temperatures, and as to the focus shifted to other planets such as Mars and moons such as Europa, then so developed a need to recognize the durability and adaptability of microorganisms to potentially thrive in such environments. In addressing the extreme environmental factors, it is therefore logical to consider both durability and adaptability as two primary factors in the determination of the limits of the extremes that would then differentially suppress microbial activity.

Durability for microorganisms is commonly recognized through the primary forms in which they are encountered. During active growth, microorganisms assume a vegetative form in which the cells are more vulnerable to extreme environments but are actively growing. After active growth, vegetative cells tend to die off, due often to the inhibitory or toxic environment created by the products of their collective growth. There are, however, two mechanisms by which vegetative cells can enter into a survival mode. These are the generation of dormancy: (1) through the cells moving into a more durable state as some form of spore involving commonly thickening of the cell walls; and (2) though the cell shrinking to a minimal size for survival with the export from the cell of all extraneous water and materials coupled with the cell wall becoming electrically neutral (Lappin-Scott et al, 1992). These two states of dormancy are known commonly as spores or ultramicrobacteria (UMB) respectively. One basic question about each of these states is how long can they survive and what triggers an end to the dormancy. For both states, the trigger to end dormancy is the arrival of suitable environmental conditions for growth. Here, the spores will now germinate, releasing vegetative cells, while the UMB will swell up into fully-functional vegetative cells. The dormancy period is itself a reflection of the durability of the dormant cell. This will be discussed separately for sporogenous microorganisms and UMB.

Spore formation amongst microorganisms has been recognized for the last century and a half because of the impact that the spores had primarily on the food industry and secondarily on the health industry as a dispersal mechanism (Lynch et al, 1979). Zappfe et al recognized in 1903 the frequency of spore formation amongst microorganisms and likened them to seeds in plants. One major difference is that the plant seed is a part of the reproductive cycle for the plant, while the microbial spore is a survival mechanism. Spores in microorganisms can be divided into two major groups. This is dependent upon whether the spore is formed within a vegetative cell (endospore) or is generated from an extension of the vegetative cell (exospore). Generally endospores are produced by one section of bacteria (Bergey's Manual, Cullimore, 2000) while exospores are commonly found among genera of the Fungi.

Endospores generally have been found to persist for longer periods of time in the natural environment than the exospores. In the surface biosphere, endospores of two genera (*Bacillus* and *Clostridium*) have been recognized as being of particular concern in the food and health industries respectively. *Clostridium botulinum* became a standard for the determination of time/temperature killing rates because of the importance of this species in the generation of botulism in foods (including canned meats and vegetables). Esty and Meyer (1922) set 121.1^{o}C (250^{o}F) as the first standard for achieving sterilization using pressurized steam (and the survivor population was reduced by twelve orders of magnitude). Over the last hundred years, this standard temperature has been adopted, with contact times governed by the bulk and characteristics of the mass to be sterilized. This contact time would never be less than 15 minutes. Exospores are, in general, more sensitive to sterilization by heat than the endospores. As a general rule, endospores will commonly survive 80^{o}C for 10 minutes, and exospores 60^{o}C for the same time.

For environmental challenges other than heat, the spores act as agents for survival through adverse conditions. Factors that can create adverse conditions, effectively killing the vegetative cells but not the spore forms, include the conditions that would follow growth (e.g., higher levels of acidity, abnormally reductive conditions, toxic daughter products) and also conditions associated with the movement of these spores through alien environments (e.g., restricted water availability, unfavourable temperatures, microbial competition, and unsuitable habitats). Survival of these spores therefore becomes dependent on these factors. As an extreme example of spore survival, Weber et al (1985) conducted trials using *Bacillus subtilis* endospores to determine the effects of very high vacuum, low temperatures and UV radiation. The authors' findings led them to project that such endospores could survive in interstellar molecular clouds for extensive periods (projected by the authors to be 4.5 to 45 million years).

In the surface biosphere, evidence of long-term survival, particularly of endospores, is becoming better understood. Anthrax as a disease caused by *Bacillus anthracis* is well-known to persist as endospores in contaminated soils. Since Koch first described the pathogen in 1877, there has been a growing understanding of the survival rates through the experimental use of endospores as a biological warfare agent.

In summary, microbial spores can be expected to survive for significant periods of time in adverse environments, including those that may be associated with containment envelopes for used nuclear fuel. Here, the concern is how long these persistent spores can survive before favourable environmental conditions become available for the regeneration and growth of vegetative cells. In 1999, the US Army Corp of Engineers produced a technical fact sheet indicating that there were critical concerns with respect to the survival of spores of *Bacillus anthracis*. Hendricksen et al (2002) examined the long-term survival of spores of the insect pathogen *Bacillus thuringiensis var kustaki* and, in a detailed study, found that these endospores would survive for seven years with some vertical movement down through the soil profile, but little lateral movement. Thus, sporogenous forms of microorganisms are likely to be a concern in the design and management of the containment envelope for used nuclear fuel. This is because of the ability of the persistent forms to be able to survive in adverse conditions for extended periods of time.

Ultramicrobacteria (UMB) is the second critical concern in determining the risk of microbiological activity within the envelope. This is because the UMB can be present as metabolically- and electrically-neutral survival cells that have a small size and a potential to persist for considerable lengths of time. Much of the biomass associable with the crust may be in a transitional (quiescent) state while moving from one sustaining environment to another. As suspended animates with a relatively small size, the UMB cells (0.1 to 0.5 microns) may be able to enter into many of the porous structures within rocks and survive in these locations for prolonged periods.

Given that two decades ago rocks would have been considered essentially sterile materials, current microbiological investigations are beginning to indicate that ultramicrobacteria may well be residing in a very passive way in the rock. If such rocks are disturbed, then these resident microorganisms could return from suspended animation to an active vegetative state. This would be likely to happen only if nutritional and environmental conditions become favorable for growth. If this does happen, their growth may create challenges. The intensity of these challenges would be very dependent upon the availability of sufficient nutrients within a nurturing environment.

Beijerinck made a perceptive statement that “everything is everywhere and the environment selects”. From the survival of microbial cells through the production of various endo- and exo- spores and UMB, there would be a high probability that the limits of the biosphere may be dictated more by the presence of these various forms of dormant survivors rather than actively metabolizing cells. Given ubiquitous omni-presence may be applicable to microorganisms, then what of the conditions under which growth can occur? How extreme is extreme to prevent microbial growth? Clearly, from the engineering design management of an effective containment envelope for used nuclear fuel, the absence of any microbiological challenges would appear to offer distinct advantages.

Extreme environments were traditionally thought to be abiotic (free of life) simply because the conditions were too extreme for the biota to survive. There is a fundamental flaw in this premise. In traditional thinking, “biota” referred to plants and animals (Concise Oxford Dictionary, 8th edition: “the animal and plant life of a region”). This definition does not include the microbial kingdom. Furthermore, conditions that may be considered extreme for plants and animals are not extreme to many microorganisms that have a greater ability to adapt, in part, because of the simple and more forgivable cell structures. Cockell (2003) summarizes this ability as follows:

> "Comets and asteroids strike the surface of the Earth, stars explode nearby, enormous volcanoes erupt, and more recently, humans litter the planet with waste. Many animals and plants go extinct during the voyage, but humble microbes, simple creatures made of a single cell, survive this journey"

Then what is too extreme for microbial survival? A few certainties and several possibilities are summarized below:

Water: Water is an essential ingredient for life to occur in the manner observed in the biosphere. Animals extract water primarily from their food, plants extract it from the environment within which they are growing (primarily soil or surrounding waters); but microbes, like plants, extract water from the surrounding environment with most of that water being stored outside of the cell in the EPS (if bacterial). Of the microorganisms, it is the fungi that are the most resistant to low availability of water. They tend to be the dominant part of the biomass when the water content in the environment is between four and 20% of saturation capacity. Bacteria tend to dominate once the water content exceeds 80% of saturation capacity. Between 20 and 80% there is a competition between bacteria and fungi with the prime advantage for the bacteria being the ability to bind water into the EPS effectively, making slimes (biofilms), while the advantage for the fungi is the bigger "arsenal" of enzymes (to break down organic nutrients) and antibiotics (to restrict competition from other organisms). Not surprisingly, it is the fungal biomass that tends to dominate soils, while bacterial biomass dominates waters. Generally, the minimum amount of water necessary to support microbial activity is thought to be between two and 4% of saturation. There are exceptions to this generalization. For example, clay may hold water in a manner that renders it unavailable for microbial activity. Solid water (ice) is generally considered to be unsupportive of microbial activity, but the act of freezing is not necessarily lethal. Silliker et al (1980) considered that:

> "Most gram-positive organisms, including *Bacillus, Clostridium, Lactobacillus, Staphylococcus, Micrococcus* and *Streptococcus* are relatively resistant to freezing and cold storage, and freezing

> will not eliminate staphylococci and other gram-positive
> organisms from foods".

Additionally, it was found that there was prolonged survival of these microorganisms in environments such as frozen chow mein and ice cream, where survival times were minimally nine months at -25°C and seven years at -23°C respectively. Growth has been recorded down to -10°C but below that temperature, there remains conjecture as to whether the observed effects are of a biochemical or cellular origin. This would indicate that water in a frozen crystalline form may still contain biocolloidal liquid-bound water that could support growth (Vinebrooke et al, 1998).

Temperature: All microorganisms can grow only within a particular temperature range that can be related to their normal habitat. In the last twenty years, there has been a revision in the approach to the selection criteria for microorganisms that may now be based on the crystalline state of the water. Drost-Hansen et al in 1979 brought forward the hypothesis that water is actually in a fluid crystalline state. They referred to the water as being vicinal and claimed that crystalline structures change form at specific temperatures. Temperature growth ranges for particular microorganisms were thought to be affected by these different crystalline forms. Critical temperatures at which vicinal water changed was: 15°C (59°F), 30 °C (86°F), 45 °C (113°F), and 60 °C (140°F). These temperatures also happen to be the common threshold temperatures for the growth of different groups of microorganisms (based upon temperature). In simplest terms, Cullimore (2002) considered that microorganisms growing below 15°C are considered to be cold-loving psychrotrophs; between 15°C and 30 °C low mesotrophs; between 30 °C and 45 °C high mesotrophs (and in particular includes the parasites of warm blooded animals, including humans); between 45 °C and 60 °C thermotrophs. Above 60 °C, hyperthermotrophic microbial activity can occur, but generally it is in specialized habitats where the temperature remains relatively constant or fluctuates in a harmonic manner. The highest temperature at which microbial activity can occur is in pressurized water at higher pressures and temperatures than boiling water at sea level. Prescott et al (1996) considered that the black smokers located in the deep ocean rifts and ridges may show evidence of bacterial activity:

> "Evidence has been presented that these bacteria can grow
> and reproduce at 115°C. It is possible that bacteria may grow
> at higher temperatures. The pressure present in their habitat is
> sufficient keep water liquid (at 265atm; seawater doesn't boil
> until 460°C"

From this review, microbial activity appears to be possible over the temperature range from at least -10 ^{o}C to greater than 110 ^{o}C.

pH: Excessively acid conditions (low pH) are extremely corrosive. Alkaline conditions (high pH values) can also be corrosive. It is therefore natural to consider that microorganisms can also suffer under very acidic or alkaline conditions. Seckbach (1999) and Prescott et al (2002) both reported microorganisms able to grow over an extreme range of acidic and alkaline conditions: extreme acidophiles, pH < 3.0 (at Red Mountain Calif filamentous bacteria lacking cell walls are reported growing at negative (<0) pH values) ; acidophiles, pH <5.0; neutrophiles, pH optimum between 6.0 and 8.0; alkalophiles, pH optimum between 8.0 and 10.0; extreme alkalophiles, growth over pH >10.0. There appears to be the potential for microorganisms to be able not just to survive, but also grow, over the full spectrum of pH with individual microorganisms adapting to specific pH ranges. Microorganisms possessing EPS have the potential to employ that coating as a buffering mechanism to protect the cells from radical pH values. From the information to date, it would appear that extremes in pH values will not eliminate microbial activities but would affect the types of growth occurring at the site.

ORP (reduction – oxidation potential): the prime effect of an oxidative or reductive regime relates to the method by which bacteria can obtain energy from the degradation of organic materials. Oxidative conditions generate respiratory activities that lead to an efficient degradation of organics, with carbon dioxide and water as two major products. Reductive conditions generate reductive activities leading to the less efficient breakdown of organics by fermentative activities, with fatty acids becoming a major product. Under very reductive conditions, these fatty acids can be reduced to methane by the methane-producing bacteria that are also able to reduce carbon dioxide to methane. For these conditions, the ORP has to be -150 millivolts or more reductive. At ORP values between -150 and 0 millivolts, alternate electron acceptors may be involved (Cullimore, 1999). If the acceptor is sulphate, then that is reduced to hydrogen sulphide. Under oxidative conditions, the ORP value in water (the prime growth medium for most microorganisms) is buffered by the limited ability of water to take up dissolved oxygen. Generally oxygen saturates between seven and 18 ppm, depending upon temperature, with colder conditions (<10^{o}C) likely to cause supersaturation of the water with oxygen. Critical minimal oxygen levels for respiratory activities appear to be at around 0.02 ppm.

Salinity: High concentrations of salt (generally considered as being equivalent to sodium chloride) are generally considered to have an impact on the forms of microbial growth. Madigan et al (1997) defined extreme halophiles to embrace all microorganisms requiring hypersaline conditions (>9% NaCl) for growth. Many extreme halophiles actually grow optimally at 12 to 23% with virtually all of these species also able to grow at the limits of saturation (32% NaCl). Cullimore (2000) reported that most bacteria are inhibited at between 5 and 8% salt with one major family (F from Section 4, *Halobacteriacaea*), including many of the extreme halophiles. The presence of high salt concentrations may therefore limit the range of microorganisms that can be metabolically active to the extreme halophiles, including the Archaea, able to thrive under these saline conditions. Deep ocean environments, such as at the site of the *RMS Titanic*, present salinity levels approaching saturation (Sagalevitch, 2002), but the variety of microorganism is diverse (Cullimore and Johnston, 2000) and complex with various microbial consortia involved in growths such as the rusticles (Cullimore et al, 2002). It may be reasonably conjectured that there are no specific concentrations of dissolved salts that could effectively prevent microbial activity in some form or other.

Pressure: Barometric or hydrostatic pressures have very significant impacts on plants and animals in that they have very little ability to adapt to orders-of-magnitude changes in either atmospheric or water depth. This is, in part, because the organisms are complex in form, with physiological and metabolic systems that are unable to compensate for the conditions associated with dramatic changes. Microorganisms are relatively simple forms of life and can adapt to radical changes in these pressures. Cullimore and Johnston (2003) reported that five cultured species of bacteria were subjected to conditions at a depth of 3,800 m at the site of the RMS *Titanic* for eighteen hours and all five species survived with losses of no more than one order of magnitude. These confined cultures had been exposed to close to saturated salt conditions with pressures of 5,400 kPa and a temperature just above the normal freezing point for water.

The fact that microorganisms are adaptable to a broader range of pressures than higher organisms has been well known for a long time. Foster et al (1958) summarized this as:

> "Generally hydrostatic pressures in the region of 600 times normal will inhibit growth, and even cause death, of many terrestrial species. On the other hand, marine bacteria near the ocean floor grow readily at such pressures. Thus it appears that the nature of

> the habitat governs to a considerable extent the response of bacteria
> to pressure."

It would be reasonable to conclude that at least some of the microorganisms subjected to these extreme pressures would be likely to survive and then grow under what would be extremely challenging conditions for any plant or animal to survive. The rich diversity of the fauna in deep ocean habitats means that adaptation can also occur there over evolutionary scales of time.

As a final note, throughout this review, the terms bacteria and Archaea are often used interchangeably. Given that the Archaea are genetically so distinct from bacteria, it is possible that they could have very different survival and dispersive strategies that we do not yet know about. This may be important, as they are the life form that dominates the deep subsurface and extreme environments.

4. Possible Natural Phenomena Affecting Microbiological Challenges

This Chapter provides a perspective on the possible natural phenomena of changing climate and its implications for more storms, heavier precipitation events, changing groundwater regimes, thawing permafrost, rising sea level, and the possibility of related microbiological implications on used nuclear fuel management and potential risks to used nuclear fuel management sites.

Natural phenomena tend to have the most dramatic effect on the surface biosphere rather than the subsurface biosphere, which is protected from these events to some degree by the soils and geosphere. The subsurface biosphere is much larger in size than the surface biosphere that is directly exposed to climatic events at the primary level of exposure (direct impact). For the subsurface biosphere, the exposure is secondary as impacts from climate change penetrate into the subsurface environment. Most significant would be changes in precipitation patterns, which, over the years, could affect the water table, raising it (in the event of increased precipitation or ascending sea levels) or lowering it (in the event of reduced precipitation or falling sea levels). The implications for a used nuclear fuel storage and/or disposal facility would be more significant if this moved the facility into a saturated from an unsaturated state. This would encourage microbiological activity of the reductive forms, leading to organic acid production, accelerated gas production and increased rates of electrolytic corrosion. Should the facility move from a saturated to an unsaturated state, the form of microbial impact would be dependent on the nature of the environment. If the atmosphere of the facility moves to an oxidative type (due to the presence of oxygen), then aerobic microbial activity would dominate with a significant fungal (mold) population and a greater amount of biomass production, with consequent effects on the transmissivity of water and gases through the containment envelope. The impact on any container may be restricted by the tendency for the aerobic biomass to buffer the water to a pH between 6.5 and 8.5, depending on the substrate being metabolized, which would have less potential corrosive effects. However, if the container embodies materials that have potential value to the microorganisms (as nutrients or electron donors) then it may be expected that the container could be structurally compromised.

Geological perturbations, such as would occur through violent realignments (earthquakes) and glaciation (compression), would have minor impacts on the subsurface biosphere, but secondary repercussions would ripple outwards from the impacted region. Such events would change the nature of the microbiological activities within the containment envelope that could affect the long-term security of the contained used nuclear fuel.

5. Possible Microbiological Implications of Contaminants that may Escape Containment

This Chapter provides a review of possible microbiological implications of any contaminants that may escape containment and their subsequent dispersion within the geosphere and biosphere.

Given that the biosphere would essentially embrace much of the geosphere where disposal is likely to placed, the pathways moving the used nuclear fuel from containment to dispersion would include:

- Corrosion of the container leading to releases of contaminants from the used fuel.
- Degradation of the integrity of the container (such as concrete) leading to the spalling and collapse of the container.
- Bioaccumulation of the contaminants within biofilms and biocolloidal structures.
- Generation of localized pockets of gas destabilizing the stored fuel and confining structures, leading to the creation of pneumatically-driven vertical flow patterns that could include sheered biofilms and biocolloids incorporating contaminants.
- Accumulation of contaminants within stabilized biological concretions (similar to rusticles) as encrusted growths. This may retard the movement of the contaminants away from the containment envelope. This form of growth is most likely at redox fronts which could be formed by the radiolytic events generated by the used nuclear fuel.
- Movement of contaminants through the passive flow of groundwater associated with the repository facility.
- Active movement of contaminants in the enveloping groundwater through the movement of the carrier biocolloidal particles that would be subject to forces resulting from: density adjustment (by particle) though gas production (up) or jettisoning of water (down); movement within electro-magnetic fields; and/or attraction to suitable niches where growth may occur.

- Accumulation of the contaminants within biofilms and biocolloids forming the biomass at the significant redox fronts on the border between the subsurface and surface biospheres where retention is likely to occur.
- Predation of the biomass by fungi, protozoa, nematodes, worms and insects leading to the contained contaminant moving into the food chain.
- Plant roots extending down into the upper reaches of the redox front interacting with the biomass and the rhizosphere (magnified microbial activity associated with plant roots). This would create a "barter" exchange process (water and nutrients going into the plant roots in exchange for oxygen and small organic molecules being released to the microorganisms) whereby contaminants may move through the rhizosphere and into the plant.
- Microbial plant rot (such as *Erwinia carotovora*) impact on the plants, destroying the tissues and causing releases of contaminants taken up into the plant back into the soil.
- Predation of the plants, leading to the movement of the contaminant from the plant to the animal consuming the plant tissues.
- Death of plants and animals, leading to the return of the contaminants to the microbial community involved in degrading the dead carcasses and vegetative matter.
- If the contaminant arrives in an aquatic environment, then there is a possibility that the contaminant may move, accumulating into biocolloids down into the sediments, which are reductive and have a high microbial content.
- Bottom-feeding fish may take up the contaminant from disturbed sediment.
- Further contamination may enter into the water column with microbially-generated gas disrupting through the sediment up into the water. It is possible that some of the bioaccumulated contaminant may be taken up by the biofilm encapsulating the gas bubble.

While the above scenarios can be related to the movement of microbiologically accumulated contaminants into the food chain, it should be recognized that the most radio-sensitive part of the food chain is the higher trophic levels where generation times are much longer and adaptability to radiological doses is less than in the microbial world.

6. Conclusion

In conclusion, the following key points should be emphasized:

1. Microbial populations will exist anywhere on earth where there is free water, nutrients and an energy gradient to exploit. All of these factors may exist within a used fuel storage or disposal facility.
2. In the process of exploiting energy gradients, microbial populations transform materials. This may result in the accelerated degradation of containment systems and affect the mobility of radionuclides and stable element contaminants from a used fuel storage or disposal facility to the surrounding biosphere.
3. The design, construction and operation of a used fuel management facility must include consideration of the role of microbial processes.

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