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Evaluation of Microbial Activity for Long-Term Performance Assessments of Deep Geologic Nuclear Waste Repositories

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Microorganisms are ubiquitous in subsurface environments and play a major role in the biogeochemical recycling of various elements. In this paper, we have developed a general approach for a systematic evaluation of microbial impact on the long-term performance of the repository. We have demonstrated that data on microbial population alone are not sufficient for the evaluation of microbial impact on repository performance and a sensible approach for such evaluation must be based on the consideration of environmental constraints on microbial reaction pathways. We have applied our approach to both the Yucca Mountain (YM) repository and the Waste Isolation Pilot Plant (WIPP). We have demonstrated that the effect of microbial activity on the near-field chemistry in the Yucca Mountain repository is negligible because of limited nutrient supply and harsh environmental conditions created by waste emplacement. Whereas for the WIPP, we have shown that, due to the presence of a large quantity of organic materials and nutrients in the wastes, a significant microbial activity can potentially be stimulated and its impact on repository performance can be evaluated with carefully designed incubation experiments coupled with performance assessment calculations. The impact of microbial gas generation on disposal room chemistry in the WIPP can be mitigated by introducing MgO as a backfill material.

1. Introduction

Numerous studies have been conducted on microbial activity related to nuclear waste disposal in deep geologic repositories. Microorganisms have been detected in backfill materials, natural analogue sites, plutonium-contaminated soils, low-level radioactive wastes, and waste-repository sites selected for high-level radioactive wastes.[1−13] West et al. surveyed microbial population distributions in water and subsoil samples from deep mines designated for disposal of high-level radioactive waste in Europe.[14, 15] They reported finding a variety of organisms that include native organisms and organisms introduced from mining operations. The presence of viable microbial populations in subsurface environments clearly suggests that, under certain conditions, significant microbial activity can be stimulated that can potentially impact the transformation and transport of radionuclide in repository environments.

The impacts of microbial activity on repository performance are several-fold:[16] (1) Microbes may be directly involved in redox reactions of multivalent radionuclides such as uranium and plutonium,[17] whose solubilities highly depend on oxidation states. (2) Microbial reactions may play an important role in regulating water chemistry (e.g. pH, Eh, and ΣCO_2) as well as secondary mineral distributions (i.e., radionuclide sorption sites). (3) Microorganisms release low molecular weight organic acids including acetate, lactate, and formate, which are able to complex with radionuclides. On the other hand, biodegradation may eliminate organic ligands and reduce the mobility of radionuclides. (4) Microbial cells may act as colloidal particles and thus facilitate radionuclide transport. (5) Additionally, microbial reactions may impact metal corrosion and therefore the integrity of the engineered barrier system. The combination of radionuclide mobility and EBS integrity ultimately controls total radionuclide release from a repository.

In this paper, we develop a general approach for a systematic evaluation of microbial impact on the long-term performance of the repository, based on the consideration of environmental constraints on microbial processes. We then apply this approach to both the Yucca Mountain (YM) repository and the Waste Isolation Pilot Plant (WIPP). We demonstrate that the effect of microbial activity on the near-field chemistry in the Yucca Mountain repository is negligible because of limited nutrient supply and harsh environmental conditions created by waste emplacement. Whereas for the WIPP, we show that, due to the presence of a large quantity of organic materials and a significant amount of nutrients in the wastes, a significant microbial activity can potentially be stimulated and its impact on repository performance can be evaluated with carefully designed incubation experiments.

2. Systematics of Repository Biogeochemistry

Population distribution vs. activity. Microbial populations surveyed at sites related to radioactive waste disposal are listed in Table 1. It can be seen from the table that microbial population varies greatly between locations within a specific site and between different sites. Microbial populations at these sites are generally lower than 10^7 cells per gram, with the exception of populations found within some bentonite materials, in which the population can be higher than 10^{11} cells per gram. Bacteria dominate the whole microbial population, with fungi only accounting for ~ 1%. Note that, at the same site, the cell density in water is much lower than that in the associated sediments, indicating the tendency for microbial cells to attach solid surfaces. Generally, it is difficult to correlate the presence of specific organisms with site conditions. The presence of a specific set of bacteria does not guarantee that they will be active; some of them may be dormant if conditions are not conducive to their active state. This implies that data on microbial population alone are not able to provide sufficient information on the geochemical impact of microbial activity.

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TABLE 1: Abundance of Microorganisms at Radioactive Waste Disposal Related Sites

Sample Source	Microorganisms Detected	Reference
Soils, Area 13, Nevada Test Site	Bacteria: 2.9 ± 0.3 to $8.0 \pm 0.7 \times 10^6$/g Fungi: 3.1 ± 0.7 to $24.6 \pm 2 \times 10^3$/g	1
Los Alamos National Labs TRU waste shallow burial site TA-54, Area C	Bacteria: aerobes: 2.3×10^6/g anaerobes: 3.3×10^6/g Fungi: 4.9×10^4/g	2
Sediment/water, Rocky Flat Plant Pond B1	Bacteria in sediments: aerobes: $3 - 300 \times 10^6$/mL anaerobes: $0.2 - 2.0 \times 10^6$/mL Bacteria in Water aerobes: $1.1 - 340 \times 10^3$/mL anaerobes: $2 - 7.8 \times 10^2$/mL	3
Waste leachate, Trench 19S, Maxey Flats, KY	Bacteria: aerobes: 2.2×10^2 CFU/mL anaerobes: 3.2×10^2 CFU/mL	4
Groundwater (5 M NaCl), Waste Isolation Pilot Plant (WIPP), New Mexico, USA	Bacteria: Far-field: $1.02 \pm 0.49 \times 10^5$ cells/mL Near-field: $1.24 \pm 0.13 \times 10^5$ cells/mL	5
Study Site Groundwater, Nevada Test Site, USA	Bacteria: 10^2 viable cells/mL	6
Granite fracture zone, Äspö Hard Rock Lab, Sweden	Bacteria: $0.44 - 9.3 \times 10^5$ cells/mL	7
Granite shear zone, Grimsel Test Site, Switzerland	Bacteria: $3.97 \pm 0.37 \times 10^3$ cells/mL	12
Yucca Mountain tuff, Nevada, USA	$0.32 - 2.0 \times 10^5$ cells/g	10
Cigar Lake uranium ore deposit, Saskatchewan, Canada – sandstone w/ clay-hosted U ore	Bacteria in groundwater: $4.7 \times 10^2 - 4.4 \times 10^4$ cells/mL Bacteria in ore: $1.4 \pm 0.9 \times 10^5$ CFU/g viable cells	17a
Wyoming bentonite	$5.32 \pm 0.34 \times 10^{11}$ cells/g; $<1.07 \pm 3.54 \times 10^2$ CFU/g viable cells	11
Avonlea bentonite	$6.29 \pm 0.75 \times 10^{11}$ cells/g; $3.48 \pm 0.56 \times 10^4$ CFU/g viable cells	11
Canadian sand and bentonite buffer material	Bacteria: 10^1 to 10^6 viable cells/g	13

Microorganisms in a natural system are highly adaptable and sufficiently diverse for carrying out any specific metabolic reaction that the environment permits. The extent of each individual microbial reaction will be limited by environmental constraints, not by the types of microbes present. A specific group of microbes will thrive only to the extent that the environment allows. This argument is consistent with the modeling approach that has been used successfully over the years for modeling biogeochemical processes in aquatic sediments.[18, 19] In this approach, microbial population is not explicitly included. A theoretical basis for this has been provided.[16] The environmental factors that can potentially affect microbial growth and activity in a repository environment include moisture, temperature, pH, Eh, availability of organic and inorganic nutrients, and radiation. The key to evaluate the geochemical impact of microbial activity on repository performance is to determine the extents of individual microbial metabolic pathways under the constraints of these environmental factors.

Heterotrophs vs. autotrophs. The metabolic pathway that a given microorganism will use depends on the energy source (light or chemical); thus, a microorganism is described as either a phototroph or chemotroph, respectively. In a deep geologic repository, the activity of phototrophs can be excluded because of the lack of a light source after closure. Among chemotrophs, heterotrophs use organic carbon as both carbon and energy sources, whereas autotrophs derive energy from the oxidation of inorganic compounds and derive cell carbon from CO_2. As shown in Figure 1, because of their different nutritional requirements, the activities of these two groups of microorganisms in a deep geologic repository are limited in different ways, depending on the ambient chemical environment and the type of wastes to be emplaced in the repository.

An autotrophic reaction is generally limited by the availability of inorganic electron donors such as H_2, NH_4^+, NO_2^-, Mn^{2+}, Fe^{2+}, H_2S, and S. To support cellular growth, autotrophs fix CO_2 through a "Calvin cycle"and require a considerable investment of energy.[20] As a result, the direct utilization of CO_2 by autotrophs in subsurface systems is probably slow. In contrast, heterotrophs show a great range of flexibility with respect to carbon sources. Some bacteria will degrade almost any reduced carbon source, whereas others will only catabolize a few carbon compounds. Heterotrophic metabolism dominates over autotrophic metabolism in many subsurface environments, even in some pristine environments where the organic carbon supply is relatively limited, since it is observed that carbonate species, which are the end products of heterotrophic metabolism, are accumulated in these natural systems.[20]

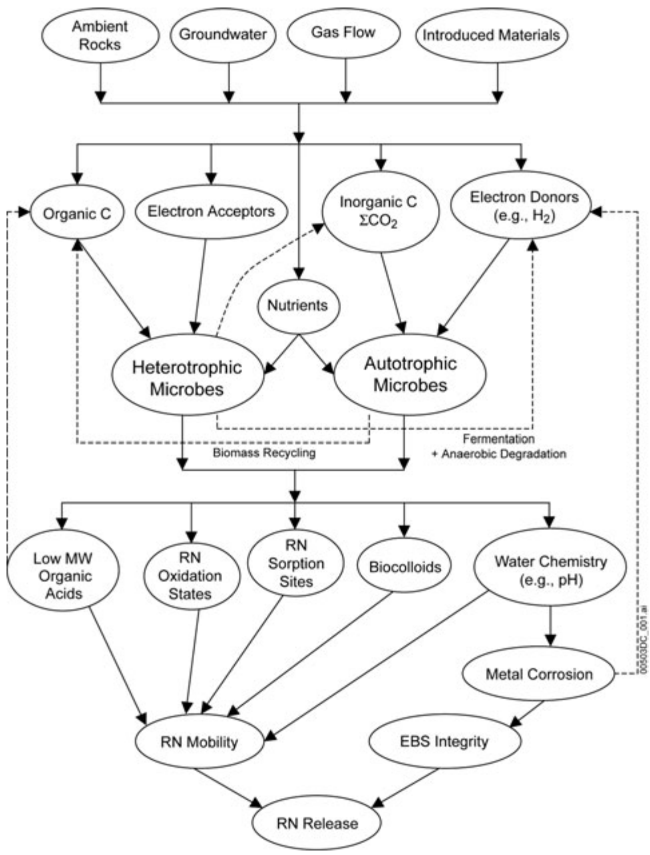

Figure 1. Systematics of Repository Biogeochemistry.

Microbially mediated redox processes. Microbial groups are known to use many redox pairs to derive their energy. If such chemical kinetic constraints exist that the rate of a given chemical reaction is sufficiently slow, bacteria can compete; thus, almost any redox pair that yields energy could be exploited. Microorganisms tend to accumulate at redox interfaces, where both metabolic electron donors and electron acceptors are available. Numerous studies have shown that microbial metabolism couples the inorganic redox chemistry of groundwaters to the oxidation of organic carbon.[21] Heterotrophs derive their energy from the oxidative breakdown of external organic substrates; hence, microbial ecologies are frequently classified in terms of dominant pathways. The principal pathways are aerobic respiration, denitrification, manganese reduction, iron reduction, sulfate reduction, fermentation, and methanogenesis. From a biochemical point of view, the energy-yielding metabolic processes involve complex electron transfer chains. From a geochemical perspective, the significant reaction is the final electron transfer to an external electron acceptor. The common electron acceptors in subsurface environments are O_2, NO_3^-, MnO_2, $Fe(OH)_3$, SO_4^{2-}, and CO_2. Fermentation does not rely on an external electron acceptor; instead, it partially oxidizes and reduces an organic substrate. The final fermentation products include CO_2, H_2, alcohols, and organic acids.

The primary pathways of organic carbon oxidation tend to occur in a predictable sequence. The most energetically favorable is aerobic respiration, followed, in order of decreasing free energy yield, by denitrification, Mn(IV) reduction, Fe(III) reduction, sulfate reduction, and methanogenesis. The differences in the energy yields of these reactions, plus the availability of electron acceptors, lead to physical (spatial or temporal) separation of redox zones in which each pathway dominates. Because aerobic respiration is the most energetically favorable reaction, aerobic bacteria have a competitive advantage over anaerobic microorganisms when a certain level of O_2 is present. This redox sequence is well established for natural systems including aquatic sediments,[19] groundwater systems,[20] and landfills.[22] This sequence imposes an important constraint on metabolic processes that can occur in a specific repository environment, and it provides a technical basis for modeling biogeochemical processes in a repository.[23, 24]

Relevant Biogeochemical Processes in radioactive repositories. The relative importance of various microbial processes in a specific repository greatly depends on the ambient environment of the repository, and the materials to be emplaced (Figure 1). A repository in unsaturated igneous rock formations (such as volcanic tuff rocks at Yucca Mountain) is generally expected to be oxidizing in its chemical environment; a repository in a hydrologically saturated zone, particularly in sedimentary rocks, could be reducing. Sedimentary rocks contain a certain amount of organic matter, which may stimulate significant microbial activities and, thus, maintain the repository and its surrounding areas in a reducing condition.

Both low-level and intermediate-level radioactive wastes contain a large portion of organic materials (e.g. cellulosics) and a significant amount of inorganic nutrients (e.g. NO_3^-). Microbial degradation of these materials has been a major concern for long-term repository performance assessment. An example is the WIPP, which is located within a salt bed in Southern New Mexico, and was designed for disposal of defense-related transuranic wastes. Microbial degradation of organic carbon-rich materials in the WIPP repository has been studied for its impact on repository pressurization and water chemistry.[24, 25] Unlike low-level and intermediate-level wastes, high-level radioactive wastes generally contain no organic materials and are thus not conducive to microbial activity.

As discussed above, autotrophs are limited by the supply of electron donors and inorganic carbon. Since carbon dioxide is present in most systems, the availability of reduced inorganic species (e.g. H_2) is expected to be a major limiting factor for autotrophs. In a nuclear-waste repository, the electron donors are derived from three sources: (1) The anaerobic oxidation of organic compounds by heterotrophic bacteria can serve as the primary source of both the reduced inorganic substrates and the inorganic carbon for autotrophic metabolisms. For instance, fermentation produces H_2 and CO_2, both of which are needed by autotrophic methanogens for synthesizing methane. (2) A large fraction of metals or metal alloys is always present in repositories, either as waste package materials or as other engineered components. Anoxic corrosion of these metals, $Fe^\circ + 2H_2O = Fe(OH)_2 + H_2$, generates hydrogen gas. And (3) reduced inorganic species may be brought in by groundwater or gas flows from the surrounding areas. Figure 1 also indicates the possibility that, through biomass recycling, a biomass synthesized by autotrophic bacteria can be used by heterotrophs.

Mobilization or immobilization of radionuclides by the activities of autotrophs (in the case of inorganic compounds) and by heterotrophs (in mixed wastes containing organics) could be significant.[4] Autotrophic bacteria such as iron and sulfur oxidizers play a significant role in the solubilization of uranium from ores and mill tailings. The biogeochemistry of uranium has been extensively studied in light of the recovery of U from ores. Microbial leaching of pyritic uranium ore is primarily indirect, and confined to the generation of the oxidizing agent, ferric sulfate, and the solvent sulfuric acid. The indirect involvement of Fe^{2+}/Fe^{3+} in the process of cyclically mediating the oxidation of the insoluble uranium oxide has been documented: $UO_2 + Fe^{3+} \rightarrow UO_2^{2+} + 2Fe^{2+}$. *T. ferroxoxidans* can also directly oxidize reduced compounds of uranium (uranous sulfate and UO_2) to their hexavalent form without the involvement of extraneous Fe^{3+}/Fe^{2+} complex as the chemical electron carrier. Iron- and sulfur-oxidizing bacteria (*T. ferrooxidans* and *T. thiooxidans*) were isolated from several uranium ores. An increase in heterotrophic microbial activity due to biodegradation of organic compounds can affect the solubility of radionuclides and metals. Heterotrophic bacteria and fungi are able to release metals from various materials, including copper-nickel concentrates, low-grade copper ore, uranium and manganese ore. Several mechanisms for heterotrophic aerobic microbial solubilization of insoluble metal have been proposed. These include organic acid production, formation of chelating agents, and metabolism of metal-associated anion. Leaching by heterotrophic organisms is entirely due to chemical reaction of excreted microbial metabolites and decomposition products.

Figure 1 provides a theoretical framework for the evaluation of the microbial activity within the repository. Note that (in addition to the processes described in the figure) temperature, water availability, and radiation will play an important role in the regulation of microbial activities within the repository during relevant time periods.

3. Evaluation of Microbial Activity in the YM Repository

Microbial population distributions. The Yucca Mountain (YM) repository is located in an unsaturated zone of welded volcanic tuff. Thus, the geochemical impact of microbial activity within the repository should be considered in the context of physical, chemical, and hydrologic conditions in vadose zones. Characterization of microbial communities has principally focused on correlating the types, numbers, and activities of present microorganisms to extant geological features, and with water and carbon availability.[10, 26–31] These studies, along with those examining the activities of subsurface isolates in pure cultures, have shown bacteria are present in all vadose zones examined thus far.

The quantity of intact phospholipid fatty acids (PLFA) is a direct indicator of viable or potentially viable cells in an environmental sample. Thus, PLFA analysis permits the direct quantification of microbial biomass in environmental samples without the need for culturing microorganisms.[32] Quantitative analysis of the total number of bacteria present in a Yucca Mountain rock sample aseptically collected from the Exploratory Studies Facility (ESF) was undertaken using this technique.[31] A single rock core was collected and cut into two pieces: one piece consists of the region facing the tunnel (proximal to the drift), and the other piece is the area reaching into the tunnel wall (distal to the drift). Using this method, it was deduced that the proximal sample contained 6×10^4 cells per gram of dry rock (3 pmole (pM) of PLFA/g dry rock); the deeper-dwelling distal sample had 4×10^4 cells per gram of dry rock (2 pM of PLFA/g dry rock), using a conversion factor of 2×10^4 cells/pM PLFA. No diglycerides, an indicator of dead bacteria, were detected. The PLFA analysis showed a preponderance of gram-negative organisms.

Subsurface environments have demonstrated high proportions of heterotrophic bacteria,[33] especially in studies of oxygenated volcanic tuff at the Nevada Test Site.[6, 27] Microbial analyses conducted in the YM Experimental Study Facility (ESF) have determined the existence of aerobic heterotrophs and autotrophs[34] including iron oxidizing, sulfur oxidizing and nitrifying organisms. Cell counts for autotrophs range between 10 and 500 cells per gram dry weight and, for the heterotrophs, between 3.2×10^4 to 2×10^5 cells per gram of tuff. The relatively small population of autotrophs reflects the oxidizing environment at Yucca Mountain, which is not conducive to sustaining autotrophic bacteria. Characterization of DNA extracted directly from YM rock was undertaken to generate a more complete roster of the organisms contained within the YM community.[35] Four hundred grams of Yucca Mountain rock were subjected to extraction. The resulting DNA was purified and subjected to a series of biochemical and genetic manipulations that included amplification of microbial 16S rDNA genes and cloning amplified genes. Two hundred rDNA clones were screened to obtain those that were unique based on restriction fragment length polymorphisms. After screening 200 rDNA clones, 65 unique clonal types, representing 65 separate types of organisms, were distinguished. Identified YM organisms span a wide phylogenetic range, and include groups of organisms known to reside in dry environments (e.g. the High Guanine (G) + Cytosine (C) Gram Positives). The great diversity of microorganisms detected at the YM site further confirms our early assertion that microorganisms in a subsurface environment are highly adaptable and sufficiently diverse for carrying out any specific metabolic reaction that the environment permits.

Temperature constraint. The temperature of the subsurface environment will limit the type of bacteria that are present, based on the optimum growth temperatures of the organisms that are present. Only a few of the identified isolates are known to withstand elevated temperatures (e.g. *P. stutzeri*, *R. pickettii*, *B. natatorius*). Only one isolate, *M. mesophilicum*, is capable of forming cysts that are desiccation resistant; *M. mesophilicum* has also been found to be dominant in soils surrounding the Chernobyl Atomic Power Station.[36] Other bacteria, if they survive, will remain in a dormant state, without any contribution to the geochemical processes of interest. Sulfate-reducing bacteria isolated from a mine in Britain and *Thiobacillus ferrooxidans* found in several sites in Europe have been subjected to repository conditions in order to establish their ability to survive under these conditions.[15] These experiments have included establishing growth curves, temperature tolerance tests, combined high pressure and temperature tolerance, and gamma-irradiation. The experimental data suggest that sulfate-reducing bacteria are more tolerant to extreme environmental conditions than the sulfur-oxidizing *Thiobacillus ferroxidans*.[15] Sulfate-reducing bacteria, strict anaerobes, are not expected to be active in the oxidizing Yucca Mountain environment. It is agreed that the maximum temperature at which known microorganisms can exist in an active state is ~120 ˚C.[37]

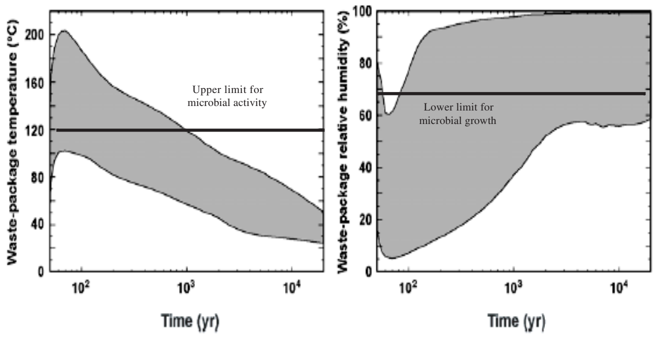

Figure 2. Thermal and Relative Humidity Histories Predicted for YM Repository and Their Constraints on Microbial Growth (modified from BSC, 2004).[38]

The in-drift thermal history in the YM repository has been predicted by a thermal-hydrologic model that accounts for heat conduction from the drift wall, into the rock matrix, resulting in vaporization and boiling, with vapor migration out of matrix blocks into fractures (Figure 2).[38] During the period of thermal perturbation resulting from waste package emplacement, the temperature in the repository drifts could exceed 120 ˚C (the upper temperature limit for the presence of microorganisms) and, for a waste package surface, could be as high as 170 ˚C. Therefore, microbes initially present in the drift will be severely limited in growth, if not totally eliminated, by heating for a few hundred years at the early stage of the repository. Microbes may migrate into the repository with infiltrating fracture flow, once temperatures decrease. Infiltrating organisms that survived the heating period may be presumed to colonize if conditions are favorable for growth. As shown in Figure 2, even after the peak temperature, the in-drift temperature will remain above 50 ˚C for the duration of the 10,000-year regulatory period. Therefore, the microbial population will be dominated by thermophiles and hyperthermophiles.

Relative humidity constraint. Water is essential for microbial growth. In order for microorganisms to grow, the relative humidity in the environment needs to be 75 to 100%.[37, 39] Many bacteria perish, sporulate, or become dormant when water activity falls below approximately 0.90.[39, 40] Some fungi and yeast can be active at lower water activity levels (on the order of 0.7 to 0.85.[37] However, these microorganisms have very small populations as compared to bacteria (Table 1) and are solely dependent on the availability of organic carbon, which will be very limited in the Yucca Mountain repository. In a full-scale test conducted at Atomic Energy Canada Ltd. (AECL), a simulated waste container (maximum heat output 85 ˚C) was buried for 2.5 years, surrounded by compacted buffer materials. Extensive microbial analysis of this test has shown that most viable organisms in the buffer material disappear around a moisture content of 15 percent (corresponding to RH 95 to 96 percent).[41]

The activity and availability of water for microbial growth in the UZ at Yucca Mountain will be dependent upon thermal-hydrologic conditions. The range of relative humidity on all waste packages is shown in Figure 2. During the thermal pulse, relative humidity on the waste package surface can be lower than 10 percent. In some realizations, relative humidity recovers (back to 100 percent) after approximately 1,000 years. In many other realizations, relative humidity remains below 90 percent, and even below 70 percent in some cases, throughout the regulatory period, thus limiting microbial growth and activity. In addition, low water activity generally corresponds to low liquid-water availability. Low saturation of liquid water in fractures on the drift wall will limit the transport growth substrates to microbial cells and, therefore, the activity of the cells.

Constraint of oxidizing environment. Bacteria that are able to grow in the presence of molecular oxygen, either in a gaseous or dissolved form, are termed aerobes; those that can grow without oxygen are anaerobes. Wang and Van Cappellen[19] have shown that a typical limiting concentration of O_2 for aquatic sediments is 1 to 30 μM (approximately 0.4 to 10 percent atmospheric oxygen), above which anaerobic microbial reactions are completely inhibited.

During the thermal pulse, water vapor will drive air mass away from the waste drift, thus resulting in depletion in oxygen concentration, which is proportional to the fraction of remaining air mass in the gas phase. A thermo-hydrological calculation shows that the air mass fraction will decrease to less than 10 percent during the thermal pulse, and will then quickly recover (back to 100 percent) after the thermal pulse, as does the oxygen concentration.[42] However, the depletion in oxygen concentration is not expected to have an impact on microbial reaction pathways, because microbial activity will be severely limited, if not completely eliminated, due to both the heating and the desiccating effects, during the thermal pulse, as discussed above. Therefore, within the time window permissive for microbial activity (i.e. with temperature less than 100 °C), the oxygen concentration in the repository will be maintained above its limiting concentration (i.e. greater than 10 percent of atmospheric value). Therefore, the overall chemical environment in the repository will be oxic, and a significant anaerobic microbial activity will be unlikely.

The assertion of an overall oxic environment in the repository is consistent with field observations.[42a] Iron and manganese oxides/oxyhydroxides are the most common mineral phases found in the fractures in both saturated and unsaturated zones. The redox state of the unsaturated zone (UZ) waters is probably dominated by the presence of oxygen in the UZ gas phase. At the two locations where such measurements have been made, the UZ gas phase has atmospheric levels of oxygen. The measured Eh values are typically high (400 to 600 mV). In addition to the oxic background environment, radiolysis of water inside the waste drift will generate hydrogen peroxide, thus adding another oxidizing agent to the water.

The oxidizing environment will also prevent the generation or accumulation of reduced inorganic species (e.g. H_2, NH_4^+, NO_2^-, Mn^{2+}, Fe^{2+}, H_2S, and S), which are a prerequisite substrate for autotrophic microbial processes. Chemical analyses of YM groundwaters from Nye County Early Warning Detection Program wells show that the waters contain only trace amounts of dissolved iron (less than 2.34 ppm) and manganese (less than 0.58 ppm) (not necessarily all in Fe^{2+} and Mn^{2+}), and NH_4^+ (less than 1 ppm). Fe^{2+} ions may be present as an intermediate product of waste package corrosion, but they are transient in time and localized in space to the corrosion front. Long-term corrosion tests evaluated waste package material coupons in various solutions under anticipated repository conditions.[35] Interestingly, even in this oligiotrophic and metallic alloy-dominated environment, the microbial population was dominated by hetrotrophic bacteria. Bacilli, known as heterotrophs, were observed to be the most numerous, representing 84 percent of the screened clones. This indicates that metal corrosion under Yucca Mountain-relevant conditions will not support any significant autotrophic microbial growth. This may be partly because abiotic Fe^{2+} oxidation is kinetically favored over microbial oxidation.

Constraints of nutrient supply. Experiments have been performed to define conducive and inhibitory environmental conditions that pertain to growth of Yucca Mountain microbial communities in modified Yucca Mountain groundwater.[31] Experimental data show that nitrogen and sulfate sources are apparently sufficient to support microbial growth, even in unconcentrated Yucca Mountain groundwater. Both phosphate and organic carbon have been shown to have significant effect on microbial growth. Phosphate increased cell densities approximately 1.5 orders of magnitude. The addition of a carbon source, glucose, resulted in increases of one order of magnitude in unconcentrated simulated groundwater medium. Yucca Mountain groundwater, however, has extremely low levels of organic carbon and phosphate. Only trace concentrations of organic carbon (up to 1.1 mg/L) have been reported in qualified measurements of Yucca Mountain groundwater. The materials introduced into emplacement drifts will not contain any organic carbon,[43] except for a trace amount of reduced carbon in metal or metal alloys. These carbons, like graphite, are expected to be refractory and, thus, will not be biodegradable. Therefore, the extremely low organic-carbon supply in the repository will limit heterotrophic microbial activity.

Radiation effect. Much of the information on the radiation resistance of microorganisms comes from the food industry, because ionizing radiation is used in many countries as a means of preserving foods. Exposing foods to low doses of ionizing radiation (less than 1 Mrad) substantially reduces the number of those microorganisms responsible for food spoilage. Many radioresistant strains of bacteria have been isolated from soil, and studies of soil microflora irradiated with long-term γ-radiation (in an open-air facility at 15, 150, 800, and 1500 krad over 18 months) showed that radiation resistance increased at all doses when compared with the radiation resistance of soil microflora from soil shielded from irradiation with lead bricks.[44] However, these microbes did not show significant growth under irradiation of higher than 5 Mrad. Barnhart et al.[45] found that approximately 68 percent of the microbial isolates from radioactive shallow land burial site soil exhibited greater resistance to γ- and β-radioactivity than did the common soil bacterium *Bacillus subtilis* to ionizing radiation. Approximately 25 percent of the isolates from nonradioactive soil were more resistant than *B. subtilis*, but all these microbial isolates were less radioresistant than *Deinococcus radiodurans*, which is resistant to γ-radiation (10^5 rad). However, radio-resistant bacteria are constantly being enriched in an environment containing nanocurie levels of γ- and β-radioactivity.[45] In the later study, the radio-resistance of 10 isolates of bacteria from a soil core (of a 40-cm-to-50-cm depth, and containing 20 nCi to 30 nCi of radioactivity), taken from a shallow land burial site at Los Alamos National Laboratory, was studied. Several of the isolates were resistant to 5×10^4 rad γ-radiation. At 7.5×10^4 rad, none of the isolates survived. A time-course experiment was conducted to evaluate the effects of γ radiation on the indigenous microbiota present in rock obtained from Yucca Mountain and the Nevada Test Site.[46] Microcosms were constructed by placing pulverized Yucca Mountain rock in polystyrene cylinders. Continuous exposure (96 h) at a dose rate of 1.63 Gray (Gy)/min was used to mimic the near-field environment surrounding waste canisters. The microbial communities were characterized after receiving cumulative doses of 0 kGy, 0.098 kGy, 0.58 kGy, 2.33 kGy, 4.67 kGy, 7.01 kGy, and 9.34 kGy. Radiation-resistant microorganisms in the pulverized rock became viable but nonculturable after a cumulative dose of 2.33 kGy.

Based on the current repository loading design, the expected maximum surface dose rate from one unbreached canister designed to contain spent nuclear fuels is 1.7 Mrad/year. Therefore, it is expected that radiation may inhibit microbial growth in the repository. This assessment is consistent with an AECL test that used a simulated waste container with a maximum heat output of 85 °C). The test has shown that the surface of nuclear fuel waste containers is "sterilized" 9 to 33 days after emplacement.[41]

Overall impact of microbial activity on YM near-field chemistry. Microbial growth and activity at Yucca Mountain are limited by multiple factors: (1) The in-drift temperatures during the thermal pulse will exceed the temperature tolerance of all known microbes for a significant portion of the repository time. (2) An oxic environment will prevail in the repository over the growth-permissive period and, therefore, prevent the generation and accumulation of reduced inorganic species that are the prerequisite for autotrophic metabolism. (3) The relative humidity and the liquid-water saturation in the repository are predicted to be low for a significant duration, thus further limiting microbial activity. And (4) the extremely low organic carbon supply in the repository will limit heterotrophic microbial activity. Due to these environmental constraints, the microbial activity in the repository is expected to be low, and its impacts on drift chemistry can be negligible.

4. Evaluation of Microbial Activity in the WIPP Repository

Environmental constraints. The Waste Isolation Pilot Plant (WIPP), located in a salt bed in southern New Mexico, is designed by U.S. Department of Energy for permanent disposal of defense-related transuranic wastes. In this high-salinity environment, microbes present in the repository are dominated by halophilic or halotolerent bacteria, with a population of $1.02 \pm 0.49 \times 10^5$ cells/mL in the far field and $1.24 \pm 0.13 \times 10^5$ cells/mL in the near field.[5] Microbes detected in the WIPP include denitrifiers, fermenters, sulfate reducers, and methanogens.[5]

Unlike the YM repository, the WIPP can be categorized as an organic carbon-rich repository. Based on the inventory estimates,[24] wastes to be emplaced to the WIPP contain a large quantity of organic materials and various nutrients: $\sim 10^9$ moles of organic carbon, 2.6×10^7 moles of nitrate, and 6.6×10^6 moles sulfate. The organic materials are dominated by cellulsics, rubbers and plastics. There is a concern for the long-term performance assessment of the repository with a potential CO_2 generation from biodegradation of organic materials, especially for the scenario in which a large volume of brine is introduced into the repository by human intrusions. The generation of CO_2 can potentially impact the mobility of actinides and the closure of disposal room.

Incubation experiments. Long-term incubation experiments (~ 10 years) were started in January, 1992, to obtain data on microbial gas generation under anticipated WIPP conditions.[8, 25] The experiments performed with simulated cellulose materials and G-seep brine collected underground in the WIPP. The G-seep brine contains $10^4 - 10^6$ bacterial cells/mL.[8] In the experiments, a certain amount of microbial inoculum was added to each sample. This inoculum was prepared from a mixrure of a variety of WIPP repository-relevant samples: sediment and brine from Nash Draw, brine from the WIPP underground workings, and inocula from a non-sterile laboratory environment. The data collected in the experiments include quantification of total gas, H_2, O_2, CO_2, N_2, N_2O, CH_4 and H_2S produced in the headspace of each sample.

The results of headspace gas analyses for anaerobic, brine-inundated samples are shown in Figure 3. The amount of CO_2 accumulated in nitrate-amended samples was systematically higher than that in samples without excess nitrate amendment, demonstrating the dependence of cellulose biodegradation on the availability of electron acceptors and nutrients. Microbes use nitrate both as an electron acceptor and as a nutrient for biomass synthesis. Nutrient-amended samples initially contained 250 μmoles of nitrate/g cellulose (equivalent to $NO_3^-/C \approx 0.007$), while nutrient-amended samples plus excess nitrate contained 1240 μmoles of nitrate/g cellulose (equivalent to $NO_3^-/C \approx 0.033$).[8]

Based on the waste inventory estimates,[24] the wastes to be emplaced in the WIPP contain 2.85×10^7 kg of total equivalent cellulose materials (or 1.05×10^9 moles of C) and 1.6×10^6 kg

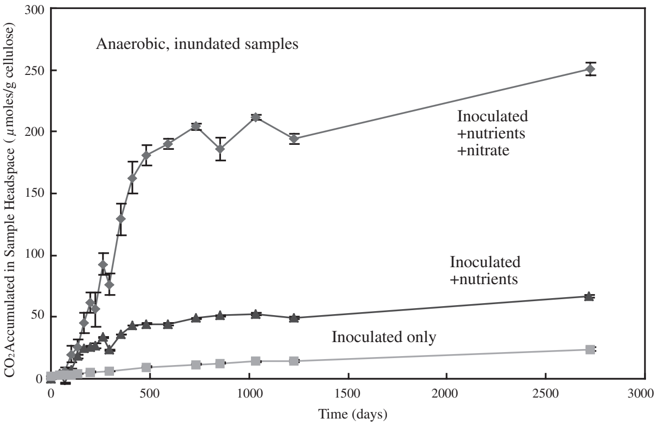

Figure 3. CO_2 production in anaerobic, brine-inundated cellulose samples. The accumulation of CO_2 in each set of samples displays two distinct gas generation phases: a rapid increase in the first 500 days followed by a long period of slow accumulation.

(or 2.6×10^7 moles of NO_3^-) of nitrate. Therefore, the average molar ratio of NO_3^- to organic C in the waste will be 0.025, slightly less than in the experiments amended with excess nitrate. Therefore, the measurements on nitrate-amended samples provide an upper bound for the microbial gas generation rate in actual wastes.

The accumulation of CO_2 in each set of samples displays two distinct gas-generation phases: a rapid transient rate in the 500 days followed by a long period of slow accumulation (Figure 3). The transition from the transient to the long-term phase is unlikely to be caused by pH changes or the accumulation of metabolites. In fact, no significant pH changes were detected during incubation.[25] In addition, the occurrence of methane (CH_4) production in amended samples without excess nitrate implies that the conditions in the experiment were not detrimental to microbial activity, since methanogens are generally extremely sensitive to environmental changes.[47] The continuous production of CO_2 in the long-term phase provides direct evidence that microbial activities in the experiments were not inhibited by metabolite accumulation. There are two possible reasons for the slow CO_2 accumulation in the long-term phase of the experiments: (1) changes in microbial communities and metabolic reaction pathways due to nitrate depletion and (2) depletion of labile constituents of the substrate.

Impact of microbial gas generation on WIPP performance. In order to mitigate the detrimental effect of microbial CO_2 generation on the WIPP performance, a sufficient amount of MgO (2×10^9 moles) will be added to the repository as a backfill.[24] MgO will react with brine and CO_2 through the following reactions:

$$MgO + H_2O \rightarrow Mg(OH)_2 \quad (1)$$

$$Mg(OH)_2 + CO_2 \rightarrow MgCO_3 + H_2O. \quad (2)$$

Reaction (2) will buffer CO_2 fugacity at $\sim 10^{-7}$ atm. This low CO_2 fugacity implies that Reaction (2) will practically remove all CO_2 from both gaseous and liquid phases. The mineral formation and brine pH resulting from MgO reactions have been calculated with computer code EQ3/6 v. 7.2a. The calculation shows that the addition of sufficient MgO into the repository will buffer pH within 9.2 to 9.9 for WIPP brines.[24] Under these chemical conditions, actinide solubility becomes minimal. Microbial gas generation has a significant impact on the evolution of disposal room pressure. The pressure buildup shown in Figure 4 is attributed to both microbial gas generation and anoxic metal corrosion.[24]

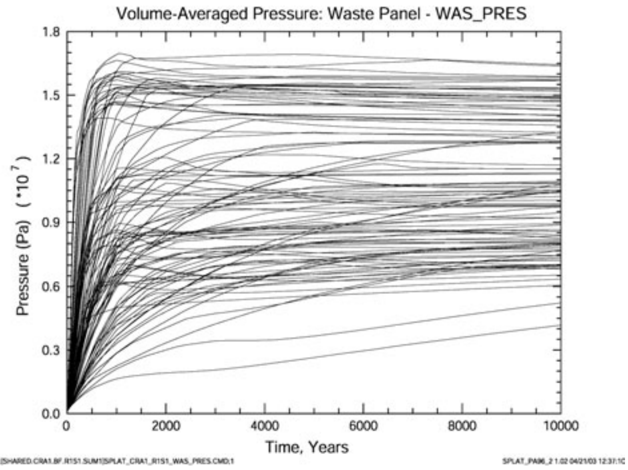

Figure 4. Evolution of Disposal Room Pressure in the WIPP Repository. The pressure buildup in the repository is partly due to microbial degradation of organic materials in the wastes.

5. Conclusions

Microorganisms are ubiquitous in subsurface environments and play a major role in the biogeochemical recycling of various elements. In this paper, we have developed a general approach for a systematic evaluation of microbial impact on the long-term performance of the repository. We have demonstrated that data on microbial population alone are not sufficient for the evaluation of microbial impact on repository performance and a sensible approach for such evaluation must be based on the consideration of environmental constraints on microbial reaction pathways. We have applied our approach to both the Yucca Mountain (YM) repository and the Waste Isolation Pilot Plant (WIPP). We have demonstrated that the effect of microbial activity on the near-field chemistry in the Yucca Mountain repository is negligible because of limited nutrient supply and harsh environmental conditions created by waste emplacement. Whereas for the WIPP, we have shown that, due to the presence of a large quantity of organic materials and nutrients in the wastes, a significant microbial activity can potentially be stimulated and its impact on repository performance can be evaluated with carefully designed incubation experiments coupled with performance assessment calculations.

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