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Scientific Analysis Administrative Change Notice

Complete only applicable items.

1. Document Number: A	ANL-EBS-MD-000038		2. Revision:	01	3. ACN:	01
4. Title: Evaluation of Potential Impacts of Microbial Activity on Drift Chemistry						
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4-2	Added information (Table 4.1-1 "Direct I <u>Parameter(s) or Infor</u> <u>Maximum surface da</u> to gamma radiation This error was identif	nputs from Project r <u>mation</u> ose rate due		Sou		3426, Table 2

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1. Document Num	ber:	ANL-EBS-MD-000038	2. Revision:	01	3. ACN:	01
4. Title: Evaluati	on of P	tential Impacts of Microbial Activity on Drift Chemistry				
6-35		Added clarification Section 6.4.7 "Radiation Effect", 5 th p "Based on the current repository load unbreached canister designed to conta 0.06 Gy/min (= 31.5 kGy/yr = 3.15 [DIRS 150442])" To: "Based on the current repository load gamma radiation from an unbreache Mrad/year) (BSC 2005 [DIRS 173420] This error was identified in CR 4728	ling design, the exp ain spent nuclear fu Mrad/yr) (conver ding design, the exp d waste package is	vels is: sion: 1 kGy = pected maxim	= 0.1 Mrad) (Pito um surface dose	nzo et al. 1999 rate due to
8-2		Citation update (Correct DIRS as appr Section 8.1 "Documents Cited", add ru "BSC (Bechtel SAIC Company) 2005 800-IED-WIS0-01301-000-00A. Las ENG.20050406.0012" This error was identified in CR 4728 Additional page 8-2a was added.	eference [173426], 5. IED Waste Pack	age Radiation		
8-2		Citation update (Correct DIRS as app Section 8.1 "Documents Cited", replace BSC 2004. Drift –Scale THC Seepage Bechtel SAIC Company. ACC: DOC. To BSC 2004. Drift –Scale THC Seepag Bechtel SAIC Company. ACC: DOC This error was identified in CR 4615 a Additional page 8-2a was added.	ce reference (16985 2 Model. MDL-NB. 20041111.0001 5 Model. MDL-N. C.20041201.0008	S-HS-000001, B S-HS-00000	Rev. 03. Las Veg	gas, Nevada:

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1. Document Number	A	NL-EBS-MD-000038	2. Revision:	01	3. ACN:	01
4. Title: Evaluation	of Pot	tential Impacts of Microbial Activity on D	rift Chemistry			
8-8		Citation update Section 8.1 "Documents Cited", referen "NIST 2003. NIST Critically Selected S Standard Reference Database 46 Versic Commerce Technology Administration. To "NIST (National Institute of Standards Selected Stability Constants of Metal C Standard Reference Database 46. Versic Maryland: U.S. Department of Comme Technology. TIC: 256840." This correction is associated with TBV- Additional page 8-8a was added.	Stability Constant, on 7.0. Gaithersbi On Order" s and Technology complexes Databa ion 8.0 for Windo crce, National Ins	s of Metal Cou urg, MD: v) 2004. NIST use, Users' Gu ows. Gaithers	NIST- U.S. Dep Critically uide. NIST burg,	

4. INPUTS

4.1 **DIRECT INPUTS**

This section documents and substantiates the direct inputs used in this report (i.e., those data and other information items that were used to develop the results or conclusions). Because the conclusions are based on environmental factors that can severely limit microbial activity in the repository, only the inputs related to the limiting environmental factors are direct inputs.

4.1.1 Direct Inputs from Project Sources

Table 4.1-1 lists direct inputs from the following Project sources:

- Project data identified by data tracking number (DTN)
- IEDs
- Analysis and model reports that support the TSPA-LA.

None of the Project sources is a product output from a canceled/superseded document.

Parameter(s) or Information	Value(s)	Source
In-drift thermal history	See Figure 6.4-1	BSC 2004 [DIRS 172463], Figure 6.5-3
Evolution of air mass fractions in drift	See Figure 6.4-2	BSC 2004 [DIRS 172463], Figure 6.5-8
Growth rates of Yucca Mountain aerobic bacteria in unconcentrated groundwater	See Figure 6.4-4	DTN: LL040801512251.115 [DIRS 171476]
Range of relative humidity on all waste packages	See Figure 6.4-5	BSC 2004 [DIRS 169565], Figure. 6.3-68
Liquid saturations in fractures at three drift-wall locations	See Figure 6.4-6	BSC 2004 [DIRS 172463], Figure 6.5-6
Growth rates of Yucca Mountain culturable aerobic bacteria as a function of macronutrient concentration	See Figure 6.4-7	DTN: LL040801512251.115 [DIRS 171476]
Largest concentration of organic carbon in qualified analyses of groundwater from locations in the vicinity of Yucca Mountain	1.1 mg/L	DTN: GS011108312322.006 [DIRS 162911], table name: S 01174_003, col. J
CO ₂ flux	See Figure 6.5-1	DTN: SN0407T0510102.017 [DIRS 170667], file: <i>gas_flux.xls</i> , worksheet: Moles per m chart
Emplacement drift committed materials have no organic components	_	BSC 2004 [DIRS 170058]
A large quantity of Fe(III) may be accumulated from oxic steel corrosion	_	BSC 2004 [DIRS 169868], Section 5.6 and Table 6.3-4

 Table 4.1-1.
 Direct Inputs from Project Sources

Parameter(s) or Information	Value(s)	Source
Under oxidizing conditions, Pu will be dominated by Pu(V) and Pu(VI) instead of Pu(IV)	_	BSC 2004 [DIRS 169425], Figure 6.5-5
Brine solutions ionic strength	10M	BSC 2004 [DIRS 169860], Section 6.1.3
Maximum surface dose rate due to gamma radiation	1160 rad/hour	BSC 2005 [DIRS 173426], Table 2

Table 4.1-1. Direct Inputs from Project Sources (Continued)	Table 4.1-1.	Direct Inputs from	Project Sources	(Continued)
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4.1.2 Direct Inputs from Outside Sources

Table 4.1-2 lists the direct inputs (from outside sources) that were obtained from investigation activities such as measurement, testing, and analysis. Of these outside sources, the National Institute of Standards and Technology (NIST) maintains the database of reaction constants that is accepted by the scientific and engineering communities as established fact, and therefore the constants do not need to be qualified in accordance with YMP procedures. Data from the other two external sources listed in Table 4.1-2 and Table 4.1-3 are not established fact and are therefore demonstrated to be qualified for their intended use in Table 4.1-4 in accordance with Section 5.2.1.1 of AP-SIII.9Q.

Table 4.1-2.	Numeric Data from Outside Sources Used as Direct Inputs
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Parameter(s) or Information	Value(s)	Source
Upper temperature limit for microbial growth	110°C	Pedersen and Karlsson 1995 [DIRS 100810]
Concentration of O ₂ for aquatic sediments above which anaerobic microbial reactions are completely inhibited	1 to 30 μ M (approximately 0.4 to 10% atmospheric oxygen)	Wang and Van Cappellen 1996 [DIRS 171057], Table 3
Water activity levels at which bacteria can grow	0.75 to 1.00	Pedersen & Karlsson 1995 [DIRS 100810]
Complexation constants	See Table 6.5-2	NIST 2003 [DIRS 171201]
Growth of Yucca Mountain bacteria	See Table 6.3-1	Horn et al. 2004 [DIRS 171058]

Table 4.1-3. Information from Outside Sources, Other Than Data, Used as Direct Inputs

Information	Source
Water is essential for microbial growth.	Horn et al. 2004 [DIRS 171058]
There is negligible long-distance biocolloid transport in porous geologic media that are not significantly fractured.	DeFlaun et al. 1997 [DIRS 171060]
Table 6.1-1 – Nutritional Requirements of Microorganisms	Pedersen and Karlsson 1995 [DIRS 100810]

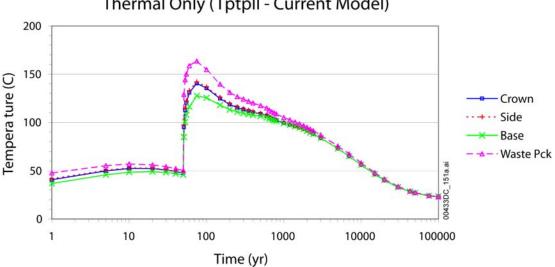
tolerance tests, combined high pressure and temperature tolerance, and gamma-irradiation. The experimental data suggest that sulfate-reducing bacteria are more tolerant to extreme environmental conditions than the sulfur-oxidizing *Thiobacillus ferroxidans* (West et al. 1986 [DIRS 171088]). Sulfate-reducing bacteria, strict anaerobes, are not expected to be active in the oxidizing Yucca Mountain environment (Section 6.4.2).

Drift Scale Coupled Processes (DST and THC) Seepage Models (BSC 2004 [DIRS 172463], Figure 6.5-3) provides the in-drift thermal history 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. The vapor moves away from the drift through the permeable fracture network by buoyancy, by the increased vapor pressure caused by heating and boiling, and through local convection. In cooler regions, the vapor condenses on fracture walls, where it drains through the fracture network either down toward the heat source from above, or away from the drift into the zone underlying the heat source. Slow imbibition of water from fractures into the matrix gradually leads to increases in the liquid saturation in the rock matrix. Under conditions of continuous heat loading due to radiation, a dryout zone may develop closest to the heat source separated from the condensation zone by a nearly isothermal zone maintained at about the boiling temperature. The predicted in-drift thermal history is shown in Figure 6.4-1.

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 (Figure 6.4-1). 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. As discussed in Section 6.4.3, during this heating period, relative humidity and water availability will also diminish; this will add another detrimental environmental factor to the survival of microorganisms.

Bacteria 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. It is shown in Figure 6.4-1 that, 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.

Bacteria are able to withstand and flourish at the highest hydrostatic pressures on the planet (Madigan et al. 2003 [DIRS 171083]). Ordinary bacteria that have not been challenged by high hydrostatic pressure during their evolution are, nevertheless, remarkably tolerant to such pressure. At a high temperature, a high hydrostatic pressure may even help microbes to survive by preventing water loss from boiling and thus maintaining sufficient liquid water for microbes to carry out necessary metabolic reactions. Because the repository is located in an unsaturated zone, the in-drift gas pressure is close to atmospheric pressure. A significant microbial activity in the repository is possible only after the first 1,000 years, when the drift temperature drops below the boiling point (approximately 96°C) (Figure 6.4-1). During the thermal event, the relative humidity is low enough to inhibit microbial growth (Section 6.4.3).



Thermal Only (Tptpll - Current Model)

Source: BSC 2004 [DIRS 172463], Figure 6.5-3.

Figure 6.4-1. TH Simulation (Tptpll): Time Profiles of Modeled Temperatures in Fractures (Similar in Matrix) at Three Drift-Wall Locations and in the Waste Package

6.4.2 **Redox Conditions**

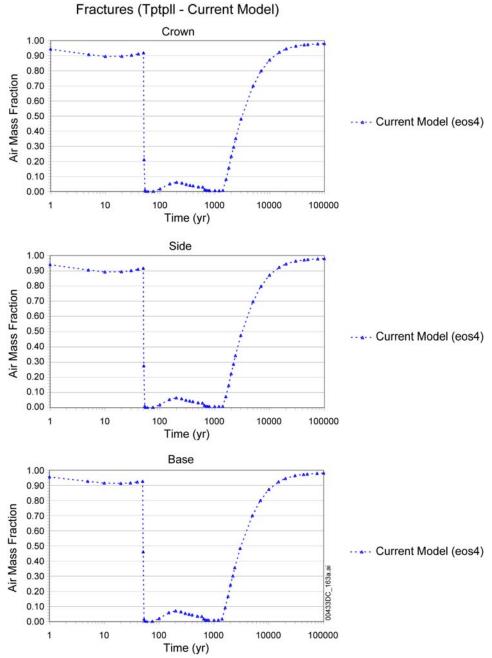
This section addresses the microbial aspects of FEP 2.1.09.06.0B (reduction-oxidation potential in drifts).

Bacteria that are able to grow in the presence of molecular oxygen, either in gaseous or dissolved form, are termed aerobes; those that can grow without oxygen are anaerobes. Table 6.4-2 shows the terms used to describe the O₂ relations of bacteria. Thus, different microbes can thrive at various redox conditions, including both the oxygenated environments that are at high redox potential, and the reducing environments in which the oxygen abundance is minimal. Wang and Van Cappellen (1996 [DIRS 171057]) have shown that a typical limiting concentration of O₂ for aquatic sediments is 1 to 30 µM (approximately 0.4 to 10 percent atmospheric oxygen), above which anaerobic microbial reactions are completely inhibited.

Туре	O₂ Relation
Aerobes	
Obligate	O ₂ is required
Facultative	O_2 is not required, but growth is better with O_2
Microaerophilic	O ₂ is required, but at lower-than-atmospheric levels
Anaerobes	
Aerotolerant	O_2 is not required, and growth is not better with O_2
Obligate (strict)	O ₂ is harmful or lethal

Table 6.4-2. Oxygen Relationship and Growth of Bacteria

Source: Madigan et al. 2003 [DIRS 171083].

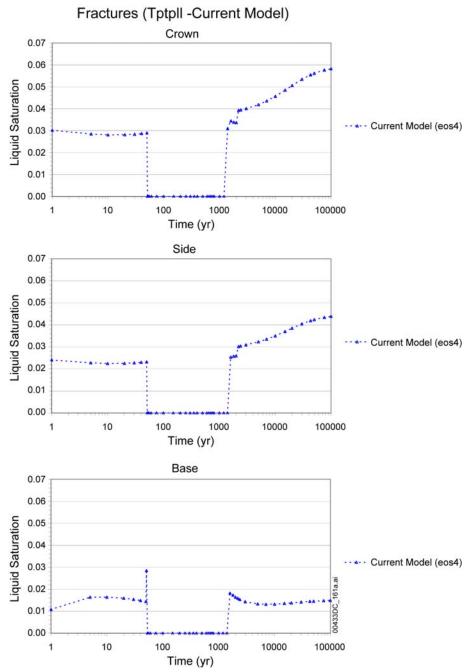


Source: BSC 2004 [DIRS 172463], Figure 6.5-8.

NOTE: Oxygen concentrations are proportional to air mass fractions.

Figure 6.4-2. Temporal Evolution of Air Mass Fractions in the Gas Phase in Fractures and Matrix at Three Drift-Wall Locations

Furthermore, 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 (Table 6.1-1). Chemical analyses of 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 Fe²⁺ and Mn²⁺), and NH₄⁺ (less than 1 ppm)



Source: BSC 2004 [DIRS 172463], Figure 6.5-6.

Figure 6.4-6. TH Simulation (Tptpll): Time Profiles of Modeled Liquid Saturations in Fractures at Three Drift-Wall Locations

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 (Pitonzo et al. 1999 [DIRS 150442]). 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 (VBNC) after a cumulative dose of 2.33 kGy. VBNC microorganisms lose the ability to grow (on media on which they have routinely been cultured) in response to the environmental stress imposed (i.e., radiation), but can be detected throughout the time course by means of direct fluorescence microscopy techniques. Two representative exopolysaccharide-producing isolates from Yucca Mountain were exposed to the same radiation regimen in sand microcosms. One isolate was much more radiation-resistant than the other, but based on culturable counts both had greater resistance than the general microbial community. However, when respiring cell counts (using only VBNC) were compared after irradiation, the results would indicate much more radiation resistance of the individual isolates and the microbial community in general. These results have significant implications for underground storage of nuclear waste, because they indicate that indigenous microorganisms are capable of surviving γ -irradiation in a VBNC state.

To further investigate the VBNC bacteria, portions of irradiated rock were placed at 4°C for 2 months, in an attempt to resuscitate the microbes to a culturable state (Pitonzo et al. 1999 [DIRS 150442]). Culturable heterotrophs were enumerated, and BIOLOG plates were used to determine the metabolic capability of the microbial community. Culturable bacteria that had previously been nonculturable were found at all doses. The number of colony types decreased from 26 in the nonirradiated control rock to between 9 and 10 in rock irradiated at doses ranging from 2.34 kGy to 9.34 kGy. BIOLOG plates indicated partial recovery of metabolic capacity in all tested samples. Using the MIDI system (Microbial ID, Inc.), fatty acid methyl ester analysis of the recovered isolates yielded three distinct groups of related bacteria. All resuscitated isolates clustered with the original nonirradiated isolates at the genus level, and 92 percent of them clustered at the species level. These results indicated that microbes were probably resuscitated from a VBNC state.

Based on the current repository loading design, the expected maximum surface dose rate due to gamma radiation from an unbreached waste package is estimated to 1160 rad/hour (=10.2 Mrad/year) (BSC 2005 [DIRS 173426], Table 2).

By comparison with the radiation dose resistance values listed in Table 6.4-7 and Table 6.4-8, 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 a nuclear fuel waste container is "sterilized" 9 to 33 days after emplacement (Stroes-Gascoyne 1996 [DIRS 171851]).

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