Irradiation of Microbes from Spent Nuclear Fuel Storage Pool Environments

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Abstract

Microbes have been isolated and identified from spent nuclear fuel storage pools at the Idaho National Engineering and Environmental Laboratory (INEEL). Included among these are *Corynebacterium aquaticum*, *Pseudomonas putida*, *Comamonas acidovorans*, *Gluconobacter cerinus*, *Micrococcus diversus*, *Rhodococcus rhodochrous*, and two strains of sulfate-reducing bacteria (SRB). We examined the sensitivity of these microbes to a variety of total exposures of radiation generated by a 6-MeV linear accelerator (LINAC). The advantage of using a LINAC is that it provides a relatively quick screen of radiation tolerance. In the first set of experiments, we exposed each of the aforementioned microbes along with four additional microbes, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Escherchia coli*, and *Deinococcus radiodurans* to exposures of 5×10^3 and 6×10^4 rad. All microbial specimens withstood the lower exposure with little or no reduction in cell population. Upon exposing the microbes to the larger dose of 6×10^4 rad, we observed two distinct groupings: microbes that demonstrate resistance to radiation, and microbes that display intolerance through a dramatic reduction from their initial population. Microbes in the radiation tolerant grouping were exposed to 1.1×10^5 rad to examine the extent of their resistance. We observe a correlation between radiation resistance and gram stain. The gram-positive species we examined seem to demonstrate a greater radiation resistance.

DISCLAIMER

Introduction:

Microbial-influenced corrosion (MIC) processes are receiving increased attention.[1-3] It is well known that MIC damage costs the petroleum industry hundreds of millions of dollars each year.[4] Additionally, the costs of MIC damage to historical buildings, monuments, works of fine art, and other antiquities are immeasurable. Though these losses are very real, our immediate concern is the possibility of MIC damage to cladding and containers holding spent nuclear fuel (SNF) housed in temporary storage pools at the Idaho Nuclear Technology Center (INTEC).

Reports of the observation of viable microbes in wet and dry SNF storage facilities is not novel, although it has not yet been unambiguously demonstrated that microbes can exist on SNF itself. Scientists in Canada have established a long-term study that has been concerned with microbes introduced during construction of the Underground Research Laboratory.[5-8] Recently, a group of our colleagues at the INEEL published an account detailing the existence of organisms on coupons placed within two of the storage pools at INTEC.[9] Though these research groups have observed microbes in storage facilities, nothing has been published thus far about the identity or radiation tolerance of the organisms. Resistance to radiation is significant because it implies that biofilms could survive on SNF.

It is well documented that some microbes such as *Deinococcus radiodurans (D. radiodurans)* survive in a radiation-rich environment.[10]. The tolerance of this organism to radiation may be related to its well-documented DNA repair mechanism, its peculiar cell wall/membrane structure, its high concentration of antioxidants, and its multiple copies of genetic information.[11] As a first step in examining possible MIC damage at INTEC, we have set out to isolate microbes from the SNF storage pools and determine their respective sensitivities to radiation.

Experimental:

Specimens were collected at two SNF storage pools, INTEC-603 and INTEC-666. The primary differences in these facilities are the age of the pool and the quality of the water within the pool. INTEC-603 is the older of the two, having been constructed in 1951-2. It is an unlined concrete basin with its water re-circulated through a closed-loop cooling system equipped with both sand and UV filtration. INTEC-666 is a stainless steel lined concrete basin that was constructed in 1980-5. The water in INTEC-666 is re-circulated through ion-exchange resins, UV irradiation, and treated by reverse osmosis in order to retain high purity. Table 1 lists important measures of the quality of the water in each facility. Samples were collected from low radiation locations within each pool. Microorganisms isolated from these samples were identified using either the Biolog technique[12] or a 16S rRNA technique.[13] Table 2 includes the identities of the bacteria with respect to INTEC location, growth conditions, and gram staining.

Each of the isolated bacteria grew from a single colony at least 24 hours prior to irradiation. Some isolates were slow growers and thus required longer incubation periods. Cell counts were determined microscopically before centrifuging the cultures (10,000 rpm for 10 minutes). Cell

pellets of each different culture were re-suspended in water and stored on ice until they were radiated. Each isolate was prepared in triplicate for irradiation.

In the course of the experiments, the microbes were irradiated for total exposures of approximately 5 x 10^3 rad, 6 x 10^4 rad, and 1.1 x 10^5 rad. The time of dose ranged from 7 minutes to 75 minutes, depending on the desired level of exposure. Following irradiation, each of the exposed samples were spread on agar-solidified growth material and colonies were counted after 24 hours and up to 10 days. Two controls were maintained throughout the irradiation study. *E. coli* without the *recA* gene was selected as our radiation sensitive control for the set of experiments. The lack of the *recA* gene prevents *E. coli* from engaging its SOS regulatory system, a complex cellular survival mechanism that repairs DNA.[11] *D. radiodurans* served as the radiation resistant control, because of its well-documented radiation tolerance. In addition, we included *P. aeruginosa* in the irradiation study because it is used in other associated MIC research.

The radiation dose was delivered using a 6-MeV LINAC in the Idaho State University LINAC Facility. Electrons within the LINAC were accelerated to a kinetic energy of 6-MeV and projected onto a tungsten target to produce the continuous bremsstrahlung and characteristic x-ray spectrum. A 0.64 cm thick aluminum plate was placed between the tungsten target and the specimen to both harden the photon beam and remove any high energy electrons that may have passed through the tungsten target. The best control of this instrument was achieved when attempts were made at controlling the absorbed dose (total exposure) delivered to a specimen. The dose rate was at best coarsely controlled due to the operational parameters that were tweaked manually by the operator during the course of an exposure. The radiation dose received by the specimen was determined using a combination of radiochromic dosimeters[14] and thimble-ionization chambers.[15] The response of the thimble-ionization chambers as a function of the magnitude of incident field strength was linear over a broad range of exposure rates. Calibration of these chambers was made versus absorbed doses in the 2 x 10^5 rad range as measured with the radiochromic dosimeters. This type of calibration correlates the positionally-dependent detectors response in units of Roentgen or Roentgen/minute, to units of absorbed dose (i.e. rad or rad/minute) for a particular photon energy spectrum. This calibration was performed within a day before or after experiments where the bacteria were irradiated.

Results:

The results from the irradiation experiments are displayed in Table 3. In this table mean populations are tabulated according to the amount of radiation absorbed. The second column represents the initial population prior to irradiation. We tracked an additional set of specimens over a six hour period in order to account for natural changes to the cell populations independent of irradiation. The value reported for each of the irradiated bacteria is corrected by a simple ratio calculation that accounts for the natural growth or death inherent to a specific microbial isolate. The data indicate about half of the microbes tested survive irradiation. Upon closer inspection it appears that of the four microbes originating from INTEC-666 only one, *C. aquaticum*, survived irradiation. Of the four microbes identified from INTEC-603, three survive irradiation. The overall trends are displayed in figure 1. This plot shows the surviving fraction of the initial cell

count as a function of the total amount of radiation absorbed by each microbial isolate. We observed two distinct trends: microbes that demonstrate a tolerance to large amounts of radiation and microbes whose populations are significantly reduced or totally killed off. The dashed diagonal line is provided to help show the separation between the two distinct sets of microbes. Points above the line represent organisms that display radiation resistance.

Discussion:

It is well documented that ionizing radiation does not kill cells directly, but acts indirectly by creating reactive free radicals.[11] These free radicals can complex, i.e. chemically alter, sensitive macromolecules in the cell leading to their inactivation. Though most of the macromolecules in the cell can be influenced in one way or another by interaction with ionizing radiation, it is the interaction with a DNA macromolecule that is often implicated in cell death. This is due to the fact that DNA is a unique molecule that often contains single copies of the genes that comprise it. Other macromolecules, such as proteins, may have several exact copies so inactivation of one will not lead to cell death. Proteins can also be re-synthesized if the genetic coding, DNA, remains intact. The role of oxygen is also significant in radiation processes. Three times as much radiation damage is observed in aerobic environments than in anaerobic conditions.[16] This is significant because our sulfate reducing bacteria (SRB) were grown, transported, and irradiated under anaerobic conditions. Therefore we believe that the radiation resistance demonstrated by the SRB's may not be a characteristic of the microbe, but be a function of the low oxygen concentration.

The variation in the radiation sensitivity of the other microbes may be due to differences that exist between gram positive and gram negative bacteria. We observed that three of the four gram-positive species (*D. radiodurans, C. aquaticum, M. diversus*, and *M. luteus*) exhibit a lower sensitivity to ionizing radiation. The exception, *R. rhodochrous*, does show radiation resistance at 6×10^4 rad, but its population is dramatically reduced by absorption of the larger total dose. It should be noted that the dose rate at the 1.1×10^5 rad level is almost twice as intense as the rates at the lower exposures. This may contribute to the observed deviation of the *R. rhodochrous* from the other three gram-positive organisms. In spore-producing gram-positive bacteria, it is known that the spores contain storage proteins that bind tightly to DNA, possibly protecting it from radiation damage.[17, 18] If all gram-positive organisms possess similar protection mechanisms that contribute to an intrinsic radiation resistance, it seems logical that larger numbers of gram-positive organisms versus gram-negative have been isolated from areas like Chernobyl, where high levels of radioactive contamination are present.[19]

We note that the radiation dose supplied by the LINAC in these experiments is more intense than "hot" SNF in the INTEC pools. This is an important point since the microbes determined to be radiation-sensitive by the bounds of our experiment are robust enough to survive within the INTEC SNF storage pools. As a final point, it should be stated that the number of organisms we have isolated and examined here is likely to represent only a small fraction of the total number of unique organisms present in the INTEC facilities, based upon our limited sampling opportunities.

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Figure Captions:

Table 1. Water Analysis at INTEC SNF storage pools INTEC-603 and INTEC-666.

Table 2. Identity, sampling origin, growth medium, and result of gram staining for each of the irradiated microbes.

Table 3. Data from the irradiation experiments. Each reported cell populations is an average value computed from three sets of triplicates, i.e. N=9. Statistical deviations are on the order of ±10%.

Figure 1. Plot of the fraction of surviving cells in each of the irradiated microbes versus the total absorbed dose in rad. Note the two groupings: microbial colonies that demonstrate resistance to the absorbed dose (above diagonal line), and those are killed off (below line).

Type of Analysis	INTEC-603	INTEC-666
Conductivity (μS / cm)	600	1.4
Chloride Concentration (ppm)	48	0.03
Nitrate Concentration (ppm)	160	NA
pH	8.5	5.7
^{60}Co (μCurie / mL)	4.5×10^{-8}	2.0×10^{-4}
^{137}Cs (μCurie / mL)	9.0×10^{-5}	2.2×10^{-6}
Total Sr (μCurie / mL)	9.8×10^{-5}	5.0×10^{-7}

Microbe Identity	Origin	Growth Medium	Gram Stain
Escherichia Coli	lab control	Luria Bertani Broth	-
Deinococcus radiodurans	lab control	Nutrient broth w/ 1% dextrose	+
Pseudomonas aeruginosa	lab addition	5% PTYG	-
Pseudomonas putida	INTEC-666	5% PTYG	-
Comamonas acidovorans	INTEC-666	5% PTYG	-
Corynebacterium aquaticum	INTEC-666	5% PTYG	+
Gluconobacter cerinus	INTEC-666	5% PTYG	-
Sulfate Reducing Bacteria A	INTEC-603	ATCC 1249 SRB media	-
Sulfate Reducing Bacteria B	INTEC-603	ATCC 1249 SRB media	-
Micrococcus diversus	INTEC-603	Nutrient broth w/ 1% dextrose	+
Rhodococcus rhodochrous	INTEC-603	5% PTYG	+
Micrococcus luteus	lab addition	5% PTYG	+

Microbe Identity	Mean Microbe Population for each absorbed dose			
	Initial	**~ 5×10^3 rad**	**~ 6×10^4 rad**	**~ 1.1×10^5 rad**
E. Coli	1.8×10^9	1.8×10^7	0	0
D. Radiodurans	9.5×10^6	7.1×10^6	3.8×10^6	9.9×10^6
P. aeruginosa	2.6×10^9	4.5×10^5	0	0
P. putida	8.0×10^8	1.5×10^7	2.7×10^2	0
C. acidovorans	5.4×10^8	2.1×10^6	0	0
C. aquaticum	2.7×10^9	9.9×10^8	7.4×10^5	8.1×10^4
G. cerinus	7.9×10^8	9.0×10^7	5.7×10^2	9.1×10^0
***SRB* A**	4.8×10^8	1.4×10^8	1.4×10^6	1.9×10^7
***SRB* B**	2.0×10^7	4.8×10^7	6.1×10^7	1.0×10^6
M. diversus	2.1×10^8	1.3×10^8	1.3×10^7	2.7×10^4
R. rhodochrous	1.5×10^8	1.8×10^8	2.1×10^5	7.7×10^0
M. luteus	9.7×10^6	3.4×10^7	3.6×10^6	2.2×10^6

Table 1 (top), Table 2 (center), and Table 3 (bottom)

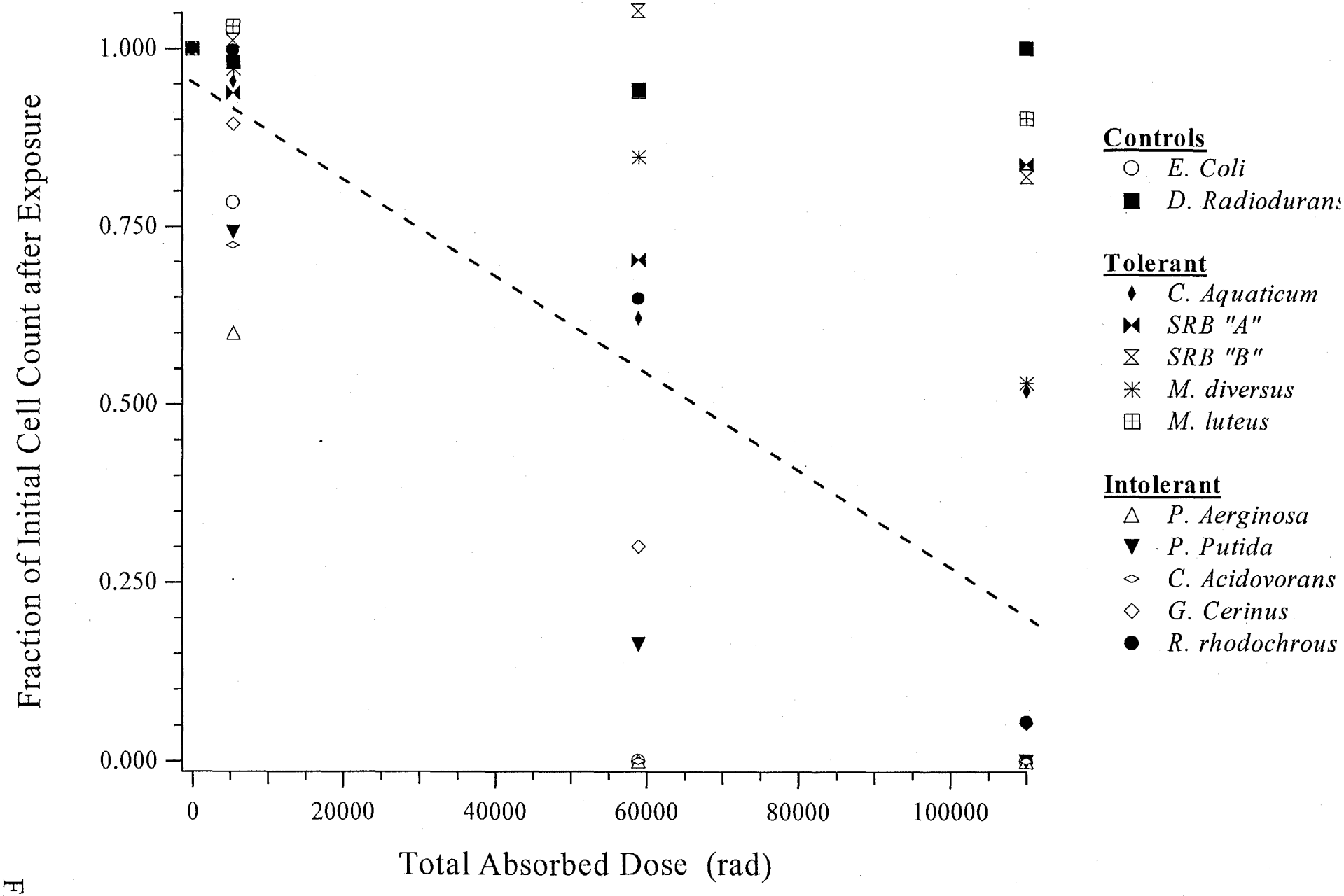
Fraction of Initial Cell Count after Exposure
1.000
0.750
0.500
0.250
0.000
0
20000
40000
60000
80000
100000
Total Absorbed Dose (rad)
Controls
E. Coli
D. Radiodurans
Tolerant
C. Aquaticum
SRB "A"
SRB "B"
M. diversus
M. luteus
Intolerant
P. Aerginosa
P. Putida
C. Acidovorans
G. Cerinus
R. rhodochrous

Figure 1