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Microbial biofilm growth on irradiated, spent nuclear fuel cladding

D.F. Bruhn [a], S.M. Frank [b,*], F.F. Roberto [a], P.J. Pinhero [a,c], S.G. Johnson [b]

[a] Science and Technology Campus, Idaho National Laboratory, Idaho Falls, ID 83415-2218, United States
[b] Materials and Fuels Complex, Department of Pyroprocessing Technology, Idaho National Laboratory, P.O. Box 1625, Idaho Falls, ID 83414-6150, United States
[c] Department of Chemical Engineering, University of Missouri, Columbia, MO 65211, United States

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ABSTRACT

A fundamental criticism regarding the potential for microbial influenced corrosion in spent nuclear fuel cladding or storage containers concerns whether the required microorganisms can, in fact, survive radiation fields inherent in these materials. This study was performed to unequivocally answer this critique by addressing the potential for biofilm formation, the precursor to microbial-influenced corrosion, in radiation fields representative of spent nuclear fuel storage environments. This study involved the formation of a microbial biofilm on irradiated spent nuclear fuel cladding within a hot cell environment. This was accomplished by introducing 22 species of bacteria, in nutrient-rich media, to test vessels containing irradiated cladding sections and that was then surrounded by radioactive source material. The overall dose rate exceeded 2 Gy/h gamma/beta radiation with the total dose received by some of the bacteria reaching 5×10^3 Gy. This study provides evidence for the formation of biofilms on spent-fuel materials, and the implication of microbial influenced corrosion in the storage and permanent deposition of spent nuclear fuel in repository environments.

1. Introduction

Biofilm formation [1–3], biofouling [4], and microbial influenced corrosion (MIC) [5] have all been observed in nuclear power plant water cooling systems and spent-fuel storage pools [6–8], and methods have been developed to mitigate adverse affects [9,10]. Microbial communities have also been characterized at high-level or transuranic (TRU) nuclear waste repository sites [11–13], and the potential for MIC of storage containers in these environments has been investigated without the presence of high-radiation fields [14–16]. However, what is less certain in high-level nuclear waste repositories is the effect of high-dose, long-term ionizing radiation on the establishment of biofilms on the surfaces of spent fuel and the importance of MIC on the long-term storage of spent nuclear fuel (SNF).

The link between microbial growth and MIC has been observed and studied on metallic systems for many years [5,17,18]. In the past it was generally believed that microbial growth on irradiated fuel assemblies or high-level waste was not possible because of the high-radiation field. However, recent evidence has shown that microorganisms are capable of surviving moderate radiation fields. At the Idaho Nuclear Technology and Engineering Center (INTEC) at Idaho National Laboratory (INL), microorganisms were collected and identified from spent-fuel storage pools [19]. At the Savannah River Site, bacteria were identified in spent nuclear fuel storage basins, including sulfate-reducing bacteria (SRB) and acid-producing bacteria, both biocorrosion-relevant microbial groups [8]. At the Department of Energy (DOE) Hanford Site, viable microorganisms were cultivated from vadose sediment samples from a high-level nuclear waste plume [20], as were microorganisms investigated at the Chernobyl Site [21].

Studies to investigate the survivability of microorganisms to high-intensity, acute radiation exposure have also been performed. In an investigation at INL, individual microbes isolated from the INTEC storage pools were exposed to acute levels of ionizing radiation using a 6 MeV linear accelerator [19]. Results showed that many exposed microbe species survived and even flourished in these high-radiation fields, which suggests that these radiation-resistant microbes could produce biofilms on waste storage containers or on spent-fuel cladding and influence the corrosion of these materials.

The purpose of the work discussed in this paper was to expand the initial studies involving the short-duration, high-intensity irradiation of individual microbe species and to explore the effects of chronic, high-dose radiation exposure on biofilm communities in conditions more characteristic of SNF storage environments. This work describes, for the first time, direct observation of microbes forming biofilms on irradiated spent-fuel cladding in high-radiation fields. The observation of this little considered phenomenon may be of significant importance to fuel handlers and waste generators alike.

* Corresponding author. Tel.: +1 208 533 7391; fax: +1 208 533 7471.
E-mail addresses: steven.frank@inl.gov (S.M. Frank), pinherop@missouri.edu (P.J. Pinhero).

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2. Experimental

The experiment was conducted in a radiological enclosure (hot cell) at the Materials and Fuels Complex (MFC) on the INL Site. The experiment involved submerging spent-fuel cladding segments, used as a support for biofilm growth, into test vessels containing nutrient-rich media with selected microbes. The test vessels were then surrounded by radioactive source material to expose the microbes to a high-dose of ionizing radiation.

The spent-fuel cladding came from fuel rods that had been removed from the Experimental Breeder Reactor-II (EBR-II) at INL. The spent-fuel rods, clad in 304 L stainless steel, had previously been chopped into the smaller segments approximately 13 mm in diameter by 13 mm in length. The fuel had been anodically dissolved from the cladding segments in an electrorefiner (ER) as part of the DOE Spent-Fuel Demonstration Project [22]. Four irradiated cladding segments and one non-irradiated segment of the same material and dimensions were used in the experiments to support biofilm growth. All cladding segments were washed in 6 N nitric acid and rinsed in sterilized water prior to insertion into the test vessels. The test vessels, 15 cm Pyrex® screw cap tubes, had previously been autoclaved before addition of media.

The microbes used in this study were originally isolated, cultured, and identified from water samples collected from INTEC spent-fuel storage pools at INL [19] and other sources listed in Table 1. The test was initiated by adding 20 mL of trypticase Soy Broth (TSB) medium and $\sim 4 \times 10^7$ cells/mL of each of the bacteria listed in Table 1 to the test vessels. The TSB medium consisted of Bactotryptone-pancreatic digest of casein 17 g/L, Soytone papaic digest of soybean meal, 10 g/L dextrose 2.5 g/L, sodium chloride 5 g/L, and dipotassium phosphate 2.5 g/L as a pH buffer. A second inoculation was also performed on two cladding segments containing 20 mL of the same medium with $\sim 1 \times 10^7$ cells/mL. No additives or buffering were added to the vessels during the duration of each experiment.

All test vessels were then transferred into the hot cells, and an irradiated cladding segment was added to each test vessel. One test vessel was used as a negative control and contained only sterile media and an irradiated cladding segment. The test vessels were placed in a steel test-tube rack and surrounded with radioactive material to simulate the radiation field associated with SNF. A positive control containing non-irradiated cladding, medium and bacteria was maintained outside of the radiation area during the study to monitor bacterial growth without exposure to radiation. Fig. 1 shows hot cell manipulation of the test equipment and Fig. 2 shows test vessels containing cladding hull segments viewed through the hot cell window.

Table 1
Bacteria used in the experiment. Several species were isolated and identified from storage pools at the Idaho Nuclear Technology Center on the INL Site in Idaho. The specific storage pools are designated 603 or 666. In addition several other species of bacteria were used in the experiment including anaerobic and sulfur-reducing bacteria.

Deinococcus radiodurans from Dr M.J. Daly, Uniformed Services University of the Health Sciences, Bethesda, MD
Pseudomonas aeruginosa American Type Culture Collection (ATCC) 14886
INL 666 isolate *Aureobacterium testaceum* (formerly *Microbacterium testaceum*)
INL 666 isolate *Pseudomonas monteilii*
INL 603 isolate #2 *Rhodococcus* sp.
INL 603 isolate #3 *Pseudonocardia saturnea* (formerly *Amycolata saturnea*)
INL 603 isolate #3A close to *Taxeobacter gelupurpurascens*
INL 603 isolate #4 *Sphingomonas* sp.
INL 603 isolate #9 close to *Sphingomonas* sp.
INL 603 isolate #11 and #12 related to *Rhodococcus* group
INL 603 isolate #13 related to *Rhizobium*
INL 603 isolate #15 *Sphingomonas* sp.
INL 603 isolate #17 *Micrococcus luteus*
INL simulated sludge isolate white related to *Agromyces fucosus*
INL simulated sludge isolate orange *Sphingomonas*
Sulfate-reducing bacterium (SRB) *Desulfovibrio desulfuricans* ATCC 27774
Sulfate-reducing bacterium (SRB) *Desulfovibrio vulgaris* ATCC 29579
INL simulated sludge isolate (SRB) *Desulfotomaculum guttoideum*
INL simulated sludge isolate Anaerobic bacterium *Clostridium subterminale*
INL simulated sludge isolate Anaerobic bacterium *Clostridium celerecrescens*

2.1. Sampling and identification of biological activity

Both the media solution and cladding segments were periodically sampled for biological activity over the course of the test period. This involved pouring a portion of the media solution from the test vessel into a new sterilized container. The cladding segment was then dropped into a sterilized stainless-steel basket. The cladding segment was rinsed with sterile water to remove any residual media solution. Two sterilized cotton swabs were then used to swab the inside and outside of the cladding segment. After swabbing, the cotton swabs were placed back into sterile containers. The swabs and media were transferred out of the hot cells for culture growth and subsequent identification.

Two of the cladding segments were sampled for biological activity after 33 days. After sampling, the two segments were cleaned by soaking them in concentrated nitric acid and then rins-

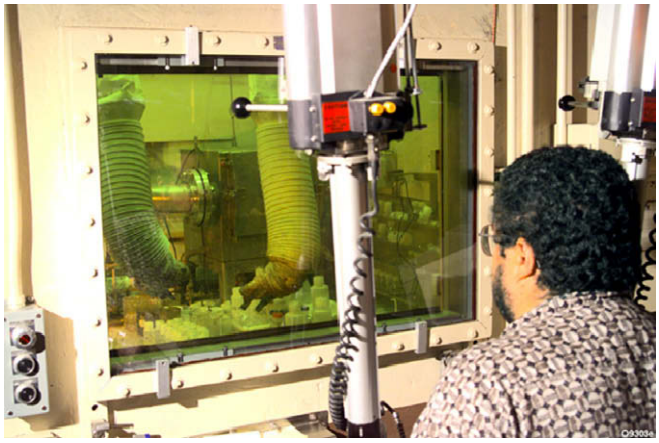

Fig. 1. Hot cell manipulation of test vessels containing microbes, nutrient-rich media, and cladding segments in a high-radiation field.

Fig. 2. Test vessels containing media and cladding segments in hot cell. The vessel on the left shows the cloudy media indicative of high biological activity after 34 days in cell for a total absorbed dose of 1.6×10^3 Gy. The vessel on the right is a control containing a cladding segment and sterile media.

ing them in sterile water. The cladding segments were then placed in new sterile test vessels that contained fresh media and microbes. The test vessels were then returned to the test-tube rack in the hot cell for the duration of the experiment. The sampling times and total radiation exposure for all cladding samples are given in Table 2.

The media solutions were serially diluted and plated on TSA (TSB with 15 g/L agar) with 1% dextrose. The swabs from the cladding were also streaked on TSA/1% dextrose plates and then transferred into Bacti SRB vials containing API anaerobic media (Sherry Laboratories, USA). The anaerobic media consisted of ammonium phosphate, dipotassium phosphate, yeast extract, sodium lactate, magnesium sulfate, and a nail (non-galvanized carbon steel) to provide iron. The Bacti SRB vials were used to culture sulfate-reducing bacteria and were serially diluted out to 10^{-8} cells/mL.

To identify what species survived the radiation exposure; colonies were isolated from the diluted media solution, the cladding segment swabs grown on TSA/1% dextrose plates, and the liquid serial dilutions of Bacti SRB vials. After single colony isolates were obtained, genomic DNA was extracted. The protocol used for identification and characterization of bacteria relies on comparative nucleic acid analysis. The genes of interest are the genes that code for the small subunit ribosomal RNA (16S rRNA). Comparative phylogenetic analysis of 16S rDNA sequences leads to the identification of microorganisms at the genus and species level. Total DNA was isolated by using the UltraClean™ Soil DNA Kit (MO BIO Laboratories, Inc., USA) as recommended by the supplier. Polymerase chain reaction (PCR) reactions for the amplification of partial 16S rDNA were performed with the primer combination 8F (5′AGAGTTTGATCCTGGCTCAG)/1492R (5′GGTTACCTTGTTACGACTT). Purified PCR products were ligated into pCR ®2.1-TOPO. Recombinant plasmid DNA was sequenced bi-directionally with infrared fluorescent-labeled primers. The sequencing reactions were analyzed on a LI-COR 4200 automated DNA sequencer. To identify the surviving bacteria the 16S rDNA sequences obtained from these isolates were compared to the 16S rDNA sequences of the bacteria isolates used to inoculate the cladding segments.

2.2. Radiation source material

The radioactive source material used to irradiate the microbes was originally EBR-II SNF. The spent fuel had either been sent to the MFC Analytical Chemistry Laboratory hot cells for dissolution and chemical analysis, or the fuel underwent treatment in the ER and then was transferred to the hot cells. The majority of the radioactive source material was dissolved fuel in liquid form. The remainder of the radioactive source material was either ER salt containing fission products from the treatment of the spent fuel, or a solid ceramic material in which the ER salt had been encapsulated. The radiation field or dose rate from SNF is dependent on many factors including fuel composition, fuel burnup rates, location and residence time in the reactor, cooling time of the fuel after removal from the reactor, and distance from the fuel source. The applied radiation field used in this experiment was approximately 2 Gy/h and is representative of SNF and other high-level nuclear waste after cooling times of approximately one year or longer after removal from the reactor core [23]. The total radioactivity associated with the spent EBR-II fuel was calculated to be 1.3×10^5 Bq/g [24].

The beta-to-gamma emission ratio for EBR-II fuel is approximately 10:1; however, for the ER salt or ceramic waste form, the beta-to-gamma ratio is approximately 20:1 due to radionuclide segregation during the ER treatment. The beta radiation field is attenuated due to shielding by the dissolver solutions and sample containers. The major fission product gamma emitter is ^{137}Cs (^{137}Cs beta decay to ^{137M}Ba followed by photon emission at 0.661 Mev), and the major fission product beta emitters are ^{90}Sr and the ^{90}Sr daughter product ^{90}Y, with beta emission energies of 0.546 and 2.281 Mev, respectively. The irradiated cladding also contributes to the total radiation field. Radionuclides in the steel material are produced by neutron activation while in the reactor, with the greatest gamma contribution from ^{60}Co (1.173 and 1.33 Mev). The inside of the cladding also has a radioactive contribution from ^{137}Cs, a fission product contaminate from the fuel.

Table 2
Results from media and cladding segment sampling for biological activity. Also shown is the duration of exposure and the total dose acquired by the microbes for each test sample and experiment number.

Sample/experiment ID	Media	Duration (days)	Control	Growth on Swab		SRB		Liquid media (cells/mL)
		Total dose (Gy)	Start at sampling (cells/mL)	In	Out	In	Out	
Cladding 1 First inoculation Experiment 1	Media/microbes	14 570	4.3×10^7 1×10^9 c	+ a	+	+ 10^5	+ 10^4	3×10^8 e
Cladding 2 First inoculation Experiment 2	Media/microbes	34 1.6×10^3	4.3×10^7 4.8×10^8 c	+ e	+ a,e	+ 10^4	+ 10^3	1.5×10^7 a,e
Cladding 2 Second inoculation Experiment 3	Media/microbes	36 1.8×10^3	4.3×10^7 2.3×10^8	+ a,e	+ a,e	+ 10^3	+ 10^5	1×10^8 a,c,e
Cladding 1 Second inoculation Experiment 4	Media/microbes	64 1.8×10^3	4.3×10^7 1×10^6	+ a	+ a	+ 10^2	+ 1–10	2×10^7 a,d
Cladding 3 Experiment 5	Media/microbes	99 4.9×10^3	4.3×10^7 1×10^5	–	–	–	–	1×10^7 b,c
Cladding 4 (Nonirradiated) Experiment 6	Media/microbes	97 4.8×10^3	4.3×10^7 1×10^5	– + a,b	– + a,b	– –	– –	1×10^7 a,b,d
Cladding 5 Control	Sterile media, no microbes	97 4.8×10^3	4.3×10^7 1×10^5	–	–	–	–	No growth

a, *Aureobacterium testaceum*; b, *Micrococcus luteus*; c, *Pseudomonas aeruginosa*; d, *Pseudomonas monteilii*; and e, *Rhodococcus* sp.

2.3. Dosimetry

Absorbed beta and gamma dose measurements were performed using LiF thermoluminescent dosimeters (TLDs). The TLDs had dimensions of 20×20 mm and were sealed in polyethylene pouches. The pouches were placed in containers similar to the test vessels containing media and cladding segments. Water was added to the containers holding the TLDs to the same approximate height as the media used in the test vessels (~30 mL). Several TLDs were used for each measurement. These were used to determine the background radiation levels in the hot cells, to measure the dose levels of the radiation fields surrounding the test vessels, and to measure the dose of one of the four irradiated cladding segments.

During each measurement, an irradiated cladding segment was removed from its test vessel, and the segment and media were sampled for biological activity as described earlier. The cladding segment was then rinsed with water and placed into one of the containers holding the TLD and water. The container was placed in the same rack location as the test vessel from which the cladding had been collected, and the rack was moved to its storage location in the hot cell and left for approximately 10 min.

After this time period, the cladding segment was removed from the vessel containing the TLD, and a second vessel with TLD but no cladding was placed in the same rack position for the same time period. After exposure, the TLDs were removed from the hot cells and transferred to a fume hood and removed from the protective pouches. The total absorbed dose from each TLD was then determined and corrected for background. In this way, the dose from the cladding segment and from the radioactive material surrounding the test vessels was determined. The uncertainty of the measured radiation dose from each TLD was approximately 10% relative standard deviation.

Dosimetry was performed shortly after the experiment was initiated and at the termination of the experiment. The first dosimetry measurement indicated an average adsorbed beta/gamma field of 1.7 Gy/h. Because it was desired to expose the microbes to the largest possible radiation field during the biofilm growth experiment, additional source material (dissolved EBR-II fuel) was placed near the test vessels. This occurred after 14 days from the start of the experiment. After the second dosimetry measurement, it was determined that the total field surrounding the irradiated cladding segments in the test vessels with irradiated cladding was 2.1 Gy/h. The non-irradiated cladding segment was calculated to have a lower field of 1.0 Gy/h after the initial dosimeter measurement and 1.4 Gy/h after the second measurement.

3. Results

Positive indication of biological activity was determined by (1) the presence of bacterial growth on culture plates after they were streaked with sterile wire loops dipped in the irradiated media solution or (2) bacterial growth on the swabs used to sample the cladding surfaces. If positive growth was observed on the culture plates, the colonies were collected for subsequent identification. Sulfate-reducing bacteria were identified by observing growth (formation of iron sulfide) in the Bacti SRB vials. Table 2 lists the microbial activity of the media and cladding segments for each sampling. The table lists culture plate (+ for growth, – for no growth), SRB growth, and media growth. Also shown is the positive control growth (no radiation exposure) at the start of each experiment and at each cladding segment sampling time, and total absorbed dose. The following describes each individual experiment's sampling procedure, the biological activity for each cladding segment sample, and the biological activity of the positive control sample.

Experiment #1, cladding segment #1, first inoculation was incubated for 14 days with a dose rate of 1.7 Gy/h for a total dose of 5.7×10^2 Gy. There was biofilm formation both inside and outside the irradiated cladding. The growth on the culture plate was very dense. Sulfate-reducing bacteria were present in the biofilm both inside and outside the cladding. Serial dilution of SRB, from the swabs used to sample the biofilm, grew to 10^5 cells on the inside and 10^4 cells on the outside of the cladding. The liquid culture had 3×10^8 cells/mL after the experiment. *Rhodococcus* sp. were isolated and identified from both the outside of the cladding and the liquid. The positive control sample was 20 mL of media inoculated at the start of the experiment and contained an unirradiated cladding segment, but was not exposure to radiation. Starting bacteria numbers were 4.3×10^7 cells/mL and had grown to 1×10^9 cells/mL with only *Pseudomonas aeruginosa* remaining after 14 days.

Experiment #2, cladding segment #2, first inoculation was incubated for 34 days with a dose rate of 1.7 Gy/h for 14 days and 2.1 Gy/h for 20 days. The total dose was 1.6×10^3 Gy. There was biofilm formation both inside and outside the irradiated cladding. Dense culture growth was observed on the streaked plates. SRB were present in the biofilm both inside and outside the cladding. Serial dilution of SRB from the swabs used to sample the biofilm grew to 10^3 cells on the inside and 10^4 cells on the outside of the cladding. The liquid culture had 1.5×10^7 cells/mL (initial 4.3×10^7 cells/mL) after the experiment. *Rhodococcus* sp. were isolated and identified from the inside and outside of the cladding. *Aureobacterium testaceum* was isolated from the outside of the cladding and the liquid. The positive control culture, which received no radiation, started at 4.3×10^7 cells/mL and had 2.3×10^8 cells/mL after 35 days.

Experiment #3, cladding segment #2, second inoculation was incubated for 36 days with a dose rate of 2.1 Gy/h. The total dose was 1.8×10^3 Gy. There was biofilm formation both inside and outside the irradiated cladding. Growth was dense on the culture plate. SRB were present in the biofilm both inside and outside the cladding. Serial dilution of SRB from the swabs used to sample the biofilm grew to 10^3 cells on the inside and 10^5 cells on the outside of the cladding. The liquid culture had 1×10^8 cells/mL after the experiment. *Rhodococcus* and *A. testaceum* were isolated and identified from the inside and outside of the cladding. *A. testaceum*, *Rhodococcus* sp., and *P. aeruginosa* were isolated from the liquid. The positive control culture, which received no radiation, started at 4.3×10^7 cells/mL and had 2.3×10^8 cells/mL after 35 days.

Experiment #4, cladding segment #1, second inoculation was incubated for 64 days with a dose rate of 2.1 Gy/h. The total dose was 3.2×10^3 Gy. There was biofilm formation both inside and outside the irradiated cladding. Growth was light on the streaked plates. SRB were present in the biofilm both inside and outside the cladding. Serial dilution of SRB from the swabs used to sample the biofilm grew to 10^2 cells on the inside and only 10 cells on the outside of the cladding. The liquid culture had 2×10^7 cells/mL after the experiment. *A. testaceum* was isolated and identified from the inside and outside of the cladding. *A. testaceum* and *P. aeruginosa* were isolated from the liquid. The positive control culture, which received no radiation, started at 4.3×10^7 cells/mL and had 1×10^6 cells/mL after 64 days.

Experiment #5, cladding segment #3 was incubated a total of 99 days: 14 days with a dose rate of 1.7 Gy/h, and 85 days with a dose rate of 2.1 Gy/h. The total dose was 4.9×10^3 Gy. There was no biofilm (including SRB) on the cladding. The liquid culture had 1×10^7 cells/mL after the experiment. *P. aeruginosa* and *Micrococcus luteus* were isolated from the liquid. The positive control culture, which received no radiation, started at 4.3×10^7 cells/mL and had 1×10^5 cells/mL after 99 days.

Experiment #6, cladding segment #4, which used a non-irradiated cladding, was incubated a total of 97 days. The radiation dose was estimated by subtracting the 0.7 Gy/h contributed by the cladding. Total exposure was thus 14 days with a dose rate of 1.0 Gy/h and 83 days with a dose rate of 1.4 Gy/h. The total dose was 3.2×10^3 Gy. There was biofilm formation both inside and outside the cladding. There were no SRB recovered from the cladding. *A. testaceum* and *M. luteus* were isolated and identified from the inside and outside of the cladding. The liquid culture had 1×10^7 cells/mL after the experiment. *Pseudomonas monteilii*, *A. testaceum*, and *M. luteus* were isolated from the liquid. The positive control culture started at 4.3×10^7 cells/mL and had 1×10^5 cells/mL after 99 days.

Negative control, cladding segment #5 was the sterile radiation control. The cladding and media were incubated 97 days and handled the same as the rest of the samples. There was no growth on the cladding and no growth in the liquid at the termination of the experiment. This demonstrated that bacteria were not introduced during the set-up and sampling in this experiment.

4. Discussion

Fig. 3 shows the overall biological activity for microbes in the media solution and for SRB from the cladding segment surface during the duration of the experiment. The total absorbed dose is also shown in Fig. 3. The media cell count (cells/mL) initially increased to 3×10^8 and then fell to $1–2 \times 10^7$, where it remained until the termination of the experiment. The SRB from the biofilm on the inside and outside of the cladding segments had maximum growth from approximately 20 to 40 days and then decreased to no observed growth by the end of the test period.

Many of the microbes used in this study were originally isolated, cultured, and identified from water samples collected from INTEC SNF storage pools at INL [19]. These microbes were probably introduced by windblown soil into the SNF storage pools. The microbes in the SNF storage pools included a large percentage of Gram-positive, high G + C content (guanine + cytosine-DNA bases) bacteria that are typical inhabitants of surface soil and vadose sediment organisms [19,20]. Colwell sampled the subsurface and surface soil at INL and found a lower number of microbes in the subsurface compared to the surface, with 84% being Gram-positive [25].

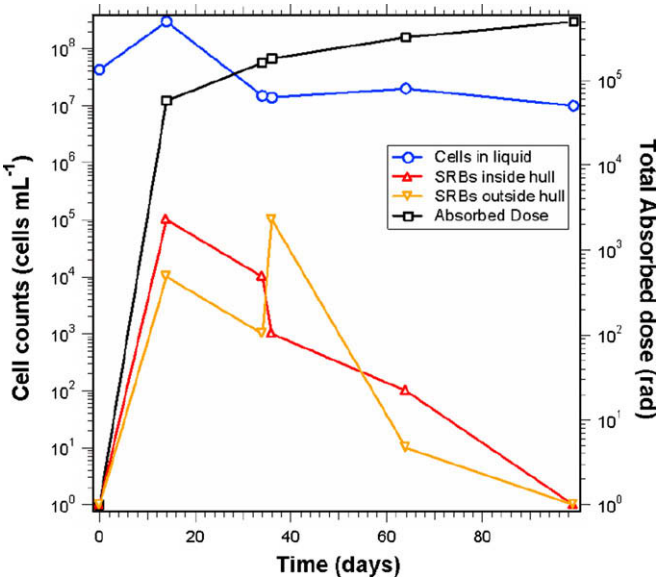

Fig. 3. Cell count versus time for absorbed dose for both microbes in media and sulfate-reducing bacteria collected from a biofilm on the cladding segment surface.

Characterization of the subsurface microbial culture collection of aerobic chemoheterothrops from DOE's Hanford Site, in Richland, Washington, USA, showed that 43% were Gram-positive, high G + C content bacteria including *Micrococcus*, *Rhodococcus*, *Arthrobacter*, and *Streptomyces* [26]. White et al. analysis of the genome of *Deinococcus*, one of the most radiation-resistant organisms known (Gram-positive, high G + C content), hints that *Deinococcus* gained some of its extraordinary survival mechanisms by adapting to harsh, dry environments [27]. *Chroococcidiopsis* has demonstrated a correlation between high resistance to desiccation and ionizing radiation [28]. Gram-positive, high G + C content bacteria were among the most common genera represented of cloned sequences of bacteria isolated from contaminated (5×10^6 Bq of cesium-137 per gram sediment) vadose zone (aerobic) sediments beneath a high-level radioactive waste tank at the DOE Hanford Site [20]. The hardy Gram-positive, high G + C content bacteria that survived this experiment, *A. testaceum*, *M. luteus*, and *Rhodococcus*, came from the harsh dry environment of INL, which is similar to that of the Hanford Site. High G + C content in bacteria has been shown to protect DNA against the damaging effects of thymine dimerization [29]. Gram-positive bacteria have increased cell wall peptidoglycan and produce catalase and superoxide dismutase, which may readily block toxic radiolytic products responsible for most of the indirect effects of ionization radiation [30].

The bacteria that tolerated the acute dose of 1.1×10^3 Gy in a previous experiment were *A. testaceum*, *M. luteus*, and anaerobic bacteria including SRB [19]. Except for *Rhodococcus* sp., these are the same bacteria that survived prolonged exposure to a total 1.8×10^3 Gy in this study. *A. testaceum*, *M. luteus*, and anaerobic bacteria including SRB also survived a higher dose of 3.2×10^3 Gy, while *Rhodococcus* sp. did not survive this dosage, although it is likely that it was out competed by faster growing microbes as opposed to radiation damage. In general, this shows a correlation between the ability to survive acute radiation doses and long-term survival at lower radiation rates amounting to higher total radiation exposure. The ability of the anaerobic bacteria, including SRB, to tolerate high-doses of ionizing radiation may be due to the lack of oxygen in the micro-niche that these bacteria inhabit. Ionizing radiation can produce reactive oxygen species, which can damage DNA, RNA, lipids, and proteins [31]. In this situation, an oxygen-free environment has less (or no) reactive oxygen species to form free radicals and reduces adverse ionizing radiation affects to the SRB.

5. Conclusion

The work presented here shows that a mixed biofilm was detected to have formed on irradiated, stainless-steel fuel cladding material. This biofilm contained Gram-positive, high G + C content, heterotrophic bacteria species identified as *A. testaceum*, *M. luteus*, and anaerobic bacteria including SRB. These biofilms survived on irradiated cladding hulls for up to 64 days with a total absorbed dose of 3.2×10^3 Gy. The formation of this biofilm, including SRB that have been implicated in enhanced corrosion of metallic materials, demonstrates that MIC is possible on waste storage containers or on spent nuclear fuel cladding.

Additionally, it was shown that various bacteria can survive in high-radiation fields of approximately 2 Gy/h in a rich media for a total dose 4.9×10^3 Gy (0.5 Mrad). Bacterial species *A. testaceum*, *Rhodococcus* sp., *P. aeruginosa*, *M. luteus*, *P. monteilii*, and SRB were isolated from the media solution with cell counts of 10^7 cells/mL after 99 days. Some of these bacteria were able to tolerate radiation exposure as much as five-times greater than tolerated in acute

radiation exposure experiments [19]. This demonstrates that bacteria can survive higher dosages of long-term, lower ionizing radiation exposure than short-term or acute ionizing radiation exposure. Long-term, lower ionization radiation exposure may allow for repair of damaged DNA before irreversible damage is sustained.

The recovery of Gram-negative organisms in the liquid media indicates that they survive as planktonic cells, but not part of a biofilm. The irradiated, stainless-steel fuel cladding material was exposed to beta and gamma radiation, and the damage caused by beta radiation may have caused cellular damage that was more difficult to repair. It appears that only the highly radiation-resistant, Gram-positive bacteria and SRB were able to survive in this environment. The lack of survival of *Deinococcus radiodurans*, which has been shown to have high-radiation resistance [27] can possibly be explained by its inability to compete in a mixed culture with faster growing *Pseudomonas* sp.

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