National Aeronautics and Space Administration

Efficacy of far-UVC light to reduce microbial bioburden during spacecraft assembly

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Program: Innovative Spontaneous Concepts

Project Objective:

•To ensure compliance with planetary protection requirements set forth by NASA, the Biotechnology and Planetary Protection group at JPL has been closely monitoring the microbial burden of spacecraft hardware since the Viking mission.

•Currently several techniques are employed as methods of microbial reduction including IPA wiping, vapor hydrogen peroxide, and dry heat microbial reduction. However these techniques are typically time consuming, disruptive to the assembly process, and susceptible to recontamination without appropriate mitigation efforts.

•The use of germicidal ultraviolet light (primarily of 254 nm) is a well-established direct approach to kill or inactivate microorganisms. Germicidal UV irradiation (UVGI) can efficiently inactivate both drug-sensitive and multi-drug-resistant bacteria, as well as different strains of viruses. However, the widespread use of germicidal ultraviolet light in any environment where human exposure is possible has been limited by the human health hazard posed by conventional germicidal UV light that is carcinogenic and cataractogenic.

•Collaborators at Columbia University have developed a novel approach utilizing far-UVC light in the 207-222 nm wavelength range to inactivate microorganisms such as MRSA (methicillin resistant staphylococcus aureus and various viruses).

•In an effort to develop this technology for PP applications, we have shown that far-UVC light is highly effective at killing hardy microorganisms including *Acinetobacter radioresistens* 50v1 and spores of *Bacillus pumilus* SAFR-032 both of which have previously been isolated from spacecraft and associated surfaces.

•The primary advantage of this technique in comparison to traditional UV light at 254 nm is the lack of negative human health impacts. Given its limited penetration depth, this wavelength of UV is effective at penetrating and killing microorganisms which are typically <1 µm, however it cannot penetrate the stratum corneum, ocular cornea, nor the cytoplasm of individual human cells.

FY18/19 Results:

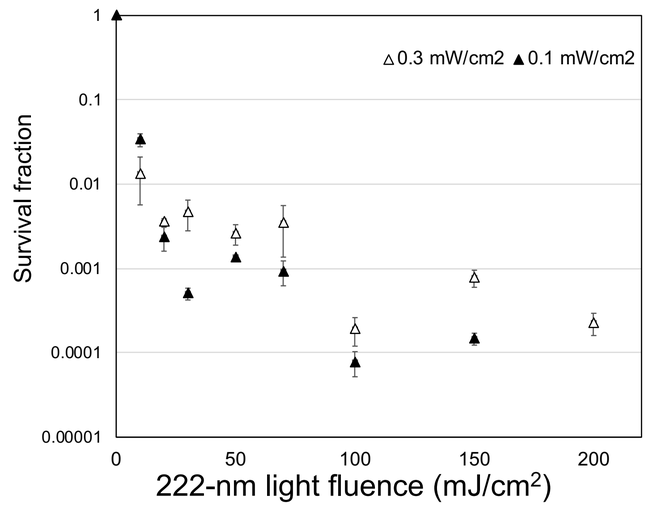

Figure 4. Survival of *B. pumilus* SAFR-032 spores exposed to different fluences of 222-nm light delivered at 0.1 or 0.3 mW/cm² (average ± SEM).

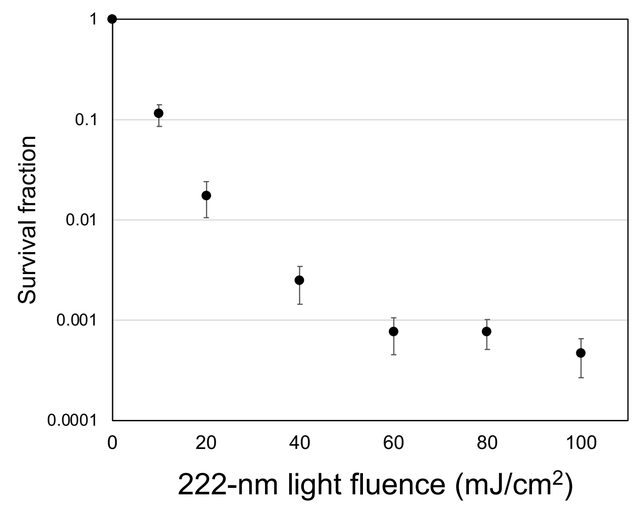

Figure 5. Survival of vegetative *A. radioresistens* 50v1 exposed to different fluences of 222-nm light (average ± SEM).

Efficacy of far-UVC light to inactivate bacterial spores and hardy vegetative cells

- Far-UVC light principally at the 202 nm wavelength with a fluence rate of 0.5 mW/cm² is effective at killing hardy cells of *Acinetobacter radioresistens* 50v1 with a putative D_{90} value of 16.9 mJ/cm² calculated for total fluences up to 60 mJ/cm²
- Far-UVC light is also effective at killing bacterial spores of *Bacillus pumilus* SAFR-032 exposed at a fluence rate of 0.3 mW/cm² produced a putative D_{90} value of 10.2 mJ/cm² calculated for values up to 40 mJ/cm².

Effects of fluence rate on the efficacy of far-UVC light

- The effect of varying fluence rate are insignificant on the putative D_{90} doses required to inactivate spores of Bacillus pumilus SAFR-032.
- There was a significant difference in D_{90} dose required to inactivate spores of *Bacillus pumilus* SAFR-032 at a fluence rate of 0.1 mW/cm² and that of *Acinetobacter radioresistens* 50v1 at 0.5 mW/cm² ($p<0.005$) however this difference could be due to either species level differences and/or effects of fluence rate.

Materials and Methods:

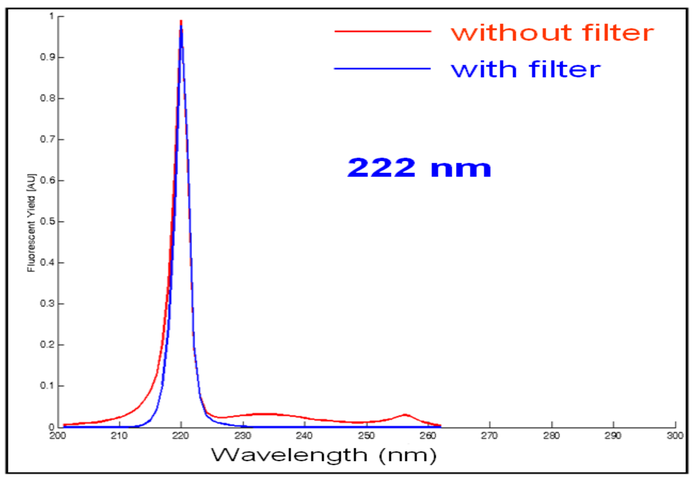

Figure 1. Measured non-filtered (red) and filtered (blue) UV spectra from our 222 nm KrCl excimer lamp.

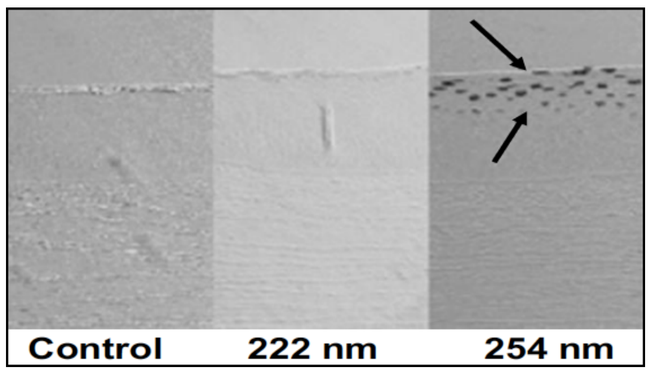

Figure 2. Bovine corneal tissue exposed to 300 mJ/cm2 of 222 nm far-UVC light or 254 nm germicidal light. Darkly stained CPD (cyclobutane pyrimidine dimers) positive nuclei are seen in the 254 nm exposed tissue, but the 222 nm exposed tissue shows no increase in CPD over zero-dose controls

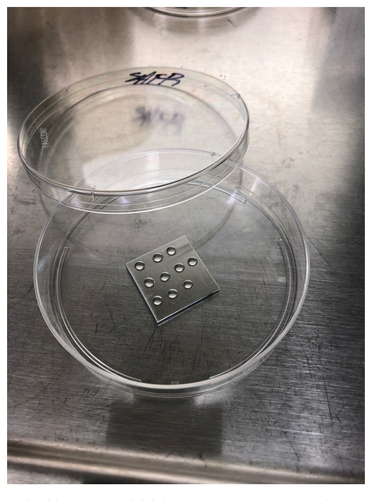

Figure 3. Aluminum 6061 coupons were used to seed the bacterial cells/spores into 10 individual spots of 10 ul each containing ~10^5 colony forming units (CFU). Spores/cells were recovered by first depositing 200 ul of polyvinyl alcohol and letting it air dry for 1 hour at room temperature then peeling the PVA film and dissolving it into 2 ml of sterile water/PBS respectively.

818-UV/DB low power UV enhanced silicon photodetector with an 843-R optical power meter (Newport, Irvine, Ca)

Discussion and Future Work:

Advantages of far-UVC light

- Requires minimal intervention by engineers to implement, and therefore does not impact the engineering scope of work.
- Can be implemented in the presence of humans throughout the assembly process, to minimize contamination during personnel handling.
- Can be used as a recontamination prevention strategy to ensure continuous microbial reduction in non-cleanroom areas such as the launch pad.
- Can be implemented in multi-project facilities and cleanrooms with varying cleanliness requirements to ensure no cross contamination of work areas occurs.

Future Work

- Perform side by side testing of bacterial spores and hardy vegetative cells using 254 nm UV light and 202 nm far-UVC light to characterize differences in efficiency
- Test a wider range of proxy spacecraft materials, facility surfaces, and ground support equipment to understand the practical applications of this technique for performing surface microbial reduction.
- Test a wider range of organisms to characterize the spectrum of resistance exhibited by various species previously isolated from spacecraft surfaces and cleanroom environments.
- Test a wider range of fluence rates to understand the appropriate implementation strategy.

National Aeronautics and Space Administration

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Publications:

Seuylemezian A, Buonanno M., Guan L., Brenner D., Welch D. The efficacy of far-UVC irradiation in inactivating spores of *Bacillus pumilus* SAFR-032 and cells of *Acinetobacter radioresistens* 50v1 on spacecraft proxy surfaces. (in draft).

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